PHARMACOKINETICS AND ANALGESIC POTENCY OF Δ⁹-TETRAHYDROCANNABINOL (THC)

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SUMMARY

It is known from the folk medicine that Cannabis may reduce pain. The aim of the pain study was to compare analgesic effects of oral delta-9-tetrahydrocannabinol (THC, dronabinol, Marinol®, main psychoactive component of the Cannabis plant) and a THC-morphine combination to morphine and placebo. This pain study was performed with 12 healthy volunteers in four different experimental models of acute pain. Additionally, side effects and vital functions were monitored and blood samples collected for the pharmacokinetic profiling of oral THC. In none of the pain models THC showed a significant analgesic effect. The THC-morphine combination showed a slight tendency to an additive effect compared to morphine alone, but this was not statistically significant. The side effects observed with THC were mainly sleepiness and mild to intermediate psychotropic side effects. The plasma concentrations of THC, analysed with gas chromatography mass-spectrometry, were very low, showed a plasma peak time of 60 to 120 min with high inter-individual variation. In addition, an extensive liver first pass metabolism could be observed leading to high metabolite-THC ratios.

In the second part of the present work the aim was to develop an application form as alternative to the Marinol® capsules. The very lipophilic THC was solubilised with Cremophor® RH 40 leading to a water-soluble THC formulation, which could be used as inhalation solution for the pulmonal administration of THC. This formulation underwent an in vitro quality assurance focussing on stability and physiological tolerability. Additionally, the particle size of the droplets in the aerosol and the output rate of the evaluated nebuliser system for the clinical application were determined.

In the third part of this work, the developed application form (inhalation solution) was used for a second clinical study with eight healthy volunteers. The pharmacokinetic properties of pulmonal THC were compared to intravenous THC and the analgesic effects were determined comparing with pulmonal placebo. With the pulmonal application form the very low bioavailability of oral THC could be increased up to 6-fold. Comparing the elimination half-lives, a 5-fold decrease of the half-life after pulmonal and intravenous THC compared to oral THC was observed, indicating that absorption is the time-determining step in the pharmacokinetic behaviour of orally administered THC. This was also reflected by the peak plasma concentration time, which occurred right at the end of the inhalation procedure of about 20 min (3 to 6-times earlier than with oral THC). Peak plasma concentrations were much higher after pulmonal than oral administration causing much less side effects, indicating that not only THC itself is responsible for the psychotropic side effects but also the known strongly psychoactive

11-hydroxy-THC. Metabolite-THC ratios were found to be much lower after pulmonal and intravenous THC than after oral THC.

The most prominent side effect of pulmonal THC was the irritation of the throat and coughing during the inhalation, which were reversible within short time after finishing the inhalation procedure.

Despite the increased bioavailability of pulmonal THC no analgesic effect resulted, suggesting that the bioavailability does not affect the efficacy in the pain reducing properties of THC. We assume that the used experimental pain models, which were all models of acute pain, were not appropriate to study the analgesic properties of THC. Further experiments are needed to evaluate the appropriate pain tests for THC and healthy subjects. In addition, it would be very interesting to investigate the analgesic effect of the pulmonal THC in patients suffering from chronic and neuropathic pain.

ZUSAMMENFASSUNG

Aus der Volksmedizin ist bekannt, dass Cannabis Schmerzen lindern kann. Das Ziel der Schmerzstudie war es, den analgetischen Effekt von Delta-9-Tetrahydrocannabinol (THC, Dronabinol, Marinol®, psychoaktive Hauptkomponente der Cannabispflanze) und einer THC-Morphin-Kombination mit Morphin und Placebo zu vergleichen. Die Schmerzstudie wurde an 12 gesunden Freiwilligen in vier verschiedenen experimentellen Akut-Schmerzmodellen durchgeführt. Zusätzlich wurden Nebenwirkungen und Vitalfunktionen überwacht und Blutproben für das pharmakokinetische Profiling des oral applizierten THC gesammelt. In keinem der Schmerzmodelle zeigte THC einen signifikanten analgetischen Effekt. Die THC-Morphin-Kombination zeigte eine leichte Tendenz zu einem additiven Effekt verglichen mit Morphin allein. Dieser Effekt war jedoch statistisch nicht signifikant. Die unter THC beobachteten Nebenwirkungen waren hauptsächlich Schläfrigkeit wie auch milde bis mittelstarke psychotropische Effekte. Die Plasmakonzentrationen des THC, die mit Gaschromatographie-Massenspektrometrie bestimmt wurden, waren sehr tief. Die maximalen Plasmakonzentrationen wurden nach 60 bis 120 min beobachtet und zeigten eine grosse interindividuelle Variation. Ausserdem wurde ein ausgeprägter First-pass Metabolismus, der zu hohen Metaboliten-THC-Quotienten führte, beobachtet.

Das Ziel im zweiten Teil der vorliegenden Arbeit war es, eine alternative Applikationsform zu den Marinol® Kapseln zu entwickeln. Das stark lipophile THC wurde mit Cremophor® RH 40 solubilisiert, was zu einer wasserlöslichen THC-Formulierung führte, die als Inhalationslösung zur pulmonalen Verabreichung verwendet werden konnte. Diese Formulierung wurde einer In-vitro-Qualitätssicherung unterzogen, die sich vorwiegend auf Stabilität und physiologische Verträglichkeit konzentrierte. Zusätzlich wurden die Partikelgrösse der Tröpfchen im Aerosol und die Freisetzungsrate des Verneblers, welcher für die klinische Applikation evaluiert worden war, bestimmt.

Im dritten Teil der vorliegenden Arbeit wurde die entwickelte Applikationsform (Inhalationslösung) in einer zweiten klinischen Studie acht gesunden Probanden verabreicht. Die pharmakokinetischen Eigenschaften von pulmonal appliziertem THC wurden mit denjenigen einer intravenösen Applikation verglichen. Gleichzeitig wurde der analgetische Effekt mit pulmonalem Placebo verglichen. Die sehr tiefe Bioverfügbarkeit von oral appliziertem THC konnte mit der pulmonalen Applikationsform bis auf das Sechsfache erhöht werden. Die Eliminationshalbwertszeiten nach pulmonaler und intravenöser Verabreichung waren im Vergleich zur oralen Form um das Fünffache verkürzt, was darauf hindeutet, dass die Absorption der geschwindigkeitsbestimmende Schritt im pharmakokinetischen Verhalten von

oral verabreichtem THC ist. Dies widerspiegelte auch der Zeitpunkt der maximalen Plasmakonzentration, der unmittelbar nach Beendigung der Inhalation (nach ca. 20 min, und somit 3 bis 6mal schneller als nach oraler Verabreichung) erreicht wurde. Die maximalen Plasmakonzentrationen waren viel höher nach pulmonaler als nach oraler Verabreichung und verursachten gleichzeitig weniger Nebenwirkungen. Dies weist darauf hin, dass nicht nur THC selber für die psychotropen Nebenwirkungen verantwortlich ist, sondern auch der stark psychoaktive Metabolit 11-Hydroxy-THC. Die Metaboliten-THC-Quotienten waren nach der pulmonalen und intravenösen Verabreichung viel tiefer als nach der oralen.

Die auffälligste Nebenwirkung von pulmonal appliziertem THC war die Irritation der Atemwege und der Hustenreiz während der Inhalation. Diese Irritation war innerhalb kurzer Zeit nach Beendigung der Inhalation reversibel.

Trotz der verbesserten Bioverfügbarkeit von pulmonalem THC konnte kein analgetischer Effekt gezeigt werden, was darauf hinweist, dass die Bioverfügbarkeit die Wirksamkeit nicht beeinflusst. Dies lässt vermuten, dass die eingesetzten experimentellen Schmerzmodelle, welche ausschliesslich Akut-Schmerzmodelle sind, zur Untersuchung der analgetischen Eigenschaften von THC nicht geeignet waren. Weitere Experimente sind erforderlich, um geeignete Schmerztests für THC an gesunden Probanden zu evaluieren. Es wäre ausserdem sehr interessant, den analgetischen Effekt von pulmonal verabreichtem THC an Patienten zu untersuchen, welche an chronischen oder auch neuropathischen Schmerzen leiden.

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INTRODUCTION AND AIM OF THE PRESENT WORK

The medicinal use of Cannabis has a very long tradition. It has been used for a variety of symptoms including for example pain, sleeplessness, and pre-menstrual symptoms [1]. The discovery of the endogenous cannabinoid system in the early 1990ies opened a broad field for research helping to understand more and more the molecular mechanisms of the Cannabis effects and the links to other modulating or regulatory systems in our body [2]. In addition, the endogenous cannabinoid system offers a lot of new targets for drug therapy [3].

Beside the research in molecular biology, the known indications of Cannabis from the folk medicine have to be clinically investigated. Some applications, e.g. against chemotherapy-induced nausea and vomiting or as antianorectic drug, of Δ^9-tetrahydrocannabinol (THC, the main psychoactive component of the Cannabis plant) are already established. Marinol®, a soft gelatine capsule containing THC dissolved in sesame oil, is a FDA-registered drug, which can be used in Switzerland for clinical research.

The analgesic effect of THC is not yet sufficiently investigated. We know from the folk medicine and anecdotal reports that Cannabis may reduce pain. It is widely used in self-medication to relieve pain of different origins such as back pain, headache, and migraine [4]. Several animal studies in different pain models show the antinociceptive effect of THC [5, 6]. There it has been shown that THC even potentiates the analgesic effects of morphine [7]. In humans there are only few controlled clinical studies and the results are not convincing. This motivated us to perform controlled clinical trials with established experimental pain tests. The aim of the pain study was to test the antinociceptive effects of oral THC and THC combined with morphine (THC-morphine) versus morphine and placebo in healthy subjects under experimental pain conditions. In addition to the pain tests the adverse effects should be monitored and plasma profiles be acquired to study the pharmacokinetics of THC and to check for a possible correlation of the plasma concentrations with analgesia and side effects.

In the second part of this work the aim was to develop an application form as alternative to the Marinol® capsules. As administration route the lung was chosen. Some pharmacokinetic studies have shown that smoked Cannabis produce early and high THC plasma levels [8, 9]. Smoking Cannabis for medical use is however not acceptable and therefore a “cold” inhalation form according to pharmaceutical standards and preventing toxic pyrolyse by-products should

be developed. This inhalation solution could also be an alternative preparation to be used in further studies with THC in other indications (e.g. migraine, chronic or neuropathic pain). The pulmonally administered inhalation solution (liquid aerosol) allows to avoid the well known extensive liver first pass metabolism [10, 11] of oral THC. The commercially available, pressure driven nebuliser system consisting of a Pari Master® (producing the air pressure) coupled to a nebuliser (either the IS-2® or the LC-Plus®), which was already used in our laboratory in a study with diacetylmorphine [12], should be evaluated for the THC inhalation solution. The very lipophilic THC should be dissolved in an aqueous vehicle using an appropriate pharmaceutical technique. The targets for the inhalation solution were the following:

- physiological tolerability (e.g. aqueous vehicle with physiological properties, well tolerable adjuvants)
- appropriate concentration of the THC to realise the shortest possible inhalation time
- appropriate properties to be nebulised with the chosen inhalation device
- appropriate properties to produce the necessary droplet size of 2-5 µm in diameter to reach the lower compartments of the lung
- appropriate stability of the THC in the inhalation solution to use the formulation for a pharmacokinetic study in humans
- properties according to the standards of the European Pharmacopeia.

In the third part of this work the developed inhalation solution should be used for a second clinical study with healthy volunteers. The aim was to investigate the pharmacokinetic properties of pulmonal THC versus intravenous THC. In addition, the antinociceptive effect of THC should be tested versus placebo by one of the four pain tests used in the first study. Side effects and vital functions should be monitored.

THEORY / LITERATURE REVIEW

1. CANNABIS SATIVA L.

1.1 Botany [13-15]

Cannabis (hemp) and Humulus (hop) are the only two genera in the family of Cannabaceae. Cannabis sativa L. grows in all warm and temperate zones except the tropical rain forests. Cannabis sativa L. is an annual plant with a life cycle of a few months. Plants of Cannabis sativa grow 1 - 5 m high and are variously branched or even unbranched if planted closely together. The whole plant is covered with minute hairs. The leaves and branches are paired at the bottom and arranged alternating higher on the stem. The leaves have a slender stalk up to about 6 cm long. They are pinnate in a digitate form. The 5 to 9 leaflets are mostly narrowly lanceolate, coarsely saw-toothed, and have a long drawn-out pointed tip.

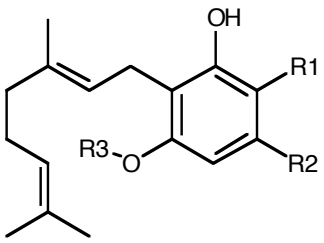
Cannabis is normally dioecious meaning that a plant has either female or male flowers. Female plants are very leavy up to the top; male plants have the leaves on the inflorescence fewer and much further apart. The male inflorescence is loosely arranged, much branched and many-flowered, standing out from the leaves, with individual flowering branches up to 18 cm long. Female inflorescences do not project beyond the leaves; they are compact, short and few-flowered, with flowers borne in pairs. The female flowers are covered with slender hairs and circular glands secreting drops of resin, which are produced most abundantly under hot conditions; in nature the function of these resin glands is presumably to protect the plant from animals.

The fruit is technically an achene. It is ellipsoid, slightly compressed, smoth, about 2.5 - 5 cm long and 2 - 3.5 cm in diameter, grayish, brownish, or variously patterned.

1.2 Constituents of Cannabis [13, 16]

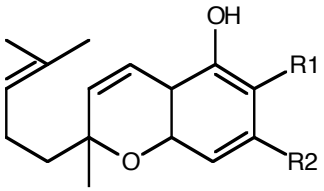
Cannabis sativa contains over 480 compounds of which more than 65 are cannabinoids (“phytocannabinoids”). The cannabinoids are the most characteristic and most specific components that are only occurring in the Cannabis plant. They are quite lipophilic, nitrogen-free, mostly phenolic, and have a C_{21}-skeleton. Biogenetically, the cannabinoids are derived from a monoterpene and a phenol or a phenolic acid, respectively. According to the difference in the terpene part of the molecule the cannabinoids can be classified in the following way: the cannabigerol[(1)]-, the cannabichromene[(2)]-, the cannabidiol[(3)]-, the tetrahydrocannabinol[(4)], and

the cannabinol[5]-type, as well as some minor cannabinoid classes. The cannabinol-type is an artefact formed by oxidation of biogenic cannabinoids that are produced during storage or extraction. The structures of the different cannabinoid types are presented in Figure 1.



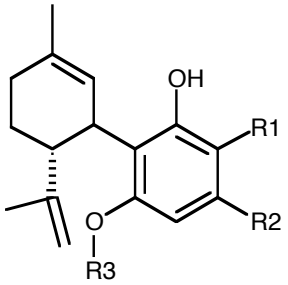
R1 = H or COOH
R2 = C_3- or C_5-sidechain
R3 = H or CH_3

(1) Cannabigerol type



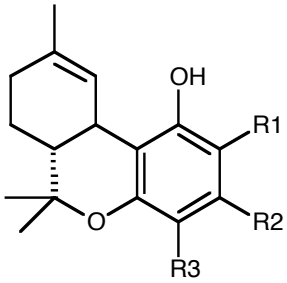
R1 = H or COOH
R2 = C_3- or C_5-sidechain

(2) Cannabichromene type



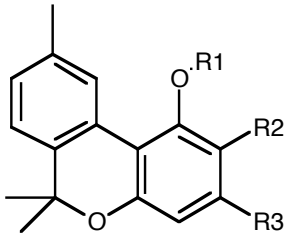
R1 = H or COOH
R2 = C_1-, C_3-, C_4-, or C_5-sidechain
R3 = H or CH_3

(3) Cannabidiol type



R1, R3 = H or COOH
R2 = C_1-, C_3-, C_4-, or C_5-sidechain

(4) Tetrahydrocannabinol type



R1 = H or CH_3
R2 = H or COOH
R3 = C_1-, C_3-, C_4-, or C_5-sidechain

(5) Cannabinol type

Figure 1: Structures of the different cannabinoid types

The circular glands produce also an essential oil, which is responsible for the characteristic smell of the hemp plant. It contains mono- and sesquiterpenes (e.g. β-caryophyllene, humulene, caryophyllene oxide, α- and β-pinene, limonene) and phenylpropane derivatives (e.g. cis- and trans-anethol, eugenol). Minor components of the hemp plant are other phenolic compounds (e.g. spiroindane, dihydrostilbene, dihydrophenanthrene derivatives), flavonoids, nitrogen-containing compounds (amines, amides), amino acids, and sugars.

1.3 Cannabis products [13, 14, 17, 18]

Cannabis resin

The resin ("Hashish") is collected mainly from the circular glands of the female inflorescence. The brownish hashish is pressed to bars or cakes. The THC content of the resin is about 2 to 10 % (or even higher, up to 25 - 30 %).

Cannabis-oil (=red oil, Indian oil)

Cannabis oil is an extract of hashish prepared by solid-liquid-extraction with an organic solvent or distillation technique. Cannabis oil can have a THC content of up to 65 %.

Cannabis herb

The herb ("Marihuana", "Marijuana") consists of the dried, cut, blooming or even fruit-bearing, green tops of the female (also male) Cannabis plant. The THC content is usually 0.5 to 2 % or even higher, up to 14 - 30 % [18].

Fatty oil of the seeds

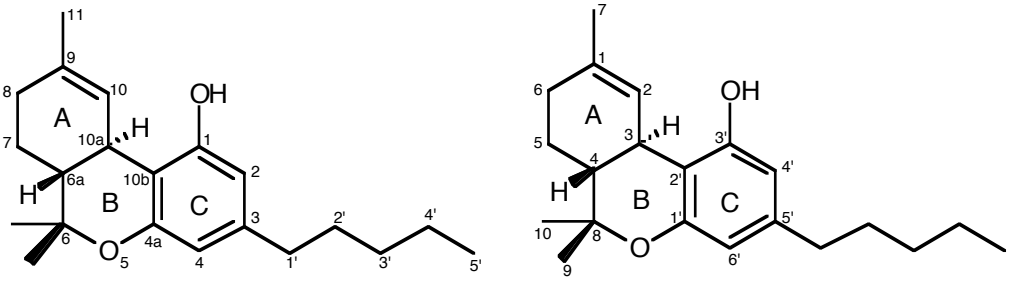
The fatty oil of the seeds is very valuable for food because it contains a lot of essential fatty acids. Provided that contamination during the extraction process can be avoided, it does not contain THC.

Swiss narcotic law for Cannabis preparations

All Cannabis preparations for the production of narcotics underlay the act of narcotics and are listed in the category of the forbidden substances [19-21]. Cannabis preparations for the production of food (e.g. fatty oil of the seed) are part of the food regulations where specific limitations of the THC content for each preparation are given (0.2 - 50 mg/kg) [22]. Hemp plants for the production of fibers or oil are regulated by decrees of the Swiss Department of Agriculture, i.e. the THC content is limited to < 0.3 % THC [23].

2. Δ⁹-TETRAHYDROCANNABINOL (THC)

2.1 Structure



Δ⁹-Tetrahydrocannabinol

Dihydrobenzopyran-numbering (IUPAC)

$C_{21}H_{30}O_2$

Δ¹-Tetrahydrocannabinol

Monoterpene-numbering

$C_{21}H_{30}O_2$

Figure 2: Structure of THC

Figure 2 shows the structure of THC using two different numbering systems. The one resulting in the Δ¹-tetrahydrocannabinol is based on the monoterpene-numbering and the other resulting in Δ⁹-tetrahydrocannabinol is based on the dihydrobenzopyran-numbering according the IUPAC (International Union of Pure and Applied Chemistry) rules. The latter is usually used nowadays. THC is the only biogenic, psychotropic substance without nitrogen.

2.2 Structure-activity relationship (SAR) at the cannabinoid receptor (CB-receptor) [2, 24]

In the late 1980ies the first cannabinoid receptor has been found and in 1990 the CB_1-receptor has been cloned. This was followed by the finding of the first endogenous ligand anandamide, the cloning of the CB_2-receptor, by the development of the first CB_1-receptor antagonist (SR 141716A), and by the finding of the second endogenous ligand 2-arachidonoyl-glycerol (2-AG). The description of the endocannabinoid system follows in chapter 2.4.2 (“Pharmacodynamics of THC”).

For the binding of THC to the cannabinoid receptor CB_1 different essential structure-activity relationships have been postulated. The dihydrobenzopyran-type structure with a hydroxyl-group at C_1 and an alkyl group C_3 is present in most active cannabinoids. Opening of the pyran ring (forming cannabidiol-type compounds) lead to a complete loss of activity at CB_1. The phenolic group at the C_1-position has to be free and the alkylic side chain with at least 5 carbon atoms at position C_3 is of considerable importance: elongation and branching results in

potentiation. A stereochemical requirement for the activity on the receptor is the trans-link of the two rings A and B at position C_{6a} and C_{10a}.

2.3 Chemical and physical data

Some physical and chemical properties of THC are listed in Table 1.

Table 1: Physical and chemical properties of THC

Systematic name [25]	Tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol
Synonym	Dronabinol
Molecular weight [25]	314.45
Molecular fomula [25]	$C_{21}H_{30}O_2$
Boiling point [25]	200°C
Property to rotate plane polarised light [25]	$[\alpha]_D^{20}$ - 150.5° (c = 0.53 in $CHCl_3$)
UV max in ethanol [25]	283 nm and 276 nm
Solubility [26]	Highly insoluble in water (~ 2.8 mg/L, 23°C) and 0.15 M NaCl (0.77 mg/L, 23°C)
	High solubility in ethanol, methanol
Octanol water partition coefficient [27]	12091
pK_a [26]	10.6
Stability [26]	Unstable in acidic solution ($t_{½}$ = 1h at pH 1.0, 55°C)
	Sensitive to light
Description [28]	Yellow resinous oil, sticky at room temperature, hardens upon refrigeration, without smell, bitter taste

2.4 Pharmacology and toxicology

2.4.1 Pharmacokinetics

Absorption and distribution [9-11, 13, 28, 29]

Inhalation of Cannabis smoke leads to a rapid absorption of THC depending on the inhalation technique and experience in smoking. Therefore, the values for the bioavailability in the literature are very variable (15 - 50 %). Oral administration leads to more erratic uptake as a result of degradation of the drug by stomach acids and extensive liver first-pass metabolism. A lipophilic vehicle can increase the absorption. The administration of THC dissolved in sesame oil (Marinol® capsules) leads to an almost complete absorption (90 - 95 %). But the measured bioavailability after orally administered Marinol® is only 10 - 20 % due to the extensive liver first-

pass metabolism [28]. The application of THC as a prodrug (THC-hemisuccinate) in suppositories shows more constant and a higher bioavailability compared to the oral application form (about two fold higher) [30].

Because of their high lipophilicity, the cannabinoids rapidly penetrate the tissues and high concentrations are found in the highly vascularised tissues shortly after drug administration. This is reflected in the high volume of distribution of about 10 L/kg [31-33]. The main distribution sites are liver, heart, lung, gut, kidney, spleen, mammary gland, placenta, adrenal cortex, thyroid, pituitary gland, and brown fat. Lower concentrations are found in brain, testis, and fetus.

Once absorbed into the blood THC becomes strongly bound to protein. Only about 3 % of the drug is in the free state. About 60 % is bound to lipoproteins (the low-density fraction), about 9 % to the blood cells, and the rest to albumin. The major monohydroxy metabolite 11-hydroxy-Δ^9-THC (THC-OH) is even more strongly bound with only 1 % remaining in the free state.

Metabolism [10, 11, 13]

The metabolism of the cannabinoids is very complex. For THC nearly 100 metabolites have been identified. All cannabinoids are good substrates of the cytochrome P450 mixed-function oxidases on account of their high lipid solubility. THC is hydroxylated at C_{11}, at C_8 and at all positions of the alkyl side-chain. C_{11} is the preferred hydroxylation site in man. In humans, cytochrome P450 isoenzyme 2C9 has been shown to catalyse the formation of the psychoactive 11-hydroxy metabolite of Δ^9-THC [34, 35] and cytochrome P450 3A to be responsible for hydroxylation at the 8β-position [35]. Following the initial hydroxylation, many of the hydroxyl groups undergo further oxidation with the major products being the formation of carboxylic groups at C_{11} and $C_{5'}$ (alkyl side-chain). Oxidation of the active metabolite THC-OH leads to the inactive metabolite 11-nor-9-carboxy-THC (THC-COOH), which is in the glucuronidated form the main metabolite found in urine. The $C_{5'}$-acid undergoes β-oxidation and related reactions result in losses of carbon atoms from the alkyl side-chain. Phase II metabolites appear to be mainly conjugates of the phase I metabolites with glucuronic acid. The O-ester-glucuronide of THC-COOH is the predominant phase II metabolite of THC. Another possible pathway is the formation of conjugates with long-chain fatty acids. Although it is a phase II reaction, it increases rather than decreases lipophilicity. These compounds, which resemble membrane constituents, are thought to be the form in which the drug may be retained within tissues for considerable periods of time.

Figure 3 shows the possible sites for the primary oxidation of THC. Figure 4 shows the main metabolic pathways for THC.

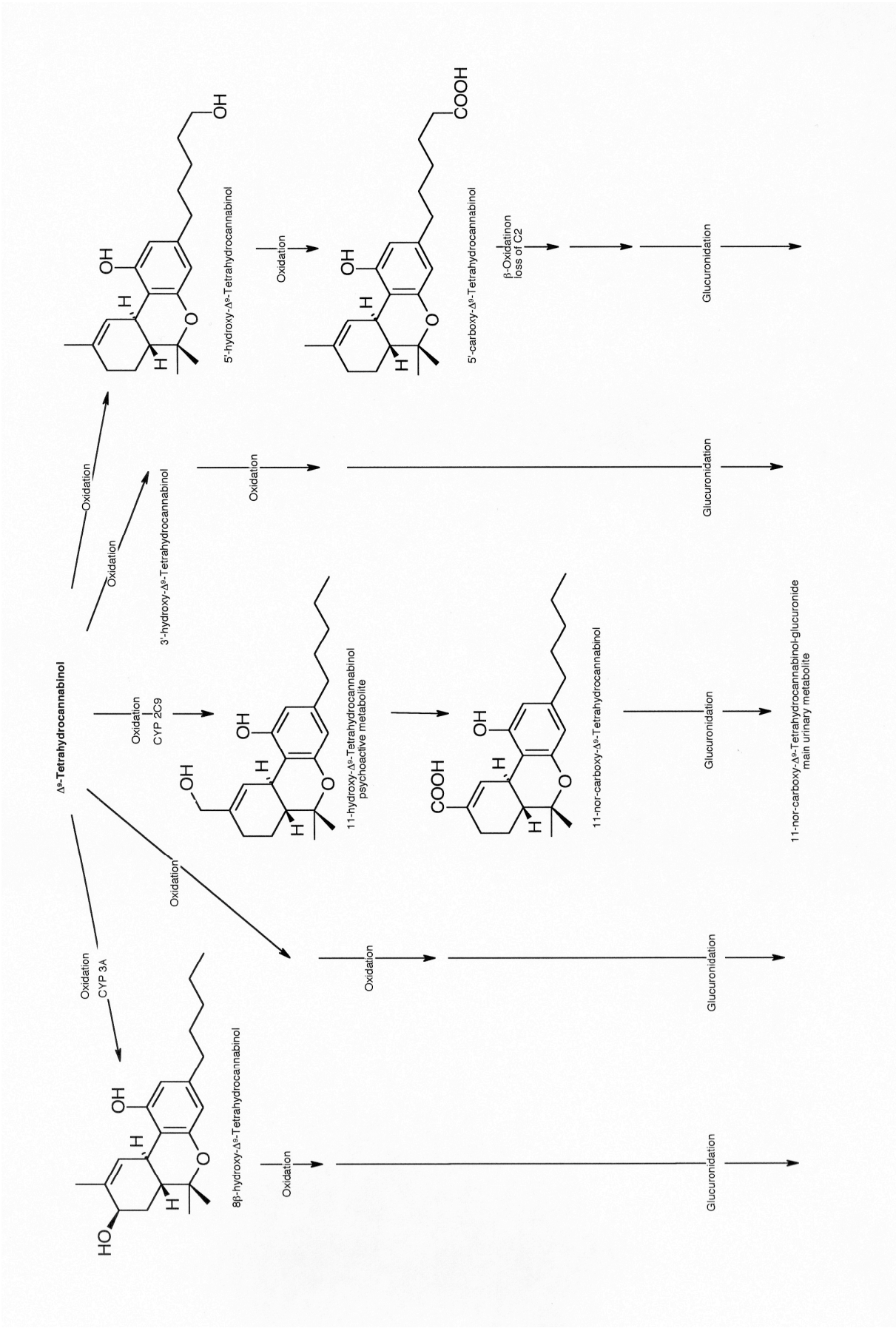


Figure 4: Main metabolic pathways for THC

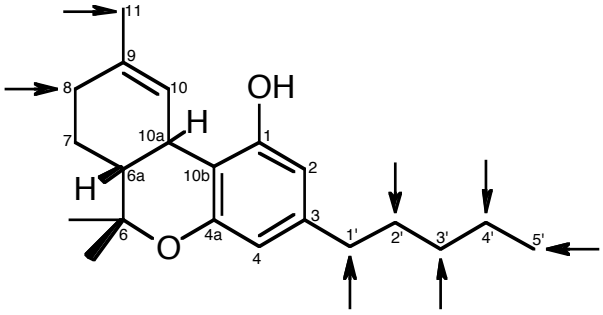


Figure 3: Sites for primary oxidation of THC

Excretion [10, 11, 13]

In common with other lipophilic drugs THC rapidly distributes between the tissues. However, the extensive deposition in fat results in a long terminal half-life. The real elimination half-life is very difficult to estimate due to the slow equilibration of the concentrations between plasma and tissue. This results in very low plasma levels, which are difficult to measure. The reported half-lives in the literature are with 1 to 4 days very variable. The complete elimination time may well reach up to 5 weeks.

THC is excreted both in urine and feces as metabolites. Feces are the preferred route in humans, with about 80 % of the excreted dose. THC undergoes an extensive enterohepatic cycle, which also contributes to the slow elimination. Most urinary metabolites are acids. The main metabolite found in urine is the THC-COOH glucuronide, which, normalised to the creatinine concentration, can be used for the detection and monitoring of drug abuse.

2.4.2 Pharmacodynamics

Cannabinoid receptors and the endogenous cannabinoid system [36, 37]

As mentioned in chapter 2.2 mammalian tissues contain two types of cannabinoid receptors, CB_1 and CB_2, both coupled to their effector systems through $G_{i/o}$ proteins. CB_1 receptors are present in the brain, the spinal cord, and in certain peripheral tissues. Some central and peripheral CB_1 receptors are located at nerve terminals where they probably modulate neurotransmitter release when activated. CB_2 receptors are expressed primarily by immune tissues, for example leukocytes, spleen, and tonsils. The discovery of CB_1 and CB_2 receptors was followed by the detection of endogenous cannabinoid-receptor ligands. Arachidonoyl-ethanolamide (anandamide) and 2-AG are the most important ones and there is evidence that both agonists can act as neuromodulators or neurotransmitters. Experiments with isolated blood

vessels have shown that anandamide and methanandamide (but no other ligand of the CB receptors) activate vanilloid receptors on sensoric neurons [38]. These findings indicate that anandamide is also an endogenous ligand for vanilloid receptors and therefore the vanilloid receptors represent possibly a cannabinoid CB_3 receptor.

The cannabinoid receptors and their endogenous ligands constitute what is now often referred to as “the endogenous cannabinoid system” or “endocannabinoid system”. The endocannabinoid system seems to play a fundamental physiological role in the regulation and modulation of processes in the central nervous system (neutrotransmitter release) and in the periphery (such as modulation of neurotransmitter release/action of autonomic and sensory fibers), as well as in the control of immunological, gastrointestinal, reproductive, and cardiovascular performance [2]. All these functions and the links to neurotransmitter systems are not yet fully understood.

Several selective synthetic agonists and antagonists have been synthesised for both CB receptors and are available for basic and clinical research.

Possible biosynthetic and catabolic pathways for anandamide are presented in Figure 5.

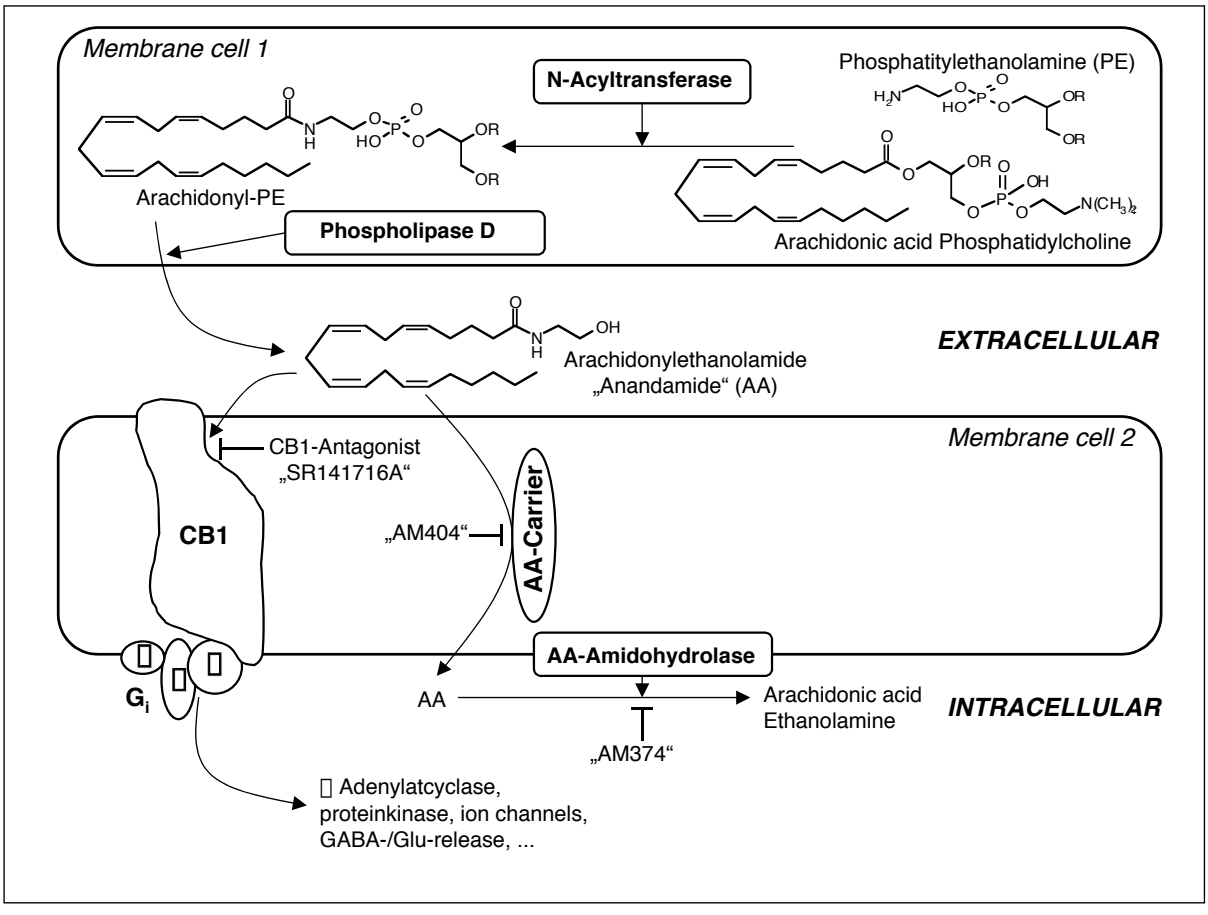


Figure 5: Possible biosynthetic and catabolic pathways for anandamide modified from Di Marzo and Petrocellis [2].

Therapeutic potential [39]

THC or Cannabis preparations are used for a broad spectrum of symptoms. But the data of the efficacy are often very limited. Well established is the antiemetic and anti-vomiting effect in chemotherapy-induced nausea and the appetite stimulating, weight increasing effect, e.g. in cancer, aids or Alzheimers disease patients. For these indications Marinol® is registered and used in the USA. Newer studies show a quite good effect of THC or Cannabis preparations (e.g. standardised extracts) in spasticity, moving disorders, and pain in patients suffering from multiple sclerosis, spinal cord injuries, or Tourette syndrome. THC is antiasthmatic and lowers intraocular pressure in glaucoma. Some first studies and case reports show THC effects against allergy, pruritus, inflammations, infections, epilepsy, depressions, bipolar disorders, anxiety, addiction, and withdrawal syndromes. Some basic research has been done in different fields: autoimmune diseases, cancer, neuroprotection, fever, and high blood pressure.

2.4.3 Side effects and toxicology

Acute toxicology [40]

The acute toxicity of THC is very low. The oral LD_{50} dose in rats was determined as 800 to 1900 mg/kg. No lethal doses could be determined in dogs (up to 3000 mg/kg administered) and monkeys (up to 9000 mg/kg). Also in men no deaths directly linked to toxic somatic effects of Cannabis have been reported so far. Nevertheless, there are different acute effects of Cannabis. Normally a Cannabis-“high” is reported to be a pleasant, relaxing experience. On the contrary there are reports of dysphoric reactions that can also lead to panic attacks. Overdosing can produce fear of death. Cannabis causes dose dependent sleepiness, mental clouding, dizziness, and confusion. It impairs cognitive functions, memory, attention, ability to react, and motor functions, and therefore also the ability to drive a vehicle.

Chronic toxicology [40, 41]

Many chronic effects of Cannabis have been described. The main risk of long-term Cannabis consumption consists of the mucous membrane damaging effect of the pyrolysis products when inhaling the smoke of a Cannabis cigarette (“joint”). There are indications that heavy Cannabis users can show slight impairment of cognitive functions and memory. THC is able to induce “temporal disintegration”, which is defined as a difficulty in regaining, coordinating, and serially indexing those memories, perceptions, and expectations that are relevant to the attainment of some goal [42]. Consuming Cannabis can trigger a latent schizophrenia in vulnerable persons without increasing the incidence of schizophrenia. The chronic toxic effects on the immune and

endocrine system are inconsistent. Also the data concerning the influence on fertility are contradictory. There is no evidence of an impairment of these functions in man. On the other hand, there are strong indications that Cannabis can cause disturbance in the development of subtle cerebral functions in a fetus of a Cannabis consuming pregnant woman resulting in impaired cognitive functions in the child. A teratogenic effect of Cannabis is unlikely.

Tolerance [43]

Tolerance is developed within short time against the receptor mediated effects of Cannabis such as the psychotropic and cardiovascular effects as well as the cognitive and psychomotor side effects. Heavy chronic Cannabis consumers report a mild withdrawal syndrome when they suddenly stop taking Cannabis, characterised by nervous agitation, hyperhydrosis, and lack of appetite. However, the Cannabis dependence is mainly considered as psychological and not somatic. The risk for Cannabis abuse in the therapeutic context is low.

Somatic side effects [40]

Acute side effects are hyposalivation (atropine-like effect), increase in heart rate, reddening of the eyes, orthostatic hypotension. Further rare side effects are headache, nausea, and vomiting. Hyposalivation and the decrease in the flood of tears can increase the risk of infections of the upper respiratory tract and the eyes.

3. CANNABIS AND PAIN

3.1 Animal data

Several animal studies have shown the analgesic effect of THC in different pain models [5, 6, 44]. In mice, subcutaneously (s.c.) and orally (p.o.) administered THC enhanced the antinociceptive effect of s.c. and p.o. morphine in the tail-flick and also in the paw-pressure test [45]. Again in mice, an inactive p.o. dose of THC (20 mg/kg) enhanced the antinociception of opioids 2.2- (for morphine) to 25.8-fold (for codeine, shift in ED_{50}) in the tail-flick test [7].

3.2 Human data

There are few experimental pain studies and they do not clearly show an analgesic effect of THC. The results are controversial. Smoked Cannabis increased pressure pain tolerance in Cannabis-naïve and Cannabis-experienced subjects compared to placebo (THC-extracted Cannabis) [46]. In a radiant heat test experienced Cannabis users reported a mild antinociceptive effect when smoking Cannabis cigarettes [46]. In thermal pain p.o. THC showed in healthy subjects with moderate side effects a reduction of pain and in subjects experiencing a “bad trip” hyperalgesia [48]. In the transcutaneous electrical stimulation pain model, smoked Cannabis had no analgesic effect, it even produced a slight hyperalgesia [49].

In clinical pain again, only few trials have been conducted and the results were equivocal [50]. Oral doses of 15 and 20 mg THC resulted in a significant reduction of cancer pain [51]. In another clinical study, the analgesic potency of THC in cancer pain was compared with codeine. The analgesic effect of 20 mg p.o. THC corresponded to that of 120 mg p.o. codeine [52] whereas i.v. administered THC did not affect pain tolerance thresholds in dental surgical pain [53]. In a double-blind, placebo-controlled, crossover trial on a chronic pain patient suffering from familial Mediterranean fever five doses of 10 mg p.o. THC (as standardised Cannabis preparation, containing 5.75% THC) per day did not reduce pain [54]. However, it significantly reduced the need for morphine (10 mg per dose) given as escape medication indicating an additive effect of THC on morphine. A case report study showed that smoked Cannabis, in low doses not causing psychotropic side effects, reduced the required opioid doses for pain management in chronic neuropathic pain in three patients by 60 to 100 % [55].

Some patients claim that Cannabis (e.g. smoked or ingested as cookie) is more effective than pure THC (e.g. dronabinol, Marinol®) for a variety of symptoms, including nausea and vomiting, wasting syndrome, and muscle spasticity [56, 57]. However, most of these assertions are based on patient reports and surveys, and have not been verified by controlled clinical trials [58]. To the best of our knowledge, no comparative data exist of the analgesic effect of THC and Cannabis. A recent study compared the subjective effects of orally administered and smoked

THC alone and THC within Cannabis preparations (brownies and cigarettes) [58]. THC and Cannabis in both application forms produced similar, dose-dependent subjective effects, and there were few reliable differences between the “THC-only” and the “Cannabis” conditions. In studies on healthy volunteers and a multiple sclerosis patient it was shown that cannabidiol (CBD) reduces the psychotropic effects of THC [59, 60] and a Cannabis-based medicinal extract [61], respectively. This could explain anecdotal reports from patients who prefer the milder forms of Cannabis containing significant levels of CBD [61]. A review of the antianxiety effects and the pharmacology of CBD is given by Partland and Russo [62] and Mechoulam et al. [63].

4. INHALATION

4.1 Physiology of the lung [64]

The lung is the organ of respiration. Its function is to maintain blood gases (nominal values of partial pressure for O_2 = 90 mm Hg, and for CO_2 = 40 mm Hg) exchanging the CO_2 of the erythrocytes with O_2 in the alveoles and so arterialising the blood. The lung divides dichotomously for about 23 generations until it reaches the alveolar sacs, which number approximately 300 millions and covers some 70 m^2. Distributed over this surface is 80 mL of blood in the alveolar capillaries, through which O_2 is given up and absorbed. The conducting airways are surrounded by smooth muscles and are innervated. They are also lined with specialised cells: some of which produce mucus, others who carry cilia. Together they form an escalator carrying mucus and deposited inhaled material upwards to maintain the lung in a sterile condition. The alveolated surface is covered with a surfactant lining (about 0.5 nm thick) which determines the normal compliance (distensibility) of the parenchyma.

4.2 Inhalation therapy [65, 66]

The administration of drugs directly into the respiratory tract is widely regarded as the method of choice for treating respiratory diseases. In addition, there has been an increase in utilising the airways and the respiratory membrane surface as a portal of entry into the body for drugs that otherwise would not be absorbed (for example because of acidic degradation of peptides such as insulin or desmopressin in the stomach) or for those drugs that suffer extensive liver first-pass metabolism after oral administration. Although some drug metabolism (e.g. by cytochromes P450) also takes place in the respiratory tract, this metabolism is of minor importance, because the presence of these enzymes and their capacity therefore are much

lower than in the liver. Some inhaled drugs are metabolised then in the lung, others are absorbed very rapidly and therefore do not undergo a metabolism in the lung.

A crucial point in the drug administration via the respiratory tract is the particle size of the inhaled droplets (liquid aerosol) or solid particles (powder aerosol). For a maximal absorption the high surface region of the alveolar compartment, which is also highly vascularised, has to be reached. Particles with a size of 2 - 5 μm are able to deposit in the alveoli. Particle with a size of 5 - 10 μm get to the upper parts of the lung, the bronchioli, and particles > 10 μm will deposit in the extrathoracic regions. Studies showed that deposition could be maximised in the lower regions of the lung by inhaling slowly and holding the breath for up to 20 seconds [67].

4.3 Novel liquid-based inhalation technology [68]

Drugs can either be administered in solid or liquid form to the lungs. Steroids in the therapy of asthma are usually powders and are administered with special devices (e.g. Turbohaler®, Diskhaler®). The today's conventional liquid inhalation devices are on one hand the pressurised metered dose inhalers (pMDI's) and on the other hand the nebulisers. pMDI's are hand-held and deliver the drug in one breath or a few breaths. The drug is either suspended or dissolved in a propellant, which is pressurised until it liquefies in a canister. Releasing a metered volume of the fluid through a control valve causes the propellant to expand and evaporate and leaves the drug in the form of a high velocity aerosol. The limitations are the high exit velocity of the drug aerosol which can lead to high levels of oropharyngeal impaction, and the need for users to coordinate the pMDI valve actuation with their breathing manoeuvre.

The nebulisers are generally larger fixed devices and deliver the drug over many breaths sometimes taking several minutes. They traditionally use compressed air directed through the liquid drug formulation in the form of a high velocity jet to produce a fine mist of droplets from an aqueous solution of the drug. Using a baffle arrangement, inhalable droplets are permitted to pass into the inhaled air stream while oversized droplets are filtered and recycled. The specific limitations of nebulisers are: relatively long treatment times, poor dose efficiency (due to residual volumes), and often lack of portability. Nebulisers of the newest generation are smaller and some even portable.

Novel liquid-based inhalation devices have been developed in the last few years. They can be grouped into three types: mechanical, vibrational, and electrostatic.

Mechanical devices usually use air compressed by a spring to aerolise a drug formulation. The Respimat® of Boehringer Ingelheim is a small, pocket-sized device (dimensions similar to a traditional pMDI) and contains the drug in an appropriate formulation without environment-polluting propellant. The drug is loaded and nebulised with a mechanical rotation of the lower

half of the device by the patient. Another example is the AERx® from Aradigm, which is a hand-held battery powered inhalation device. It utilises a unit dose of drug stored in a blisterpack. When loaded by the user a piston compresses the blister, a heat seal is opened, and the drug is forced through a polymeric nozzle plate producing the aerosol. This device is controlled by a microprocessor.

Vibrational devices can either be ultrasonically driven or membrane-vibrated. The Premaire® from Sheffield Pharmaceuticals is a pocket-sized ultrasonic nebuliser. The device utilises an ultrasonic atomising horn, which vibrates at an ultrasonic frequency and aerosolises a liquid dose into a fine, low-speed aerosol mist. Vibrating-membrane devices use a perforate membrane in contact with a reservoir of fluid, which vibrates at ultrasonic frequencies. Jets of fluid are drawn through the holes in the membrane where surface tension and hydrodynamic effects then break these jets into a steam of precisely controlled droplets. Examples for this technology are the Touch Spray® from ODEM LTD, the e-Flow® from PARI GmbH, and AeroDose® from Aerogen.

An example for the electrostatic technology is the Mystic® device from Battelle Pulmonary Therapeutics. It uses electrohydrodynamic nebulisation, which is a process of turning a liquid into a spray of fine droplets using a strong electric field.

Most of the novel liquid devices have been shown to be capable of delivering considerably more of the emitted dose to the lung and of minimising oropharyngeal impaction, compared with conventional devices, because of the much greater control of the droplet size and a much lower-velocity aerosol than pMDIs.

5. SOLUBILISATION [69, 70]

Solubilisation is a process to increase the solubility of lipophilic drugs in water (e.g. lipophilic vitamins, hormones). For this purpose surface-active substances are used to dissolve the drugs into clear, eventually opalescent solutions. These so called surfactants are amphiphilic substances consisting of hydrophilic as well as lipophilic parts within the molecule. There are three groups of surfactants: the anionic surfactants (the surfactant molecule is negatively charged forming a salt with e.g. sodium ions), the cationic surfactants (the surfactant molecule is negatively charged forming a salt with e.g. chloride), and the non-ionic surfactants (without charge). In pharmaceutical technology mainly the non-ionic type is used. Added in a certain concentration (the critical micelle concentration, CMC) into water, they have the property to form micelles in solution. Micelles are molecular aggregates of surfactant molecules with the incorporated drug, called association colloids. These particles are usually 5 - 15 nm in diameter, spherical in shape in a surfactant concentration near the CMC and mostly laminar in a higher

surfactant concentration underlying a continuous dynamic equilibrium. Another characteristic property, beside the CMC, of each surfactant is the hydrophilic-lipophilic-balance (HLB), characterising the amphiphilic properties of non-ionogenic surfactants. Surfactants with an HLB of 1 to 10 are predominantly lipophilic whereas surfactants with an HLB of 10 to 20 are predominantly hydrophilic. The HLB can be calculated with the following equation:

$$HLB = 20 \left(1 - \frac{S}{A}\right)$$

S: Saponification number of the ester

A: Acid number of the acid

Thus the non-ionogenic surfactants can be classified with the HLB system. Table 2 gives an overview.

Table 2: Classification of surfactants using the HLB system [70]

HLB	Use	Example (HLB) of the nonionic surfactant family
0 – 3	Antifoaming agent	Sorbitan trioleate, Span® 85 (1.8)
3 – 8	Water in oil emulsifier	Sorbitan mono-oleate, Span® 80 (4.3)
7 – 9	Wetting agent	Sorbitan mono-palmitate, Span® 40 (6.7)
8 – 19	Oil in water emulsifier	Polyoxyethylene-20-sorbitan-mono-stearate, Tween® 60 (14.9)
13 – 16	Detergent	Polyoxyethylene-4-sorbitan-mono-laurylate, Tween® 21 (13.3)
14 – 19	Solubilising agent	Polyoxyethylene-20-sorbitan-mono-oleate Tween® 80 (15)

The capacity of a certain surfactant to solubilise a drug can be increased using the cosolvation method. Alcohols, mainly polyols (e.g. glycerol, sorbitol), incorporate into the palisade layer of the micelle forming hydrogen bonds with the micelle constituents. The micelle stabilising effect increases with the number of hydroxyl groups of the co-solubiliser.

MATERIALS AND METHODS

1. PHARMACODYNAMIC STUDY WITH ORAL THC (PAIN STUDY)

1.1 Quality assurance of clinical test preparations

Quality assurance was done with the Marinol® capsules, which were used by the Pharmacy of the University hospital to prepare the test substances for the pharmacodynamic study. Quality assurance was also done with THC-hemisuccinate suppositories, which were used for a spasticity study at the REHAB (Rehabilitation Center for Spinal Cord Injury and Head Injury, Basel).

1.1.1 Marinol® (THC, dronabinol; capsules)

Standards, chemicals, and solvents

Table 1: Standards for the quality assurance of Marinol®

Abbreviations	Name	Lot-No.	Supplier
THC	Δ^9-Tetrahydrocannabinol	135.1B25.6L4	Lipomed, Arlesheim, CH
THC-d_3	(-)-Δ^9-THC-d_3	FYC-30965-21-A	Radian, Austin, USA

All solvents and chemicals used were of HPLC or analytical quality obtained from Merck AG, Basel (CH) or Fluka Chemie AG, Buchs (CH).

Instrumentation

The gas chromatography-mass spectrometry system (GC-MS) consisted of a HP GC 5890 Series II gas chromatograph with a 7673 autosampler and a G1512A autosampler controller, a HP 5972 mass-selective detector (MSD), a Vectra 486/66 XM computer with Chemstation Software G1046A Rev. A.00.00 (HP 1989-1994).

Method

Sample preparation

Three Marinol® capsules (soft gelatine capsules with sesame oil as vehicle, Unimed Pharmaceuticals, Inc., Deerfeld, IL, USA; provided by the Swiss Federal Office for Public Health) were randomly sampled from the batch Lot. 990468B (exp. Febr. 2001) used for the pain study. 1000 µL of ethanol were added to one capsule (containing 10 mg THC) in a 5 mL sample vial with screw cap. The capsule was then punctured several times with a sharp wire. After sonicating for 10 min (the capsule and the oil was not dissolved) the extract was

centrifuged for 5 min at 1500 rpm. 100 µL of the supernatant was diluted to 10.0 mL with ethanol. 30 µL of this solution and 30 µL of the internal standard solution containing 100 ng/µL Δ^9-THC-d_3 (in ethanol) were transferred into a GC vial insert and vortexed.

Chromatographic conditions

Column	DB-5 MS column (J&W Scientific, Folsom, CA, provided by MSP, Köniz, CH), 25 m x 0.2 mm I.D., film-thickness 0.33 µm
Carrier gas	Helium
Constant flow	0.2 mL/min
Oven	170 °C (1.0 min) to 250°C at 8.0 °C/min; 250 °C (20.0 min)
Injection volume	1 µL, splitless
Injector temperature	250 °C
Transfer line temperature	280 °C
Detection mode	Full scan monitoring (50 to 650 m/z)

Validation

Peak identification (selectivity / specificity)

Chromatographic selectivity: The retention times of the analyte and the internal standard were identified with the corresponding standards. A blank run was performed using an extract of an aliquot of the capsule matrix (sesame oil) to exclude any interferences.

Spectroscopic selectivity: The identity of the signal was determined with characteristic ions for the analyte and the internal standard, respectively. Additionally, the spectra were compared to the spectra of the online library [71].

Calibration and linear range

The quantification of THC was done with internal standard method using the ratio of the characteristic and intensive ion of THC (m/z 314) and the corresponding ion of the internal standard THC-d^3 (m/z 317). Calibration was done with the standards dissolved in ethanol in the concentrations of 30, 50 and 70 µg/mL. The constant concentration of the internal standard was 50 µg/mL. Each calibrator was measured twice.

Recovery

The recovery was not determined. It was assumed that 100 % of the THC was extracted from the sesame oil matrix with the chosen method.

Intra- and interday precision and accuracy

The intraday precision was determined preparing and measuring 4 samples for each concentration (30, 50, and 70 µg/mL) within the same day. Mean, (absolute and relative) standard deviation (SD and RSD), and accuracy were calculated.

The interday precision was determined preparing and measuring 6 samples for each concentration (30, 50, and 70 µg/mL) at different days within 1 month. Mean and (absolute and relative) standard deviation (SD and RSD) were calculated.

1.1.2 THC-hemisuccinate suppositories (rectal THC-HS)

The content of THC-hemisuccinate (THC-HS) in suppositories used in a spasticity study at the REHAB Basel was determined.

Standards, chemicals, and solvents

The THC-hemisuccinate (THC-HS) was originally synthesised by ElSohly Laboratories (ELI), Inc., Oxford MS, USA, and provided by Prof. M. ElSohly, Oxford MS [2].

THC-hemiglutarate (THC-HG) was synthesised in our laboratory according to the method of ELI used for the preparation of THC-HS [72].

All solvents and chemicals used were of HPLC- or analytical quality obtained from Merck AG, Basel (CH) or Fluka Chemie AG, Buchs (CH).

Instrumentation

HPLC HP 1090-system consisting of a Hewlett-Packard (HP) 1090M Series II Liquid chromatograph with a 1040 autosampler, a 1040M photodiode array detector (DAD), a Vectra 486/66 XM computer with HPLC Chemstation Rev. A.03.03. Software (HP 1990-1995).

Method

Sample preparation

The total weight of the suppository was determined and an aliquot of 200 mg taken for the quantitative analysis. The sample and 10.0 mL internal standard solution, containing 270 µg/mL of THC-HG in methanol, were added to a 10 mL sample vial with screw cap and heated at 50 °C for 5 min shaking vigorously (vortexed and sonicated alternately). Then the vial was put into ice to let the suppository matrix precipitate. 10 µL of the supernatant was then injected into the HPLC.

Chromatographic conditions

Stationary phase:	124 x 4 mm I.D. LiChroCART® column, packed with LiChrospher® 100 RP-18, particle size 5 µm and a 8 x 4 mm I.D. precolumn, packed with the same material (Merck AG, Basel, CH)
Mobile phase:	Solvent A: Bidistilled water containing 5.5 % (v/v) acetic acid
	Solvent B: Methanol
Gradient:	0-10 min, 85 % B, isocratic
	Run time: 10 min; post run time: 15 min
Flow:	1.1 mL/min
Oven temperature:	45 °C
Detection:	Quantification wavelength for THC-HS and THC-HG (IS): 280 nm (Online registration of the UV spectra from 190-350 nm (DAD))

Calibration

For the quantification of the THC-HS content of the suppositories the internal standard method was used (THC-HG). Calibrators were prepared in methanol using 4 different concentrations as presented in Table 2.

Table 2: Calibrators for the quantification of THC-HS: concentration of the analytes and ratios of the amounts

Calibrator	Amount of THC-HS [µg/mL]	Amount of THC-HG (IS) [µg/mL]	Ratio (THC-HS/THC-HG)
1	160	550	0.2909
2	405	370	1.0946
3	600	480	1.2500
4	590	170	3.4706

1.2 Subjects and study design (pain study)

Twelve healthy volunteers participated in this randomised, placebo-controlled, double-blind, crossover study which was carried out in the pain laboratory of the Department of Anaesthesiology at the University Hospital of Bern. The subjects were informed about the risks of the study, gave their written informed consent, and were paid for participating. Exclusion criteria were past or existing drug abuse (including alcohol and prescription drugs; Cannabis and opiate immunoassay urine tests before each session), known or suspected hypersensibility to cannabinoids or opioids, pregnancy (urine test before first session), and positive past history of any psychiatric disorders. The subjects were not allowed to take analgesics, alcohol and caffeinated beverages 48 h before and during the study, and were asked to refrain from driving

up to 12 h after the study. The study has been approved by the Ethics Committee of the Faculty of Medicine, University of Bern, the Intercantonal Drug Control Office (IKS), and the Federal Office for Public Health (study protocol, volunteer information etc. see appendices I). Each subject received either 20 mg THC (dronabinol, Marinol), 30 mg morphine hydrochloride (Schweizerhall Pharma, Basel, CH), a mixture of 20 mg THC and 30 mg morphine hydrochloride, or placebo as a single oral dose on empty stomach. The blinding of the test medications was performed by enclosing the 20 mg- or placebo Marinol® capsules in another, dark-coloured gelatine capsule, and adding either 30 mg mannitol or 30 mg morphine. The 30 mg morphine capsules were similarly prepared. Caffeine free beverages were allowed 1 h, and light, but not standardised meals 3 h post-dosing. The between-session washout phases were at least 7 days. To get the subjects familiar and comfortable with the testing procedures (reaction test, 4 pain tests including cold-, heat-, pressure-, and electrical stimulation-test, described later), each session began with a training phase. Then the baselines were recorded and the pain tests performed in a random order every hour up to 8 h post drug. Pressure and heat pain were not determined at time points 5, 6, and 7 h post drug to prevent pulp and skin damage. Side effects were monitored before each set of pain tests. Blood (5-10 mL) was collected in all four sessions through a peripheral vein catheter at baseline, 0.5, 1, 2, 4, and 8 h post drug. The heparinised blood samples were centrifuged and the plasma instantly deep-frozen and stored at -20 °C until analysis.

1.3 Pain tests

Pressure

Pressure pain tolerance thresholds were determined on the center of the pulp of the second and third finger of the right hand with an electronic pressure algometer (Somedic AB, Stockholm, S) [73-75]. A probe with a surface area of 0.28 cm^2 was used, and the pressure increase rate was set to 30 kPa sec^{-1}. Pain tolerance was defined as the point when the subject felt the pain as intolerable. For determination of the tolerance thresholds, the mean of two consecutive measurements was used.

Heat

The computer-driven Thermotest (Somedic AG, Stockholm, S) was used [76, 77]. A thermode with a surface of 25 x 50 mm was applied to the volar surface of the forearm, in the middle of a line joining the elbow to the wrist fold. The temperature of the thermode was continuously increased from 30 to a maximum of 52 °C at a rate of 2.0 °C sec^{-1}. The subject was

asked to press a button when perceiving the heat as painful (pain detection) and when feeling the heat as intolerable and not wanting the heat to be further increased (pain tolerance). At that point the temperature was recorded and the thermode cooled to 30 °C. The thermode was also cooled to 30 °C in the case when the tolerance threshold was not reached at 52 °C. 52 °C was then considered as pain tolerance threshold. Three consecutive measurements were performed for both pain detection and tolerance thresholds. The average of the last two values were computed and evaluated for data analysis.

Cold

A standardised 2-min ice water test (ice cold immersion test) was used [75, 78, 79]. Before immersion, the skin temperature on the thenar of the left hand was measured. The left hand was then immersed in ice-saturated water (0.6 ± 0.2 °C). If pain was considered as intolerable (pain tolerance) before 2 min had elapsed, the subject could withdraw the hand. Perceived pain intensity was rated continuously with an electronic visual analogue scale (VAS), and recorded on a computer. Peak pain (maximal VAS score), area under the pain intensity-time curve, and mean pain (mean VAS score) were determined. If the hand was withdrawn before the end of 2 min, pain intensity was considered to be maximal until the end of the 2-min period (for calculation of the area under the curve (AUC)).

Transcutaneous electrical stimulation (single, repeated)

Two bipolar surface Ag/AgCl-electrodes (Dantec, Skovlunde, DK) were placed on the shaved skin of the shin, 14 cm distal to the patella. The electrode surface was 7 x 4 mm, the distance between the two electrodes 1.5 cm. A train of 5 square-wave impulses was delivered from a computer-controlled constant current stimulator (University of Aalborg, DK). Each of these impulses lasted 1 msec. The whole duration of the train of 5 impulses was 25 msec, so they were perceived as a single stimulus. For the single electrical stimulation this train was given once, and for the repeated electrical stimulation this train was repeated 5 times, at the same intensity and a frequency of 2 Hz (i.e. every 0.5 sec) [80, 81]. The current intensity was increased stepwise 1 mA until the stimulus was perceived as painful. For the single electrical stimulation the pain detection threshold was defined as the minimum stimulus intensity eliciting a subjective pain. For the repeated electrical stimulation the pain detection threshold was defined as the minimum stimulus intensity eliciting a subjective increase in perception during the 5 stimulations, so that the last 1-2 impulses are perceived as painful.

1.4 Monitoring of side effects and vital functions

A 10-cm VAS (see appendix I) was used to asses sedation, psychological (euphoria, hallucinations, disorientation, altered perception etc.) and somatic side effects (heart and digestive problems). The episodes of vomiting were noted. Haemoglobin oxygen saturation (pulse oximetry), blood pressure, and heart rate were recorded. To determine the reaction time, a 1000 Hz tone was delivered from a computer with random intervals of 3-8 sec, and simultaneously a timer was started. The volunteer was told to press a button as fast as possible after the tone. The reaction time was defined at the time from the tone until the subject pressed the button. The mean value of five consecutive measurements was calculated.

1.5 Determination of morphine and -metabolites in plasma by high performance liquid chromatography with photodiode array (HPLC-DAD) and fluorescence detection (HPLC-FLD)

Standards, chemicals, and solvents

Table 3: Standards for the quantification of morphine and its metabolites

Abbreviations	Name	Lot-No.	Supplier
M-HCl	Morphine hydrochloride	408038	Hänseler AG, Herisau (CH)
M6G	Morphine-6-β-D-glucuronide	57.1B11.2	Lipomed, Arlesheim (CH)
M3G	Morphine-3-β-D-glucuronide	51F-3831	Sigma, Buchs (CH)
EM	Ethylmorphine hydrochloride	101101	Grogg Chemie, Bern (CH)

Bidistilled water, Department of Clinical Research, University of Bern (CH)
Acetonitrile gradient grade (ACN), LiChrosolv®, Merck AG, Basel (CH)
Trifluoroacetic acid for UV-Spectroscopy (TFA), Uvasol®, Merck AG, Basel (CH)
Methanol LiChrosolv®, Merck AG, Basel (CH).

All other solvents and chemicals used were of HPLC- or analytical quality obtained from Merck AG, Basel (CH) or Fluka Chemie AG, Buchs (CH).

Instrumentation

The HPLC HP 1090-system consisted of a Hewlett-Packard (HP) 1090M Series II Liquid chromatograph with a 1040 autosampler, a 1040M photodiode array detector (DAD), a 1046A fluorescence detector (FLD), and a Vectra 486/66 XM computer with HPLC Chemstation Rev. A.03.03. Software (HP 1990-1995).

Method

Sample preparation

The plasma samples were purified with solid phase extraction (SPE) columns of the type Chromabond C-18 ec, 3 mL, 500 mg. The sample preparation was done using an ASPEC robotic system (Automatic Sample Preparation with Extraction Columns; Gilson, Villiers Le Bel, F) according to the scheme presented in Table 4.

Table 4: SPE of the plasma samples for the sample preparation of the morphine session, modified method of Bourquin et al. [82]

Conditioning	Methanol	10 mL
	ACN 40 % (V/V) in phosphate buffer 0.01 M, pH 2.1	5 mL
	Bidistilled water	10 mL
Loading of the sample	Plasma	1.5 mL
	Internal standard (EM 0.001 % (w/v))	150 µL
	Carbonate buffer 0.5 M, pH 9.3	4.5 mL
	→ an aliquot of 4.1 mL (corresponding 1.0 mL plasma) was loaded onto the column	
Washing	Carbonate buffer 0.005 M, pH 9.3	20 mL
	Bidistilled water	0.5 mL
	ACN 40 % (v/v) in phosphate buffer 0.01 M, pH 2.1	0.35 mL
Elution	ACN 10 % (v/v) in phosphate buffer 0.01 M, pH 2.1	1 mL
	ACN 70 % (v/v) in phosphate buffer 0.01 M, pH 2.1	1 mL

A new batch of SPE columns resulted in slightly different extraction properties, which made a further optimisation of the extraction procedure necessary. The extraction was consequently manually done according to the scheme presented in Table 5. This alternative extraction method was used for the plasma samples of the THC-morphine session, which were after that analysed with HPLC-FLD.

Table 5: SPE of the plasma samples for the sample preparation of the THC-morphine session

Conditioning	Methanol	10 mL
	ACN 40 % (v/v) in phosphate buffer 0.01 M, pH 2.1	5 mL
	Bidistilled water	10 mL
Loading of the sample	Plasma	1.0 mL
	Internal standard (EM 0.001 % (w/v))	100 µL
	Carbonate buffer 0.5 M, pH 9.3	3.0 mL
	→ this mixture was loaded completely onto the column	
Washing	Carbonate buffer 0.005 M, pH 9.3	20 mL
	Bidistilled water	0.4 mL
	ACN 40 % (v/v) in phosphate buffer 0.01 M, pH 2.1	0.25 mL
Elution	ACN 10 % (v/v) in phosphate buffer 0.01 M, pH 2.1	1 mL
	ACN 70 % (v/v) in phosphate buffer 0.01 M, pH 2.1	1.2 mL

The eluate was evaporated under nitrogen at 43 °C, redissolved in 120 µL of bidistilled water containing 0.05 % TFA (mobile phase A of the chromatographic system), and sonicated for 10 min. The extract was filtrated through an Eppendorff pipette tip containing a small piece of Kleenex® into a 200 µL vial insert and 12 µL of the filtrate injected onto the HPLC-column.

Chromatographic conditions

HPLC-DAD

Stationary phase: 125 x 2 mm I.D. column, packed with Nucleosil-50 EC, particle size 5 µm and a 8 x 3 mm I.D. precolumn, packed with Nucleosil-120 C_8, particle size 3 µm, (both Macherey-Nagel, Oensingen, CH)

Mobile phase: Solvent A: Bidistilled water containing 0.05 % (v/v) TFA

Solvent B: ACN containing 0.05 % (v/v) TFA

Gradient: 0-1 min, 3.5 % B isocratic; 1-6 min, 3.5-13 % B linear; 6-7 min, 13-15 % B linear; 7-20 min, 15 % B isocratic; 20-21 min, 15-100 % B linear; 21-24 min, 100 % B isocratic; 24-25 min, 100-3.5 % B linear

Run time: 25 min; post run time: 15 min

Flow: 330 µL/min

Oven temperature: 45 °C

Detection: Wavelength for the quantification of morphine, its metabolites, and

	the internal standard (EM): UV 210 nm
	(online recording of the UV spectra from 190-350 nm (DAD))
HPLC-FLD	
Stationary phase:	125 x 2 mm I.D. column, packed with Spherisorb-80 ODS-1 (C_{18}), particle size 3 µm and a 8 x 3 mm I.D. precolumn, packed with Spherisorb-80 ODS-1 (C_{18}), particle size 3 µm, (both Macherey-Nagel, Oensingen, CH)
Mobile phase:	Solvent A: Bidistilled water containing 0.05 % (v/v) TFA
	Solvent B: ACN containing 0.05 % (v/v) TFA
Gradient:	0-5.5 min, 4 % B isocratic; 5.5-9 min, 4-15 % B linear; 9-9.2 min, 15-25 % B linear; 9.2-18 min, 25 % B isocratic; 18-19 min, 25-100 % B linear; 19-21 min, 100 % B isocratic; 21-22 min, 100-4 % B linear
	Run time: 22 min; post run time: 15 min
Flow:	300 µL/min
Oven temperature:	40 °C
Detection:	Wavelength for the quantification of morphine, its metabolites, and the internal standard (EM): 343 nm (excitation wavelength: 227 nm, 220 Hz; response time 1.0 sec)
	343 nm quantification wavelength (amplification factor: 15)

Validation

Peak identification (selectivity / specificity):

HPLC-DAD:

Chromatographic selectivity: The peaks of the analytes (M, M6G, M3G) and the internal standard (EM) were assigned by the corresponding standards. Negative- (extracted blank plasma) and positive-control samples (spiked and extracted blank plasma) were analysed to exclude any interferences.

Spectroscopic selectivity: The identity of the signal was determined by comparing the UV-spectra in the range of 205-325 nm with the spectra of the standards. The recorded UV-spectra were also used for the peak purity check.

HPLC-FLD:

Chromatographic selectivity: The peaks of the analytes (M, M6G, M3G) and the internal standard (EM) were assigned by the corresponding standards. Negative- (extracted blank

plasma) and positive-control samples (spiked and extracted blank plasma) were analysed to exclude any interferences.

Spectroscopic selectivity: The identity of the signal was determined by using dual detection comparing the UV-spectra in a range of 205-325 nm and the fluorescence (detection at 343 nm after excitation at 227 nm) with the spectra of the standards. The recorded UV-spectra were also used for the peak purity check.

Calibration and linearity (HPLC-DAD and HPLC-FLD)

Blank plasma was spiked with different concentrations of standards. After the sample preparation with SPE the samples were analysed using the described method. For each analyte calibrators of 20, 50, and 100 ng/mL were used. In addition a calibrator of 500 ng/mL was used for M and M6G, and a calibrator of 1000 ng/mL for M3G. The concentration of the internal standard (EM) was 1000 ng/mL.

Recovery and precision

For the determination of the recovery blank plasma was spiked 5 times with different concentrations of analytes (20-100 ng/mL). The samples were prepared and analysed with the described method. For the interday precision blank plasma was spiked with 20 ng/mL of each analyte, prepared, and analysed at 5 different days within a month.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were determined with spiked blank plasma. The LOD was defined at the concentration where the peaks could be clearly integrated with a signal to noise ratio of 3 to 1. The LOQ was defined at the concentration where a control sample of 20 ng/mL (HPLC-DAD) and 10 ng/mL (HPLC-FLD), respectively, was within the range of ± 20 % of the calibration.

1.6 Determination of THC and -metabolites in plasma by gas chromatography mass-spectrometry (GC-MS)

Standards, chemicals, and solvents

Table 6: Standards for the quantification of THC and its metabolites

Abbreviations	Name	Lot-No.	Supplier
THC	□^{9}-Tetrahydrocannabinol	135.1B25.6L4	Lipomed, Arlesheim, CH
THC-d_3	(-)-□^{9}-THC-d_3	FYC-30965-21-A	Radian, Austin, USA
THC-OH	(±)-11-Hydroxy-□^{9}-THC	34703-81B	Radian, Austin, USA
THC-OH-d_3	(±)-11-Hydroxy-□^{9}-THC-d_3	31534-49A	Radian, Austin, USA
THC-COOH	(±)-11-nor-9-Carboxy-□^{9}-THC	31533-70A	Radian, Austin, USA
THC-COOH-d_3	9-Carboxy-11-nor-□^{9}-THC-5'-H_3	3983-59	Research Triangle Institute, NC, USA

□-Glucuronidase, Type IX-A, Escherichia choli, from Sigma, Fluka Chemie AG, Buchs (CH), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) from Fluka Chemie AG, Buchs (CH). All other solvents and chemicals used were of GC or analytical quality obtained from Merck AG, Basel (CH) or Fluka Chemie AG, Buchs (CH).
The immunoaffinity resin slurry was obtained from ElSohly Laboratories, Inc., Oxford, MS, USA.

Instrumentation

The GC-MS system consisted of a HP GC 5890 Series II gaschromatograph with a 7673 autosampler and a G1512A autosampler controller, a HP 5972 mass-selective detector (MSD), a Vectra 486/66 XM computer with Chemstation Software G1046A Rev. A.00.00 (HP 1989-1994).

Method

Hydrolysis and extraction procedure for the plasma samples

The preparation of the plasma samples was done using an immunoaffinity extraction procedure [83]. In a 10 mL sample vial with screw cap 0.5 mL of plasma was spiked with 25 □L of a methanolic solution containing 1 □g/mL of THC-d_3, THC-OH-d_3, and THC-COOH-d_3. To each vial 2 mL of 0.1 M potassium phosphate buffer (pH 6.8) was added followed by 200 □L of a 25'000 units/mL solution of □-glucuronidase in the same buffer (a total of 5'000 units). The vial was then vortexed, closed tightly, and incubated at 37 °C for 16 h. After cooling the sample to room temperature 1 mL of immunoaffinity resin slurry was added. The tubes were closed tightly, placed on a test tube mixer operated at a speed of 30 rpm, and mixed for 45 min. The mixture was poured into a frit filter cartridge (preconditioned with 3 mL of methanol and 3 mL of

bidistilled water) that was positioned onto a vacuum manifold (Adsorbex SPU, model EM 6500, series 5055, 16-2-1988, Merck, Darmstadt, D) and the liquid was allowed to pass through under a slight vacuum. The resin was washed with 3 mL of phosphate saline buffer (pH 7.0), two times with 3 mL of bidistilled water, followed by 3 mL of 10 % acetone in bidistilled water, and then dried under vacuum for 2 min. The elution of the analytes was performed using 5 portions of 0.5 mL methanol, allowing the solvent to flow through under gravity into a 10 mL tube. The eluate was then evaporated to dryness at 45 °C under nitrogen. The residue was derivatised with 60 µL of BSTFA containing 1 % TMCS and by heating at 70 °C for 30 min. After cooling to room temperature, the samples were transferred to GC vial inserts and the vials were capped. The trimethylsilyl (TMS) derivatives (dissolved in the excess of derivatisation reagent) were directly injected on the GC-MS.

Chromatographic Conditions

Column	DB-5 MS column (J&W Scientific, Folsom, CA; provided by MSP, Köniz, CH), 25 m x 0.2 mm I.D., film-thickness 0.33 µm
Carrier gas	Helium
Constant flow	0.2 mL/min
Oven	200 °C (0.5 min) to 280 °C at 30.0 °C/min, 280 °C (13.5 min)
Injection volume	2 µL, splitless
Injector temperature	250 °C
Transfer line temperature	280 °C
Detection mode	Single ion monitoring (SIM, the ions are listed in Table 7)

Table 7: Monitored ions for the quantification of THC and its metabolites (TMS derivatives)

Analyte	Quantitation ion	Qualifying ion
THC-TMS	371	343
THC-d_3-TMS	374	389
THC-OH-TMS_2	371	474
THC-OH-d_3-TMS_2	374	377
THC-COOH-TMS_2	371	488,473
THC-COOH-d_3-TMS_2	374	491

Validation

Peak identification (selectivity / specificity):

Chromatographic selectivity: The peaks of the analytes (THC, THC-OH, THC-COOH) were assigned by the corresponding standards and deuterated standards (THC-d_3, THC-OH-d_3, THC-COOH-d_3). Negative- (extracted blank plasma) and positive-control samples (spiked and extracted blank plasma) were analysed to exclude any interferences.

Spectroscopic selectivity: Peak identification was performed in the SIM mode by the qualifyer ions listed in Table 7.

Calibration and linearity

Blank plasma was spiked with different concentrations of standards. After extraction the samples were analysed using the described method. For each analyte calibrators of 100, 20, and 10 ng/mL were used. In addition to that a calibrator of 2 ng/mL was used for THC and THC-OH. For THC-COOH two additional calibrators, 200 and 500 ng/mL, were used. The concentration of the internal standards was 50 ng/mL each.

Recovery, intra- and interday precision, and accuracy

For the determination of the recovery 5 samples of blank plasma were spiked with 20 ng/mL of each analyte. The samples were analysed as described above, and the area under the peaks were compared with the areas of identically concentrated standard solutions.

For the determination of the intraday precision, 5 samples of blank plasma were spiked with 20 ng/mL of each analyte and prepared within the same day according to the method described above. Mean, standard deviation (SD and RSD), and accuracy were calculated.

For the determination of the interday precision, 5 samples of blank plasma were spiked with 20 ng/mL of each analyte, and analysed at 5 different days within a month.

Limit of detection and limit of quantification

The LOD and the LOQ were determined with spiked blank plasma. The LOD was defined at the concentration where the peaks could be clearly integrated with a signal to noise ratio of 3 to 1. The LOQ was defined at the concentration where a control sample of 2 ng/mL for THC and THC-OH and 10 ng/mL for THC-COOH, respectively, was within the range of ± 20 % of the calibration.

1.7 Statistical analysis

Statistical analysis was performed independently for each pain test. Differences of the baseline values in the four different sessions of each volunteer were excluded with the Friedman's test. Then for each session the mean results of all subjects and time-points were calculated. The three verum sessions were then compared to the placebo session using the Wilcoxon signed ranks test. $P < 0.05$ was considered as significant.

1.8 Calculation of the pharmacokinetic parameters

For the calculation of the pharmacokinetic parameters the TopFit software (version 2.0) was used [84]. The data were evaluated as mean of the 12 volunteers. Non-compartmental analysis was performed for the calculation of the parameters (half-life, AUC).

2. DEVELOPMENT OF WATER SOLUBLE THC FORMULATIONS

2.1 Extraction, purification, and quality assurance of THC from extract ELB-11-98 for the in vitro experiments

Standards, chemicals, and solvents

Table 8: Standards for the development of water soluble THC formulations

Abbreviations	Name	Lot-No.	Supplier
THC	Δ^9-Tetrahydrocannabinol	135.1B25.6L4	Lipomed, Arlesheim, CH
THC-d_3	(-)-Δ^9-THC-d_3	FYC-30965-21-A	Radian, Austin, USA

Petroleum ether, boiling range 40-80 °C, (extra pure, containing n-hexane), Merck AG, Basel (CH); diethylether, Merck AG, Basel (CH).
All other solvents and chemicals used were of HPLC or analytical quality obtained from Merck AG, Basel (CH) or Fluka Chemie AG, Buchs (CH).
Extract ELB-11-98: Ethanolic extract of Swiss Cannabis prepared earlier in our lab for analytical purposes.

Instrumentation

The GC-MS system consisted of a HP GC 5890 Series II gaschromatograph with a 7673 autosampler and a G1512A autosampler controller, a HP 5972 mass-selective detector (MSD), and a Vectra 486/66 XM computer with Chemstation Software G1046A Rev. A.00.00 (HP 1989-1994).

The HPLC HP 1090-system consisted of a Hewlett-Packard (HP) 1090M Series II Liquid chromatograph with a 1040 autosampler, a 1040M photodiode array detector (DAD), and a Vectra 486/66 XM computer with HPLC Chemstation Rev. A.03.03. software (HP 1990-1995).

The column chromatography (CC) fractions were collected with a LKB fraction collector, LKB 7000 Ultro Rac, LKB Produkter AB, Bromma (S).

The medium pressure chromatograhy (MPLC) system consisted of a Büchi chromatography pump (type: B-681) with a Büchi fraction collector (type: B-684), Büchi AG Flawil (CH).

Qualitative and quantitative characterisation of the extract ELB-11-98 with GC-MS

A methanolic solution (sonicated for 30 sec at room temperature) of the extract ELB-11-98 with a concentration of 240 ng/mL was used for the quantitative and qualitative characterisation.

30 µL of this solution was mixed with 30 µL of the internal standard solution (100 µg/mL THC-d_3) and injected into the GC-MS.

Chromatographic conditions GC-MS

Column	DB-5 MS column (J&W Scientific, Folsom, CA), 25 m x 0.2 mm I.D., film-thickness 0.33 µm
Carrier gas	Helium
Constant flow	0.2 mL/min
Temperature program	170 °C (1.0 min) to 250 °C at 8.0 °C/min, 250 °C (20.0 min)
Injection volume	1 µL, splitless
Injector temperature	250 °C
Transfer line temperature	280 °C
Detection mode	Full scan monitoring (50 to 650 m/z)

For the quantification of THC in the extract ELB-1-98 three calibrators were used: 80, 53, and 26.7 µg/mL of THC (each with 50 µg/mL internal standard).

For the identification of other peaks their mass spectra were compared with those of the online library [71] and literature [85].

Starting experiments with thin layer chromatography (TLC)

TLC was used to monitor the fractionation of the following CC and MPLC purification of the extract ELB-11-98, respectively.

Chromatographic conditions

Stationary phase	High performance thin layer chromatography (HPTLC) silica gel 60 plates 254, 5 x 10 cm, (Merck, Darmstadt, D)
Mobile phase	Petroleum ether / diethylether (1:1), saturated atmosphere
Spot volume	2 µL
Visualisation	UV at 254 nm Spraying with anisaldehyde reagent[1] followed by heating (80 °C for 30 min)

[1] Anisaldehyde reagent: 0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, 5 mL of conc. sulfuric acid.

Purification of the extract ELB-11-98 with column chromatography (CC) and medium pressure liquid chromatography (MPLC)

In a first step 300 mg of the extract ELB-11-98 were purified using an open column according to the conditions described below. The collected fractions were screened with TLC and the THC containing fractions were pooled. After evaporation of the solvents with the vacuum distiller, purity was checked with GC-MS. In a second step, follow-up purification was done with MPLC according to the conditions described below. Again the collected fractions were screened, pooled, the solvent evaporated, and the purified THC analysed with GC-MS and additionally HPLC-DAD (methods described below).

Chromatographic conditions for CC

Column and stationary phase	Length: 40 cm, diameter: 3 cm, filled with silica gel 60, 0.063-0.200 mm (Merck, Darmstadt (D) 1.07734.1000) in petroleum ether / diethylether 95:5; after each run the column was refilled completely with new silica gel.
Mobile phase	200 mL petroleum ether / diethylether 95:5 300 mL petroleum ether / diethylether 80:20
Flow	1 drop/sec
Sample volume	300 mg extract ELB-1-98 dissolved in 10 mL petroleum ether / diethylether 95:5
Forerun	200 mL
Fractionation	50 fractions (6 mL each) with the fraction collector
Monitoring	TLC

Chromatographic conditions for MPLC

Column and stationary phase:	Length: 45 cm, diameter: 3.5 cm (+ precolumn: length 10 cm, diameter 1 cm), filled with 130 g of LiChroprep® Si 60, 25-40 µm (Merck, Darmstadt, D), Art.Nr. 1.09390.1000) in petroleum ether / diethylether 95:5; after each run a washing procedure (described below) was performed and after 5 runs the column was completely refilled with new silica gel.	
Mobile phase:	300 mL of petroleum ether / diethylether 95:5	
	100 mL of petroleum ether / diethylether 90:10	
	300 mL of petroleum ether / diethylether 80:20	
	200 mL of petroleum ether / diethylether 50:50	→ washing
	200 mL of diethylether	→ washing
	250 mL of methanol	→ washing
Flow:	→ Pressure 1.5 bar	
	→ 5 mL/min	
Sample volume:	300 mg of prepurified extract dissolved in 10 mL of petroleum ether / diethylether 95:5	
Forerun:	90 mL	
Fractionation:	60 fractions (6 mL each) with the fraction collector	
Monitoring:	TLC	

Quality assurance with GC-MS and HPLC

To monitor the efficiency of the extraction and to characterise the quality of the obtained THC GC-MS and HPLC-DAD was used. For GC-MS the sample concentration was 150 µg/mL and for HPLC-DAD 120 µg/mL.

Chromatographic conditions for GC-MS

The conditions were the same as for peak identification in the extract ELB-11-98.

Chromatographic conditions for HPLC-DAD

Stationary phase:	124 x 4 mm I.D. Spherisorb® ODS I column, particle size 3 µm and a 8 x 4 mm I.D. precolumn, packed with the same material (both Macherey-Nagel AG, Oensingen, CH)	
Mobile phase:	Solvent A:	Bidistilled water containing 0.05 % (v/v) formic acid
	Solvent B:	ACN containing 0.05 % (v/v) formic acid
	0-15 min, 60 % B isocratic	
	Run time: 15 min; post run time: 15 min	
Flow:	1.0 mL/min	
Oven temperature:	40 °C	
Injection volume:	10 µL	
Detection:	DAD 210 nm	
	Online recording of the UV spectra from 190-350 nm (DAD)	

2.2 Development and validation of the THC inhalation solution

Standards, chemicals, and solvents

Table 9: Standards for the quantification of THC in the developed water soluble THC formulations

Abbreviations	Name	Lot-No.	Supplier
THC	Δ^9-Tetrahydrocannabinol	135.1B48.1L1	Lipomed, Arlesheim, CH
phen	Phenanthren	11015AU	Aldrich, Schnellendorf, D

Table 10: Chemicals for the preparation of the inhalation solution

Abbreviations	Name	Lot-Nr.	Supplier
-	Purified THC out of the extract ELB-11-98 (the ethanolic extract of Swiss Cannabis prepared earlier in our lab for analytical purposes)	-	-
THC	Δ^9-Tetrahydrocannabinol, dronabinol	300.802	THC Pharm, Frankfurt am Main (D)
	Cremophor® RH 40, Polyoxyl 40 Hydrogenated Castor Oil (Ph. Eur.)	54-2535	BASF GmbH, Ludwigshafen (D)
	Cremophor® EL, Polyoxyl 35 Hydrogenated Castor Oil (Ph. Eur.)	80-4326	BASF GmbH, Ludwigshafen (D)
	Alcohol benzylicus (Ph. Eur.)	2002.02.0379	Hänseler AG, Herisau (CH)
NaAsc	Sodium ascorbate (USP)	1167110	Pharmacy of the University Hospital, Bern (CH)
NaH_2PO_4	Sodium dihydrogen phosphate dihydrate (Ph. Eur.)	010008	Pharmacy of the University Hospital, Bern (CH)
EtOH	Ethanolum absolutum	412613/1 62100	Fluka Chemie AG, Buchs (CH)

Bidistilled water, Department of Clinical Research, University of Bern (CH);
methanol LiChrosolv®, Merck AG, Basel (CH).
All other solvents and chemicals were of HPLC or analytical quality obtained from Merck AG, Basel (CH) or Fluka Chemie AG, Buchs (CH).

Instrumentation

The HPLC HP 1090-system consisted of a Hewlett-Packard (HP) 1090M Series II Liquid chromatograph with a 1040 autosampler, a 1040M photodiode array detector (DAD), and a Vectra 486/66 XM computer with HPLC Chemstation Rev. A.03.03. software (HP 1990-1995).

Certoclav, heating plate (Jura type 1074), light bulb (TUNGSRAM, 100 watt, 235-245 V, E27, "dim", Austria)

HPLC method

Sample preparation

To 100 µL of the respective formulation 100 µL of internal standard solution containing 2 mg/mL of phen in EtOH were added and diluted to 1.0 mL. 10 µL were then injected on the HPLC-DAD.

Chromatographic conditions (modified from the method of Brenneisen et al. [86])

Stationary phase	125 x 4 mm I.D. column, packed with Spherisorb ODS I, particle size 3 µm and a 8 x 4 mm I.D. precolumn, packed with the same material (Macherey-Nagel, Oensingen, CH)
Mobile phase	Solvent A: Bidistilled water containing 1 % (V/V) acetic acid Solvent B: Methanol 0-16 min, 77.5 % B isocratic; post run time: 15 min
Flow	0.6 mL/min
Oven temperature	40 °C
Detection	Wavelength for the quantification of THC with the internal standard method (phen as internal standard): UV 230 und 280 nm; online recording of the UV spectra from 190-350 nm (DAD)

Validation

Peak identification (selectivity / specificity)

Chromatographic selectivity: The peaks of THC and the internal standard phen were assigned by the corresponding standards. Adjuvants were tested for interference.

Spectroscopic selectivity: The identity of the signal was determined by comparing the UV spectra in a range of 205-325 nm with the spectra of the standards. The recorded UV spectra were also used for the peak purity check.

Calibration and linearity

The calibrators were prepared using standard solutions. Concentrations of 1000, 500, 250, and 100 µg/mL of THC were used. The internal standard concentration was 200 µg/mL.

Intra- and interday precision and accuracy

For the determination of the intra- and interday precision standard solutions were used. For the intraday precision 4 samples of each concentration (1000, 500, 250, and 100 µg/mL) were

prepared and measured within the same day. Mean, standard deviation (SD, RSD), and accuracy were calculated.

For the interday precision (SD, RSD) 5 samples of each concentration (1000, 500, 250, and 100 □g/mL) were prepared at different days within 1 month and analysed.

Limit of quantification and limit of detection

The LOQ and the LOD were not determined because the method was used for the quantification of the THC content in galenic formulations and therefore the concentration range not critical.

Pilot solubilisation experiments

The solubilisation experiments were all done using 1.8 mL-GC vials and making 1 mL-formulations. The solvent of an aliquot of the ethanolic THC stock solution (containing 5 mg/mL of THC) was evaporated under nitrogen at room temperature to get the necessary amount of pure THC. The appropriate amount of Cremophor® was then added and the mixture heated to 63 °C for 20 min in a water bath. The aqueous phase was heated separately. After that the two phases were mixed together and shaken vigorously until a clear solution was obtained. The solutions were then allowed to cool, examined for residues of THC, and finally analysed with the HPLC method described before. The THC peak areas were compared with those of the ethanolic THC standard solution.

The different solubilisation experiments are summarised in Table 11.

Table 11: Solubilisation experiments

Experiment	THC [mg/mL]	Solvent	Cremophor® RH 40 concentration in % [w/v]	Cremophor® EL concentration in % [w/v]
1	1	Bidistilled water	-	-
	1	EtOH	-	-
	1	Bidistilled water	5	-
	1		10	-
	1		15	-
	1		-	5
	1		-	10
	1		-	15
2	2	EtOH	-	-
	2	Bidistilled water	2.5	-
	2		5	-
	2		10	-
	2		-	2.5
	2		-	5
	2		-	10
3	3	EtOH	-	-
	3	Bidistilled water	2.5	-
	3		5	-
	3		10	-
	3		-	2.5
	3		-	5
	3		-	10
4	3	Bidistilled water	7.5	-
	3		5	-
	3		4	-
	3		3	-
	3		2	-
	3		1	-

Follow-up experiments with different pH in the heat stress test

To compare the stability of the formulation the next experiments were done at different pH conditions, buffered and not buffered, followed by a stress test in the certoclav (stress test conditions described below). The THC concentration was set to 3 mg/mL, Cremophor concentrations to 5 %. The buffer capacity[2] ( ) of the phosphate buffer was 0.03 each time. The formulations at 4 different pH values (6.0, 6.8, 7.4, and 8.0), buffered and non-buffered,

[2] Equation to calculate the buffer capacity :

$$ = 2.3\ C\ \frac{K_a\left[H_3O^+\right]}{\left(K_a + \left[H_3O^+\right]\right)^2}$$

C: total buffer concentration

respectively, were compared by analysing the following variables before and after the stress test with the described HPLC method: area of the THC and the cannabinol peaks (CBN, degradation product of THC due to oxidation), and the ratio of the areas.

Conditions of the stress tests

Heat

The formulations in the 1.8 mL GC vials were capped and left in the certoclav at 1 bar, 121 °C, 20 min steam. After this treatment the vials were shaken for 5 min to re-emulsify the two separated phases. After cooling to room temperature the samples were analysed using the described HPLC method.

Light

The formulations in the 1.8 mL GC vials were capped and put on a piece of plastic (distance to the table: 6 cm) covered with a white sheet of paper. In a distance of 45 cm the lamp was installed containing a 100 W light bulb. The measured temperature on the piece of plastic was 27 °C and the measured light intensity 12 500 lux. The test time was set to 6 and 24 h, respectively. After cooling to room temperature the samples were analysed using the described HPLC method.

Statistical experiment

A statistical experiment was done using a factorial 2^3-design [87, 88]. The null hypothesis (H_0) was that there is no influence of the adjuvants on the stability of the formulations and that there are no interactions between the adjuvants leading to additional influence on the stability of the formulations. The factors and levels of the 2^3-design were defined as listed in Table 12.

Table 12: Definition of factors and levels for the 2^3-design

Factors		Levels	
		+	-
A	pH (buffered)	8.0	7.4
B	Cremophor® RH 40 conc. [% w/v]	6	3
C	NaAsc conc. [% w/v]	0.2	0.05

Basing on these definitions the scheme presented in Table 13 was arranged for the experiments.

Table 13: Scheme for the 2^3-design

	Level of the factors			Level of the interactions			
	A	B	C	AB	AC	BC	ABC
(1)	-	-	-	+	+	+	-
a	+	-	-	-	-	+	+
b	-	+	-	-	+	-	+
ab	+	+	-	+	-	-	-
c	-	-	+	+	-	-	+
ac	+	-	+	-	+	-	-
bc	-	+	+	-	-	+	-
abc	+	+	+	+	+	+	+

Symbols: a, b, c Upper level of the factors A, B, and C (not marking the lower level)
(1) All factors on the lower level

This scheme led to the different formulations for the statistical experiment. The composition of the 8 experiments is presented in Table 14.

Table 14: Formulations for the statistical experiment (3mg/mL THC added)

No.	pH buffered	Cremophor® RH 40 concentration in % [w/v]	NaAsc concentration (antioxidant) in % [w/v]
(1)	7.4	3	0.05
a	7.4	6	0.05
b	8.0	3	0.05
ab	8.0	6	0.05
c	7.4	3	0.2
ac	7.4	6	0.2
bc	8.0	3	0.2
abc	8.0	6	0.2

The THC concentration was set to 3 mg/mL. All the formulations were analysed immediately after preparation, after the heat stress test, and finally after the light stress test (6 h and 24 h) with the described HPLC method. Statistical assessment was done using Yates-analysis [87]. The endpoint was the ratio of the peak areas of THC and CBN, respectively, after the stress tests.

Final formulation with adjuvants

The final formulation with all adjuvants (buffer, antioxidant, conservant) is presented in Table 15.

Table 15: Final formulation of the THC inhalation solution

Component	Amount	[% (w/v)]
THC	30.0 mg	0.3
Cremophor® RH 40	500.0 mg	5.0
NaAsc	5.0 mg	0.05
Benzyl alcohol	100.0 mg	1.0
Phosphate buffer (pH 7.4, 84.2 mM)	to 10.0 mL	

The THC and the Cremophor® RH 40 were heated together in a water bath at 63 °C for 10 min. Two third of the phosphate buffer, also heated to 63 °C, were then incorporated in the mixture by shaking. After cooling to room temperature the benzyl alcohol, the NaAsc, and the remaining phosphate buffer were added to the mixture. The clear, yellowish solution was then sonicated for 30 s and finally filtrated through a 0.22 µm filter under aseptic conditions.

Quality assurance of the final formulation (in vitro)

Stability

Quantification of the THC content was done every 4 to 9 days using the described HPLC method.

Viscosity

The measurement of the viscosity was done by the pharmacy of the University Hospital Bern according to the standards of the European Pharmacopeia [89]. The measurement was carried out with the placebo solution.

Osmolality

The measurement of the osmolality was done by the pharmacy of the University Hospital Bern according to the standards of the European Pharmacopeia [89].

pH

The pH value of the formulation was determined three times using a potentiometer.

Particle size distribution

The measurement was performed with a Malvern Mastersizer X equipped with a 100 mm lens. For the calculation of the particle size Malvern Software with the algorithm for volume distribution, polydisperse aerosol, and the 2QAA-model representing water in air was used. To minimise light scattering the room was darkened during the measurements. Temperature and humidity remained constantly at 23 °C and 40 %, respectively. For the sample analysis the solution was nebulised continuously into the laser beam and continuously removed by a vacuum cleaner. The obscuration was held on a value of approximately 10-30 %. The particle size distribution was determined with the vehicle solution with both nebulisers (n = 10) and with the THC solution with the LC-Plus nebuliser (n = 5).

Output rates

For the determination of the output rate of THC from the pressure driven PARI® Master coupled to the LC Plus nebuliser the conditions developed earlier by our group were used [12] (Figure 1). The PARI® Master was connected to the tubing followed by the interrupter and the nebuliser (either the LC-Plus- or the IS-2-nebuliser) equipped with an inspiratory valve (LC-Plus). The nebuliser was connected to a PARI filter set containing a filter pad collecting the aerosol. The filter set was coupled with an expiratory valve filled with a bowl of glass wool to collect the small amount that is not retained by the filter pad. This valve was then again connected with the adult's mouthpiece (with a second expiratory valve) leading to a 3 L hand pump representing the lung and simulating the "breathing" (3 Liter Calibrated Syringe, Sensor Medics Corporation, Yorba Linda, CA, USA; provided by the Dept. of Pneumology, University Hospital, Bern).

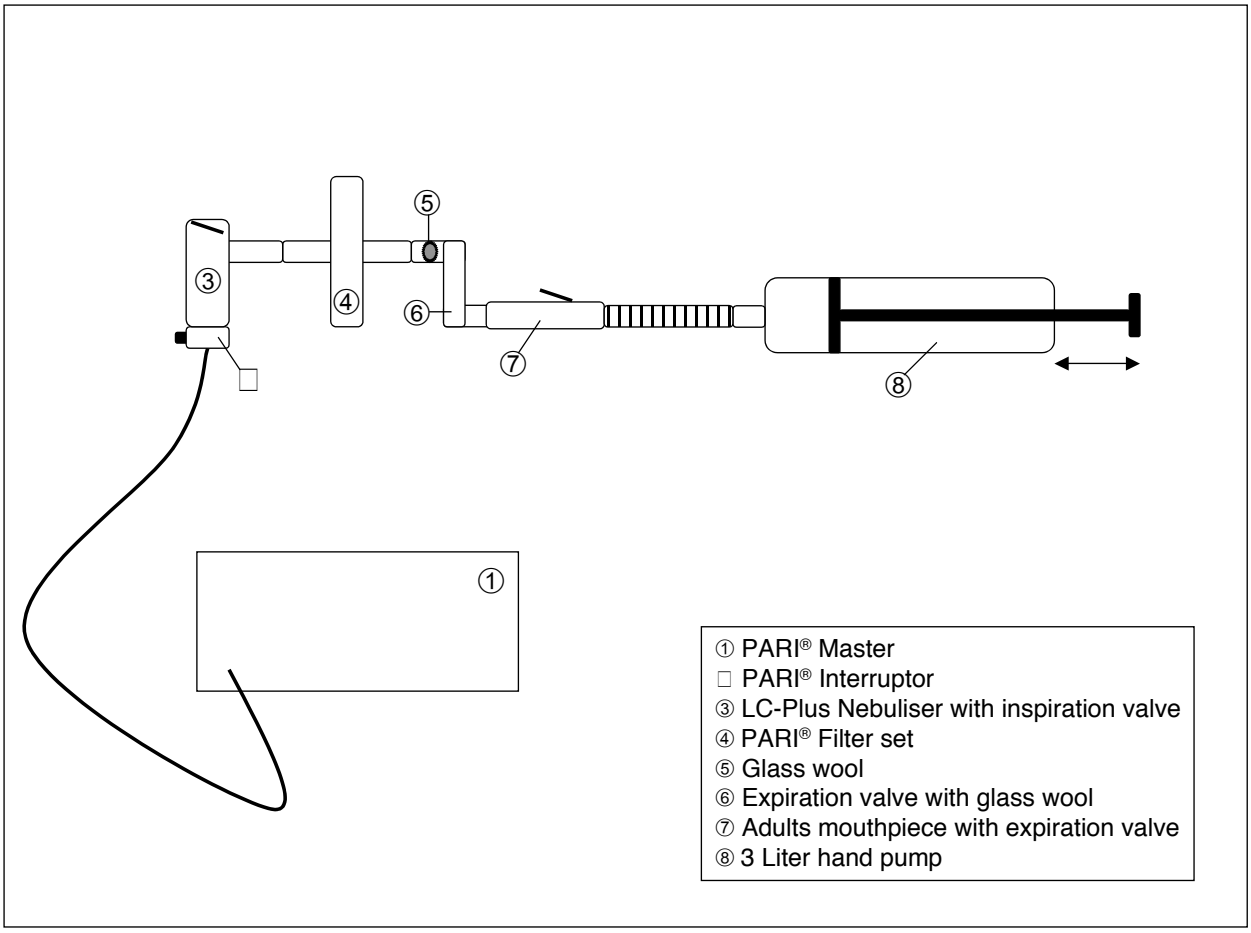


Figure 1: Scheme of the experimental arrangement for the determination of the output rate

Samples of 2 mL (n = 3) and 3 mL (n = 3) were nebulised with each of the nebulisers. The inhalation was simulated manually with the hand pump (velocity 1 breath/10 sec, simulating optimal inhalation). The filter pad and the glass wool which collected the aerosol were then extracted using the following procedure: the filter pad and the bowl of glass wool were lyophilised for 15 h, transferred to a 200 mL beaker, and 20 mL of ethanol added. After sonicating for 5 min the filter pad and the bowl of glass wool were again extracted with 30 mL of ethanol. The two extracts were combined, evaporated to a volume of about 2 mL, and rediluted to 10.0 mL. Then to 1.0 mL of this extract 100 □L of the internal standard solution containing 2 mg/mL phen in ethanol were added. 10 □L were injected into the HPLC-DAD.

2.3 Preparation and validation of the THC injection solution

Standards, chemicals, and solvents

Table 16: Chemicals for the preparation of the injection solution

Abbreviations	Name	Lot-No	Supplier
THC	Δ^9-Tetrahydrocannabinol, Dronabinol	300.802	THC Pharm, Frankfurt am Main (D)
Tween® 80	Polysorbatum 80 (Ph. Eur.)	020077	Pharmacy of the University Hospital, Bern (CH)
NaAsc	Sodium Ascorbate (USP)	1167110	Pharmacy of the University Hospital, Bern (CH)
NaCl	Sodium chloride (Ph. Eur.)	010042	Pharmacy of the University Hospital, Bern (CH)
EtOH	Ethanolum absolutum	412613/1 62100	Fluka Chemika AG, Buchs (CH)

Bidistilled water, Department of Clinical Research, University of Bern (CH).
All other solvents and chemicals used were of HPLC or analytical quality obtained from Merck AG, Basel (CH) or Fluka Chemie AG, Buchs (CH).

Composition and preparation of the THC injection solution

The THC injection solution was prepared using the formulation of Olsen et al. [90] adding NaAsc as an antioxidant for better stability. The composition of the formulation is presented in Table 17.

Table 17: Composition of the THC injection solution

Component	Amount	[% (w/v)]
THC	10.0 mg	0.1
EtOH abs.	500.0 µL	5.0
Tween® 80	150.0 mg	1.5
NaAsc	10.0 mg	0.1
NaCl solution (0.9 % w/v)	to 10.0 mL	

The THC was dissolved in the EtOH and Tween® 80, then added to NaAsc dissolved in 1 mL of the NaCl solution. The remaining sodium chloride solution was finally added to the mixture. The clear, yellowish solution was then sonicated for 30 sec and filtrated through a 0.22 µm filter under aseptic conditions.

Quality assurance of the injection solution

Stability

Quantification of the THC content was done every 3 to 9 days using the described HPLC method.

Osmolality

The measurement of the osmolality was done by the pharmacy of the University Hospital Bern according to the standards of the European Pharmacopeia [89].

pH

The pH value of the formulation was determined three times using a potentiometer.

Sterility

The absence of microbial contaminants was confirmed by the pharmacy of the University Hospital Bern using the method of the European Pharmacopeia [89].

3. PHARMACOKINETIC STUDY WITH PULMONAL AND INTRAVENOUS THC

3.1 Subjects and study design (pharmacokinetic study)

Eight healthy volunteers were admitted to this randomised, placebo controlled (only inhalation), double-blind, crossover study which was carried out in the Clinical Investigation Unit (CIU) of the University Hospital of Bern. The subjects were informed about the risks of the study, gave their written informed consent, and were paid for participating. Exclusion criteria were past or existing drug abuse (including alcohol and prescription drugs; Cannabis urine test before each session), known or suspected hypersensibility to cannabinoids, pregnancy (urine test before first session), positive past history of any psychiatric disorders, and lung diseases. Each subject had to pass the lung function tests including vital capacity and forced expiratory volume in one second. The subjects were not allowed to take analgesics, alcohol, and caffeinated beverages 48 h before and during the study and were asked to refrain from driving up to 24 h after the study. The study was approved by the Local Ethics Committee, the Swiss Agency for Therapeutic Products (Swissmedic), and the Federal Office for Public Health (study protocol, volunteer information etc. see appendix II). In the first and second session, each subject received randomly and double-blinded either the THC (0.053 mg/kg b. wt.) or the placebo inhalation aerosol. In the third session THC was administered i.v. (0.053 mg/kg b. wt.) over a time period of 2 min. The between-session washout phases were at least 7 days. To familiarize the subjects with the pain test and the VAS, each session began with a training phase, during which the subjects performed a pain test and a 5-min inhalation training with the placebo aerosol. This was followed by the recording of the baseline (vital functions, side effects scores, and pain test). After administration of the THC and placebo preparations vital functions and side effects were recorded and ice water pain determined at 20, 40, 60, 120, 240, and 480 min. Blood (5 mL) was collected in all three sessions through a peripheral vein catheter at baseline, 5, 10, 20, 40, 60, 120, 240, and 480 min after administration of the test medications. The heparinised blood samples were centrifuged and the plasma instantly deep-frozen and stored at - 20 °C until analysis.

3.2 Inhalation procedure

The pressure-driven inhalation device PARI® Master and the PARI® LC-plus nebuliser with interrupter were used. The subjects were instructed to inhale deeply with a breath frequency of 1 breath per 10 sec waiting 3 to 5 sec before expiration. The subjects were instructed to continue until all the inhalation solution had been inhaled. Inhalation time and any residue left in the nebuliser compartment were measured.

3.3 Composition and preparation of the clinical test substances

Verum inhalation solution

The verum inhalation solution was prepared using the composition and method described in chapter 2.2 ("Development and validation of the THC inhalation solution"), 1 to 2 weeks before the respective sessions.

Placebo inhalation solution

The placebo inhalation solution was exactly prepared like the verum solution only lacking the THC, 1 to 2 weeks before the respective sessions.

Injection solution

The injection solution was prepared using the composition and method described in chapter 2.3 ("Preparation and validation of the THC injection solution") 1 to 2 weeks before the respective sessions.

3.4 Pain test

The same cold test as in the first pain study (ice cold immersion test, described in chapter 1.3 “Pain tests”) was used.

3.5 Monitoring of side effects and vital functions

A 10-cm VAS (see appendix II) was used to asses psychological and somatic side effects, such as sedation, euphoria, anxiety, nausea, vertigo, headache, irritation of airways etc.. Haemoglobin oxygen saturation (pulse oximetry), blood pressure, and heart rate were recorded.

3.6 Determination of THC and -metabolites in plasma by gas chromatography mass-spectrometry (GC-MS)

Standards, chemicals, and solvents

Table 18: Standards for the quantification of THC and its metabolites

Abbreviations	Name	Lot-No	Supplier
THC	□^{9}-Tetrahydrocannabinol	135.1B48.1L1	Lipomed, Arlesheim, CH
THC-d_3	□^{9}-THC-d_3	315.1B1.1L1	Lipomed, Arlesheim, CH
THC-OH	(±)-11-Hydroxy-□^{9}-THC	34703-81B	Radian, Austin, USA
THC-OH-d_3	(±)-11-Hydroxy-□^{9}-THC-d_3	31534-49A	Radian, Austin, USA
THC-COOH	(±)-11-nor-9-Carboxy-□^{9}-THC	31533-70A	Radian, Austin, USA
THC-COOH-d_3	(±)-11-nor-9-Carboxy-□^{9}-THC-D_3	35002-42B	Radian, Austin, USA

□-Glucuronidase, Type IX-A from Escherichia choli, Sigma, Buchs (CH);
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) from Fluka Chemie AG, Buchs (CH).
All other solvents and chemicals used were of HPLC or analytical quality obtained from Merck AG, Basel (CH) or Fluka Chemie AG, Buchs (CH).

Instrumentation

The GC-MS system consisted of a HP GC 5890 Series II gaschromatograph with a 7673 autosampler and a G1512A autosampler controller, a HP 5972 mass-selective detector (MSD), and a Vectra 486/66 XM computer with Chemstation Software G1046A Rev. A.00.00 (HP 1989-1994).

Method

Hydrolysis and extraction procedure for the plasma samples

The hydrolysis of the plasma samples was done using the method of Feng et al. [83]. In a 10 mL tube 0.6 mL of plasma was spiked with 30 □L of a methanol solution containing 1 □g/mL THC-d_3, THC-OH-d_3, and THC-COOH-d_3. To each tube 2.44 mL of 0.1 M potassium phosphate buffer (pH 6.8) followed by 200 □L of a 25'000 units/mL solution of □-glucuronidase in the same buffer (a total of 5'000 units) were added. The tube was then vortexed, capped, and incubated at 37 °C for 16 h. The sample was cooled to room temperature and extracted automatically with an ASPEC XL (Automatic Sample Preparation with Extraction columns) robotic system equipped with a Dilutor 402 (Gilson, Villliers Le Bel, F), using the method of Moeller et al. [91] and Bakerbond C_{18} SPE columns (Stehelin & Cie AG, Basel, CH) (Table 19).

Table 19: SPE of the plasma samples

Conditioning	Methanol	6 mL
	Bidistilled water	3 mL
Sample	An aliquot of 2.725 mL (corresponding to 0.5 mL plasma) of the hydrolysed mixture was loaded onto the column	2.725 mL
Washing	Bidistilled water	3 mL
	0.25 M Acetic acid	3 mL
	Bidistilled water	3 mL
	Acetone	0.075 mL
Drying	Air	2 mL
Elution	Acetone	0.5 mL
	Acetone	0.5 mL
	Acetone	0.5 mL

The eluate was evaporated to dryness at 50 °C under nitrogen. The residue was then derivatised using the method of Feng et al. [83]. 60 µL of BSTFA containing 1 % TMCS was added to the residue and vortexed. The tube was capped and heated at 70 °C for 30 min. The sample was then cooled to room temperature, transferred to a GC vial insert, capped, and injected into the GC-MS.

Chromatographic conditions

Column	DB-5 MS column (J&W Scientific, Folsom, CA, provided by MSP, Köniz, CH), 25 m x 0.2 mm I.D., film-thickness 0.33 µm
Carrier gas	Helium
Constant flow	1.2 mL/min
Temperature program	200 °C (0.5 min) to 280 °C at 5.0 °C/min, 280 °C (5 min)
Injection volume	2 µL, splitless
Injector temperature	250 °C
Transfer line temperature	280 °C
Detection mode	SIM monitoring (the ions are listed in Table 20)

Table 20: Monitored ions for the quantification of THC and its metabolites (TMS derivatives)

Analyte	Quantitation ion	Qualifying ion
THC-TMS	371	343
THC-d_3-TMS	374	389
THC-OH-TMS_2	371	474
THC-OH-d_3-TMS_2	374	377
THC-COOH-TMS_2	371	488, 473
THC-COOH-d_3-TMS_2	374	491

Validation

Peak identification (selectivity / specificity):

Chromatographic selectivity: The peaks of the analytes (THC, THC-OH, THC-COOH) and the corresponding deuterated internal standards (THC-d_3, THC-OH-d_3, THC-COOH-d_3) were assigned by standards. Negative control - (extracted blank plasma) and positive control-samples (spiked blank plasma) were analysed to exclude any interferences.

Spectroscopic selectivity: Peak identification was performed in the SIM mode by the qualifyer ions listed in Table 20.

Calibration and linearity

Blank plasma was spiked with different concentrations of standards. After extraction the samples were analysed using the described method. For each analyte the following calibrators were used in duplicates: 0.4, 1, 2, 5, 10, 20, 40, and 100 ng/mL plasma. For THC, additional calibrators containing 200 and 300 ng/mL plasma were used. The concentration of the internal standards was 50 ng/mL each.

Recovery, intra- and interday precision, and accuracy

For the determination of the recovery 6 samples of blank plasma were spiked with different concentrations (2-150 ng/mL) of each analyte. The samples were prepared and analysed with the described method, and the area of the respective peaks compared with the area of the peaks of a standard measured without sample preparation.

For the determination of the intraday precision 5 samples of blank plasma were spiked with 4 and 100 ng/mL of each analyte, respectively, then extracted and analysed within the same day according to the described method. Mean, standard deviation (SD, RSD), and accuracy were calculated.

For the determination of the interday precision 5 samples of blank plasma were spiked with 4 and 100 ng/mL of each analyte, respectively, and analysed at 5 different days within one month.

Limit of detection and limit of quantification

The LOD and LOQ were determined with spiked blank plasma. The LOD was defined as the concentration where the peaks could be clearly integrated with a signal to noise ratio of 3 to 1. The LOQ was defined as the concentration where a control sample of 0.4 ng/mL was within the range of ± 20 % of the calibration.

3.7 Calculation of the pharmacokinetic parameters

Plasma concentrations versus time were used to estimate pharmacokinetic parameters, including plasma peak concentrations (C_{max}), time to reach peak plasma concentration (t_{max}), and area under the concentration-time curve (AUC). Based on a non-compartment model, all pharmacokinetic parameters were assessed by use of standard calculation procedures performed by the TopFit® (version 2.0) computer software [84]. AUC up to the time corresponding to the last measurable concentration ($AUC_{0\text{-}tlast}$) was calculated by numeric intergration using the linear trapezoidal rule. Values for C_0 (extrapolated) were determined by linear regression of the logarithmically transformed concentration values back to the time point 0. The value of the elimination rate constant, λ_z, was determined by using TopFit® software, applying a non-compartmental analysis technique that focused on the terminal linear phase of semilogarithmic plots of the individual plasma concentration-time data. The elimination half-life ($t_{1/2}$) was calculated using the following equation: $t_{1/2} = 0.69315/\lambda_z$. The bioavailability (F) of inhaled THC compared to i.v. THC was calculated using the following equation: $F = (AUC_{inhal.}/AUC_{i.v.})/(dose_{i.v.}/dose_{inhal.})$. The clearance (CL) and volume of distribution (V_z) were calculated using the i.v. data and the following equations: $CL = dose_{i.v.}/AUC_{i.v.}$; $V_z = CL/\lambda_z$.

A second analysis of the i.v. data was done with the PKAnalyst® software (Version 1.0) [92] using a two-compartment model (model # 8, two compartments with bolus input and first-order output, micro-constants as input) for the fitting. Clearance CL and distribution volume V_z were manually calculated using the following equations: CL = dose / $AUC_{0\text{-}480min}$, V_z = CL / elimination rate constant.

RESULTS

1. PHARMACODYNAMIC STUDY WITH ORAL THC (PAIN STUDY)

1.1 Quality assurance of clinical test preparations

1.1.1 Marinol® (THC, dronabinol; capsules)

Validation

Peak identification (selectivity / specificity)

The blank run showed no interferences at the retention times of the analyte and the internal standard. Retention time and ions for THC were 20.3 min (20.0 - 21.0 min) and m/z 314, 299, and 271, respectively. Retention time and ions for THC-d_3 were 20.4 min (20.0 - 21.0 min) and m/z 317, 302, and 274, respectively.

Calibration and linearity

Table 1 shows the data of the calibration.

Table 1: Calibration data for the quantification of THC in Marinol® capsules

Analyte	Retention time [min]	m[1)]	b[1)]	Correlation coefficient r
THC	20.3	0.0223	- 0.0829	0.9994

[1)]y = mx + b; x: amount of THC; y: ratio of the areas under the peak

The calibration was linear in the concentration range of 30 to 70 µg/mL THC. The present method was consequently used to determine the THC content of the Marinol® capsules used in the pain study.

Recovery

The recovery was not determined. It was assumed that 100 % of the THC was extracted from the capsule and the sesame oil matrix.

Intra- and interday precision and accuracy

Table 2 shows the results for the intraday precision and the accuracy. Table 3 shows the results for the interday precision.

Table 2: Intraday precision and accuracy for the quantification of THC in Marinol® capsules

Added THC [µg/mL]	Found [Mean, µg/mL]	SD [µg/mL, n = 4]	RSD [%, n = 4]	Accuracy [%]
30	29.01	0.55	1.1	- 3.3
50	51.60	1.17	1.6	3.2
70	70.01	2.94	2.7	0.01

Table 3: Interday precision for the quantification of THC in Marinol® capsules

Added THC [µg/mL]	SD [µg/mL]	RSD [%]
30 (n = 6)	0.74	2.6
50 (n = 6)	0.46	4.1
70 (n = 6)	0.85	1.2

Limits of detection and quantification

The LOD and LOQ were not determined because the method was used for the quantification of the THC content in the 10 mg-Marinol® capsules and therefore the range of the concentration far above the LOD/LOQ.

Quality assurance of Marinol®

Table 4 shows the results of the quality assurance of the Marinol® capsules used in the pain study.

Table 4: Quality assurance of 10 mg-Marinol® capsules

	Measured THC content in mg	Difference to the declared content of 10 mg
Capsule 1	10.8 mg	+ 8.0 %
Capsule 2	10.9 mg	+ 9.0 %
Capsule 3	11.0 mg	+ 10.0 %

The THC content of the three capsules was in the ± 10 % range of the declared content and thus in agreement on the regulations of the European Pharmacopeia [89].

1.1.2 THC-hemisuccinate suppositories (rectal THC-HS)

Calibration

The calibration data are presented in Table 5.

Table 5: Calibration data for the quantification of THC hemisuccinate in suppositories

Analyte	Retention time [min]	m[1)]	b[1)]	Correlation coefficient r
THC-HS	4.47	1.0685	0.0217	0.9994

[1)]y=mx + b; x: ratio of amount of the concentrations (THC-HS vs. THC hemiglutarate); y: ratio of the areas under the peak

Results of the quality assurance

The analysis of 6 suppositories showed that a high amount of the THC-HS was already hydrolysed. The results are presented in Table 6.

Table 6: THC-HS quantification in suppositories

Sample	Type / Lot	THC-HS content [mg]	% of the declared content	Comment
1	5 mg / VSu0698	3.0	60 %	**out** of the tolerated limits of ± 10%[1)]
2	5 mg / VSu0698	3.1	62 %	**out** of the tolerated limits of ± 10%
3	10 mg / VSu0798	7.8	78 %	**out** of the tolerated limits of ± 10%
4	10 mg / VSu0798	9.4	94 %	**within** the tolerated limits of ± 10%
5	20 mg / VSu0898	17.8	89 %	**out** of the tolerated limits of ± 10%
6	20 mg / VSu0898	16.9 mg	85 %	**out** of the tolerated limits of ± 10%

[1)] Prescribed limits of the European Pharmacopeia: ± 10 % [89]

The THC-HS content of the suppositories of batch 1 - 3 and 5 - 6 were not within the ± 10 % range of the declared content and thus not in agreement of the regulation of the European Pharmacopeia [89]. In consequence, batch 1-3 and 5-6 could not be used for clinical purposes within the REHAB Basel project due to significant hydrolysis of THC-HS to THC.

1.2 Subjects and study design

The subjects were all Cannabis-naïve. Six females (21 - 38 years; 70 ± 8 kg b.wt.) and 6 males (18 - 47 years; 74 ± 7 kg b.wt.) were admitted.

1.3 Pain tests

Pressure

In the pressure test, where pain tolerance thresholds were measured, no significant analgesic effect of THC was observed compared to placebo (Figure 1). On the other hand, morphine alone increased the pain tolerance threshold significantly compared to placebo ($p = 0.01$).

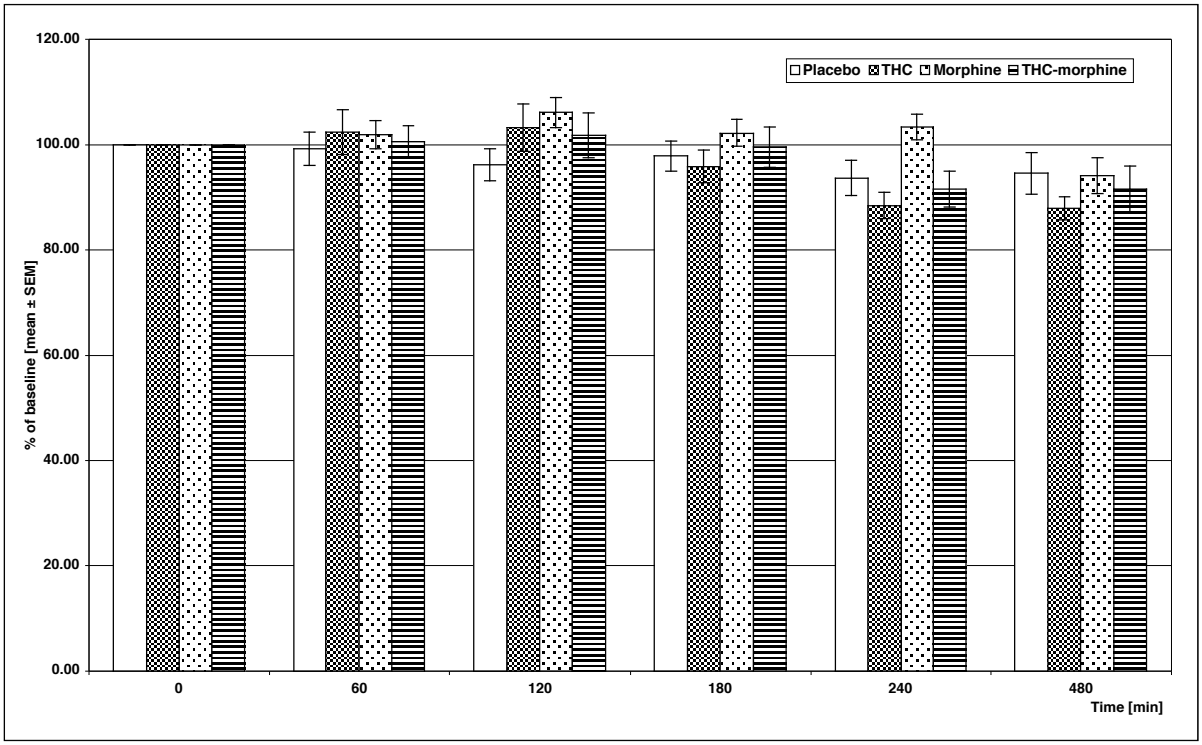


Figure 1: Pain tolerance of THC, morphine, THC-morphine, and placebo in the pressure test: values > 100 % of baseline (mean ± SEM) indicate analgesia, values < 100 % hyperalgesia.

Heat

As shown in Figure 2 and 3, THC did not produce any analgesic effect in the heat test, neither alone nor in combination with morphine. Morphine alone had no effect.

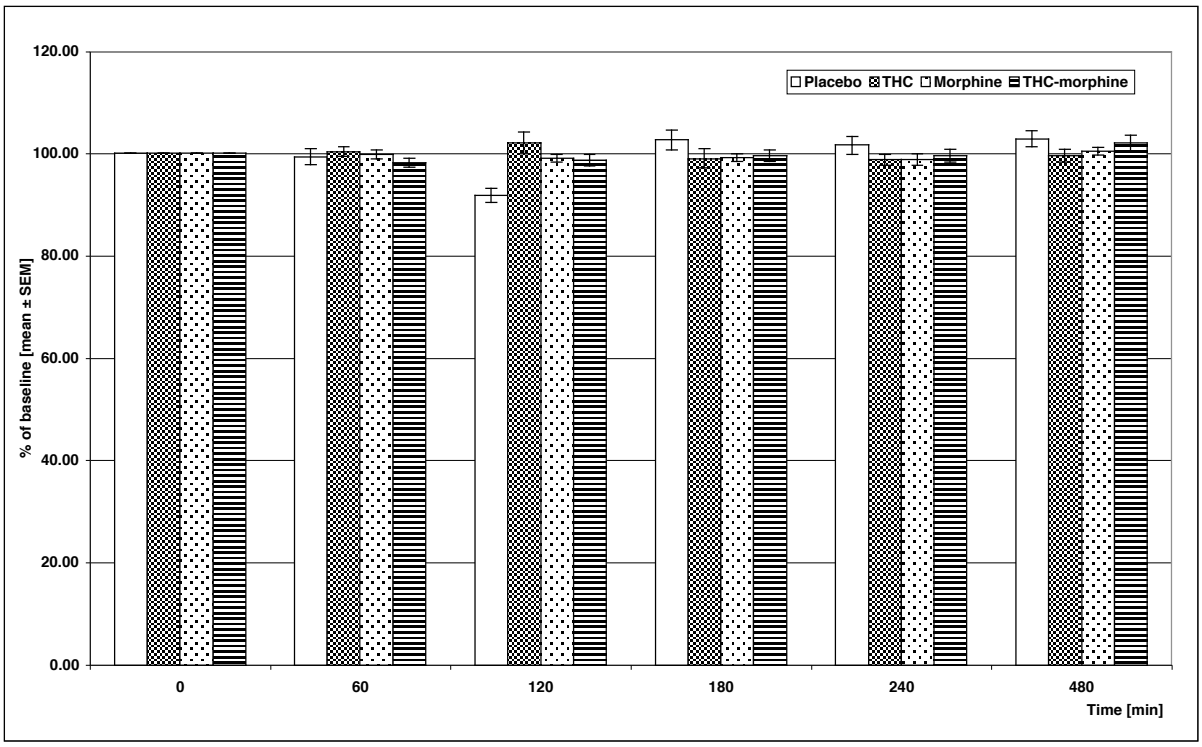


Figure 2: Pain detection threshold of THC, morphine, THC-morphine, and placebo in the heat test: values > 100 % of baseline (mean ± SEM) indicate analgesia, values < 100 % hyperalgesia.

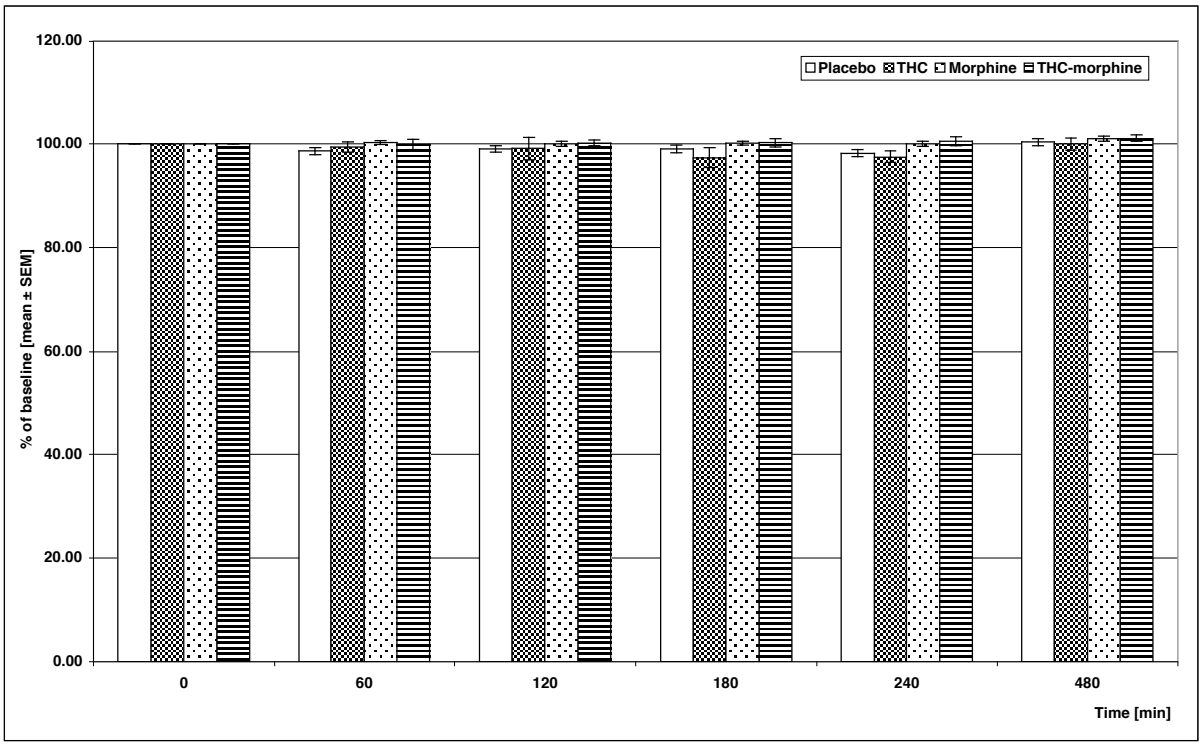


Figure 3: Pain tolerance threshold of THC, morphine, THC-morphine, and placebo in the heat test: values > 100 % of baseline (mean ± SEM) indicate analgesia, values < 100 % hyperalgesia.

Cold

Figure 4 represents the area under the pain-intensity time curve (total pain, AUP) in the cold test (ice cold immersion test). The AUP's of THC and THC-morphine showed no significant difference compared to placebo. Morphine alone significantly (p = 0.014) reduced AUP, whereas THC alone increased AUP, an effect completely neutralised when combining THC with morphine. This hyperalgesia was not significant. The same effect was observed for the mean pain value. The peak pain value was significantly reduced by morphine (p = 0.017) and the THC-morphine combination (p = 0.046), but not with THC alone.

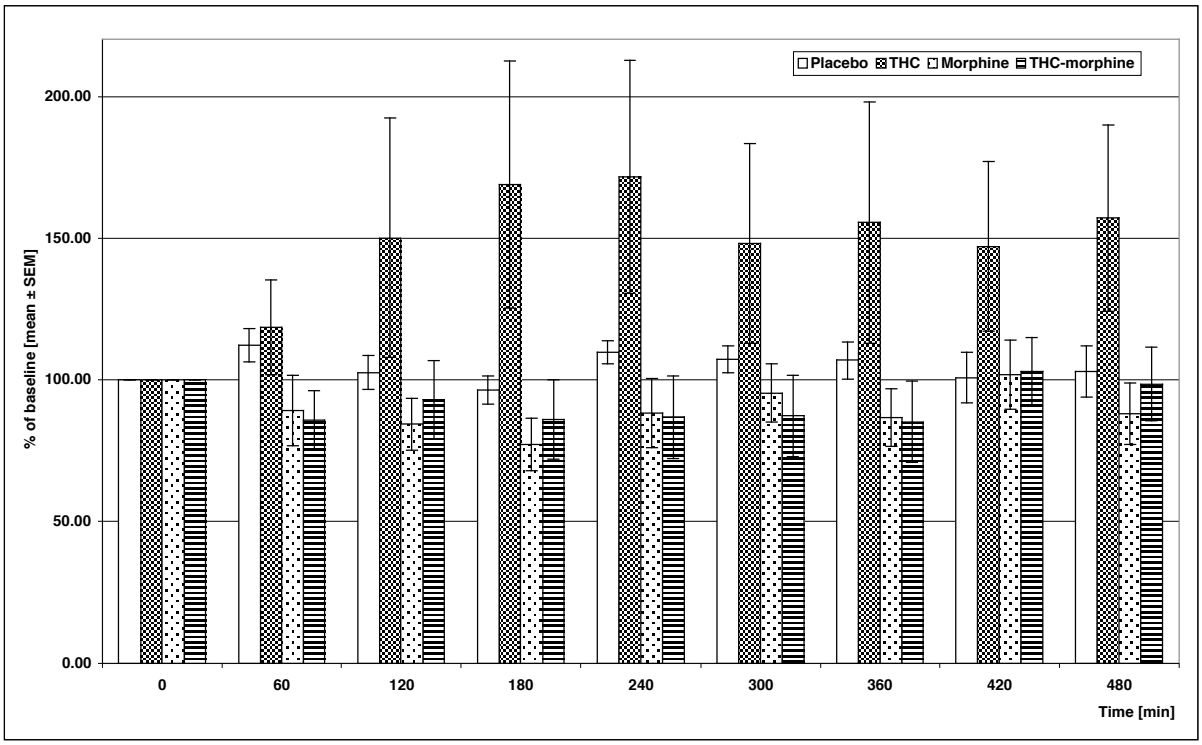


Figure 4: Pain tolerance of THC, morphine, THC-morphine, and placebo in the ice cold immersion test; values > 100 % of baseline (mean ± SEM) indicate hyperalgesia, values < 100 % analgesia.

Transcutaneous Electrical Stimulation (single, repeated)

In the single mode of the transcutaneous electrical stimulation (Figure 5) no significant analgesic effect of THC and THC-morphine was observed. However, a slightly additive effect of THC in combination with morphine compared to morphine alone at most of the observation points could be seen. Morphine significantly increased the pain detection threshold value (p = 0.008). In the repeated mode (Figure 6) THC in combination with morphine was again additively effective in the pain detection compared to morphine alone and even produced a statistically significant analgesic effect compared to placebo (p = 0.042). Morphine alone

showed again a significant increase of the pain detection threshold value compared to placebo ($p = 0.004$). THC alone did not significantly reduce pain. It again caused a slight, not significant hyperalgesia in the second part of the session (time point 4 to 8 h post drug) compared to placebo.

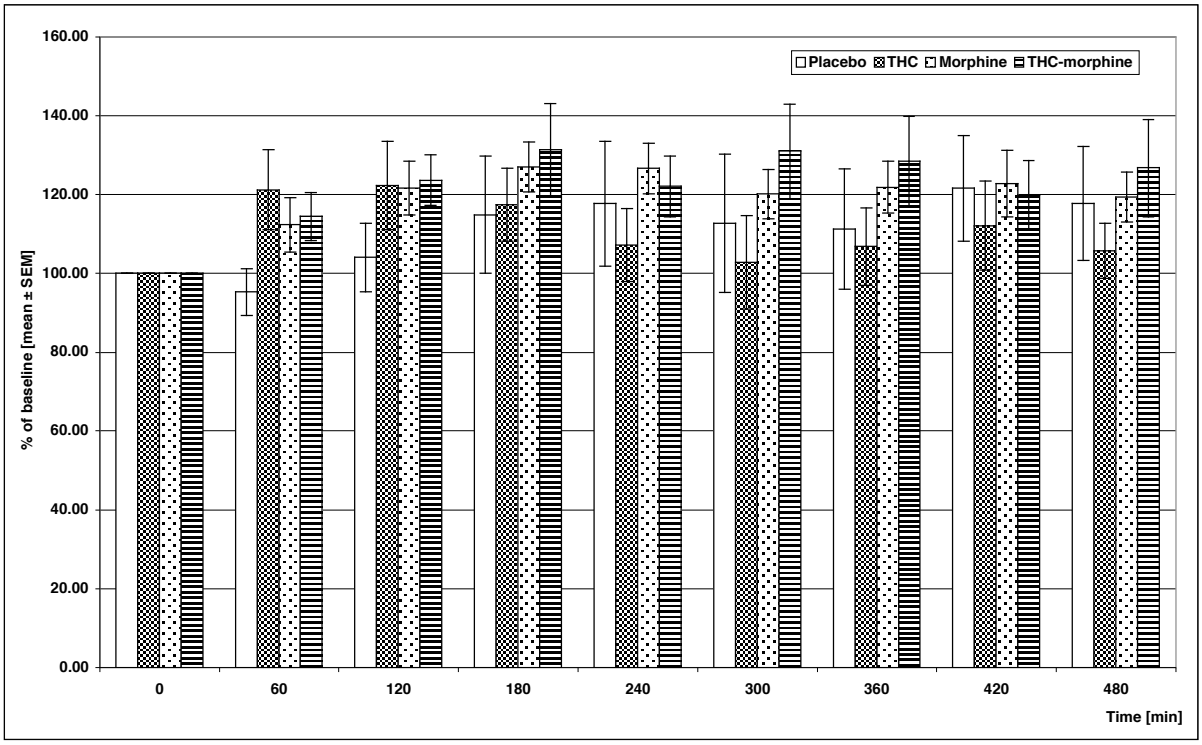


Figure 5: Pain detection threshold of THC, morphine, THC-morphine, and placebo after single transcutaneous electrical stimulation; values > 100 % of baseline (mean ± SEM) indicate analgesia, values < 100 % hyperalgesia.

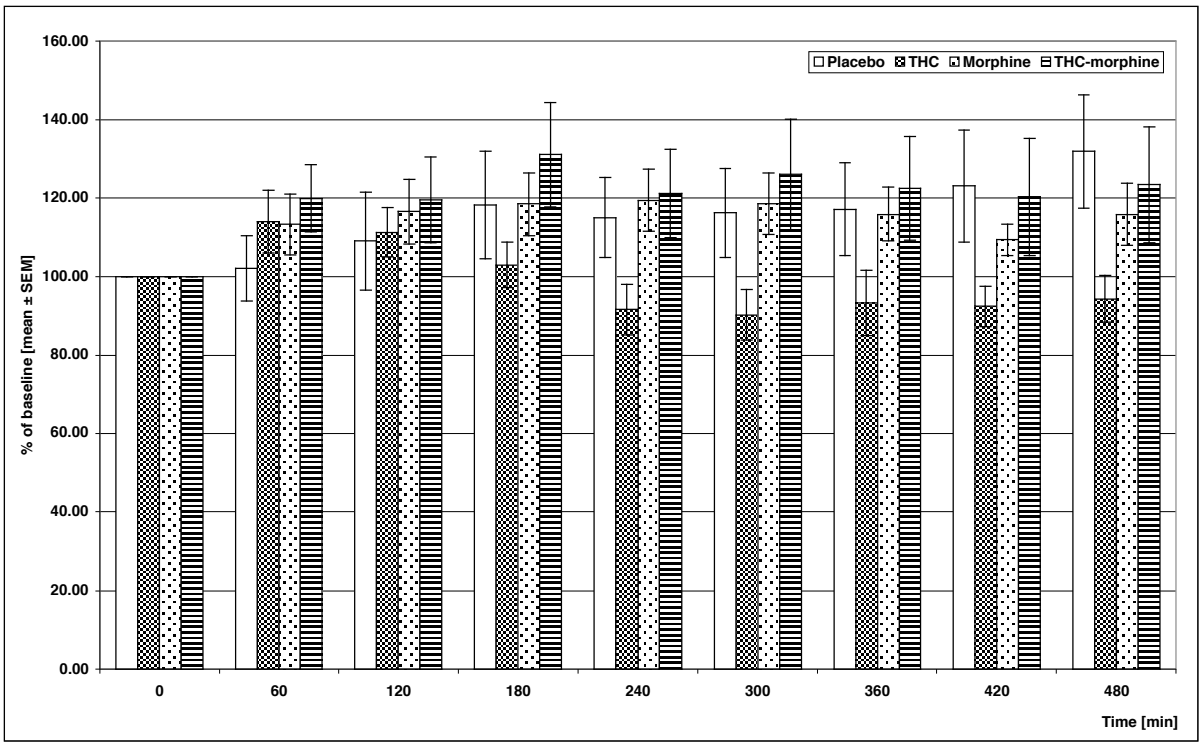


Figure 6: Pain detection threshold of THC, morphine, THC-morphine, and placebo after repeated transcutaneous electrical stimulation; values > 100 % of baseline (mean ± SEM) indicate analgesia, values < 100 % hyperalgesia.

1.4 Monitoring of side effects and vital functions

The side effects, summarised in Table 7, were usually mild. Most of the subjects felt sleepy and confused after the administration of THC and THC-morphine. They also reported altered inner and outer perception, feelings of anxiety and aggression. Interestingly, the euphorigenic and hallucinogenic effects of THC were reduced when combining with morphine (Figures 7 & 8).

Table 7: Psychological and somatic side effects (peak VAS %, mean ± SEM of all subjects) after THC, morphine, THC-morphine, and placebo (n = 12)

Side effect	Placebo		THC		Morphine		THC-Morphine	
	f[1]	VAS [%]	f[1]	VAS [%]	f[1]	VAS [%]	f[1]	VAS [%]
Sleepiness	12	70 ± 31	12	82 ± 20	12	64 ± 33	12	85 ± 20
Euphoria	1	66 ± 0	9	54 ± 34	3	16 ± 11	5	21 ± 29
Irritation	1	12 ± 0	5	44 ± 34	1	6 ± 0	5	28 ± 31
Anxiety	0	-	4	54 ± 43	0	-	3	22 ± 32
Tenseness and aggressiveness	1	10 ± 0	4	57 ± 39	2	14 ± 5	2	49 ± 66
Confusion and disorientation	1	2 ± 0	7	58 ± 31	0	-	8	13 ± 9
Change of inner perception	2	16 ± 4	10	66 ± 30	5	19 ± 9	9	61 ± 37
Change of outer perception	0	-	8	53 ± 28	0	-	4	41 ± 36
Hallucinations	0	-	6	64 ± 29	0	-	5	39 ± 37
Strange thoughts, ideas, moods	0	-	7	51 ± 40	1	13 ± 0	3	43 ± 50
Nausea	0	-	5	25 ± 17	3	27 ± 7	6	11 ± 8
Headache	2	23 ± 8	6	63 ± 28	5	33 ± 33	5	36 ± 29
Difficulties in breathing	0	-	6	30 ± 33	2	18 ± 17	4	22 ± 23
Heart problems (tachycardia)	0	-	6	48 ± 37	0	-	1	97 ± 0
Digestive problems	0	-	5	25 ± 19	3	13 ± 12	4	8 ± 6
Dry mouth	5	21 ± 20	12	76 ± 28	8	31 ± 22	10	51 ± 37
Vertigo	3	6 ± 2	11	51 ± 35	5	12 ± 10	9	34 ± 33
Vomiting	0	-	0	-	4	-	2	-
Orthostatic disorder	0	-	0	-	1	-	2	-

[1] f: Frequency of side effect per 12 subjects

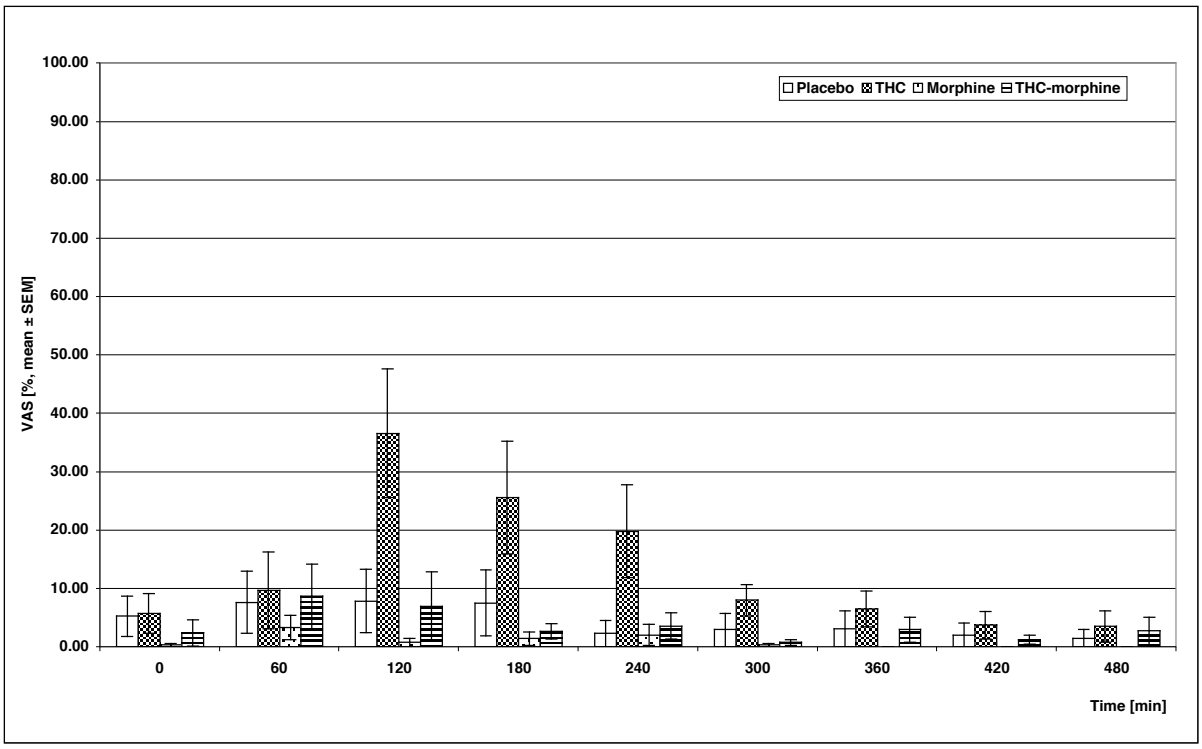


Figure 7: Side effect "euphoria" (VAS %, mean ± SEM) after THC, morphine, THC-morphine, and placebo.

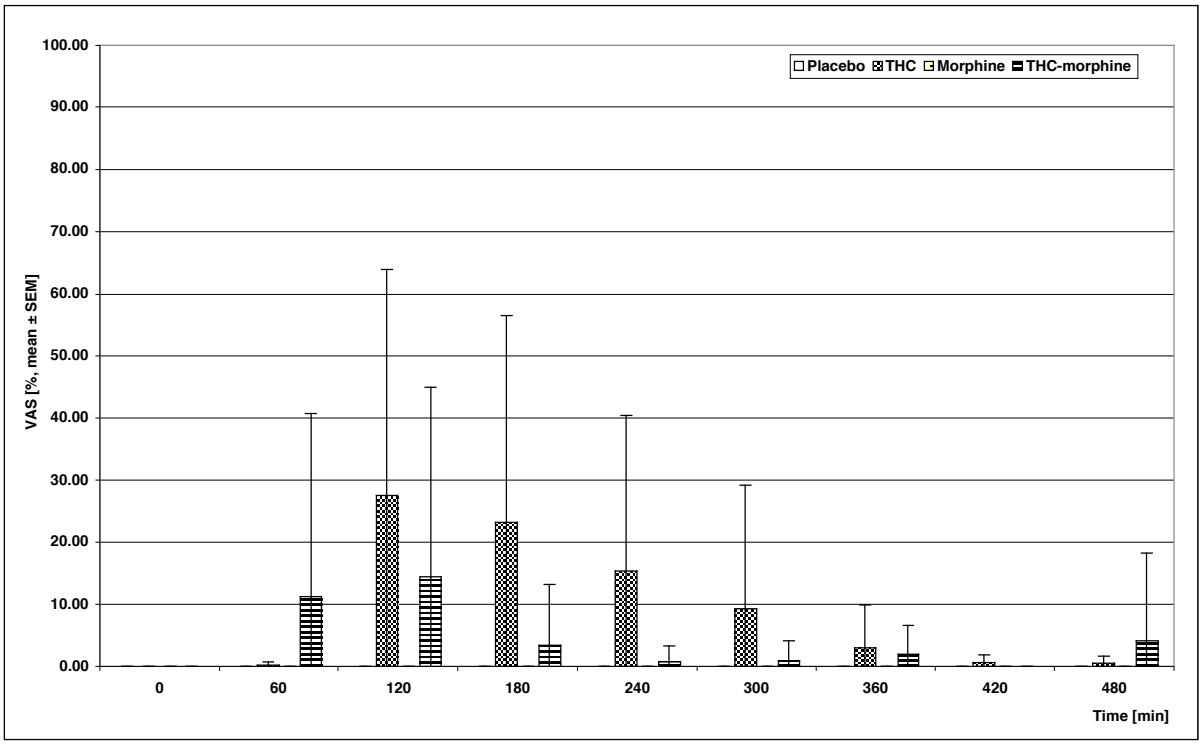


Figure 8: Side effect "hallucinations" (VAS %, mean ± SEM) after THC, morphine, THC-morphine, and placebo.

The reaction time was not significantly impaired by any of the test preparations (Figure 9). Systolic (116 ± 4 to 101 ± 3 mm Hg) as well as diastolic blood pressure (65 ± 3 to 54 ± 2 mm Hg) decreased significantly only after THC-morphine compared to placebo. With 63 ± 3 to 87 ± 4 THC alone increased significantly the heart rate, whereas the haemoglobin oxygen saturation was only significantly reduced after THC-morphine.

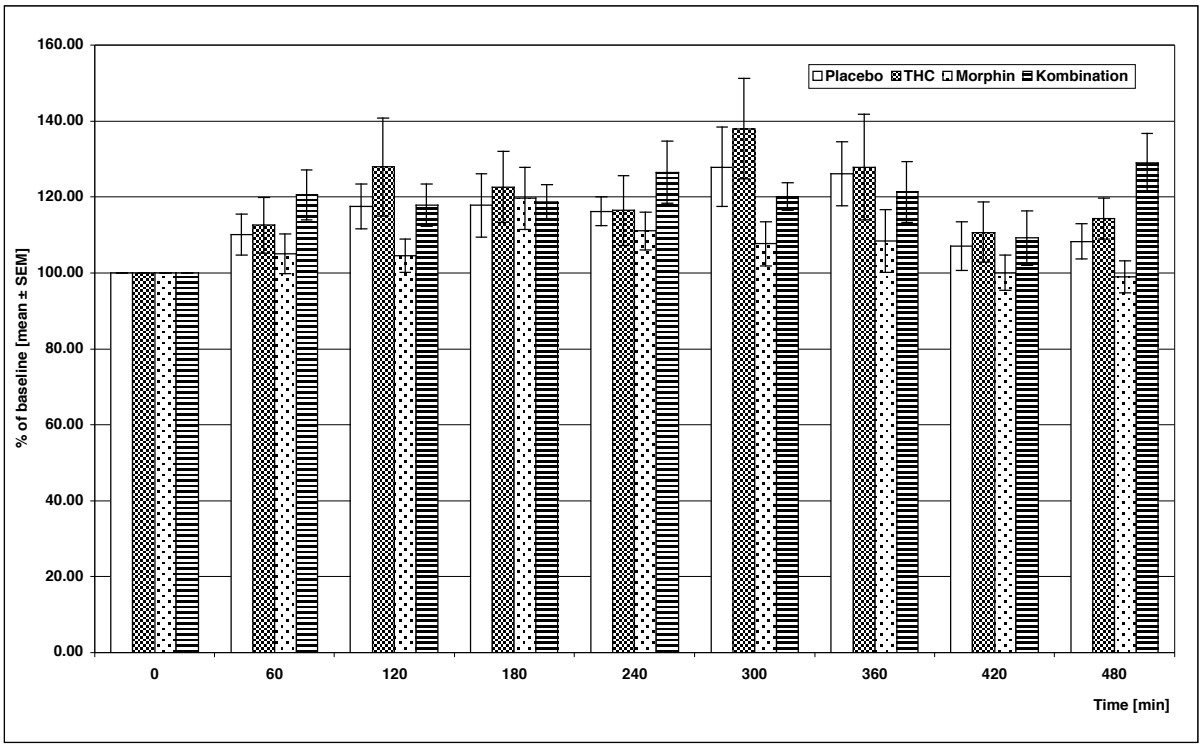


Figure 9: Reaction time % of baseline (mean ± SEM) after THC, morphine, THC-morphine, and placebo.

1.5 Determination of morphine and -metabolites in plasma by HPLC-DAD and HPLC-FLD

Validation

Peak identification (selectivity / specificity)

HPLC-DAD:

Blank and positive control samples showed no interferences with the analytes and the internal standard. The retention times of the analytes are presented in Table 8, paragraph “Calibration and linearity”.

HPLC-FLD:

Blank and positive control samples showed no interferences with the analytes and the internal standard. The retention times of the analytes are presented in Table 9, paragraph “Calibration and linearity”.

Calibration and linearity

HPLC-DAD:

Table 8 gives an overview of the calibration results.

Table 8: Calibration data for the quantification of morphine and its metabolites in plasma (HPLC-DAD)

Analyte	Retention time [min]	m[1)]	b[1)]	Correlation coefficient r
M3G	2.75	1.35664	0.06873	0.9999
M6G	3.81	1.63370	-0.00779	0.9988
M	4.40	1.28137	0.01607	0.9985

[1)] y = mx + b; x: ratio of the concentrations; y: ratio of the areas under the peak

The data show linearity in the calibrated range of 20 to 500 ng/mL for M and M6G and 20 to 1000 ng/mL for M3G, respectively.

HPLC-FLD:

Table 9 gives an overview of the calibration results.

Table 9: Calibration for the quantification of morphine and its metabolites in plasma (HPLC-FLD)

Analyte	Retention time [min]	m[1)]	b[1)]	Correlation coefficient r
M3G	5.15	0.79240	0.00799	0.9992
M6G	7.28	0.26299	0.00485	0.9997
M	9.59	0.54045	0.00171	0.9995

[1)] y = mx + b; x: ratio of the concentrations; y: ratio of the areas under the peak

The data show linearity in the calibrated range 20 to 500 ng/mL for M and M6G and 20 to 1000 ng/mL for M3G, respectively. The current method was used for the acquisition of pharmacokinetic data and therefore the range of calibration had to be selected in order to include the highest plasma levels of the morphine metabolites as well as the lowest plasma levels of the analytes in the elimination phase. Different experiments showed a LOQ of 20 ng/mL (DAD) and 10 ng/mL (FLD), respectively, for each of the analytes. The upper LOQ was equal to the highest calibrator (1000 ng/mL).

Recovery and precision

Table 10 shows the results for the two quantification methods.

Table 10: Recovery and precision of the quantification of morphine and its metabolites in plasma

Analyte	Recovery [%] HPLC-DAD (n = 5)	Recovery [%] HPLC-FLD (n = 5)	Precision (RSD, %; n = 5) HPLC-DAD	Precision (RSD, %; n = 5) HPLC-FLD
M3G	68.4	66.3	6.3	7.4
M6G	87.3	65.7	9.7	9.8
M	67.3	46.1	7.5	8.3
IS	87.3	42.8	-	-

Limit of detection

The LOD was found to be 20 (DAD) and 10 ng/mL (FLD), respectively. At this concentration levels the peaks could clearly be detected and integrated.

Limit of quantification

The LOQ was 20 (DAD) and 10 ng/mL (FLD), respectively, corresponding to the lowest calibrator.

Plasma levels

The measured morphine plasma concentrations after oral administration of 30 mg of morphine alone ranged from 0 to 11.2 ± 2.2 ng/mL peaking at 60 min. The M6G plasma concentrations were 16.5 ± 6.8 to 97.5 ± 14.4 ng/mL, mostly peaking at 120 min. The M3G levels were 98.7 ± 23.2 to 707.8 ± 64.2 ng/mL, with peaks from 60 to 120 min. Figure 10 shows the mean morphine, M6G, and M3G plasma concentrations after the oral administration of morphine plotted against time on a semilogarithmic scale.

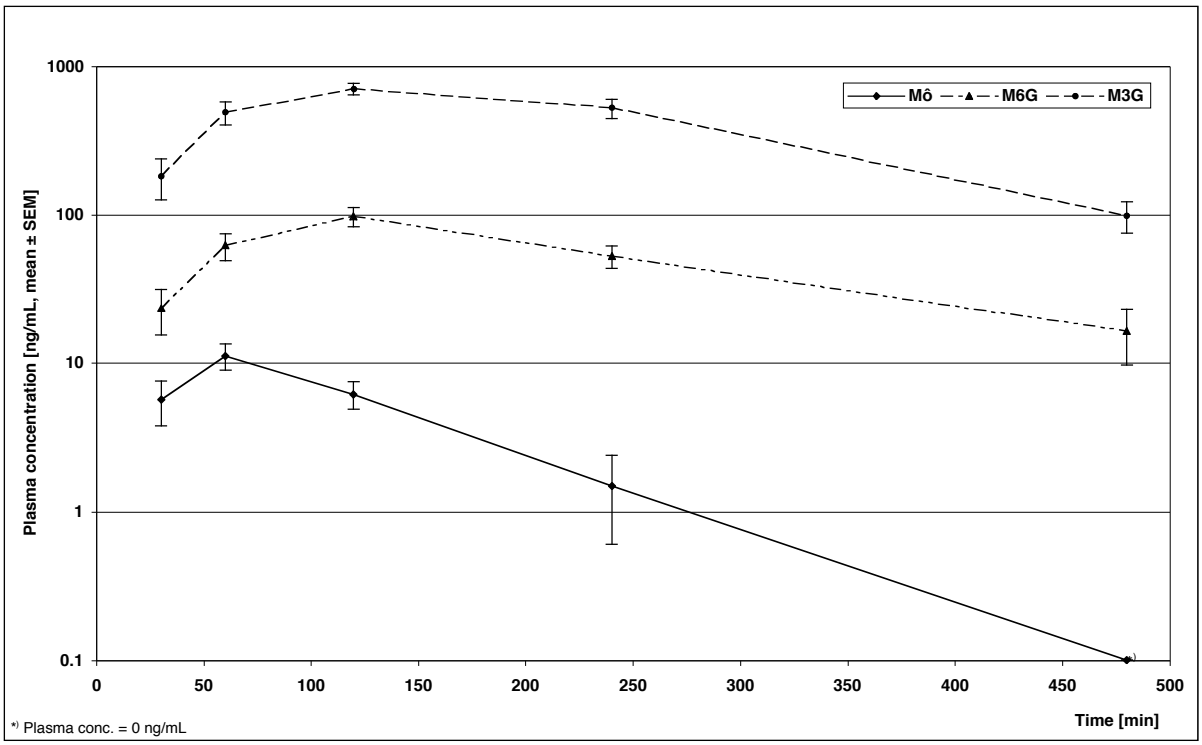


Figure 10: Plasma concentration of morphine and its main metabolites M6G and M3G after oral administration (n=12).

After oral administration of THC-morphine the morphine, M6G, and M3G levels were 0 to 14.1 ± 3.3, 13.3 ± 5.8 to 143.3 ± 12.3, and 129.4 ±16.3 to 561.8 ± 46.5 ng/mL, respectively. Figure 11 shows the mean morphine, M6G, and M3G plasma concentrations after the oral administration of THC-morphine plotted against time on a semilogarithmic scale.

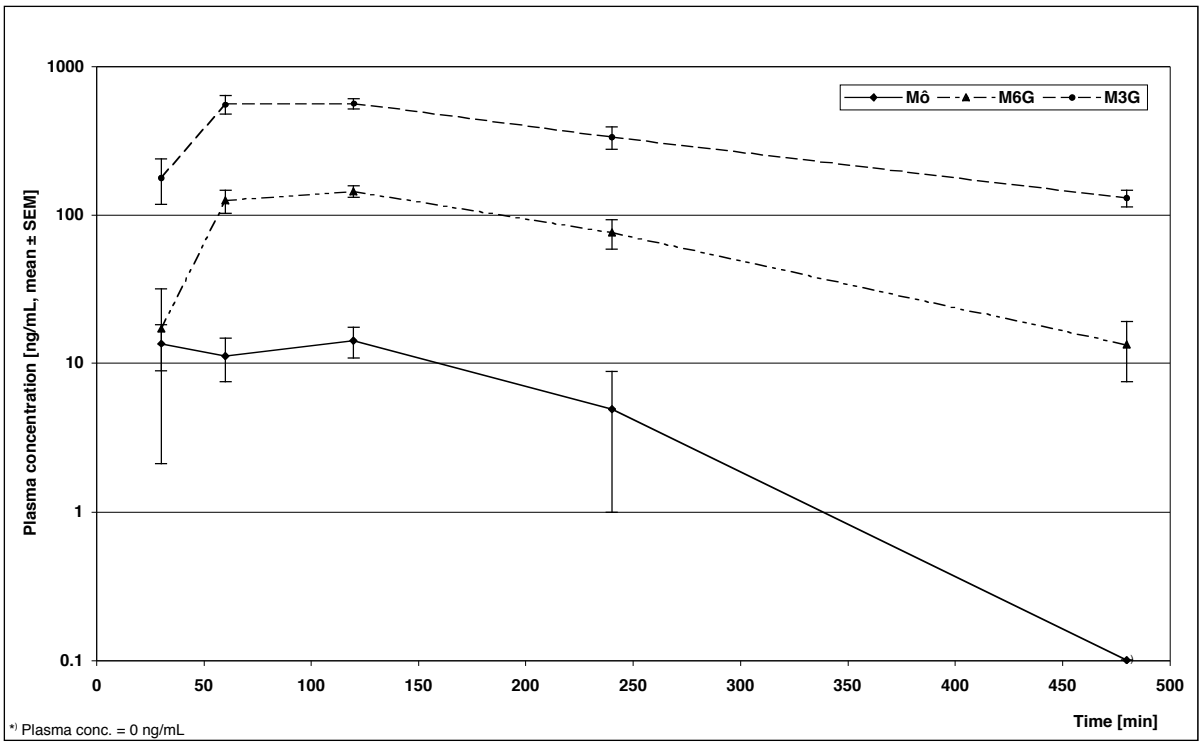


Figure 11: Plasma concentration of morphine and its main metabolites M6G and M3G after oral administration in combination with THC (n=12).

1.6 Determination of THC and -metabolites in plasma by GC-MS

Validation

Peak identification (selectivity / specificity)

Blank and positive control samples showed no interferences with the analytes and the internal standard (for a typical chromatogram see appendix III). The corresponding retention times and ions are summarised in Table 11.

Table 11: Retention times and monitored ions for THC and its metabolites (TMS derivatives)

Analyte	Retention time [min]	Quantitation ion	Qualifying ion
THC-TMS	8.27 (8.25-8.30)	371	343
THC-d_3-TMS	8.23 (8.20-8.25)	374	389
THC-OH-TMS_2	11.53 (11.51-11.56)	371	474
THC-OH-d_3-TMS_2	11.48 (11.46-11.50)	374	377
THC-COOH-TMS_2	14.57 (11.55-11.60)	371	488, 473
THC-COOH-d_3-TMS_2	14.50 (14.48-15.53)	374	491

Calibration and linearity

Table 12 gives an overview of the calibration results.

Table 12: Calibration data for the quantification of THC and its metabolites in plasma (TMS derivatives)

Analyte	Retention time [min]	m[1)]	b[1)]	Correlation coefficient r
THC-TMS	8.27	0.0577	0.0823	0.9983
THC-OH-TMS_2	11.53	0.0464	0.0413	0.9992
THC-COOH-TMS_2	14.57	0.0216	0.1568	0.9995

[1)] y = mx + b; x: ratio of the concentrations; y: ratio of the areas under the peak

The data show good linearity in the calibration range of 2 to 100 ng/mL for THC and THC-OH and 10 to 500 ng/mL for THC-COOH, respectively. The current method was used for the acquisition of pharmacokinetic data and therefore the range of calibration had to be selected in order to include the highest plasma levels of THC and its metabolites as well as the lowest plasma levels of the analytes in the elimination phase. Different experiments showed a LOQ of 2 ng/mL for THC and THC-OH and 10 ng/mL for THC-COOH, respectively. The upper LOQ was equal to the highest calibrator (100 and 500 ng/mL, respectively).

Recovery, intra- and interday precision, and accuracy

Table 13 summarises the results of the intraday precision. Table 14 shows the results of the recovery and the interday precision.

Table 13: Intraday precision and accuracy for the quantification of THC and its metabolites in plasma (TMS derivatives)

Analyte	Conc. added [ng/mL]	Conc. found [mean, ng/mL] (n = 5)	SD [ng/mL]	RSD [%]	Accuracy [%]
THC-TMS	20	19.76	0.95	4.80	- 1.2
THC-OH-TMS_2	20	19.96	0.25	1.26	- 0.2
THC-COOH-TMS_2	20	20.99	0.54	2.59	+ 4.9

Table 14: Recovery and interday precision for the quantification of THC and its metabolites in plasma (TMS derivatives)

Analyte	Recovery [%] (n = 5)	Precision (RSD %; n = 5)
THC-TMS	84.4	3.96
THC-d_3-TMS	87.3	
THC-OH-TMS_2	89.6	4.35
THC-OH-d_3-TMS_2	90.5	
THC-COOH-TMS_2	85.2	6.96
THC-COOH-d_3-TMS_2	87.3	

Limit of detection

The LOD was found to be 2 ng/mL for THC-TMS and THC-OH-TMS_2 and 10 ng/mL for THC-COOH-TMS_2, respectively. At these concentration levels the peaks were clearly detectable and could be integrated.

Limit of quantification

The LOQ was 2 ng/mL for THC-TMS and THC-OH-TMS_2 and 10 ng/mL for THC-COOH-TMS_2, respectively, corresponding to the lowest calibrator.

1.7 Plasma levels and phamacokinetics

The measured THC plasma concentrations after oral administration of 20 mg of THC ranged from 1.1 ± 0.9 to 7.2 ± 1.8 ng/mL, with a maximum at 60 or 120 min. The THC-OH plasma concentrations were 0.3 ± 0.3 to19.7 ± 1.8 ng/mL, mostly peaking at 120 min, and the THC-COOH levels were 1.7 ± 1.7 to 241.4 ± 19.3 ng/mL, peaking at 120 or 240 min. Figure 12 shows mean THC, THC-OH, and THC-COOH plasma concentrations after the oral administration of THC plotted against time on a semilogarithmic scale.

The ratio of the parent drug THC to its psychoactive metabolite THC-OH was at most time points 0.5 - 1 to 1.

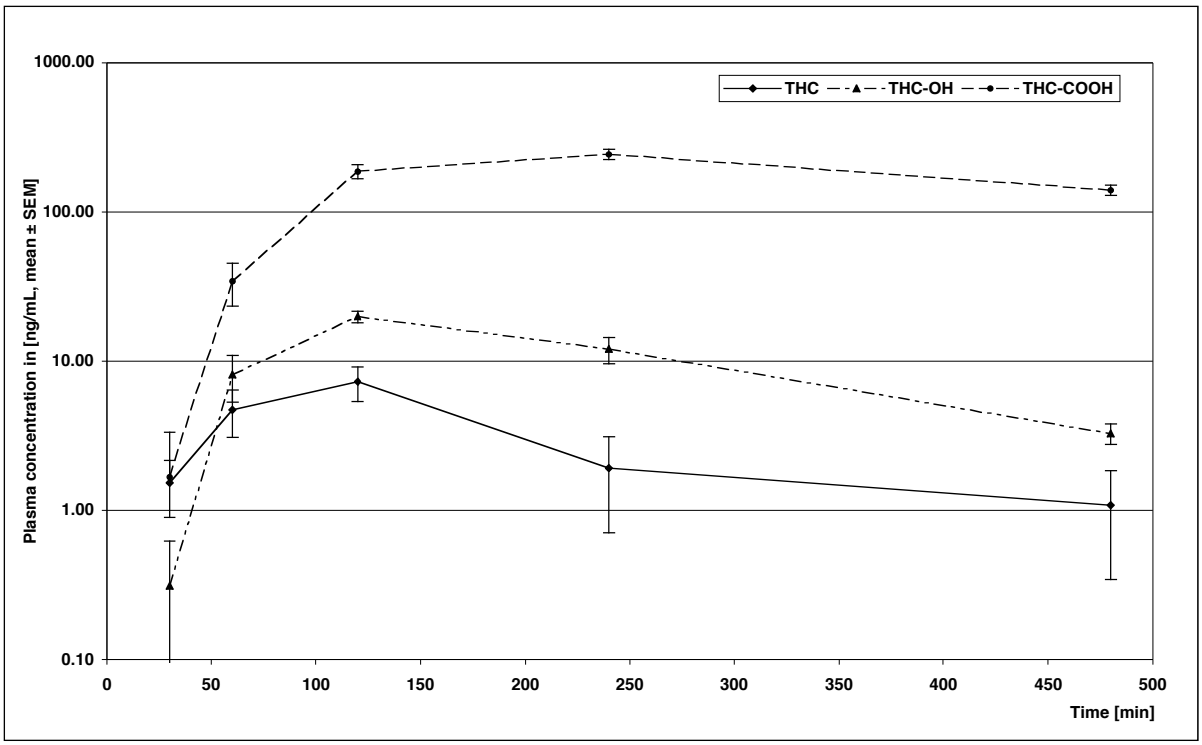


Figure 12: Plasma concentration of THC and its main metabolites THC-OH and THC-COOH after oral administration (n=12).

After oral administration of THC-morphine the THC, THC-OH, and THC-COOH levels were 4.0 ± 1.4 to 6.7 ± 2.1, 0.2 ± 0.2 to 7.9 ± 2.4, and 0 to 134.7 ± 18.8 ng/mL, respectively. Figure 13 shows mean THC, THC-OH, and THC-COOH plasma concentrations after the oral administration of THC-morphine plotted against time on a semilogarithmic scale.

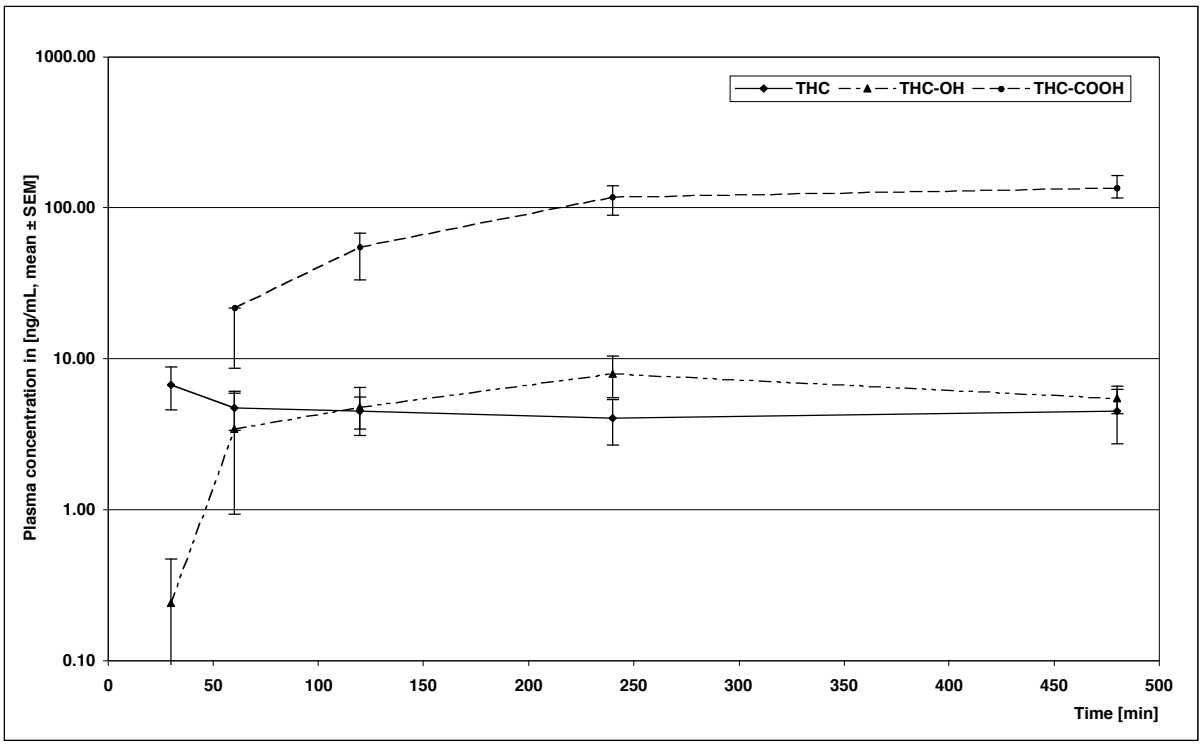


Figure 13: Plasma concentration of THC and its main metabolites THC-OH and THC-COOH after oral administration in combination with morphine (n=12).

Table 15 gives an overview of the pharmacokinetic parameters of orally administered THC calculated with the plasma concentration time curve data of the mean of the twelve volunteers.

Table 15: Pharmacokinetic parameters of orally administered THC

Parameter	THC	THC-morphine
t_{max} [min]	120	30
C_{max} [ng/mL]	7.19	6.66
$t_{1/2}$ [min]	299	367
$AUC_{0\text{-}480min}$ [ng·min/mL]	1377	2076

2. DEVELOPMENT OF WATER SOLUBLE THC FORMULATIONS

2.1 Extraction, purification, and quality assurance of THC from extract ELB-11-98 for the in vitro experiments

Quantitative and qualitative characterisation of the extract ELB-11-98 with GC-MS

The quantification of THC in the extract ELB-11-98 resulted in a THC content of 42 %. Impurities were characterised as very small amounts of other cannabinoids such as cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG) etc. Presumably there were also some organic solvent residues and some substances not detectable by GC-MS present.

Pilot experiments with TLC

The TLC system showed a good separation of the different components of the ethanolic THC extract with an Rf of THC of 0.7. Due to the insufficient visualisation of the analytes with UV light at 254 nm, HPTLC plates were sprayed with anisaldehyde reagent and heated. The spots then showed a red to purple colour.

Purification of the extract ELB-11-98 with CC and MPLC

The purification with CC yielded in pre-purified THC-fractions with a THC content of 75 to 85 %. The average yield was 46 %. The follow-up purification with MPLC led to THC fractions with a THC content of 95 %. The average yield of the purest THC fractions was 70 %.

Quality assurance with GC-MS and HPLC

The GC-MS chromatogram of the pure THC fractions showed either no or only minor (0-1 %) contaminating by-products. The HPLC chromatogram showed only minor (1.36 - 3.3 %) contamination, too. The THC content of 95 % was sufficient enough for the further use in the in vitro experiments.

2.2 Development and validation of the THC inhalation solution

Validation of the HPLC method

Peak identification (selectivity / specificity)

The adjuvants showed no interferences with THC and the internal standard. The retention time for the THC is presented in Table 16.

Calibration and linearity

Table 16 gives an overview of the calibration results.

Table 16: Calibration data for the quantification of THC in water soluble THC formulations

Analyte	Retention time [min]	m[1]	b[1]	Correlation coefficient r
THC	6.90	0.05804	0.00074	0.9999

[1] y = mx + b; x: ratio of the concentrations; y: ratio of the areas under the peak

The calibration graph was linear in the concentration range of 100 to 1000 µg/mL THC.

Intra- and interday precision and accuracy

Table 17 shows the results of the intraday precision and Table 18 for the interday precision.

Table 17: Intraday precision of the quantification of THC in water soluble THC formulations

Concentration of THC [µg/mL]	Mean [µg/mL]	SD [µg/mL] (n = 4)	RSD [%]	Accuracy [%]
1000	1002.5	8.44	0.84	+ 0.25
500	500.7	4.20	0.84	+ 0.14
250	247.8	1.90	0.77	- 0.88
100	99.7	2.47	2.47	-0.27

Table 18: Interday precision of the quantification of THC in water soluble THC formulations

Concentration of THC [µg/mL]	SD [µg/mL] (n = 5)	RSD [%]
1000	2.04	0.20
500	7.99	1.61
250	1.19	0.48
100	2.42	2.42

Limit of quantification and limit of detection

The determination of LOQ and LOD was not necessary because the method was used for the quantification of THC in galenic formulations where the concentration was far above the LOQ and LOD.

Pilot solubilisation experiments

The experiment with pure water showed insoluble residues at the bottom of the vial, a turbid solution, and the AUC of the THC peak was about 0.4 % of that of the ethanolic reference solution (= 100 %).

In the first series of experiments with the solubilisers and the lowest THC concentration (1mg/mL) the resulting solutions were clear, no residue could be seen in the GC vial, and the

AUC of the THC peaks were comparable to that of the ethanolic reference solution. There was no difference between the single experiments. Both types of Cremophor® (EL and RH 40) in each concentration (5, 10, and 15 %) were able to fully solubilise the THC. In the next series the THC concentration was therefore increased to 2 mg/mL and the Cremophor® concentrations were decreased to 2.5, 5, and 10 %. The formulations with 2.5 % of the solubilisers showed residues at the bottom and at the wall of the GC vial. The AUC of the THC peaks were nevertheless not much decreased compared to that of the ethanolic reference solution. The formulations with 5 % of the Cremophors® showed again very small residues at the wall of the GC vial which could be due to incomplete covering of the THC with the solubiliser during the heating process. The AUC of the THC peaks were comparable to that of the ethanolic reference solution. The formulations with 10 % of the solubilisers formed a clear solution with no residues and the AUC of the THC peaks were again comparable to that of the ethanolic reference solution. To determine the limit of solubilisation, the THC concentration was once more increased to a level of 3 mg/mL, and the solubiliser concentrations were left constant at 5 and 10 %, respectively. The formulations with Cremophor® RH 40 showed clear solutions. A very small residue remained at the wall of the GC vial in the 5 % Cremophor® formulation which could be again due to the incomplete inclusion of the THC by the solubiliser during the heating process. The AUC of the THC peaks were comparable to that of the ethanolic reference solution. The formulations with Cremophor® EL showed a different result. The 10 % formulation produced a clear solution with no residues. The 5 % formulation showed small residues at the wall of the GC vial and a decreased AUC of the THC peak compared to the ethanolic reference solution.

To complete the solubilisation experiments another series was done only with the better solubiliser, the Cremophor® RH 40, to determine the limit of the solubilisation rate with a THC concentration of 3 mg/mL. Up to a Cremophor® concentration of 3 % the solubilisation of the THC was incomplete. With 4, 5, and 7.5 % of Cremophor® RH 40 the solution was clear and there was no residue seen neither at the bottom nor at the wall of the GC vial. To be sure of a complete solubilisation process 25 % more solubiliser were added, and the further experiments done with a solubiliser concentration of 5 % to solubilise 3 mg/mL THC.

Follow-up experiments with different pH in the heat stress test

The results of these experiments are summarised in Figure 14. The buffered formulations showed an advantage in stability compared to the non-buffered formulations. This result was very clear in the formulations with Cremophor® EL. When comparing the buffered formulations, Cremophor® RH 40 showed an advantage in stability compared to Cremophor® EL. Comparing

the different buffered formulations with Cremophor® RH 40 with the different pH, the formulation with pH 8.0 showed the lowest decrease of the ratio of THC vs. its oxidation product cannabinol (CBN). These results indicated a better stability in not acidic pH ranges. Garret and Hunt [26] reported a rapid degradation in acidic solution ($t_{½}$ = 1 h at pH 1.0, 55°C). For the next experiments, the formulations with Cremophor® RH 40 at pH 7.4 and 8.0 were further examined and optimised.

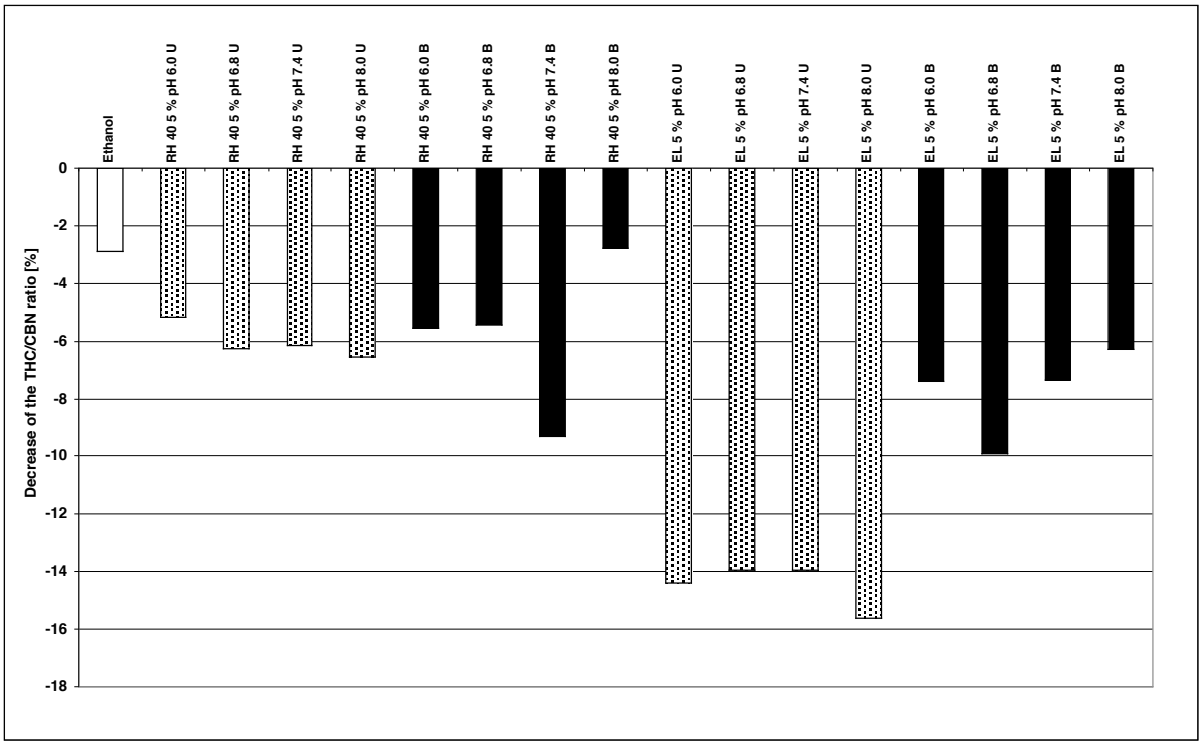


Figure 14: Ratio of THC/CBN (AUC of the peaks in the HPLC chromatogram) normalised to the value before the heat stress test of the different formulations. Grey bars represent non-buffered formulations, black bars buffered formulations.

Statistical experiment

To investigate the influence of different adjuvants on the stability of the formulation, a factorial design experiment (a 2^3-design) was carried out.

The Yates-analysis after the heat stress test is presented in Table 19.

Table 19: Yates-analysis after the heat stress test

	Factors			Interactions				Endpoint (THC/CBN)
	A	B	C	AB	AC	BC	ABC	
(1)	-	-	-	+	+	+	-	5.9718
a	+	-	-	-	-	+	+	5.7937
b	-	+	-	-	+	-	+	5.7775
ab	+	+	-	+	-	-	-	4.6848
c	-	-	+	+	-	-	+	5.9368
ac	+	-	+	-	+	-	-	5.8125
bc	-	+	+	-	-	+	-	5.6944
abc	+	+	+	+	+	+	+	4.7284
4-fold effect	4A	4B	4C	4AB	4AC	4BC	4ABC	
	-2.3611	-2.6296	-0.0557	-1.7563	0.1805	-0.0235	0.0729	
Sum of squares	$(4A)^2$	$(4B)^2$	$(4C)^2$	$(4AB)^2$	$(4AC)^2$	$(4BC)^2$	$(4ABC)^2$	
	5.5748	6.9148	0.0031	3.0846	0.0326	0.0006	0.0053	
F_{calc}	$(4A)^2/(4ABC)^2$	$(4B)^2/(4ABC)^2$	$(4C)^2/(4ABC)^2$	$(4AB)^2/(4ABC)^2$	$(4AC)^2/(4ABC)^2$	$(4BC)^2/(4ABC)^2$		
	1051.85	1304.68	0.58	582.00	6.15	0.11		
Significance: $F_{calc} > F_{tab}$ [1)]	$F_{calc} > F_{tab}$ *	$F_{calc} > F_{tab}$ *	$F_{calc} < F_{tab}$ n.s.	$F_{calc} > F_{tab}$ *	$F_{calc} < F_{tab}$ n.s.	$F_{calc} < F_{tab}$ n.s.		

[1)] F_{tab} = 161.44 (degrees of freedom: 1, 1; p-value: 0.05)

The statistical analysis showed that the two factors A and B had a significant effect on the stability of the formulation. Also the interaction between the two factors A and B had a significant effect on the stability. No changes of the statistical results in the Yates-analysis were found after the additional first and second light stress test respectively (data not shown).

The endpoint values of the 8 different experiments showed a negative influence of the upper level of the two factors A and B on the stability of the formulations, meaning that pH 7.4 and the lower concentration of the solubiliser (3 %) showed better stability. The amount of the added antioxydant (NaAsc) showed no significant influence on the stability of the formulation.

Development of the final THC inhalation solution

The findings of the statistical experiments lead to the following conclusions for the final composition of the formulation: lower concentration of the solubiliser (3 %), physiological pH 7.4, lower concentration of NaAsc 0.05 %. To be sure of a complete solubilisation process 25 % more solubiliser (5 %) and a conservant (benzylalcohol) in adequate concentration was added leading to the final formulation as presented in Table 20.

Table 20: Final formulation of the THC inhalation solution

Component	Amount	[%, w/v]
THC	30.0 mg	0.3
Cremophor® RH 40	500.0 mg	5.0
NaAsc	5.0 mg	0.05
Benzylalcohol	100.0 mg	1.0
Phosphate buffer (pH 7.4, 84.2 mM)	ad 10.0 mL	

Quality assurance

Appearance

The THC inhalation solution was clear, yellowish, and free of floating particles.

Stability

The THC content of the inhalation solution, stored at 4 °C and protected from light, was within the ± 5 % range during 83 days. The results are presented in Figure 15.

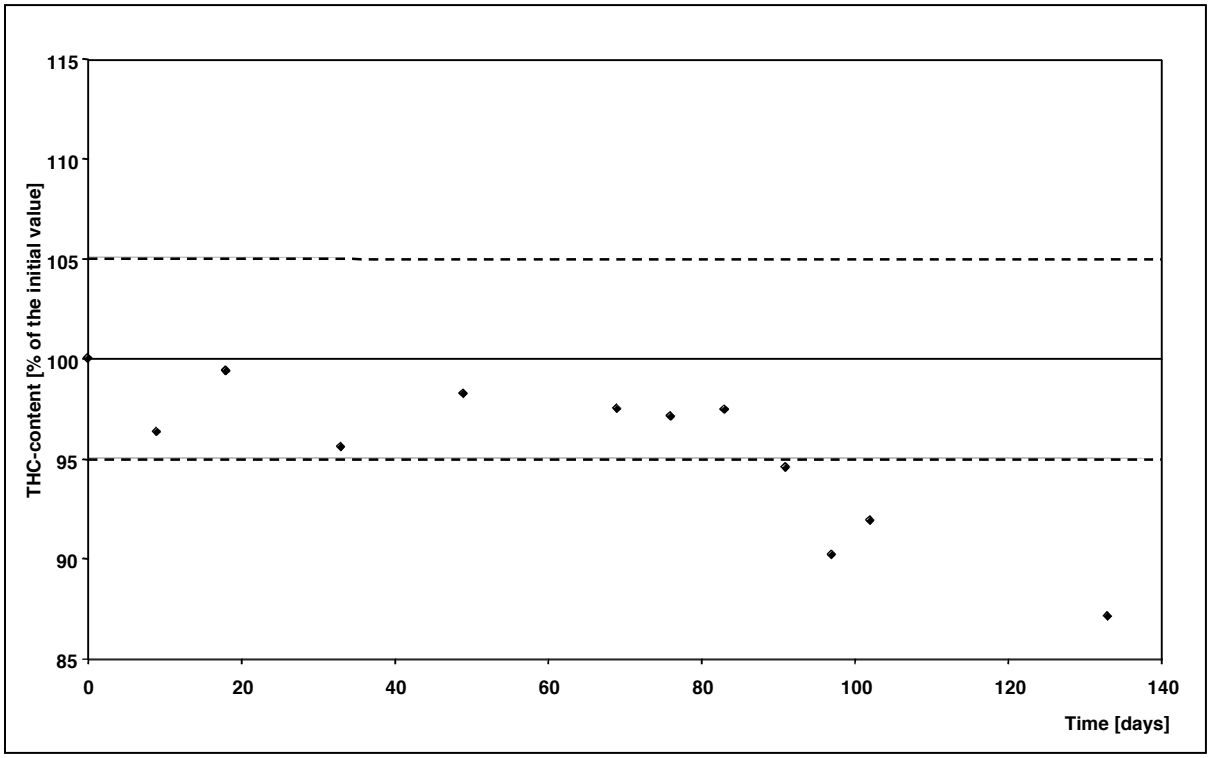


Figure 15: Stability of the inhalation solution (THC-content in % of the initial value) stored at 4°C and protected from light.

Viscosity

The dynamic viscosity was 1.578 mPas (n = 4).

Osmolality

The osmolality was 550 mOsm/kg (n = 3).

pH

The pH was 7.40 (n = 3).

Particle size distribution

Table 21 lists the results of the measurement of the particle size. Figure 16 shows the distribution of the particle size.

Table 21: Particle size of the aerosolised THC inhalation solution

Nebuliser system	PARI Master / IS-2 nebulizer	PARI Master / LC-Plus nebulizer
Placebo solution	2.5 ± 0.14	3.5 ± 0.27
Particle size [□m] (median ± SD, n = 10)		
Verum solution	n.d.	3.8 ± 0.32
Particle size [□m] (median ± SD, n = 5)		

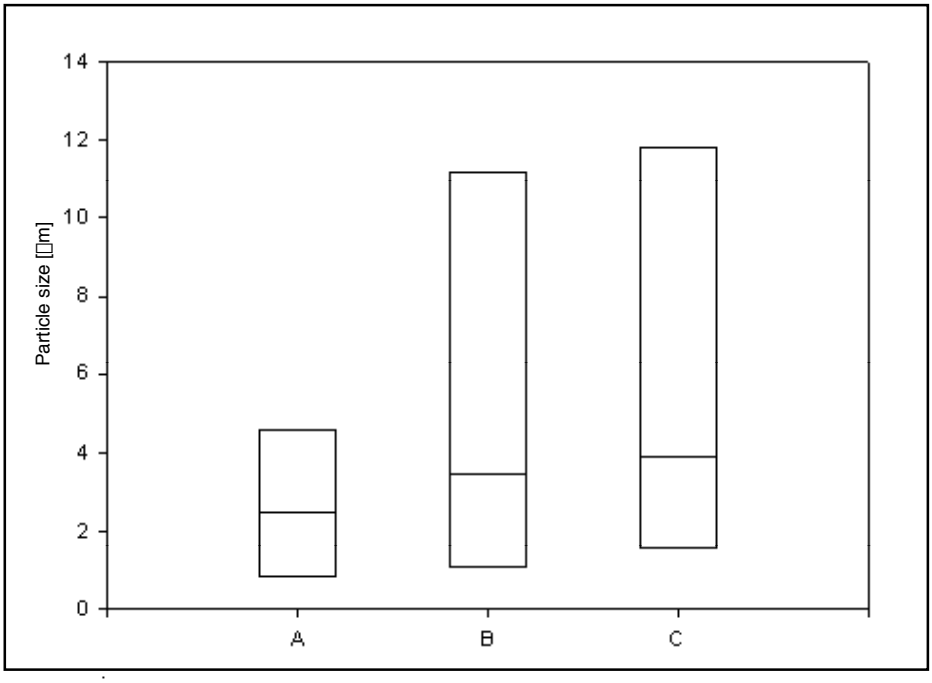


Figure 16: Particle size distribution of the two different nebulisers measured with either verum or placebo solution (median with its 10- and 90-percentiles, respectively; A: IS-2 nebuliser, placebo solution; B: LC-Plus nebuliser, placebo solution; C: LC-Plus nebuliser, verum solution).

Output rates and output time

Table 22 shows the results of the output rates and output times of the two different nebulisers tested.

Table 22: Output rates and output times of THC nebulised with the two different nebulisers

Nebuliser system	PARI Master / LC-Plus nebuliser	
Concentration	3 mg/mL THC	
Volume nebulised	2 mL	3 mL
Output rates [%] (mean ± SD, n = 3)	62.4 ± 3.2	64.5 ± 6.0
Output time [min] (mean ± SD, n = 3)	9.5 ± 0	14.2 ± 0.3
Nebuliser system	PARI Master / IS-2 nebuliser	
Concentration	3 mg/mL THC	
Volume nebulised	2 mL	3 mL
Output rates [%] (mean ± SD, n = 3)	57.1 ± 1.0	60.6 ± 3.6
Output time [min] (mean ± SD, n = 3)	12.8 ± 0.8	21.7 ± 0.8

Consequently, for the clinical study we decided to use the LC-Plus nebuliser because of the higher output rate, the shorter output time, and the appropriate particle size distribution.

2.3 Preparation and validation of the THC injection solution

Quality assurance

Stability

The THC content of the injection solution, stored at 4 °C and protected from light, was within the ± 5 % range at least during one month. The results are presented in Figure 17.

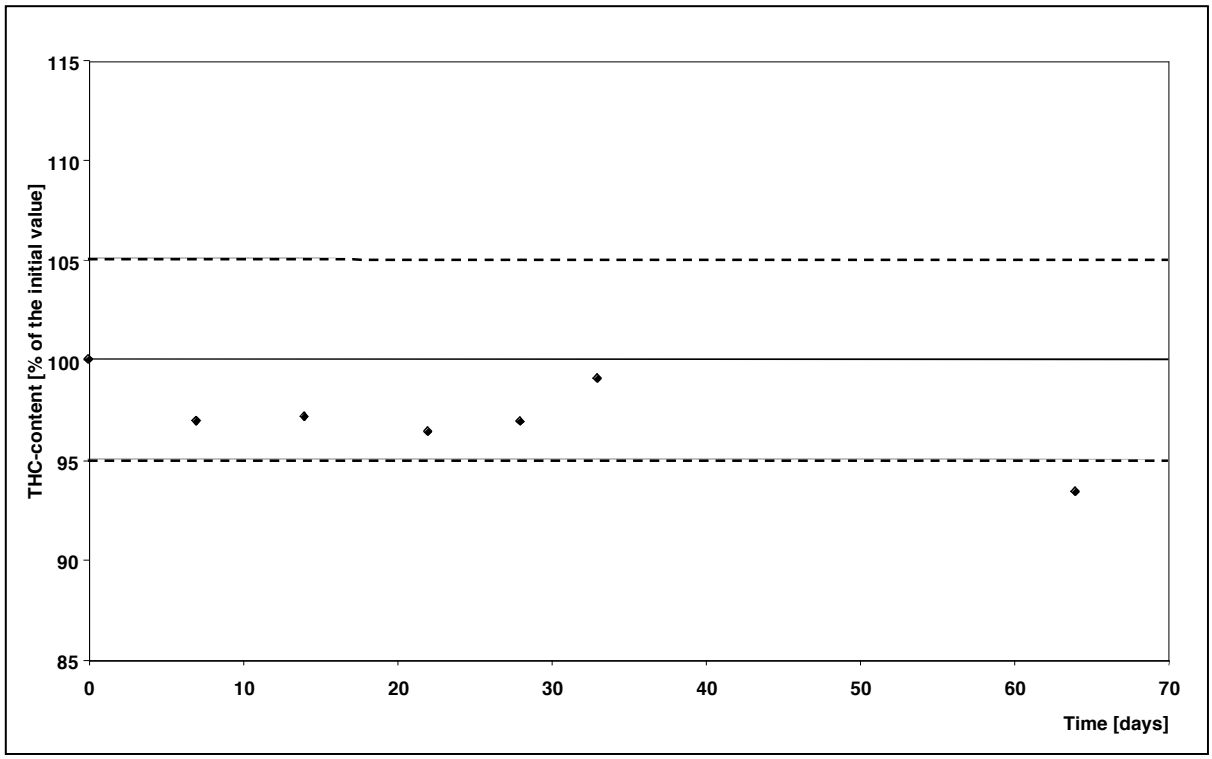


Figure 17: Stability of the injection solution (THC-content in % of the initial value) stored at 4°C and protected from light.

Osmolality

The osmolality was 321 mOsm/kg (n = 3).

pH

The pH was 7.40 (n = 3).

3. PHARMACOKINETIC STUDY WITH PULMONAL AND INTRAVENOUS THC

3.1 Subjects

The subjects were all Cannabis-naïve and non-smokers. Four females (26 - 35 years; 60 ± 8 kg b.wt.) and 4 males (27 - 50 years; 80 ± 5 kg b.wt.) were included. All of the subjects showed normal vital capacity and normal forced expiratory volume in one second in the lung function tests.

3.2 Preparation of the clinical test preparations

The properties of the 2 formulations were according to the standards of the European Pharmacopeia. The i.v. formulation passed the sterility test.

3.3 Pain test

Figure 18 shows the results of the ice water test (ice cold immersion test) plotted as mean of the eight subjects. No significant analgesic effect of pulmonal or i.v. THC compared to placebo could be observed.

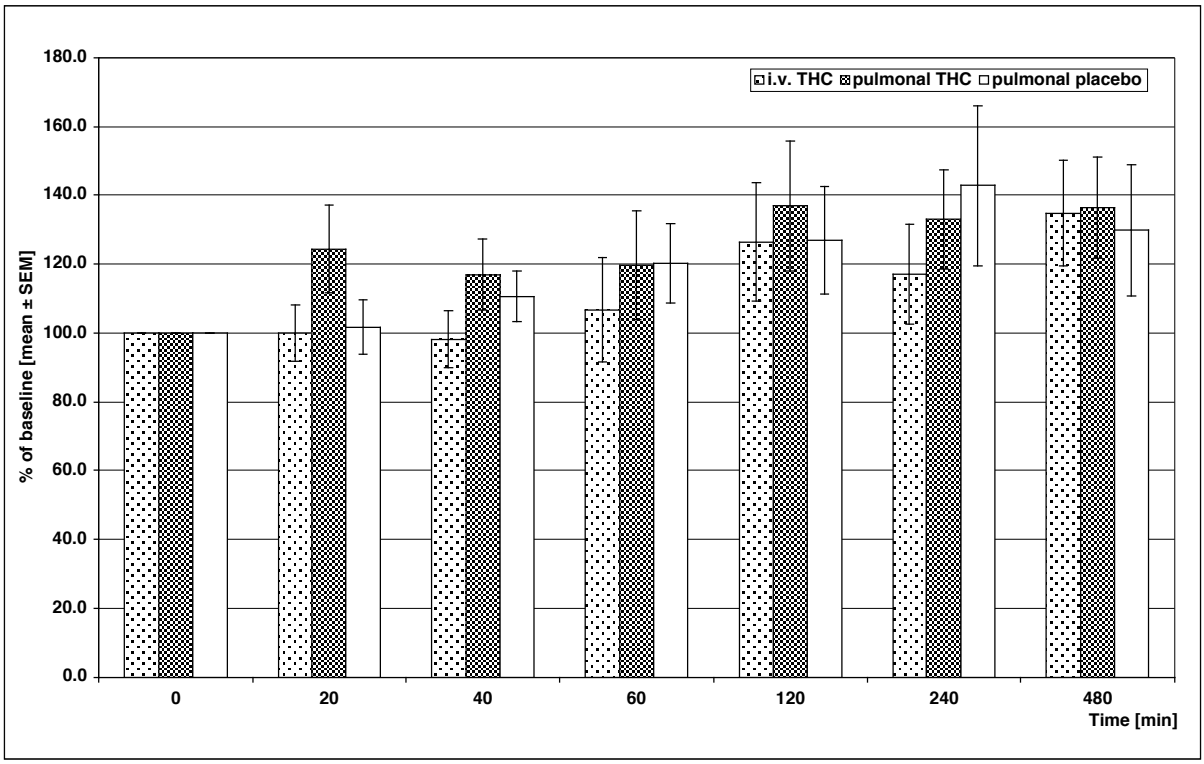


Figure 18: Pain tolerance of i.v THC, pulmonal THC, and pulmonal placebo in the ice cold immersion test; values > 100 % of baseline (mean ± SEM) indicate hyperalgesia, values < 100 % analgesia.

3.4 Monitoring of side effects and vital functions

The observed psychological and somatic side effects are listed in Table 23.

Table 23: Psychological and somatic side effects (peak VAS %, mean ± SEM of all subjects) after pulmonal and i.v. THC, and pulmonal placebo (n = 8)

Side effect	Placebo (pulmonal)		THC (pulmonal)		THC (intravenous)	
	f[1]	VAS [%]	f[1]	VAS [%]	f[1]	VAS [%]
Sleepiness	5	50 ± 16 %	8	64 ± 8 %	7	86 ± 4 %
Euphoria	3	19 ± 8 %	5	51 ± 16 %	7	57 ± 14 %
Irritation	1	4 ± 0 %	3	15 ± 5 %	6	38 ± 13 %
Anxiety	2	6 ± 1 %	1	14 ± 0 %	6	45 ± 13 %
Tenseness and aggressiveness	1	5 ± 0 %	2	9 ± 2 %	6	45 ± 13 %
Confusion and disorientation	2	6 ± 0 %	4	33 ± 20 %	8	80 ± 6 %
Change of inner perception	2	9 ± 2 %	5	42 ± 18 %	8	87 ± 3 %
Change of outer perception	1	6 ± 0 %	3	31 ± 17 %	8	65 ± 9 %
Hallucinations	-	-	2	27 ± 25 %	7	52 ± 15 %
Strange thoughts, ideas, moods	1	6 ± 0 %	1	15 ± 0 %	6	47 ± 11 %
Nausea	-	-	4	26 ± 7 %	7	46 ± 14 %
Headache	1	20 ± 0 %	5	40 ± 14 %	8	48 ± 10 %
Difficulties in breathing	-	-	5	24 ± 10 %	5	59 ± 17 %
Irritation of the throat, coughing	2	7 ± 3 %	8	70 ± 9 %	3	27 ± 20 %
Irritation of the upper resp. tract	-	-	5	53 ± 19 %	4	30 ± 17 %
Heart problems (tachycardia)	-	-	3	8 ± 1 %	8	45 ± 13 %
Digestive problems	-	-	2	7 ± 3 %	5	36 ± 16 %
Dry mouth	2	19 ± 12 %	3	24 ± 13 %	8	83 ± 10 %
Vertigo	1	10 ± 0 %	5	38 ± 6 %	8	75 ± 8 %
Vomiting	0	-	0	-	0	-
Orthostatic disorder	0	-	0	-	0	-

[1] f: Frequency of side effects per 8 subjects

In the THC inhalation session all subjects reported irritation of the throat and coughing during the inhalation (partly impairing inhalation efficiency) (Figure 19). This adverse effect was reversible within 30 min after finishing inhaling. It was not observed in the placebo inhalation session. The psychotropic effects of the THC aerosol were usually very mild. The i.v. application caused much more prominent side effects, i.e. strong psychotropic symptoms, increased heart

rate, and dry mouth. Figure 20 shows the hallucinogenic effect of i.v. THC and pulmonal THC vs. pulmonal placebo.

Blood pressure was not changed by any of the test preparations, whereas both pulmonal and i.v. THC increased heart rate significantly compared to placebo (data not shown).

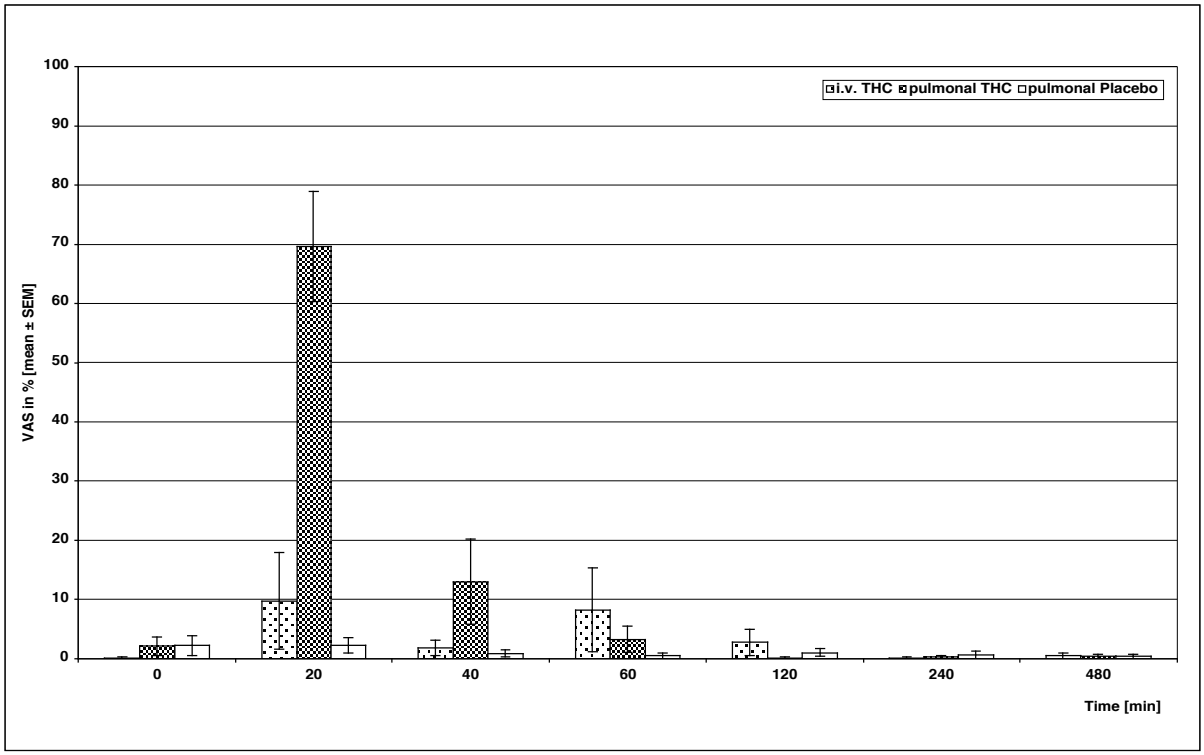


Figure 19: Side effect "irritation of the airways, coughing" (VAS %, mean ± SEM) after i.v. THC, pulmonal THC, and pulmonal placebo.

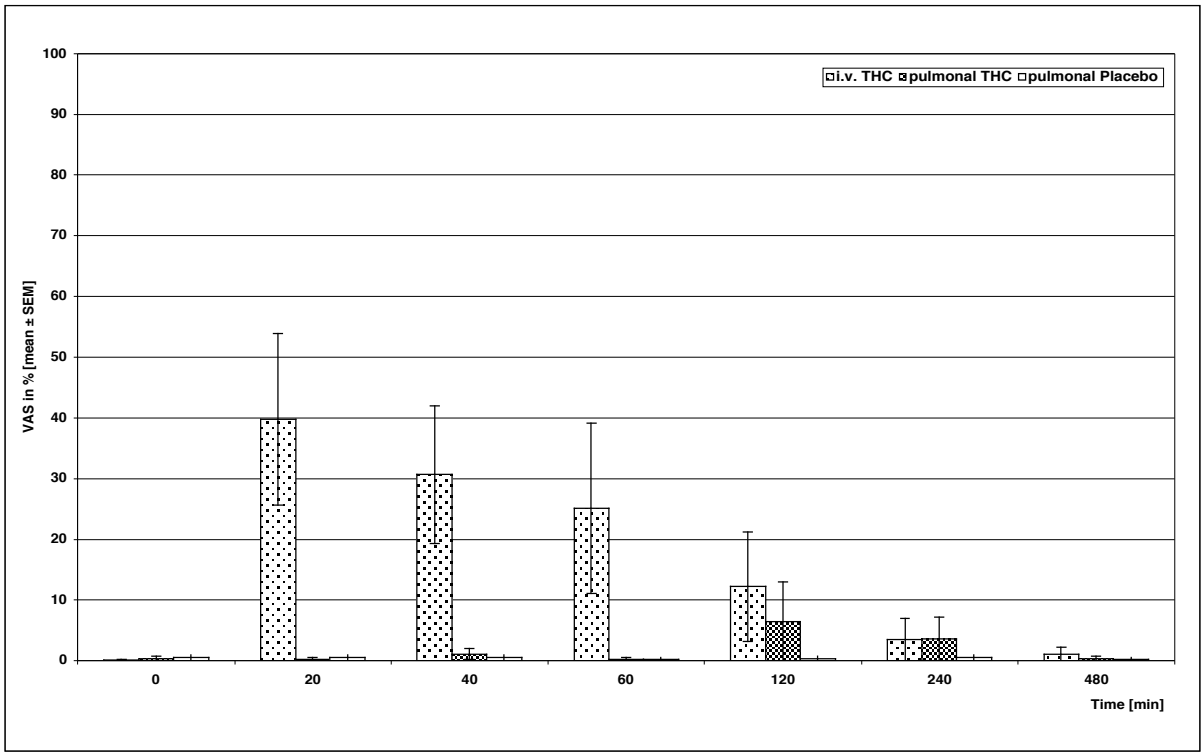


Figure 20: Side effect "hallucinations" (VAS %, mean ± SEM) after i.v. THC, pulmonal THC, and pulmonal placebo.

3.5 Determination of THC and -metabolites in plasma by GC-MS

Validation

Peak identification (selectivity / specificity)

Blank and positive control samples showed no interferences with the analytes and the internal standard (for a typical chromatogram see appendix IV). The corresponding retention times and ions are summarised in Table 24.

Table 24: Retention times and monitored ions for THC and its metabolites (TMS derivatives)

Analyte	Retention time [min]	Quantitation ion	Qualifying ion
THC-TMS	11.87 (11.85 - 11.93)	371	343
THC-d_3-TMS	11.85 (11.80 - 11.88)	374	389
THC-OH-TMS_2	15.39 (15.36 - 15.44)	371	474
THC-OH-d_3-TMS_2	15.36 (15.32 - 15.40)	374	377
THC-COOH-TMS_2	17.44 (17.40 - 17.48)	371	488, 473
THC-COOH-d_3-TMS_2	17.40 (17.36 - 17.44)	374	491

Calibration and linearity

To reach sufficient linearity two calibration curves for THC, from 0.4 to 20 and 20 to 300 ng/mL plasma were determined. Table 25 gives an overview of the calibration results.

Table 25: Calibration data for the quantification of THC and its metabolites in plasma (TMS derivatives)

Analyte	Retention time [min]	m[1)]	b[1)]	Correlation coefficient r
THC-TMS (0.4 - 20 ng/mL)	11.87	1.3918	0.0111	0.9994
THC-TMS (20 - 300 ng/mL)	11.87	1.3455	0.1851	0.9984
THC-OH-TMS_2	15.39	1.1193	0.0107	0.9988
THC-COOH-TMS_2	17.40	1.1988	0.0106	0.9992

[1)] y = mx + b; x: ratio of the concentrations; y: ratio of the areas under the peak

The data show good linearity in the calibration range of 0.4 to 20 ng/mL for the lower concentrations of THC and in the range of 20 to 300 ng/mL for the higher concentrations of THC. The method was linear in the calibrated concentration range of 0.4 to 100 ng/mL for THC-OH and THC-COOH. The current method was used for the aquisition of plasma profiles and therefore the range of calibration had to include the highest plasma levels of THC and -metabolites as well as the lowest plasma levels in the elimination phase. Different experiments showed a LOQ of 0.4 ng/mL for THC, THC-OH, and THC-COOH. The upper LOQ was corresponding to the highest calibrators (300 ng/mL for THC and 100 ng/mL for the metabolites).

Recovery, intra-and interday precision, and accuracy

Table 26 summarises the data of the intraday precision and Table 27 of the recovery and the interday precision.

Table 26: Intraday precision and accuracy of the quantification of THC and its metabolites in plasma (TMS derivatives)

Analyte	Conc. added [ng/mL]	Conc. found [Mean ng/mL] (n = 5)	SD [ng/mL]	RSD [%]	Accuracy [%]
THC-TMS	4	4.08	0.17	4.10	+ 2.1
THC-TMS	100	103.05	1.09	1.06	+ 3.1
THC-OH-TMS_2	4	4.04	0.22	5.50	+ 1.0
THC-OH-TMS_2	100	102.77	1.13	1.11	+ 2.8
THC-COOH-TMS_2	4	4.02	0.17	4.15	+ 0.5
THC-COOH-TMS_2	100	102.30	1.45	1.42	+ 2.3

Table 27: Recovery and interday precision of the quantification of THC and its metabolites in plasma (TMS derivatives)

Analyte	Recovery [%] (n = 6)	Precision [RSD, %] (n = 5)	
		4 ng/mL plasma	100 ng/mL plasma
THC-TMS	91.3	9.83	3.90
THC-d_3-TMS	90.6		
THC-OH-TMS_2	87.6	4.11	2.85
THC-OH-d_3-TMS_2	86.8		
THC-COOH-TMS_2	77.9	9.09	3.75
THC-COOH-d_3-TMS_2	76.3		

Limit of detection

The LOD was found to be 0.4 ng/mL for THC and -metabolites. At this concentration level the peaks were clearly detectable and could be integrated.

Limit of quantification

The LOQ was 0.4 ng/mL for THC and -metabolites, corresponding to the lowest calibrator.

3.6 Plasma levels and pharmacokinetics

Figure 21 and 22 show the plasma profiles of THC and its two metabolites THC-OH and THC-COOH following pulmonal and i.v. administration, respectively. The mean plasma level of pulmonal THC after 10 min was 18.7 ± 7.4 ng/mL (mean ± SEM) with a mean duration of the inhalation procedure of 23 ± 3 min. As can be seen in Figure 21, the peak plasma levels of 18.9 ± 5.0 ng/mL were measured at 20 min. Then, the plasma concentrations decreased rapidly to 6.1 ± 4.0 ng/mL after 1 h and 2.4 ± 1.7 ng/mL after 2 h. Peak plasma levels of the two main metabolites THC-OH and THC-COOH were 1.38 ± 0.31 ng/mL occurring at 40 min and 10.0 ± 2.85 ng/mL mostly peaking at 120 min, respectively.

The plasma levels 5 min after the i.v. injection of THC (0.053 mg/kg b.wt.) ranged from 81.6 to 640.6 ng/mL (271.5 ± 61.1 ng/mL) (Fig. 22). Then, the plasma levels decreased rapidly to a mean concentration of 95.6 ± 28.2 ng/mL at 10 min, 38.3 ± 10.6 ng/ml at 20 min, 20.1 ± 5.3 ng/mL after 1 h, and 9.0 ± 3.0 ng/mL at 2 h. Peak plasma levels of THC-OH and THC-COOH were 9.13 ± 0.84 ng/mL occuring at 5 or 10 min and 36.66 ± 3.75ng/mL occuring at 60 min, respectively. Figure 23 shows the mean THC plasma concentrations after the i.v. and pulmonal administration plotted against time on a semilogarithmic scale.

The ratio of THC to its psychoactive metabolite THC-OH was at most time points 2 - 15 to 1 for pulmonal and 5 - 10 to 1 for i.v. THC, respectively.

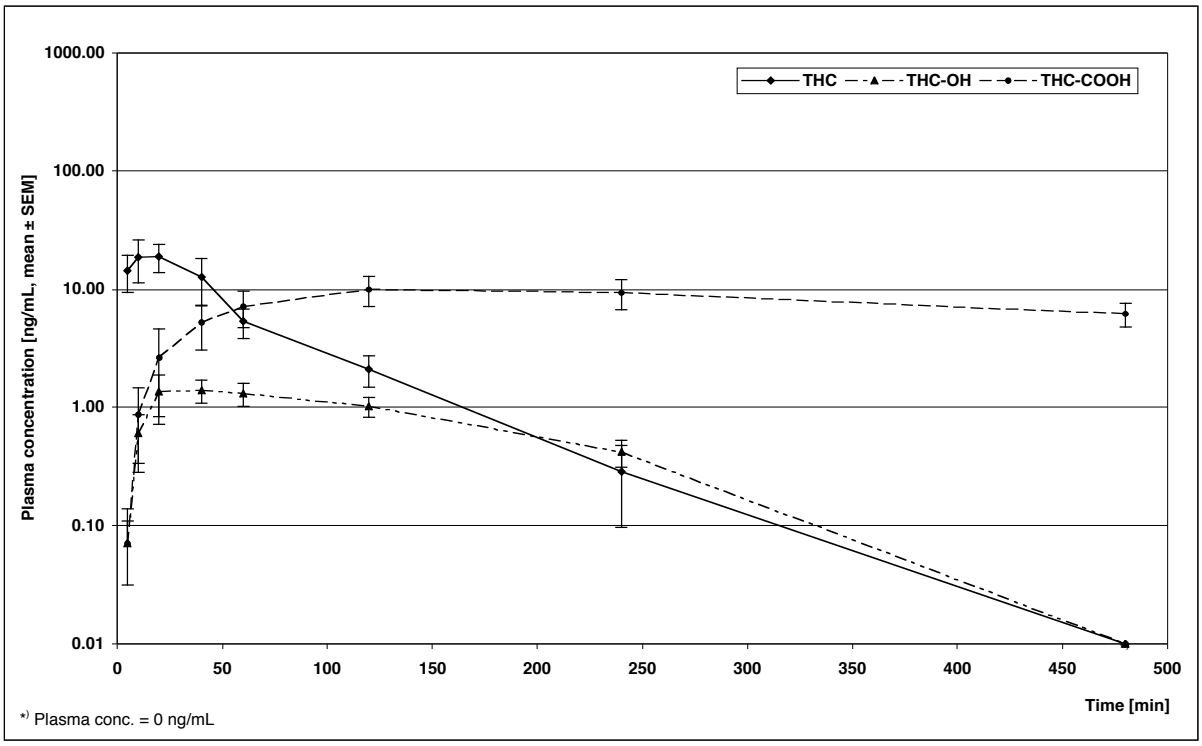


Figure 21: Plasma concentration of THC and its main metabolites THC-OH and THC-COOH after pulmonal THC (n=8).

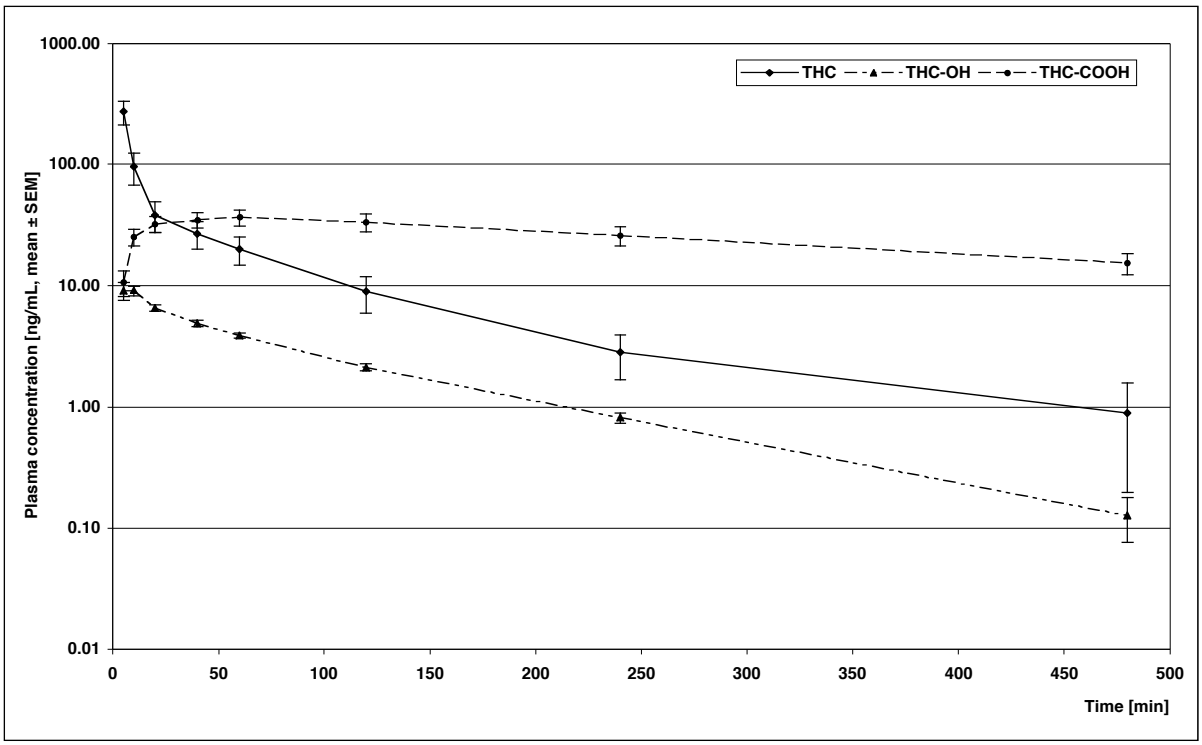


Figure 22: Plasma concentration of THC and its main metabolites THC-OH and THC-COOH after i.v. THC (n=8).

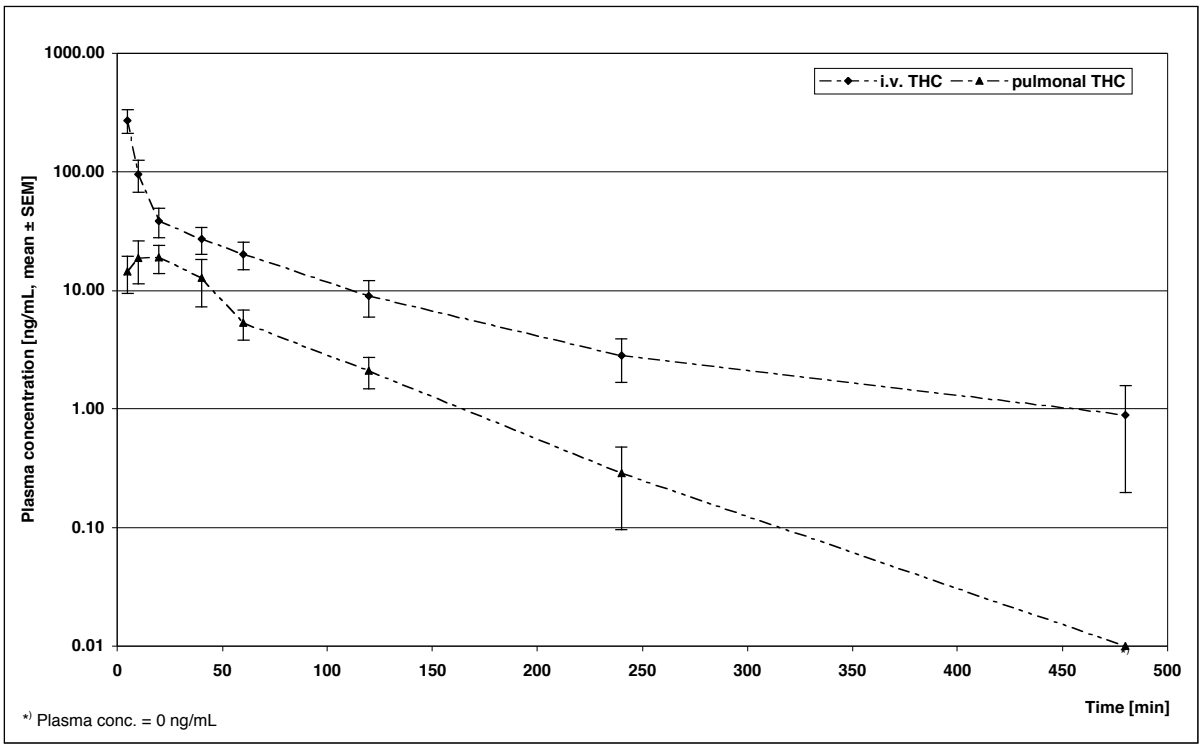


Figure 23: THC plasma concentration after i.v. and pulmonal administration (n=8).

Table 28 a and 28 b summarise the pharmacokinetic parameters for i.v. and pulmonal THC calculated with the TopFit software [84]. Table 29 presents the results of an alternative calculation of the i.v. data using the PKAnalyst software [92] fitted for a two-compartment model.

Table 28a: Pharmacokinetic parameters of i.v. and pulmonal THC

Subject		Intravenous THC		Pulmonal THC			
No	Gender	Dose [mg]	AUC_{0-480} [ng*min*mL^{-1}]	Dose [mg]	AUC_{0-480} [ng*min*mL^{-1}]	Bioavailability F [%]	Half-life $t_{1/2}$ [min]
1	f	3.3	5560.5	3.71	2527.7	40.4	43
2	m	4.2	3434.3	2.98	693.6	28.5	46
3	m	4.24	4556.3	4.08	2596.8	59.2	64
4	m	4.53	5824.3	4.56	1256.5	21.4	41
5	f	2.4	19244.9	2.34	67.9	0.4	18
6	f	2.92	3402.5	2.97	527.8	15.3	44
7	f	3.5	10506.8	3.29	361.3	3.7	84
8	m	4.0	2588.1	4.03	1580.6	60.6	31
Mean ± SEM (f & m)			6889.8 ± 1967.1		1201.5 ± 342.1	28.7 ± 8.2	46 ± 7
Mean ± SEM (f)			9678.7 ± 3518.4		871.2 ± 560.3	14.9 ± 9.1	47 ± 14
Mean ± SEM (m)			4100.9 ± 701.9		1531.9 ± 399.5	42.4 ± 10.2	45 ± 7

Table 28b: Pharmacokinetic parameters of i.v. THC

Subject		Intravenous THC			
No	Gender	Distribution volume V_z [L]	Clearance CL [mL/min]	Half life $t_{1/2}$ [min]	Elimination rate constant λ_z [$\cdot 10^{-2}$]
1	f	98.6	570	120	0.578
2	m	89.2	1220	51	1.370
3	m	66.3	930	49	1.400
4	m	51.7	777	46	1.500
5	f	30.8	174	133	0.566
6	f	65.1	857	53	1.320
7	f	40.7	333	185	0.813
8	m	121.0	1540	54	1.280
Mean ± SEM (f & m)		70.4 ± 10.8	800.1 ± 158.5	73 ± 12	1.100 ± 0.140
Mean ± SEM (f)		58.8 ± 15.1	483.5 ± 148.7	95 ± 17	0.820 ± 0.180
Mean ± SEM (m)		82.1 ± 15.1	1116.8 ± 168.4	50 ± 2	1.390 ± 0.050

Table 29: Pharmacokinetic parameters of i.v. THC fitted to a two-compartment model

Subject		Intravenous THC							
No	Gender	Dose [mg]	AUC_{0-480} [ng*min*mL^{-1}]	alpha $t_{1/2}$ [min]	beta $t_{1/2}$ [min]	Elimination rate constant λ_z [. 10^{-2}]	Correlation	Distribution Volume V_z [L]	Clearance CL [mL/min]
1	f	3.3	7166.8	1.66	99.2	0.699	0.999703	65.9	460.5
2	m	4.2	3717.1	2.58	48.3	1.436	0.999897	78.7	1129.9
3	m	4.24	5638.9	1.93	40.6	1.706	0.999970	44.1	751.9
4	m	4.53	6538.8	2.29	41.6	1.665	0.999983	41.6	692.8
5	f	2.4	16655.6	3.25	62.8	1.110	0.999974	13.1	144.1
6	f	2.92	3706.9	2.20	42.4	1.634	0.999925	48.2	787.7
7	f	3.5	9376.3	2.88	61.4	1.129	0.999785	33.1	373.3
8	m	4.0	2458.1	3.01	43.0	1.612	0.999923	100.9	1627.3
Mean (f & m)			6907.3	2.47	54.0	1.373	0.999895	53.2	745.9
(SEM)			(1598.8)	(0.20)	(7.1)	(0.128	(0.000035)	(9.8)	(164.3)
Mean			9266.4	2.50	66.5	1.141	0.999847	40.1	441.4
(SEM, f)			(2737.4)	(0.35)	(11.9)	(0.191)	(0.000062)	(11.2)	(133.3)
Mean			4588.2	2.45	43.4	1.605	0.999943	66.3	1050.5
(SEM, m)			(922.2)	(0.23)	(1.7)	(0.059)	(0.000020)	(14.3)	(215.3)

DISCUSSION AND CONCLUSIONS

1. PHARMACODYNAMIC STUDY WITH ORAL THC (PAIN STUDY)

A multimodel, well established experimental pain test battery [93-95] was used to cover different types of pain. On one hand the more superficial pain in the electrical stimulation and heat test, and on the other hand the more deep pain in the pressure and cold test (ice cold immersion test). An oral formulation of THC (dronabinol) registered in some countries under the trade mark of Marinol®, was used although this administration route was not ideal in a pharmacokinetic point of view. The 8-h study period enabled to register also effects at time points where mainly the metabolites were present in the plasma.

THC did not produce any analgesia after pressure stimulation. Interestingly, it seemed even to antagonise morphine analgesia. This could be the result of a hyperalgesic effect of THC that has not been detected with THC alone, but appeared when THC was combined with morphine.

In the heat test (pain tolerance threshold) THC produced a significant hyperalgesia. One problem in this test was the temperature limit of 52°C. Some volunteers had already a baseline of 52°C. A similar hyperalgesic effect of THC on thermal pain was also reported in habitual Cannabis users participating in a pain study with Cannabis cigarettes [96]. Heat pain was recently found to be inadequate for detecting opioid-induced analgesia [95].

Hyperalgesia after THC was also observed with the cold test. Interestingly, the combination with morphine totally reversed this effect to an analgesic effect, which was comparable to that of morphine. The cold test is well established to measure opioid-induced analgesia, but it is unclear whether it is also suited for non-opioid analgesics. Jones et al. showed the opiate sensitivity of the cold test but also the apparent insensitivity of the model for non-steroidal anti-inflammatory drugs [78]. We assume that hyperalgesia measured in the cold test was due to an outlier, which also caused the wide variability.

The results of both the single and the repeated transcutaneous electrical stimulation showed a tendency to an additional analgesic effect of the THC-morphine combination compared to morphine alone. In the repeated mode the analgesic effect of THC-morphine was even statistically significant.

Our findings indicate that the analgesic effect of THC and morphine, which was in all our experiments gender-independent, is much influenced by the pain model used.

Luginbuhl et al. [95] also reported that the experimental pain profile differed for substances like alfentanil, xenon, and nitrous oxide. This illustrates the benefit of a multimodel stimulation in the investigation of the analgesic properties of new drugs. None of the experimental pain tests used in this study produces inflammation or tissue damage. Different animal studies have shown an increased analgesic effect of THC in models of inflammatory pain [97]. It has previously been reported that in rats cannabinoid CB_1 receptors are upregulated in chronic neuropathic pain and therefore could lead to an increased analgesic effect of THC in chronic pain [98]. In a retrospective study with patients suffering from chronic pain of different origins, 3 out of 6 patients could reduce their pain to a satisfactory level with a dose of THC of 5 to 20 mg/day, whereas the other 3 patients had to stop the medication due to lack of analgesia or intolerable side effects [99]. Therefore, we cannot rule out that THC would have an analgesic effect after induction of inflammation, tissue or nerve damage. However, up to now no adequate test models for healthy subjects are available.

The side effects of THC were common and normally not severe. There was one volunteer out of twelve who reported disliking very much the psychotropic effects of THC. We assume that a strong aversion to the psychotropic effect leads to a discomfort and therefore possibly also to increased sensitivity to pain. This phenomenon could be observed in the ice cold immersion test where one subject showed a nice correlation between hyperalgesia and anxiety. Von Graffenried et al. [100] found anxiety (and also other psychological factors like mood) to be a factor that might be responsible for the unreliable results obtained in experimentally induced pain in man especially for mild analgesics. If anxiety towards pain tests played a role, even when not using psychotropic drugs, anxiety feelings produced by the psychotropic THC could have an additional influence on the outcomes of pain tests. Many of the psychotropic side effects of THC (euphoria, hallucinations, confusion etc.) were lowered when combining with morphine. On the other hand THC was also influencing the side effects of morphine. Nausea and vomiting was decreased in the combination session compared to the morphine session. This could be due to the well-known antiemetic effect of THC [101]. The reduction of this common side effect of opioids would be a great benefit in the therapy of chronic pain. Although the subjects were Cannabis-naïve the typical psychotropic side effects of THC made the true blinding of the study impossible. A psychoactive placebo could be used to improve the blinding, but the inactivity of the placebo regarding the investigated parameters should be determined.

To reach optimal sensitivity in the determination of the plasma concentration of THC and its two main metabolites THC-OH and THC-COOH, an immunoaffinity extraction procedure with specific antibodies for THC and -metabolites after enzymatic hydrolysis was used followed by

derivatisation and analysis with GC-MS [83]. The very specific extraction procedure produced samples, which were almost free of impurities.

Although THC is almost completely absorbed (90-95 %) after oral administration [28, 31] the plasma profiles after 20 mg THC are characterised by very low levels of THC and high levels of the two main metabolites THC-OH and THC-COOH. The levels were similar to those measured in an earlier study after administration of oral THC [30]. Due to the combined effects of extensive first pass hepatic metabolism, pre-systemic elimination in the gut, and high lipid solubility (volume of distribution V = 10 L/kg b.wt.), only 10 to 20 % of an oral dose is reaching the systemic circulation [28, 31]. The metabolites were detectable already 30 min post drug indicating the rapid liver first pass metabolism. The microsomal hydroxylation is catalised by cytochrome P450 isoenzymes [11]. THC-OH is psychoactive whereas the dominating metabolite THC-COOH is inactive. In glucuronidated form the latter is the main urinary excretion product of THC [10]. A correlation between the THC plasma levels and the pharmacological profiles could only be observed related to the side effects. It is not known whether THC-OH has analgesic properties, too.

These very low plasma levels after 20 mg p.o. THC did not allow to perform the pharmacokinetic analysis by using the individual plasma curves (except for AUC, C_{max}, t_{max}). Therefore, it was based on the plasma concentration time curve out of the mean data of the twelve volunteers.

The analysis of the plasma samples for the quantification of morphine and its main metabolites M6G and M3G caused a lot of problems. The change to a new batch of SPE columns resulted in a complete loss of the recovery of the analytes and made further optimisation of the extraction procedure inevitable. Recovering the analytes as much as possible unfortunately lead to increased matrix effects and chromatographic interferences. To separate these impurities from the analytes the HPLC column and the detecting system had to be changed.

The plasma profiles after the administration of 30 mg morphine showed only low levels of morphine, but high (M6G) to very high (M3G) concentrations of its glucuronidated metabolites. Like THC, phase-II biotransformation by first pass metabolism starts within minutes after administration. M3G is the dominating urinary excretion product of morphine [102], but only M6G exhibits analgesic effects [103].

2. DEVELOPMENT OF WATER SOLUBLE THC FORMULATIONS

To investigate whether another application form with a better bioavailability than the oral formulation could increase the analgesic effect of THC, an inhalation solution of THC was developed. To get optimal physiological tolerability the aim was to develop an aqueous inhalation solution. Due to the very low water solubility of the drug [26], we had to add a solubiliser to the formulation in order to get the necessary THC concentration. For this purpose Cremophors® were evaluated, for clinical use approved solubilisers for topical or oral application forms.

First experiments showed good solubilisation properties for Cremophor® RH 40 and Cremophor® EL. By adding 5 % of the solubiliser a THC concentration of 3 mg/mL could be achieved. The inclusion of the THC by the solubiliser in the heating process turned out to be a very crucial step in the production of the micellar solution regarding the completeness of the solubilisation.

The stress tests with the buffered and non-buffered formulations at four different pH values showed differences in the stability of THC. As known from literature [26] THC was less stable under acidic conditions than in formulations with higher pH. Buffered formulations showed great advantages in the stability of the THC compared to the non-buffered solutions. In addition to that the formulations with Cremophor® RH 40 showed slight advantages in stability than the formulations basing on Cremophor® EL. These findings and also the fact that Cremophor® RH 40 has much better properties concerning taste and odour (not soapy) lead to the decision to perform the further experiments with Cremophor® RH 40 only.

To further investigate the influence of the solubiliser, the pH, and the addition of an antioxidant on the stability of THC in the aqueous formulation, a statistical experiment was carried out. The 2^3-design [69, 88] with additional Yates-analysis allowed to investigate the influence of the different factors on the chosen endpoint, in this case the stability of the THC (expressed as THC/CBN ratio; CBN being the oxidative degradation product of the THC). The analysis showed a significant advantage of the lower concentration of the solubiliser and the physiological pH compared to pH 8, whereas the concentration of the sodium ascorbate did not influence the stability of the formulation. The antioxidant was added because both the THC and the solubiliser are sensitive to oxygen. The chemically compatible benzylalcohol was added as a conservant.

For the use in the pharmacokinetic study this formulation, consisting of THC, Chremophor® RH 40, sodium ascorbate, and benzylalcohol in phosphate buffer at physiological pH, had to undergo an in vitro quality assurance. The tests according to the European Pharmacopeia [89] (pH, osmolality, viscosity, etc.) showed acceptable results for clinical purposes. An appropriate

stability resulted when storing the solution at 4°C and protected from light. The THC content stayed in the ± 5 % range for over 80 days that allowed to prepare the solutions some weeks in advance of the trial.

For the pulmonal application of a drug the particle size of the droplets in the aerosol produced by the nebuliser system is of crucial importance. The analysis of the particle size distribution with laser diffractometry was performed with two nebulisers used already in earlier studies with opiates [12]. The IS-2 and the LC-Plus nebuliser, both pressure driven and coupled to the Pari Master apparatus (producing the air pressure), showed both the necessary median particle size smaller than 5 μm in diameter. This droplet dimension is needed to reach the lower compartments of the lung [66]. The determination of the output rate and time was then the next important step to examine if the concentration of 3 mg/mL inhalation solution would be sufficient to administer the target dose of 4 mg/75 kg b.wt. in a time which would be appropriate for the use in the clinical study. The LC-Plus nebuliser showed with 63 % a slightly higher output rate than the IS-2 nebuliser (59 %). However, with the LC-Plus resulted a much shorter output time (4.75 min/mL) than with the IS-2 nebuliser (7 min/mL). Consequently, taken all this parameters in account, the LC-Plus nebuliser was used for the study.

For the determination of the absolute bioavailability of THC a THC injection solution was prepared using the formulation of Olsen et al. [90] and adding sodium ascorbate as a stabiliser. The THC inhalation solution could not be used due to the risk of an anaphylactic reaction when injecting Cremophor® intravenously [104].

3. PHARMACOKINETIC STUDY WITH PULMONAL AND INTRAVENOUS THC (PHARMACOKINETIC STUDY)

The pulmonal application of nebulised THC seems to be a promising mode for the clinical use of THC. The pulmonal bioavailability of 28.7 ± 8.2 % reached with our application device was significantly higher compared to the oral administration, where the bioavailability was found to be 5 to 20 % [8, 9, 31]. Some volunteers even showed a bioavailability of over 40 %. Most of the subjects reached plasma levels comparable to those of i.v. THC at 10 and 20 min. Peak plasma levels of THC were already observed before the end of the inhalation procedure.

Regarding the plasma concentrations of the THC metabolites THC-OH and THC-COOH, similar patterns for pulmonal and i.v. THC were observed. The THC to THC-OH ratios found in

the present study, 5 - 10 to 1 (i.v. THC) and 2 - 15 to 1 (pulmonal THC), and in an earlier study [105], 0.5 - 1 to 1 (oral THC), confirm the findings reported by Wall et al. for i.v. THC [31]. The significantly lower formation of the psychoactive THC-OH after pulmonal THC, due to the absent first pass metabolism, results in remarkably less intensive psychotropic side effects compared to oral THC. This is an important fact regarding the development of future THC application forms.

The plasma concentration time plot of the i.v. administration showed first a distribution phase with a very rapid decrease of the THC plasma levels followed by the elimination phase with a much longer terminal plasma elimination half-life, compatible with a two-compartment elimination kinetic, which has been described before for THC by Wall et al. [31] and Huestis [9].

Similar pharmacokinetic data were obtained when using the two- and non-compartment calculation model for the i.v. data. The results were in agreement with those from earlier studies (reviewed by Grothenhermen [29]).

The placebo aerosol was very well tolerated indicating a good tolerability of the vehicle with the adjuvants used for the solubilisation and stabilisation of the formulation. Nevertheless, irritation of the airways and coughing after pulmonal THC was observed for all subjects, meaning that THC itself caused these adverse effects. Coughing impaired the inhalation procedure and therefore most likely also the bioavailability, which would probably be higher with a less irritating formulation of THC. The irritations were reversible within a short time after the end of inhalation indicating no lasting damage of the mucosa. This particular effect of THC was also demonstrated by Tashkin et al. [106]. It is very difficult to hypothesise what happens when the THC containing micelles get in contact with the surface of the airways. We assume that the micelles release the THC due to diluting effects of the surfactant and mucus and therefore THC is able to irritate. As the micellar formulation used in this study did not prevent mucosa irritation, other techniques should be tested, for example the use of liposomes or microencapsulation. Among other adverse effects in the inhalation session were very mild psychotropic symptoms and headache. Higher C_{max} and very rapid increase of the concentration in the central nervous system were responsible for the more pronounced adverse effects of i.v. THC, which were mainly of psychotropic nature.

THC did not reduce pain in the ice water test, as it was the case in our preceding pain study with oral THC [105]. As postulated before [105], this indicates that not the low oral bioavailability of THC is responsible for the lack of analgesia. It is assumed, that the ice water test is not the right model to determine an analgesic effect of THC.

4. SUMMARY OF PHARMACODYNAMIC AND PHARMACOKINETIC PROPERTIES OF ORAL, PULMONAL, AND INTRAVENOUS THC

After pulmonal THC much higher plasma levels resulted than after oral THC. The bioavailability of pulmonal THC showed an up to six-fold increase compared to the oral application form used in the first pain study. The elimination half-life of oral THC was much longer (5-fold increase) than after i.v. or pulmonal THC indicating that absorption is the time-determining step in the pharmacokinetics of oral THC. A further disadvantage of the oral administration was the plasma peak time. The highest THC plasma concentrations were found at 60 or 120 min post drug indicating a quite high inter-individual variability. With pulmonal THC peak plasma concentrations resulted already 20 min post drug, actually at the end of the inhalation procedure, in all of the eight subjects. Peak plasma concentrations were much higher after pulmonal than oral administration causing much less side effects indicating that not only THC itself is responsible for the psychotropic side effects but also the known strongly psychoactive metabolite THC-OH.

Despite the increased bioavailability of pulmonal THC no analgesic effect could be provoked suggesting that the bioavailability does not affect the efficacy in the pain reducing properties of THC. We assume that our experimental pain models, which were all models of acute pain, were not the right ones for studying the analgesic properties of THC. Further experiments are needed to evaluate the appropriate pain tests for THC and healthy subjects.

5. OUTLOOK

The THC inhalation solution could be an ideal formulation for the rapid onset of action. The novel liquid-based inhalation devices (see “Theory and Literature Review”, chapter 4.3) would be suitable for its administration.

To achieve a better tolerable formulation other pharmaceutical techniques are required for the solubilisation of THC in an aqueous vehicle. Micro- or nanoemulsions could be used or even nanoencapsulation of THC. When using nanoencapsulation, THC would be dissolved in a suitable vehicle oil which then would be encapsulated by a special technique forming particles of a few nanometers in diameter. THC would be absorbed within these nanocapsules not getting into contact with the surface of the airways.

An easy to handle, pocket-sized and portable inhalation device would be an option to further study the therapeutic potential of pulmonal THC. The pulmonal aerosol should be the

application form of first choice in acute pain situations, like migraine or spasms, where a rapid onset of action is of great therapeutic relevance.

It can be disputed whether the right experimental pain models were used to measure the analgesic effect of THC and whether THC reduces acute pain actually. Therefore THC should be tested too with other pain models, such as models for chronic pain (nowadays not available), or to investigate the effect in patients suffering from chronic or neuropathic pain.

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APPENDICES

I. Study protocol (incl. volunteer information) and VAS questionnaire of the pain study

II. Study protocol (incl. volunteer information) and VAS questionnaire of the pharmacokinetic study

III. Typical chromatogram of the determination of THC and its metabolites in plasma of the pain study

IV. Typical chromatogram of the determination of THC and its metabolites in plasma of the pharmacokinetic study

V. Paper pain study

VI. Paper pharmacokinetic study

I. Study protocol (incl. volunteer information) and VAS questionnaire of the pain study

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The analgesic effect of delta-9-tetrahydrocannabinol alone and in combination with morphine in healthy subjects

S T U D Y P R O T O C O L L

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Introduction

Cannabis (Cannabis sativa L., Cannabaceae) has been used as medicinal plant for thousands of years against pains, muscle spasms, rheuma, malaria, opstipation etc. (see ref. 1-3 for lit. reviewing the therapeutic potential of Cannabis and cannabinoids). Some anecdotal reports about Cannabis as analgesic exist from the 19th century. Reynolds, a famous British neurologist and physician of Queen Victoria, was enthousiastic about Cannabis as pain medicament: „In almost all painful maladies I have found it is by far the most useful of drugs... It is especially so in cases of...neuralgia of the fifth nerve...tumor of brain...thickening of spinal meninges...the lithning pains of the ataxia patient... and migraine." The US Pharmacopeia of 1888 recommended the use of Cannabis as analgesic.

Among the more than 400 identified Cannabis constituents the ***cannabinoids*** (about 60) are the compounds of pharmacological interest. Most research in animals and humans has been done with ***(-)-delta-9-tetrahydrocannabinol (THC)*** and cannabidiol (CBD). THC is the dominating cannabinoid of „Drug-Type Cannabis" and responsible for its psychoactivity, whereas CBD is the dominating cannabinoid in „Fiber-Type Cannabis". It is not psychactive. Synthetic THC has been registered by FDA under the international name of ***Dronabinol*** for the treatment of anorexia of AIDS patients and nausea/vomiting associated with cancer chemotherapy. It is sold in the US under the trade name of ***Marinol®*** as 5, 10 and 15 mg capsules. The medicinalisation of THC in the US lead to a rescheduling by WHO and UN, allowing the therapeutic use of THC under medicinally strictly controlled conditions. The Swiss Narcotic Law did not (yet) follow and only allows today the use of THC for clinical research with a special permit of the Federal Office of Public Health. Such a study is ongoing at the Rehabilitation Center for Paralyzed Patients in Basel (Rehab). The clinical use of Cannabis plant products is still forbidden in Switzerland.

Until 1987 the mechanism of action of THC was attributed to unspecific membrane binding and interaction with the serotonin and prostaglandin synthesis. The first ***cannabinoid receptor*** (CB-1) has been discovered in the rat (and later also in mammalians) [4]. It is mainly located in the brain (substantia nigra, cerebellum etc.). A peripheral cannabinoid receptor system (CB-2) was then detected 1993 in rat spleen [5]. THC has among the natural cannabinoids as agonist the highest binding affinity to CB-1. An ***endogenous ligand*** („Anandamide-I") was first identified in 1992 [6], followed by other arachidonic acid ethanolamides binding to the receptor [7, 8]. The

physiological role of the cannabinoid receptors and ligands is not yet clear. One speculates, that they might play a role in the coordination of psychomotoric functions, memory, emotions, immunomodulation, sleep rhythm, antiinflammation and analgesia.

Several ***animal studies*** have shown the analgesic effect of THC [9-13], some only with very high doses (10-40 mg/kg). In rodents THC was equipotent with morphine or even 3 times more effective. In a recent animal study a low inactive dose of THC (20 mg/kg) was co-administered orally with morphine to mice resulting in enhanced morphine-induced anti-nociception, i.e. 7.6-fold shift in ED_{50}. In the tail-flick latency test codeine, methadone, oxymorphone and hydromorphone showed significant ED_{50} shifts with potency ratios of 25.8, 4.1, 5.0 and 12.6, respectively [14]. In a study with rhesus monkeys THC (0.1-10 mg/kg i.m.) dose-dependently increased antinociception, which was reversable by the specific cannabinoid receptor antagonist SR 141716A [15]. A recent study with rats showed that analgesia produced by a synthetic THC derivative and morphine involves similar brainstem circuitry (rostral ventro-medial medulla, RVM) and that cannabinoids are indeed centrally acting analgesics but with a new mechanism of action [16].

The number of controlled clinical trials with THC is limited and the results somewhat equivocal. Oral THC (5-20 mg) and placebo were compared in 10 cancer patients with moderate chronic pains. 15 and 20 mg THC produced significant pain relief, but with more central side-effects (drowsiness, mental clouding) than small doses. The effect peaked at 3 h and was still near maximum after 6 h [17]. Oral THC (10, 20 mg) and codeine (60, 120 mg) were compared in 36 patients with cancer pain. Analgesic efficacy was equivalent with the two drugs and both THC 20 mg and codeine 120 mg gave significant pain relief compared with placebo [18]. The conclusion was that THC is not suited as standard pain medicament but can be used in small doses for co-medication. In a placebo-controlled study 54 outclinic patients with tumor-induced pains received THC orally. The majority of patients were sedated, relaxed, less depressive but did not report pain reduction [19]. Oral THC had an analgesic effect vs. placebo in healthy subjects after thermally induced pain [20]. No significant analgesic effects from 2 doses of i.v. THC (0.22, 0.44 mg/kg) were found in 10 subjects undergoing dental surgery [21]. The pain level was increased after pressure- and electrostimulation when THC was administered i.v. The side-effects were anxiety and dysphoria [22]. No analgesic effect could be observed in healthy subjects after i.v. doses of 1.5 and 3 mg THC; 10 mg diazepam and placebo were used as control [23]. End-stage cancer patients reported no significant reduction of pain after 0.15 and 0.3 mg

THC per kg b.wt. [24]. Pain relief and reduction of analgesics-co-medication could be observed in a paralyzed patient with spasticity after the oral and rectal administration of 10 and 5 mg THC, respectively [25]. A patient with chronic pain resulting from familial mediterranean fever obtained in a double-blind placebo-controlled crossover trial 50 mg oral THC (as standardized Cannabis preparation) daily and 10 mg morphine. The difference in daily analgesic consumption between active and placebo phase was found to be highly significant [26]. For further literature reviewing the analgesic potency of THC and Cannabis see ref. [27-30].

Aim and relevance of study, hypothesis

Morphine as clinically well established hypno-analgesic may produce relevant dose-dependent acute (respiratory depression etc.) and chronic side-effects (opstipation etc.), tolerance and psychic and physic dependence. It shows poor efficacy on pain syndromes associated with nerve damage. It is postulated from animal data that the combination with THC increases the efficacy of morphine allowing to reduce the dosage and therefore the risk of side-effects and tolerance development. After single oral, sub-psychotropic doses of THC, morphine and a combination of these two substances the analgesic potency will be measured in healthy subjects using standardized pain tests as well as patient monitors and visual analog scales (VAS) to record potential CNS, respiratory and cardiovascular side-effects. Pharmacokinetic parameters will be acquired for correlating plasma concentrations and effects including also metabolites. If the hypotheses of this study are confirmed, therapeutic strategies including THC could be developed, particularly for the treatment of pain states in which the currently available therapies either are ineffective or cause unacceptable side-effects.

Methods

Study design

The study will be performed as double-blind, cross-over trial. Each volunteer will be tested a 4 different sessions, separated by a minimum 7-days-interval. Each subject receives one oral dosage of THC, morphine, THC plus morphine and placebo in a random order. The analgesic potency is tested by using 4 pain tests. Psychotropic side-effects are monitored by a standar-dized

questionnaire. For the pharmacokinetic profiling blood samples are collected imme-diately before each pain test series through an intravenous catheter inserted in a large vein in the cubital fossa.

Subjects

15 paid healthy volunteers, male and female medical students will participate in the study. Exclusion criterias are: existing or earlier drug abuse (including alcohol and medicaments), known or suspected hypersensibility to cannabinoids or opioids, pregnancy. Subjects are not allowed to use non-steroidal antiinflammation drugs, paracetamol and other analgesics 48 h before and during the study. Written informed consent will be obtained from all subjects.

Substances and dosages

Each subject receives orally (empty stomach) on each experimental session either placebo, 20 mg THC (Marinol®), 30 mg morphine sulfate (MST Continus®) or 20 mg THC plus 30 mg morphine sulfate.

Pharmacodynamics

Experimental pain tests (order randomised)

All tests will be applied to the right side. Each test series includes heat, ice water, pressure and transcutaneous electrical stimulation (single and repeated stimulation). These tests will be performed within each series in a randomized order. The volunteers will first try all tests for training. When they are familiar with the testing procedure, baseline recordings of all tests will be performed. Then the test drug will be administered. The test series will be performed every hour, from 1 to 10 h after administration of the drug.

Heat Stimulation. The heat stimulation [31] will be applied to the volar surface of the forearm, in the middle of a line joining the elbow to the wrist fold. Heat pain thresholds will be determined using a computerized version of the Thermotest (Somedic AB, Stockholm, Sweden). The thermode consists of series-coupled Peletier elements and measures 25 mm x 50 mm. A baseline temperature of 30ºC (± 0.2ºC) and a 2.0ºC/s rate of change (heating and return to baseline) will be used. To avoid skin damage a maximum limit of 52ºC will be set. The volunteer will be informed

to press a button when he/she begins to perceive the heat as painful (pain detection threshold) and when he/she finds the heat intolerable and does not want the heat to be further increased (pain tolerance threshold). These temperatures are recorded, and the thermode automatically cools to the baseline temperature. Three consecutive measurements will be performed for both detection and tolerance thresholds. The average of the last two values will be computed and considered for the data analysis.

Ice water test. A two minutes ice water test [32] will be used. The hand is immersed in ice saturated water (1.5 ± 1.0°C). If the pain is considered intolerable before two minutes have elapsed, the volunteer can withdraw the hand, and the elapsed time will be noted. Perceived pain intensity will be continuously rated with an electronic visual analogue scale (VAS) coupled to a pen recorder. The area under the pain intensity/time curve will be determined. If the hand is withdrawn before the end of the two minutes, the pain intensity will be considered to be maximal until the end of the period.

Pressure Pain. Pressure stimulation [33] will be applied to the center of the pulpa of the 2nd and 3rd toe. An electronic pressure algometer (Somedic AB, Stockholm, Sweden), whose probe has a surface area of 64 mm^2, will be used. The pressure will be increased from 0 at a rate of 30 kPa/s to a maximum pressure of 1500 kPa. The volunteer will be informed to press a button when he/she begins to perceive the pressure as painful (pain detection threshold) and when he/she finds the pressure intolerable and does not want the pressure to be further increased (pain tolerance threshold). If the threshold will be above 1500 kPa, this value will be considered as threshold. The mean of 2 determinations of both pain detention and tolerance thresholds from the 2nd and 3rd toe will be considered for data analysis.

Transcutaneous Electrical Stimulation. Two bipolar surface Ag/AgCl-electrodes (inter-electrode distance approximately 2 cm) will be placed in the innervation area of the sural nerve (foot, just distal to the lateral malleolus). Electrophysiological (flexion reflex) and psychophysical (perception of pain) thresholds will be determined. The electrophysiological signal produced by the flexion reflex will be recorded from bipolar Ag/AgCl-electrodes placed over the middle of the biceps femoris and the rectus femoris muscles. A 25 ms, train-of-five, 1 ms, square-wave impulse (perceived as a single stimulus) will be delivered from a computer-controlled constant current

stimulator (University of Aalborg, Denmark). The current intensity will be increased from 1 mA in steps of 1 mA until a pain sensation (psychophysical threshold) or a flexion reflex (electrophysiological threshold) will be evoked. These thresholds will be defined as single stimulus thresholds. The above-mentioned stimulus burst will be repeated 5 times with a frequency of 2 Hz [34] to elicit temporal summation. Temporal summation occurs when the repetition of a stimulus causes increased pain perception, probably as a result of sensitization of spinal cord neurons [35]. The current intensity will be increased from 1 mA in steps of 1 mA until the summation threshold will be reached. Summation threshold will be defined as the stimulus intensity eliciting an increase in perception of current intensity (psychophysical threshold) or an increase in amplitude of the last 1 or 2 reflexes (electrophysiological threshold) during the 5 stimulations.

For all the above measurements, if the threshold will be above a maximal current of 80 mA, the threshold will be defined as 80 mA. Three consecutive measurements will be performed, and the average of the last two values will be considered for data analysis.

Side effects

A 10 cm visual analog scale (VAS) will be used to assess sedation, psychotropic effects and nausea. The episodes of vomiting will be noted. Hemoglobin oxygen saturation (pulse oximetry), expired CO_2 (via nasal catheter), blood pressure and heart rate will be recorded. To determine the reaction time, a 1000 Hz tone will be delivered from a computer with randomized intervals of 3 to 8 seconds, and a timer will be simultaneously started. The volunteer will be instructed to press a button as fast as possible after the tone. The reaction time will be defined as the time from the tone until the volunteer presses the button. The mean of five consecutive measurements will be calculated. All the above parameters will be recorded immediately before each test series.

Pharmacokinetics

Plasma profiles of THC and THC metabolites (11-carboxy-THC, 11-hydroxy-THC) will be performed by gas-chromatography/mass spectrometry (GC/MS) [25]. Plasma profiles of morphine and morphine metabolites (morphine-6-glucuronide, morphine-3-glucuronide) will be performed by HPLC [36]. The pharmacokinetic data evaluation includes C_{max}, t_{max}, $t_{1/2}$, V and CL.

Ethical Aspects, Permits

The toxicity of THC is extremely low. Fatal intoxications after either recreational or therapeutic use have never been reported. According to the manufacturer of Marinol®, the human lethal dose after i.v. injection is estimated to be 30 mg/kg. CNS side-effects (sedation, dysphoria, anxiety etc.) only occur after oral doses of THC higher than 20 mg [37]. A psychic dependence may develop only after long-term use and high dosages [37]. The study requires a special THC permit of the Federal Office of Public Health.

Bern, 25. November 1998 / cannabis / ifai-studienprotokoll.doc

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Die analgetische Wirkung von delta-9-Tetrahydrocannabinol allein und in Kombination mit Morphin

VersuchsteilnehmerInnen-Information, Einverständniserklärung

Einleitung, Zielsetzung

Die Cannabispflanze wird seit Jahrtausenden u.a. gegen Schmerzen und Muskelkrämpfe eingesetzt. In der Schweiz ist in den letzten Jahren eine zunehmende volksmedizinische (illegale) Verwendung von Cannabisprodukten zu beobachten. Der Hauptwirkstoff ist das delta-9-Tetrahydrocannabinol (THC), welches in den USA als Marinol® registriert ist. Der schmerzhemmende Effekt von THC und die Potenzierung der Morphinwirkung ist in verschiedenen Tierstudien gezeigt worden, während die Resultate aus den wenigen kontrollierten Humanversuchen kontrovers sind. Das Ziel dieser doppelblind und placebokontrolliert durchgeführten Studie an gesunden ProbandInnen ist die Messung der Schmerzhemmung von oral verabreichtem THC allein oder in Kombination mit Morphin anhand von verschiedenen standardisierten Schmerztests.

Versuchsablauf

Am jeweils rund 12 Stunden (08.00-20 h) dauernden Versuch könne MedizinstudentInnen sowie Angehörige des DKF teilnehmen, welche nicht Alkohol-, Medikamenten-, Opiat- oder Cannabis-abhängig sind/waren, keine Überempfindlichkeit gegenüber Cannabinoiden und Opiaten aufweisen

und nicht schwanger sind. Jede(r) VersuchsteilnehmerIn erhält an 4 verschiedenen Tagen *nüchtern* eine orale Dosis von 20 mg THC, 30 mg Morphin, eine Kombination von 20 mg THC und 30 mg Morphin oder Placebo. Zwischen den einzelnen Versuchen liegen mindestens 7 Tage. Gemessen wird während 10 Stunden der schmerzstillende Effekt nach Hitze-, Druck-, Kälte- und Elektrostimulation. Gleichzeitig werden anhand von Befragungsprotokollen und Monitoren allfällige psychische und physische Nebenwirkungen aufgezeichnet. Vor jedem Schmerztest wird zur Bestimmung der Blutspiegel und Metaboliten von THC und Morphin über einen Venenkatheter eine Blutprobe entnommen. Während des ganzen Versuches ist ein Arzt/eine Ärztin und eine Pflegeperson anwesend. Selbstverständlich werden Sie während der gesamten Versuchsdauer von uns verpflegt. Der Versuch findet im Schmerzlabor des Institutes für Anästhesie und Intensivmedizin (IFAI) statt. Die aus dem Versuch gewonnenen medizinischen und persönlichen Daten werden anonymisiert verarbeitet, sind vertraulich und nicht öffentlich zugänglich. Die persönlichen Daten können von den zuständigen Behörden und von befugten Personen während einer Inspektion eingesehen und geprüft werden.

Nebenwirkungen

Die Toxizität des THC ist extrem niedrig. Allenfalls auftretende kardiovaskuläre Nebenwirkungen (Blutdruck- und Pulsveränderungen) sind harmlos (falls Probleme in der Nacht nach dem Versuch auftreten → Tel. Versuchsleiter). Unerwünschte psychische, reversible Effekte (Sedation, Angst etc.) treten erst in höheren Dosen auf. Ein Abhängigkeitsrisiko besteht nur bei Langzeitanwendung. Morphin kann Übelkeit, Verstopfung, Blutdruck- und Stimmungsveränderungen sowie eine Atemdepression verursachen. Ein Abhängigkeitsrisiko besteht ebenfalls nur nach längerem Einsatz.

Verpflichtung

Sie haben das Recht, jederzeit vom Versuch zurückzutreten. 48 h vor und während den einzelnen Sitzungen dürfen keine nichsteroidalen Entzündungshemmer und Analgetika sowie kein Alkohol eingenommen werden. Bis 12 h nach dem Versuch darf kein Fahrzeug geführt werden.

Versicherung

Für klinische Versuche an gesunden Probanden besteht eine pauschale Haftpflichtversicherung der Universität Bern.

Entschädigung

Sie werden für die Versuchsteilnahme mit insgesamt 1000.-- Franken entschädigt (250.-- pro Sitzung).

Einverständniserklärung

Ich habe die VersuchsteilnehmerInnen-Information gelesen und Sinn und Inhalt der Studie verstanden. Ich erhielt Gelegenheit, Fragen zum Versuchsablauf und zu allfälligen Nebenwirkungen zu stellen.

Bern, den .....

.....

Unterschrift VersuchsteilnehmerIn

Unterschriften Versuchsleiter:

.....

Prof. Dr. A. Zbinden

.....

Prof. Dr. R. Brenneisen

.....

Dr. M. Curatolo

THC/Morphin-Analgesie-Studie

Proband/in: Nr.
Versuchsdatum: / /
Session:

Messzeitpunkt: t 0 (Baseline)

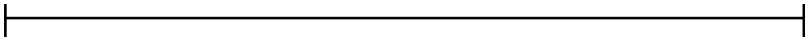
Nebenwirkungen-0

Bitte beantworten Sie folgende Fragen durch Markierung der skalierten Linie mit einem *Strich*:

1. Ich fühle mich angenehm ruhig und entspannt.

0% 100%
überhaupt nicht sehr stark

2. Ich kann mich gut konzentrieren.



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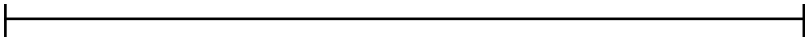
3. Ich fühle mich schläfrig.



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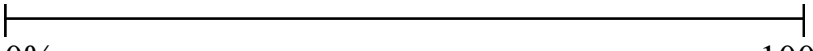
4. Ich fühle mich euphorisch.



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5. Ich fühle mich verstimmt.

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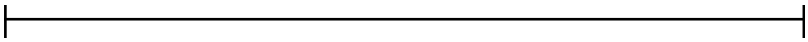
6. Ich habe Angstgefühle.



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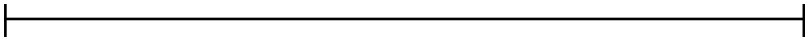
7. Ich fühle mich innerlich gespannt, aggressiv.



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8. Ich fühle mich verwirrt, desorientiert.

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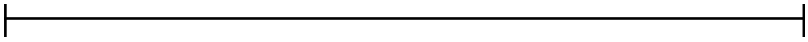
9. Ich nehme mich selbst als verändert wahr.



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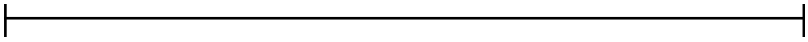
10. Ich nehme meine Umgebung als verändert wahr.



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11. Ich habe optische, akustische oder andere Halluzinationen.

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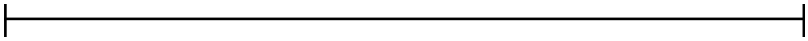
12. Ich nehme Stimmungen, Gedanken, innere Bilder wahr, die mir nicht vertraut sind.



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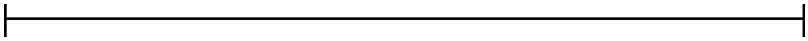
13. Ich empfinde Übelkeit.



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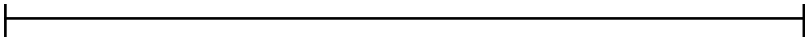
14. Ich habe Kopfschmerzen.



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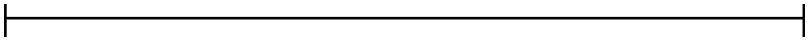
15. Ich habe Atembeschwerden.



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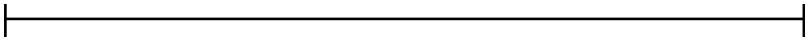
16. Ich habe Herzbeschwerden (z.B. Herzrasen, Herzstechen).



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17. Ich habe Magen-, Darmbeschwerden.

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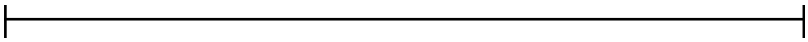
18. Ich empfinde Mundtrockenheit.



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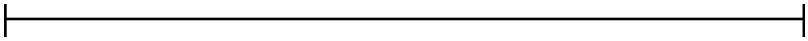
19. Ich habe Schwindelgefühle.



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20. Ich habe andere Beschwerden, nämlich:

II. Study protocol (incl. volunteer information) and VAS questionnaire of the pharmacokinetic study

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Pharmakokinetik und –dynamik des pulmonal applizierten delta-9-Tetrahydrocannabinols (THC-Flüssigaerosol)

Studienprotokoll

1. Einführung

Bei der geplanten pharmakokinetischen und -dynamischen Studie handelt es sich um die Fortsetzung des inzwischen abgeschlossenen Projektes „The analgesic effect of delta-9-tetrahydrocannabinol alone and in combination with morphine in healthy subjects". Für den allgemeinen Background zu THC und Analgesie sei deshalb auf dieses am 7.12. 1998 bewilligten Sudienprotokolls (Gesuch Nr. 195/98) verwiesen.
Nachdem sich im Rahmen dieser Schmerzlaborstudie gezeigt hat, dass die orale Applikation von THC in Form von Marinol®-Kapseln in pharmakokinetischer Hinsicht nicht ideal ist (intensiver Lebermetabolismus, tiefe Bioverfügbarkeit etc.), drängt sich die klinische Prüfung anderer Anwendungsformen auf. Alternativ bieten sich Suppositorien, welche allerdings auch einem First-Pass-Effekt unterliegen, Lungenaerosole, Sublingualsprays (zur Zeit in klinischer Erprobung bei GW Pharmaceuticals, GB), Hautpflaster (eher für Depotanwendung geeignet, klinische Versuche laufen in den U.S.A.) sowie Injektionslösungen an. Cannabiszigaretten sind medizinisch-ethisch nicht vertretbar und im Gegensatz zu den USA in der Schweiz für die Forschung nicht zugelassen. Bereits kommerziell

zugänglich sind einige Inhalatoren (z.B. Vapormed Vaporizer Volcano). Diese Geräte sind allerdings wissenschaftlich nicht oder nur ungenügend validiert und eignen sich nur zur „heissen" (Heissluft, Infrarotlampe etc.) Verdampfung von Cannabis. Aus toxikologischen (keine Bildung von Pyrolyseprodukten), galenischen (definierte Partikelgrösse etc.) und technischen Gründen (validierte Inhalatorgeräte auf dem Markt) ist die „kalte" Inhalation in Form von THC-Flüssigaerosolen zu bevorzugen. Die Aerosolproduktion erfolgt dabei mittels Pressluft oder Ultraschall. Die intravenöse Applikation von THC wurde bereits an Tieren und Menschen getestet. Dabei stellte die, wie bei der Herstellung der Flüssigaerosole, extrem schlechte Wasserlöslichkeit des THC (0.003 mg/mL) ein zu lösendes galenisches Problem dar.

2. Ziel der Studie, Studiendesign

Im Rahmen des geplanten Projektes soll nun ein bereits in unserem Labor entwickeltes und in vitro validiertes THC-Flüssigaerosol pulmonal an 8 gesunden ProbandInnen im Vergleich mit i.v. appliziertem THC getestet werden. Diese Phase-I-Pilotstudie, bestehend aus insgesamt 3 Sessionen (Flüssigaerosol-Verum, Flüssigaerosol-Placebo, Injektionslösung) umfasst primär pharmakokinetische Messungen (Plasmaspiegel, AUC, Bioverfügbarkeit, Halbwertszeit etc.) sowie ein Nebenwirkungs-Monitoring (Lungen-verträglichkeit, Vitalfunktionen). Zur Beurteilung der analgetischen Potenz soll ein einfacher Schmerztest (Eisbad) durchgeführt werden, was eine Placebokontrolle und Doppelblindbedingungen erfordert.

3. Methoden

3.1 Probanden

- 4 männliche und 4 weibliche, freiwillige, bezahlte ProbandInnen, welche bereits an der Schmerzlaborstudie teilgenommen haben und

dem Studienleiter bekannt sind (DKF-Angehörige, MedizinstudentInnen). Die ProbandInnen werden vor Versuchsbeginn in der Abteilung für Pneumologie (Prof. Bachofen) einem Lungenfunktionstest unterworfen.

- Einschlusskriterien: die ProbandInnen müssen gesund sein, über intakte Lungenfunktionen verfügen und dürfen 48 h vor, während und 24 h nach dem Versuch keinen Alkohol, keine Drogen und Medikamente konsumieren. Bis 24 h nach dem Versuch sind das Führen von Fahrzeugen und das Bedienen von Maschinen nicht erlaubt. Die ProbandInnen müssen die Einverständniserklärung unterschreiben, dies nachdem sie ausführlich über die Studie informiert worden sind und die ProbandInnen-Information gelesen haben.
- Ausschlusskriterien: Konsum von Alkohol, Drogen oder Medikamenten (insbesondere Analgetica) vor und/oder während der Studie; positiver Cannabisnachweis im Urin (Test jeweils kurz vor Sessionsbeginn); Schwangerschaft (Test kurz vor Studienbeginn).

3.2 Versicherung

Die VersuchsteilnehmerInnen sind durch die Universität Bern pauschalversichert.

3.3 Testsubstanzen, Dosierungen

- THC-Flüssigaerosol:
  - Rezeptur: 30.0 mg THC (THC Pharm, D-Frankfurt/Main; Analysenzertifikat des Herstellers vorhanden; Nachkontrolle im DKF), 500.0 mg Cremophor RH 40 (Emulgator; BASF; Toxizitätsdaten vorhanden), 5.0 mg Natriumascorbat (Vit. C, Antioxydans; Pharmakopöe-Qualität), 100.0 mg Benzylalkohol (Konservierungsmittel; Pharmakopöe-Qualität), Phosphatpuffer pH 7.4 ad 10.0 mL. Die 0.3%-Flüssigaerosole werden jeweils unter GMP-Bedingungen

in der Inselspital-Apotheke frisch hergestellt und sterilfiltriert. Die Qualitätskontrolle (THC-Gehaltsbestimmung) erfolgt im DKF.

- Dosierung: 0.053 mg THC pro kg KG (4 mg THC pro 75 kg) in Form von 2.2 mL THC-Flüssigaerosol.

• Placebo-Flüssigaerosol:

Mit Ausnahme des Wirkstoffes THC identische Zusammensetzung wie THC-Flüssigaerosol. Herstellung unter GMP-Bedingungen in der Inselspital-Apotheke.

Die Verblindung der beiden Flüssigaerosole übernimmt ebenfalls die Inselspital-Apotheke.

• THC-Injektionslösung:

- Rezeptur: 10 mg THC, 150.0 mg Polysorbatum 80 (Tween® 80), 10.0 mg Natriumascorbat, Ethanol abs. 500 µL, NaCl 0.9% pH 7.4 ad 10.0 mL. Die 0.1%-Injektionslösungen werden jeweils kurz vor den Versuchen unter GMP-Bedingungen in der Inselspital-Apotheke frisch hergestellt und sterilfiltriert. Die Qualitätskontrolle (THC-Gehaltsbestimmung) erfolgt im DKF.
- Dosierung: 0.053 mg THC pro kg KG (4 mg THC pro 75 kg).

3.4 Studienablauf

• Die Studie wird in den Räumlichkeiten der Clinical Investigation Unit (CIU) am Inselspital Bern unter der Überwachung eines Arztes und einer Forschungsschwester durchgeführt. Eine Session dauert jeweils 9h (8-17h).

• Session 1: Trainingsversuch (Instruktion Inhalationsinstrument/ -technik) mit Placebo-Flüssigaerosol; anschliessend Verum- oder Placeboversuch mit 0.053 mg THC pro kg KG (4 mg THC pro 75 kg) pulmonal in Form von 2.2 mL THC-Flüssigaerosol oder 2.2 mL Placebo-Flüssigaerosol. Die standardisierte Applikation (1 Zug/10 sec, 10 min Inhalationsdauer) erfolgt mittels eines druckluftbetriebenen Vernebler-gerätes (PariMaster®-LC Plus). 5 min vor (Basislinie), 5, 10, 20, 40, 60,

120, 240 und 480 min nach Applikation werden mittels eines Venenkatheters 5-10 mL Blut entnommen, zentrifugiert und sofort tiefgefroren. Zum Zeitpunkt der Blutentnahmen werden jeweils ein Analgesie-Test (Eiswasser) sowie ein Nebenwirkungs-Monitoring (Vitalfunktionen, Lungenverträglichkeit, psychotrope Effekte etc. ➡ Beilage) durchgeführt.

- Session 2: 0.053 mg THC pro kg KG (4 mg THC pro 75 kg) pulmonal in Form von 2.2 mL THC-Flüssigaerosol oder 2.2 mL Placebo-Flüssigaerosol. Blutentnahmen und Messungen wie bei Session 1.
- Session 3: 0.053 mg THC pro kg KG (4 mg THC pro 75 kg) in Form einer intravenösen Injektionslösung (10 mg THC/10 mL). Blutent-nahmen und Messungen wie bei Session 1.
- Bioanalytik und pharmakokinetisches Profiling: Quantifizierung des THC und seiner Hauptmetaboliten (11-Hydroxy-THC, 11-Nor-Carboxy-THC) in Plasma mittels Gaschromatographie-Massenspektrometrie. Auf Basis der resultierenden THC-Plasmakonzentrations-Zeit-Profile werden dann dessen Areas Under the Curve (AUC), Bioverfügbarkeit (F), Plasma-peaks (C_{max}), Zeitpunkt der Plasmapeaks (t_{max}), terminale Plasmahalb-wertszeiten ($t_{1/2z}$), Verteilung (V) und Clearence (CL) berechnet.

Bern, den / / 2002

Prof. Dr. pharm. R. Brenneisen
DKF
(Studienleiter)

Bern, den / / 2002

Dr. med. S. Russmann
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(Prüfarzt)

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Pharmakokinetik und –dynamik des pulmonal applizierten delta-9-Tetrahydrocannabinols (THC-Flüssigaerosol)

Zusammenfassung

1. Einleitung

Bei der geplanten Phase-I Studie mit 2 neuen THC-Applikationsformen handelt es sich um die Fortsetzung der Schmerzlaborstudie „The analgesic effect of delta-9-tetrahydrocannabinol alone and in combination with morphine in healthy subjects" (Projekt Nr. 195/98, 7.12.1998). Nachdem sich im Rahmen dieser Schmerzlaborstudie gezeigt hat, dass die orale Applikation von THC in Form von Marinol®-Kapseln (synthetisches THC) in pharmakokinetischer Hinsicht nicht ideal ist (intensiver Lebermetabo-lismus, niedrige Bioverfügbarkeit etc.), drängt sich die klinische Prüfung anderer, bevorzugt nicht invasiver Anwendungsformen auf.

2. Ziel der Studie, Fragestellungen

Im Rahmen des geplanten Projektes soll nun ein in unserem Labor entwickeltes THC-Flüssigaerosol pulmonal an 8 gesunden ProbandInnen im Vergleich mit i.v. THC getestet werden. In den 3 Sessionen (Aerosol-Verum, -Placebo, Injektionslösung) werden primär ein pharmakokinetisches Profiling sowie Nebenwirkungs-Monitoring durchgeführt. Mittels Eisbadtest wird zudem doppelblind und vs. Placebo die analgetische Wirkung gemessen.

3. Versuchsplan, -dauer

An dieser Phase-I Pilotstudie werden in 3 je 9-stündigen Sessionen 4 gesunde Probandinnen und 4 Probanden teilnehmen. Der klinische Versuch findet in der Clinical Investigation Unit (CIU) des Inselspitals unter Dauerüberwachung eines Arztes und einer Krankenschwester statt. Die ProbandInnen erscheinen um 8 h nüchtern in der CIU, werden nach der Trainingsphase (Instruktion Inhalationsgerät/-technik) venenkathetrisiert und an den Monitor zur Messung der Vitalfunktionen (Blutdruck, Puls, Sauerstoffsättigung) angeschlossen. Bei Probandinnen wird vor Versuchsbeginn ein Schwangerschaftstest und bei allen VersuchsteilnehmerInnen vor jeder Session ein Cannabis-Urintest durchgeführt. Um 8:30 h, nach Sammeln der 1. Blutprobe (Baseline) sowie Ausfüllen der Nebenwirkungsprotokolle (Visual Analog Scales, VAS), inhalieren die ProbandInnen unter Doppelblindbedingungen und nach vorgängiger Anleitung mittels Druckluft-Vernebler ein THC-Aerosol enthaltend 0.053 mg THC pro kg KG (4 mg THC pro 75 kg) oder ein Placebo-Aerosol oder erhalten eine intravenöse Injektion von 0.053 mg THC pro kg KG (4 mg THC pro 75 kg). Über einen Venenkatheter werden insgesamt 9 Blutproben zu je rund 10 mL gesammelt, Nebenwirkungen mittels VAS erfasst und die Vitalfunk-tionen aufgezeichnet. Zum Zeitpunkt der Blutentnahmen wird jeweils auch ein Schmerztest (Eiswasser) durchgeführt.

Die Studie dauert voraussichtlich 8 Monate und soll im Juli 2002 oder sofort nach Vorliegen der Bewilligungen (inkl. Swissmedic, BAG) beginnen.

4. Einschluss-, Auschlusskriterien

- 4 männliche und 4 weibliche, freiwillige, bezahlte ProbandInnen, welche bereits an der Schmerzlaborstudie teilgenommen haben und dem Studienleiter bekannt sind (DKF-Angehörige, Medizinstudentinnen).
- Einschlusskriterien: die ProbandInnen müssen gesund sein, über intakte Lungenfunktionen verfügen und dürfen 48 h vor, während und 24 h nach dem Versuch keinen Alkohol, keine Drogen und Medikamente

konsumieren. Bis 24 h nach dem Versuch sind das Führen von Fahrzeugen und das Bedienen von Maschinen nicht erlaubt. Die ProbandInnen müssen die Einverständniserklärung unterschreiben, dies nachdem sie ausführlich über die Studie informiert worden sind und die ProbandInnen-Information gelesen haben.

- Ausschlusskriterien: Konsum von Alkohol, Drogen oder Medikamenten (insbesondere Analgetica) vor und/oder während der Studie; positiver Cannabisnachweis im Urin, Schwangerschaft.

5. Risikoabschätzung

Die Toxizität des THC ist extrem niedrig. Allenfalls auftretende Blutdruck- und Pulsschwankungen sind harmlos. Unerwünschte psychische Effekte (Sedation, Angst etc.) sind reversibel und treten erst in höheren Dosen auf. Ein Abhängigkeitsrisiko besteht nur bei Langzeitanwendung hoher Dosen und missbräuchlichem Konsum zu Rauschzwecken. Dies gilt auch für das (seltene) Auftreten von Entzugssymptomen.

6. Studienleitung

Prof. Dr. pharm. Rudolf Brenneisen, Leiter Labor „Phytopharmakologie, Bioanalytik und Pharmakokinetik", Departement Klinische Forschung (DKF).

Bern, den 13.6.2002

Prof. Dr. pharm. R. Brenneisen
DKF

Universität Bern
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Pharmakokinetik und –dynamik des pulmonal applizierten delta-9-Tetrahydrocannabinols (THC-Flüssigaerosol)

VersuchsteilnehmerInnen-Information

Einleitung und Zielsetzung

Die Cannabispflanze wird seit Jahrtausenden u.a. gegen Schmerzen und Muskelkrämpfe eingesetzt. In der Schweiz, anderen europäischen Ländern sowie in den USA ist in den letzten Jahren eine zunehmende volksmedi-zinische Verwendung von Cannabisprodukten zu beobachten. Eine ausreichende klinische Datenlage besteht allerdings nur für das in den USA als Marinol® registrierte Dronabinol (synthetisches delta-9-Tetrahydro-cannabinol, THC). Nachteil der Marinol®-Kapseln ist der sehr intensive, nach Resorption aus dem Darmtrakt rasch einsetzende Abbau des THC in der Leber. Aus diesem Grunde sind alternative galenische Applikations-formen notwendig, bei denen dieser Lebereffekt nicht vorhanden oder weniger ausgeprägt ist. Die klinische Studie mit einem pulmonal appli-zierten THC-Flüssigaerosol und einer intravenösen THC-Injektionslösung, an der Sie nun teilnehmen, ist eine Folgestudie zur

abgeschlossenen Schmerzstudie mit oral verabreichtem THC (Marinol®). Sie soll primär dazu dienen, die Blutspiegel des THC und seiner Metaboliten nach Inhalation und Injektion zu vergleichen, die schmerzhemmende Wirkung anhand eines Kältetests zu messen sowie allfällige psychische und physische Nebenwirkungen zu erfassen.

Versuchsablauf

An den jeweils 9 h (8-17 h) dauernden Versuchssessionen können MedizinstudentInnen sowie Angehörige des DKF teilnehmen, welche nicht alkohol-, medikamenten- oder drogenabhängig sind/waren und nicht schwanger sind (Urintest). Vor Studienbeginn werden Sie in der Abteilung für Pneumologie einem Lungenfunktionstest unterworfen. Vor jeder Session wird ein Cannabis-Urintest durchgeführt.

Vor dem ersten Versuch durchlaufenen Sie eine Trainingsphase, die dazu dient, Sie mit dem Inhalationsinstrument und der Inhalationstechnik vertraut zu machen. Nach Anweisung inhalieren Sie dann unter standardisierten Bedingungen (1 Zug/10 sec, 10 min Inhalationsdauer) mittels eines druckluftbetrieben Verneblergerätes eine Lösung von 0.053 mg THC pro kg Körpergewicht (entsprechend 4 mg THC pro 75 kg) oder eine Lösung ohne Wirkstoff (Placebo) oder man injiziert Ihnen intravenös eine Lösung von 0.053 mg THC pro kg (4 mg THC pro 75 kg). Zur Bestimmung der Blutspiegel von THC und dessen Metaboliten werden über einen Venenkatheter zu definierten Zeitpunkten pro Session insgesamt 9 Blutproben zu je 5-10 mL entnommen. Die Messung des schmerzhemmenden Effektes erfolgt mittels Eiswasser-Test jeweils zum Zeitpunkt der Blutentnahmen. Gleichzeitig werden anhand von Befragungsprotokollen und Monitoren allfällige psychische und physische Nebenwirkungen aufgezeichnet.

Während des ganzen Versuches ist ein Arzt und eine Forschungsschwester anwesend. Selbstverständlich werden Sie während

der gesamten Versuchsdauer von uns verpflegt. Der Versuch findet in der Clinical Investigation Unit (CIU) des Inselspitals Bern statt.
Die aus dem Versuch gewonnenen medizinischen und persönlichen Daten werden anonymisiert verarbeitet, sind vertraulich und nicht öffentlich zugänglich. Die persönlichen Daten können aber von den zuständigen Behörden und von befugten Personen während einer Inspektion eingesehen und geprüft werden.

Nebenwirkungen

Die Toxizität des THC ist extrem niedrig. Allenfalls auftretende kardio-vaskuläre Nebenwirkungen (Blutdruck- und Pulsveränderungen) sind harmlos. Falls Probleme in der Nacht nach dem Versuch auftreten ➡ Tel. Versuchsleiter: 031-352 41 25 od. 079-300 83 29. Unerwünschte psychische, reversible Effekte (Sedation, Angst etc.) treten meist erst in höheren Dosen auf. Ein Abhängigkeitsrisiko kann bei zweimaliger Appli-kation ausgeschlossen werden.

Verpflichtung

Sie haben das Recht, jederzeit vom Versuch zurückzutreten. 48 h vor und während des Versuches dürfen kein Alkohol sowie keine Medikamente und Drogen eingenommen werden. Bis 24 h nach dem Versuch darf kein Fahr-zeug gelenkt und keine Maschinen bedient werden.

Versicherung

Der Versuchsleiter Prof. Dr. R. Brenneisen ersetzt Ihnen Schäden, die Sie gegebenenfalls im Rahmen des klinischen Versuchs erleiden. Zu diesem Zweck hat Prof. Dr. R. Brenneisen zu Ihren Gunsten im Rahmen der „Probandenversicherung der Universität Bern" eine Versicherung bei der „Allianz Suisse Versicherungen" abgeschlossen.

Entschädigung

Sie werden für die Versuchsteilnahme nach Beendigung der Session 3 mit insgesamt 750 Franken entschädigt.

Bern, den 13. Juni 2002

Prof. Dr. R. Brenneisen
DKF
Versuchsleiter

E I N V E R S T Ä N D N I S E R K L Ä R U N G

- **Bitte lesen Sie dieses Formular sorgfältig durch.**
- **Bitte fragen Sie, wenn Sie etwas nicht verstehen oder wissen möchten.**

Nummer der Studie	:	
Titel der Studie	:	**Pharmakokinetik und -dynamik des pulmonal applizierten delta-9-Tetrahydrocannabinols (THC-Flüssigaerosol)**
Ort der Studie	:	Dep. Klinische Forschung (DKF), Universität Bern und Clinical Investigation Unit (CIU), Inselspital Bern
Stud.leiter, Prüfarzt	:	Prof. Dr. pharm. R. Brenneisen, Dr. med. S. Russmann
Versuchsperson	:	
Geburtsdatum	:	Geschlecht :

- Ich wurde vom unterzeichnenden Arzt mündlich und schriftlich über die Ziele, den Ablauf der Studie mit THC-Aerosolen und -Injektionslösungen, über die zu erwartenden Wirkungen, über mögliche Vor- und Nachteile sowie über eventuelle Risiken informiert.
- Ich habe die zur oben genannten Studie abgegebene schriftliche Information vom 13.6.2002 gelesen und verstanden. Meine Fragen im Zusammenhang mit der Teilnahme an dieser Studie sind mir zufriedenstellend beantwortet worden. Ich kann die schriftliche Information behalten und erhalte eine Kopie meiner schriftlichen Einverständniserklärung.
- Ich hatte genügend Zeit, um meine Entscheidung zu treffen.
- Ich bin darüber informiert, dass eine Versicherung Schäden deckt, falls solche im Rahmen der Studie auftreten.
- Ich bin einverstanden, dass die zuständigen Fachleute des Studienauftraggebers, der Behörden und der Ethikkommission zu Prüf- und Kontrollzwecken in meine Originaldaten Einsicht nehmen dürfen, jedoch unter strikter Einhaltung der Vertraulichkeit.
- Ich nehme an dieser Studie freiwillig teil. Ich kann jederzeit und ohne Angabe von Gründen meine Zustimmung zur Teilnahme widerrufen, ohne dass mir deswegen Nachteile bei der weiteren medizinischen Betreuung entstehen. In diesem Fall werde ich zu meiner Sicherheit abschliessend medizinisch untersucht.
    - Ich bin mir bewusst, dass während der Studie die in der Information genannten Anforderungen und Einschränkungen einzuhalten sind. Im Interesse meiner Gesundheit kann mich der Prüfarzt jederzeit von der Studie ausschliessen.

Bern, den	Unterschrift der Versuchsperson
Bern, den	Unterschrift des Studienleiters
Bern, den	Unterschrift des Prüfarztes

THC-Kinetik-Studie

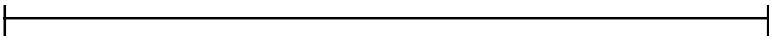
Proband/in: Nr.
Versuchsdatum:
Session:

Messzeitpunkt: t 0

Nebenwirkungen-0

Bitte beantworten Sie folgende Fragen durch Markierung der skalierten Linie mit einem *Strich* (falls 0% oder 100% zutreffen, bitte entsprechend einkreisen):

1. Ich fühle mich angenehm ruhig und entspannt.



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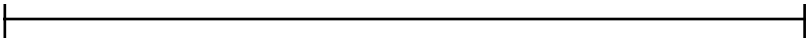
2. Ich kann mich gut konzentrieren.



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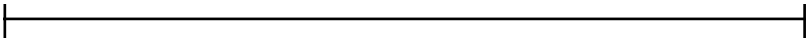
3. Ich fühle mich schläfrig.



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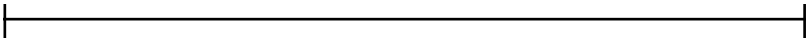
4. Ich fühle mich euphorisch.



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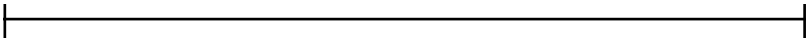
5. Ich fühle mich verstimmt.



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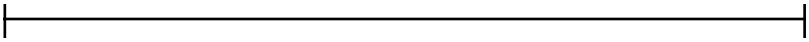
6. Ich habe Angstgefühle.



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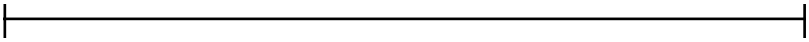
7. Ich fühle mich innerlich gespannt, aggressiv.



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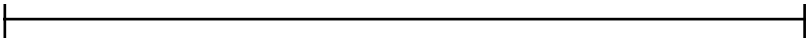
8. Ich fühle mich verwirrt, desorientiert.



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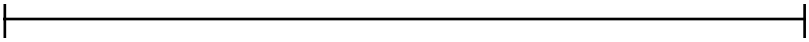
9. Ich nehme mich selbst als verändert wahr.



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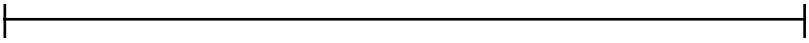
10. Ich nehme meine Umgebung als verändert wahr.



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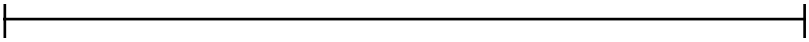
11. Ich habe optische, akustische oder andere Halluzinationen .



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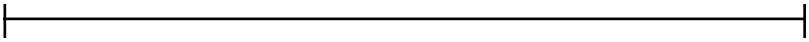
12. Ich nehme Stimmungen, Gedanken, innere Bilder wahr, die mir nicht vertraut sind.



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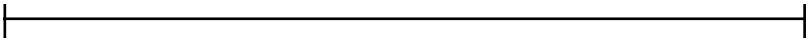
13. Ich empfinde Übelkeit.



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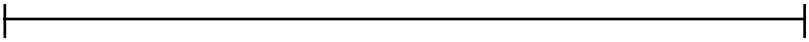
14. Ich habe Kopfschmerzen.



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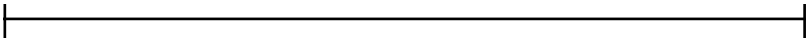
15. Ich habe Atembeschwerden.



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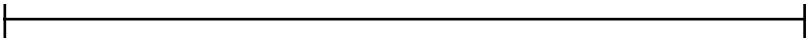
16. Ich habe Husten / Hustenreiz.



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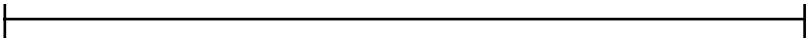
17. Meine Atemwege sind irritiert / gereizt / brennen.



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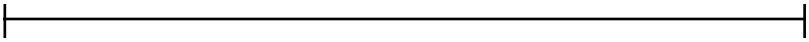
18. Ich habe Herzbeschwerden (z.B. Herzrasen, Herzstechen)



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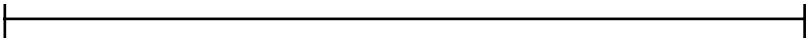
19. Ich habe Magen-, Darmbeschwerden .



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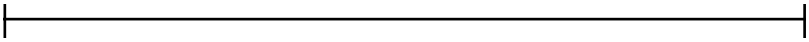
20. Ich empfinde Mundtrockenheit.



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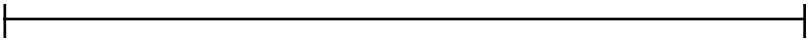
21. Ich habe Schwindelgefühle.



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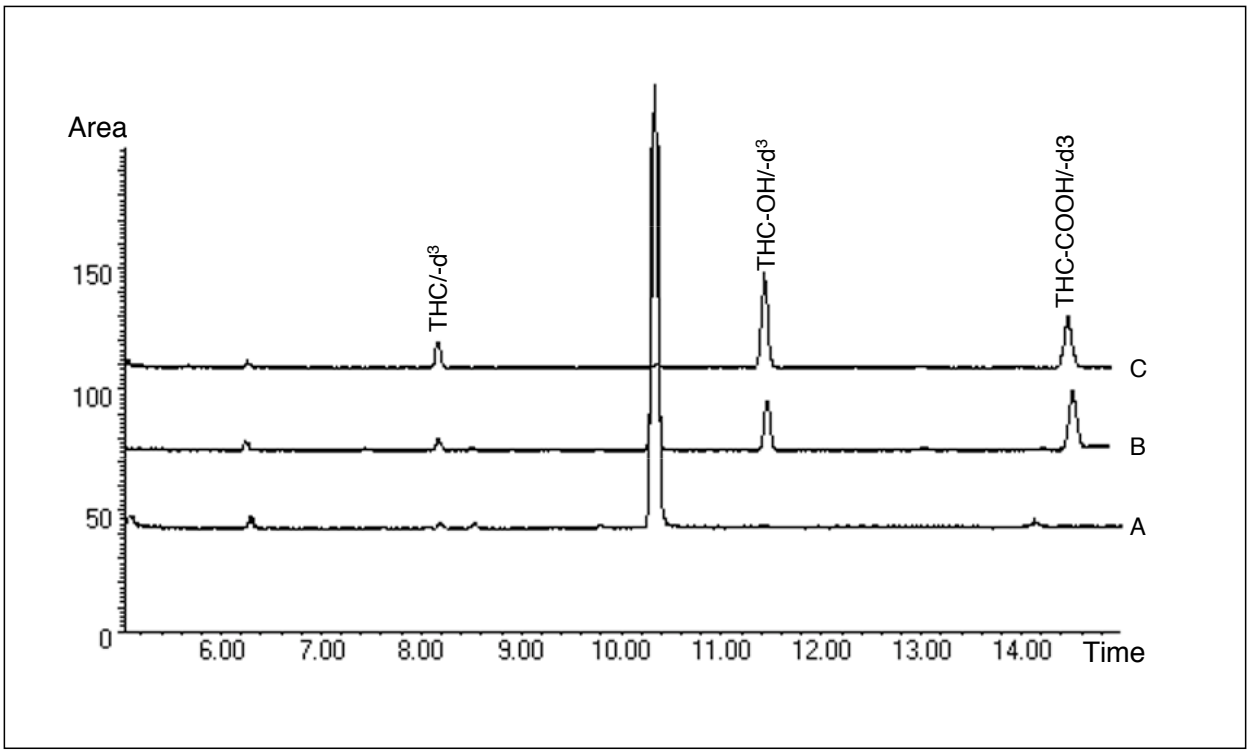
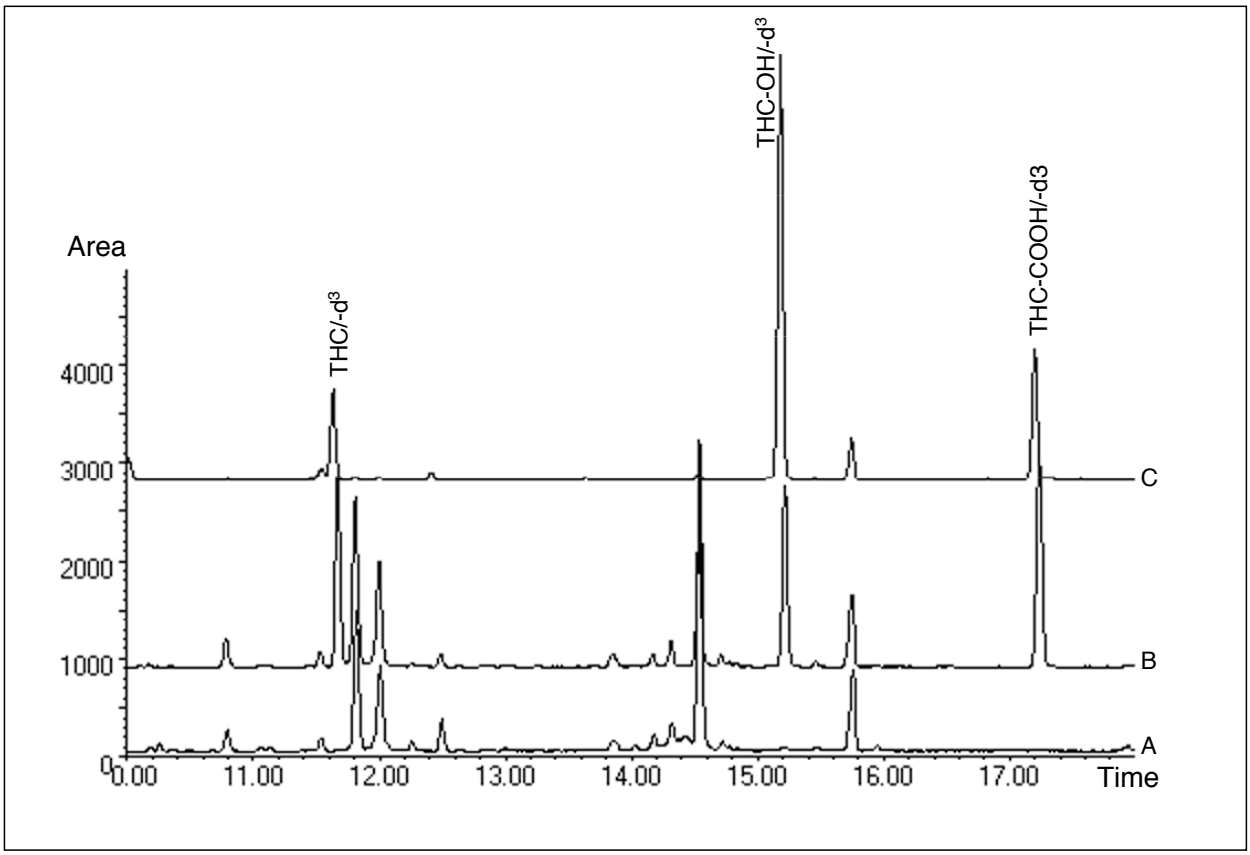
22. Ich habe andere Beschwerden, nämlich :

III. Typical chromatogram of the determination of THC and its metabolites in plasma of the pain study



Appendix III: Typical chromatogram of the determination of THC and its metabolites in plasma of the pain study. A: blank plasma, ion 371; B: real sample (oral 60 min), ion 371; C: blank plasma spiked with internal standard, ion 374.

IV. Typical chromatogram of the determination of THC and its metabolites in plasma of the pharmacokinetic study



Appendix IV: Typical chromatogram of the determination of THC and its metabolites in plasma of the kinetic study. A: blank plasma, ion 371; B: real sample (i.v. 10 min), ion 371; C: blank plasma spiked with internal standard, ion 374.

V. Paper pain study

Pain 105 (2003) 79–88

PAIN

www.elsevier.com/locate/pain

The analgesic effect of oral delta-9-tetrahydrocannabinol (THC), morphine, and a THC-morphine combination in healthy subjects under experimental pain conditions

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Abstract

From folk medicine and anecdotal reports it is known that Cannabis may reduce pain. In animal studies it has been shown that delta-9-tetrahydrocannabinol (THC) has antinociceptive effects or potentiates the antinociceptive effect of morphine. The aim of this study was to measure the analgesic effect of THC, morphine, and a THC-morphine combination (THC-morphine) in humans using experimental pain models. THC (20 mg), morphine (30 mg), THC-morphine (20 mg THC + 30 mg morphine), or placebo were given orally and as single doses. Twelve healthy volunteers were included in the randomized, placebo-controlled, double-blinded, crossover study. The experimental pain tests (order randomized) were heat, cold, pressure, single and repeated transcutaneous electrical stimulation. Additionally, reaction time, side-effects (visual analog scales), and vital functions were monitored. For the pharmacokinetic profiling, blood samples were collected. THC did not significantly reduce pain. In the cold and heat tests it even produced hyperalgesia, which was completely neutralized by THC-morphine. A slight additive analgesic effect could be observed for THC-morphine in the electrical stimulation test. No analgesic effect resulted in the pressure and heat test, neither with THC nor THC-morphine. Psychotropic and somatic side-effects (sleepiness, euphoria, anxiety, confusion, nausea, dizziness, etc.) were common, but usually mild.

Keywords: Delta-9-tetrahydrocannabinol; Morphine; Experimental pain; Antinociception; Plasma levels; Pharmacokinetics

1. Introduction

From the folk medicine and anecdotal reports it is known that Cannabis may reduce pain. It is widely used in self-medication to relieve pain of different origins such as back pain, headache, and migraine (Ogborne et al., 2000). Several animal studies have shown the analgesic effect of delta-9-tetrahydrocannabinol (THC) in different pain models (Lichtman and Martin, 1997; Smith et al., 1998a; Vivian et al., 1998). In mice, subcutanously (s.c.) and orally (p.o.) administered THC enhanced the antinociceptive effect of s.c. and p.o. morphine in the tail-flick and also in the paw-pressure test (Smith et al., 1998b). Again in mice, an inactive p.o. dose of THC (20 mg/kg) enhanced the antinociception of opioids 2.2- (for morphine) to 25.8-fold (for codeine, shift in ED_{50}) in the tail-flick test (Cichewicz et al., 1999).

Few human trials have been conducted and the results were equivocal (Campbell et al., 2001). Oral doses of 15 and 20 mg THC resulted in a significant reduction of cancer pain (Noyes et al., 1975a). In another clinical study, the analgesic potency of THC in cancer pain was compared with codeine. The analgesic effect of 20 mg p.o. THC corresponded to that of 120 mg p.o. codeine (Noyes et al., 1975b). Intravenously administered THC did not affect pain tolerance thresholds in dental surgical pain (Raft et al., 1977). In a double-blind, placebo-controlled, crossover trial on a chronic pain patient suffering from familial Mediterranean fever five doses of 10 mg p.o. THC (as standardized Cannabis preparation,

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containing 5.75% THC) per day did not reduce pain (Holdcroft et al., 1997). However, it significantly reduced the need for morphine (10 mg per dose) given as escape medication indicating an additive effect of THC on morphine.

Some patients claim that Cannabis is more effective than THC (e.g. dronabinol, Marinol®) for a variety of symptoms, including nausea and vomiting, wasting syndrome, and muscle spasticity (Grinspoon and Bakalar, 1997; Joy et al., 1999). However, most of these claims are based on patient reports and surveys, and have not been verified by controlled clinical trials (Wachtel et al., 2002). To the best of our knowledge, no comparative data exist of the analgesic effect of THC and Cannabis. A recent study compared the subjective effects of orally administered and smoked THC alone and THC within Cannabis preparations (brownies, cigarettes) (Wachtel et al., 2002). THC and Cannabis in both application forms produced similar, dose-dependent subjective effects, and there were few reliable differences between the THC-only and whole-plant conditions. In studies on volunteers and a multiple sclerosis patient it was shown that cannabidiol (CBD) reduces the psychotropic effects of THC (Zuardi et al., 1982, 1995) and a Cannabis based medicinal extract (Notcutt et al., 2001), respectively. This could explain anecdotal reports from patients who prefer the milder forms of Cannabis containing significant levels of CBD (Notcutt et al., 2001). A review of the antianxiety effects and the pharmacology of CBD is given in Partland and Russo (2001) and Mechoulam et al. (2002).

In experimental pain the results are controversial. Smoked Cannabis increased pressure pain tolerance in Cannabis-naïve and Cannabis-experienced subjects compared to placebo (THC-extracted Cannabis) (Milstein et al., 1975). In a radiant heat test experienced Cannabis users reported a mild antinociceptive effect when smoking Cannabis cigarettes (Greenwald and Stitzer, 2000). In thermal pain p.o. THC showed in healthy subjects with moderate side-effects a reduction of pain and in subjects experiencing a 'bad trip' hyperalgesia (Zeidenberg et al., 1973). In the transcutaneous electrical stimulation pain model, smoked Cannabis had no analgesic effect, it even produced a slight hyperalgesia (Hill et al., 1974).

It was the aim of the present study to test the antinociceptive effects of oral THC and THC combined with morphine (THC-morphine) versus morphine and placebo in healthy subjects and under experimental pain conditions. Plasma profiles were acquired to study the pharmacokinetics of THC and look for a possible correlation with analgesia and side-effects.

2. Materials and methods

2.1. Subjects and study design

Twelve healthy, Cannabis-naïve volunteers (six females, age 25 ± 7 years, weight 70 ± 8 kg; six males, 27 ± 11 years, 74 ± 7 kg; all right handed) participated in this randomized, placebo-controlled, double-blind, cross-over study which was carried out in the pain laboratory of the Department of Anaesthesiology at the University Hospital of Bern. All pain tests were performed by the same investigator. The subjects were informed about the risks of the study, gave their written informed consent, and were paid for participating. Exclusion criteria were past or existing drug abuse (including alcohol and drugs; Cannabis and opiate urine tests before each session), known or suspected hypersensibility to cannabinoids or opioids, pregnancy (urine test before first session). The subjects were not allowed to take analgesics, alcohol, and caffeinated beverages 48 h before and during the study and were asked to refrain from driving up to 12 h after the study. The study has been approved by the Ethics Committee of the Faculty of Medicine, University of Bern. Each subject received either 20 mg THC (dronabinol, Marinol® soft gelatine capsules; Unimed Pharmaceuticals, Inc., Deerfield, IL, USA), 30 mg morphine hydrochloride (Schweizerhall Pharma, Basel, Switzerland), a mixture of 20 mg THC and 30 mg morphine hydrochloride, or placebo as a single oral dose on empty stomach. The blinding of the test medications was performed by enclosing the 20-mg or placebo Marinol® capsules in another, dark-colored gelatine capsule and adding either 30 mg mannitol or 30 mg morphine hydrochloride. The 30-mg morphine capsules were similarly prepared. Caffeine-free beverages were allowed 1 h, and light, but not standardized meals 3 h post-dosing.The between-session washout phases were at least 7 days. To get the subjects familiar and comfortable with the testing procedures, each session began with a training phase. Then the baselines were recorded and the pain tests performed in a random order every hour up to 8 h post drug. Pressure and heat pain were not determined at timepoints 5,6 and 7h post drug to present skin damage. Side-effects were monitored before each set of pain tests. Blood (5–10 ml) was collected in all four sessions through a peripheral vein catheter at baseline, 0.5, 1, 2, 4, and 8 h post drug. The heparinized blood samples were centrifuged and the plasma instantly deep-frozen and stored at −20°C until analysis.

2.2. Pain tests

2.2.1. Pressure

Pressure pain tolerance thresholds were determined on the center of the pulp of the second and third finger of the right hand with an electronic pressure algometer (Somedic AB, Stockholm, Sweden) (Brennum et al., 1989, 1992; Petersen-Felix et al., 1994). A probe with a surface area of 0.28 cm^2 was used, and the pressure increase was set to 30 kPa s^{-1}. Pain tolerance was defined as the point when the subject felt the pain as intolerable. For determination of the tolerance thresholds, the mean of two consecutive measurements was used.

2.2.2. Heat

The computer-driven Thermotest (Somedic AB, Stockholm, Sweden) was used (Fruhstorfer et al., 1976; Arendt-Nielsen et al., 1996). A thermode with a surface of 25 × 50 mm was applied to the volar surface of the forearm, in the middle of a line joining the elbow to the wrist fold. The temperature of the thermode was continuously increased from 30 to a maximum of 52°C at a rate of 2.0°C s^{-1}. The subject was asked to press a button when perceiving the heat as painful (pain detection) and when feeling the heat as intolerable and not wanting the heat to be further increased (pain tolerance). At that point the temperature was recorded and the thermode cooled to 30°C. The thermode was also cooled to 30°C in the case when the tolerance threshold was not reached at 52°C. 52°C was then considered as pain tolerance threshold. Three consecutive measurements were performed for both pain detection and tolerance thresholds. The average of the last two values were computed and evaluated for data analysis.

2.2.3. Cold

A 2-min ice cold immersion test was used (Jones et al., 1988; Sindrup et al., 1993; Petersen-Felix et al., 1994). Before immersion, the skin temperature on the thenar of the left hand was measured. The left hand was then immersed in ice-saturated water (0.6 ± 0.2°C). If pain was felt as intolerable (pain tolerance) before 2 min had elapsed, the subject could withdraw the hand. Perceived pain intensity was rated continuously with an electronic visual analogue scale (VAS) and recorded on a personal computer. Peak pain, area under the pain intensity-time curve, and mean pain were determined. If the hand was withdrawn before the end of 2 min, pain intensity was considered to be maximal until the end of the 2-min period (for calculation of area under the curve).

2.2.4. Transcutaneous electrical stimulation (single, repeated)

Two bipolar surface Ag/AgCl-electrodes (Dantec, Skovlunde, Denmark) were placed on the shaved skin of the shin, 14 cm distal to the patella. The electrode surface was 7 × 4 mm, and the distance between the two electrodes was 1.5 cm. A train of five square-wave impulses was delivered from a computer-controlled constant current stimulator (University of Aalborg, Denmark). Each of these impulses lasted 1 ms. The whole duration of the train of five impulses was 25 ms, so they were perceived as a single stimulus. For the single electrical stimulation this train was given once, and for the repeated electrical stimulation this train was repeated five times, at the same intensity and a frequency of 2 Hz (i.e. every 0.5 s) (Arendt-Nielsen et al., 1994; Curatolo et al., 2000). The current intensity was increased stepwise 1 mA until the stimulus was perceived as painful. For the single electrical stimulation the pain detection threshold was defined as the minimum stimulus intensity eliciting a subjective pain. For the repeated electrical stimulation the pain detection threshold was defined as the minimum stimulus intensity eliciting a subjective increase in perception during the five stimulations, so that the last one to two impulses were perceived as painful.

2.3. Monitoring of side-effects and vital functions

A 10-cm VAS was used to assess psychological (euphoria, hallucinations, disorientation, altered perception, etc.) and somatic side effects (heart and digestive problems, etc.). The episodes of vomiting were noted. Hemoglobin oxygen saturation (pulse oximetry), blood pressure, and heart rate were recorded. To determine the reaction time, a 1000 Hz tone was delivered from a computer with random intervals of 3–8 s, and simultaneously a timer was started. The volunteer was told to press a button as fast as possible after the tone. The reaction time was defined as the time from the tone until the subject pressed the button. The mean value of five consecutive measurements was calculated.

2.4. Bioanalytics

Plasma concentrations of THC and its metabolites 11-nor-9-carboxy-THC (11-COOH-THC) and 11-hydroxy-THC (11-OH-THC) were determined by gas-chromatography/mass spectrometry (GC/MS) according to the method of Feng et al. (2000). Plasma concentrations of morphine and its metabolites morphine-6-glucuronide (M-6-G) and morphine-3-glucuronide (M-3-G) were determined by high performance liquid chromatography (HPLC) using a modified method described previously (Bourquin et al., 1999). Major modifications involved: (i) the washing (20 ml of 0.005 M carbonate buffer pH 9.3, 0.4 ml of bidistilled water and 0.25 ml of acetonitrile-0.01 M phosphate buffer pH 2.1 40:60 v/v) and elution steps (1.0 ml of acetonitrile-0.01 M phosphate buffer pH 2.1 10:90, followed by 1.2 ml of acetonitrile-0.01 M phosphate buffer pH 2.1 70:30) in the solid-phase sample extraction procedure; (ii) the HPLC column (125 × 2 mm i.d. column and a 8 × 3 mm i.d. precolumn, both packed with Spherisorb-80 ODS-1 3 μm (Macherey-Nagel, Oensingen, Switzerland); (iii) the multi-step gradient (0–5.5 min, 4% B, isocratic; 5.5–9 min, 4–15% B, linear; 9–9.2 min, 15–25% B linear; 9.2–18 min, 25% B, isocratic; 18–19 min, 25–100% B, linear; 19–21 min, 100% B, isocratic; 21–22 min, 100–4% B, linear. (A) Bidistilled water, containing 0.05% (v/v) trifluoro acetic acid (TFA), (B) acetonitrile, containing 0.05% (v/v) TFA; reconditioning time 15 min, flow rate 300 μl/min); and (iv) detection and quantitation with a fluorescence detector at 343 nm (excitation wavelength of 227 nm; 220 Hz; response time 0.5 s). The limit of quantification (LOQ) for THC and its metabolites was 2 ng/ml for THC and 11-OH-THC and 10 ng/ml for 11-COOH-THC, respectively. The LOQ for morphine and its metabolites was 10 ng/ml.

2.5. Statistics

Statistical analysis was performed independently for each pain test. Differences of the baseline values in the four different sessions of each volunteer were excluded with the Friedman's test. Then we calculated for each session the mean results of all subjects and time-points. The three verum sessions were then compared to the placebo session using the Wilcoxon signed ranks test. $P < 0.05$ was considered as significant.

3. Results

3.1. Pain tests

3.1.1. Pressure test

In the pressure test, where pain tolerance thresholds were measured, no significant analgesic effect of THC and THC-morphine was observed compared to placebo (Fig. 1). On the other hand, morphine alone increased the pain tolerance significantly compared to placebo ($P = 0.01$).

3.1.2. Heat test

THC did not produce any analgesic effect in the heat test, neither alone nor in combination with morphine (data not shown). Morphine alone had no effect.

3.1.3. Cold test

Fig. 2 represents the area under the pain-intensity time curve (total pain) in the cold test (ice cold immersion test). The area under the pain-intensity time curves of THC and THC-morphine showed no significant difference compared to placebo. Morphine alone significantly ($P = 0.014$) reduced the cold pain (AUC). THC alone showed an increase of the pain (AUC), which was completely neutralized when combining with morphine. This hyperalgesia was not significant. The same effect was observed for the mean pain value. The peak pain value was significantly reduced by morphine ($P = 0.017$) and THC-morphine ($P = 0.046$) but not with THC alone.

3.1.4. Transcutaneous electrical stimulation

In the single mode of the transcutaneous electrical stimulation (Fig. 3A), no significant analgesic effect of THC and THC-morphine was observed. However, a slight additive effect of THC-morphine compared to morphine alone at most of the measuring time points could be seen. Morphine significantly increased the pain detection threshold ($P = 0.008$). In the repeated mode (Fig. 3B) THC-morphine was again additively effective in the pain detection compared to morphine alone and even produced a statistically significant analgesic effect compared to placebo ($P = 0.042$). THC alone did not significantly reduce pain. It again caused a slight, not significant hyperalgesia in the second part of the session (timepoint 4 h up to 8 h post drug) compared to placebo.

3.2. Side-effects and vital functions

The side-effects, summarized in Table 1, were usually mild. Most of the subjects felt sleepy (Fig. 4) and confused after the administration of THC and THC-morphine. They also reported altered inner (Fig. 5) and outer perception, feelings of anxiety and aggression. Interestingly, the euphorigenic and hallucinogenic effects of THC were reduced when combining with morphine. Other side-effects were nausea and vomiting, dizziness, headache, reduced rate of breathing, tachycardia, and dry mouth. The reaction time was not significantly impaired with any of the test substances.

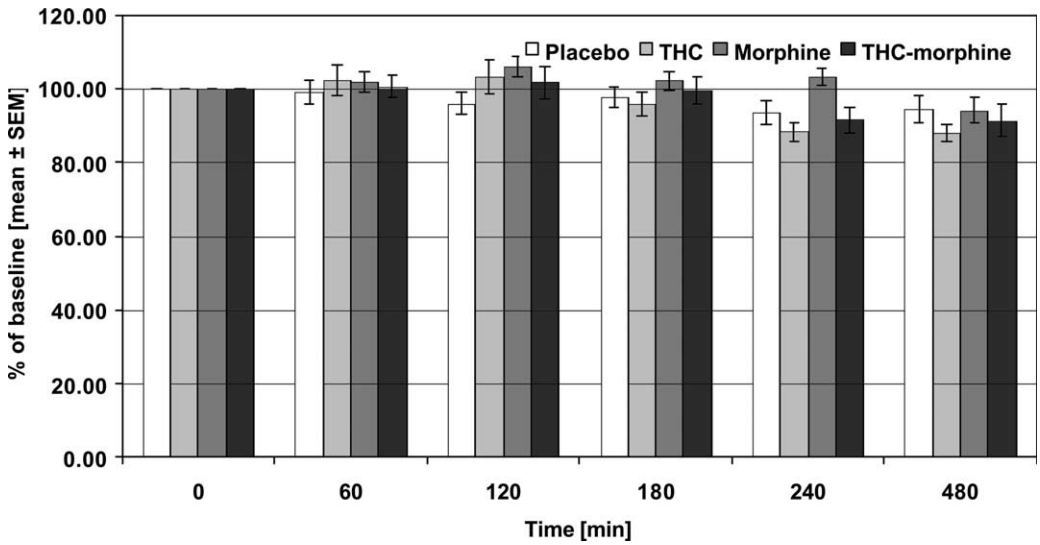

Fig. 1. Pain tolerance of THC, morphine, THC-morphine, and placebo in the pressure test; values > 100% of baseline (mean ± SEM) indicate analgesia, values < 100% hyperalgesia. The effect of morphine was statistically significant versus placebo.

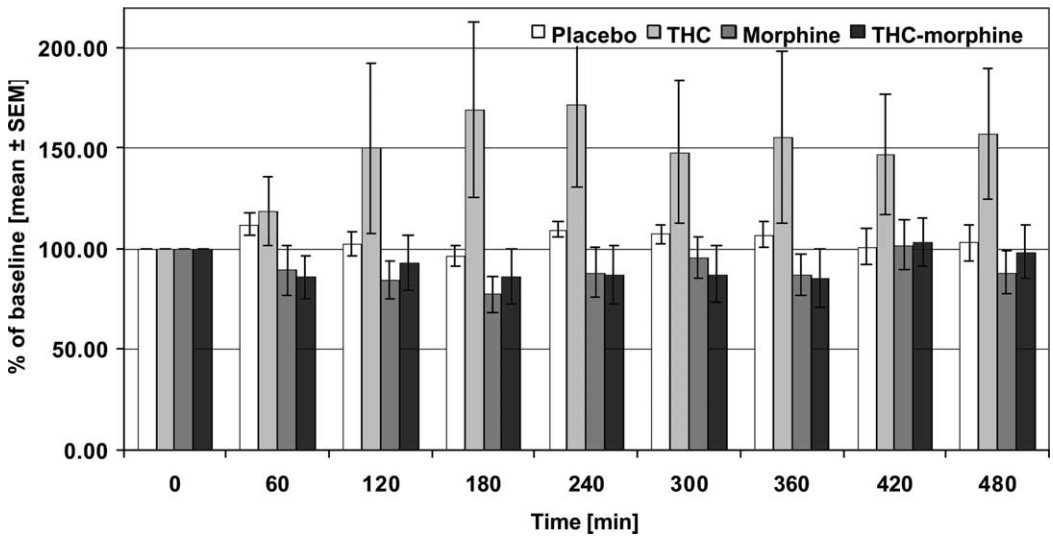

Fig. 2. Pain tolerance of THC, morphine, THC-morphine, and placebo in the cold test (ice cold immersion test); values >100% of baseline (mean ± SEM) indicate hyperalgesia, values <100% analgesia. The effect of morphine was statistically significant versus placebo.

Systolic (116 ± 4–101 ± 3 mmHg) as well as diastolic blood pressure (65 ± 3–54 ± 2 mmHg) decreased significantly only after THC-morphine compared to placebo. With 63 ± 3–87 ± 4 THC alone increased significantly the heart rate, whereas the hemoglobin oxygen saturation was only significantly reduced after THC-morphine.

3.3. Plasma levels

Fig. 6 shows the plasma profiles of THC and its main metabolites after 20 mg oral THC. The THC plasma levels ranged from 1.1 ± 0.8 to 7.2 ± 2.0 ng/ml (mean ± SEM), with the maximum concentrations at 60 or 120 min. The 11-OH-THC plasma levels were 0.3 ± 0.3–19.7 ± 2.0 ng/ml, mostly peaking at 120 min, and the 11-COOH-THC levels 1.7 ± 1.8–241.4 ± 21.1 ng/ml, peaking at 120 or 240 min. After administration of THC-morphine the THC, 11-OH-THC and 11-COOH-THC levels were 4.0 ± 1.4–6.7 ± 2.1, 0.2 ± 0.2–7.9 ± 2.4 and 0–134.7 ± 18.8 ng/ml, respectively. THC and metabolites were not detectable in any of the baseline samples.

Table 1
Psychological and somatic side-effects (peak VAS %, mean ± SEM of all subjects) after THC, morphine, THC-morphine, and placebo

Side effects	Placebo		THC		Morphine		THC-morphine	
	n	VAS (%)	*n*	VAS (%)	*n*	VAS (%)	*n*	VAS (%)
Sleepiness	12	70 ± 31	12	82 ± 20	12	64 ± 33	12	85 ± 20
Euphoria	1	66 ± 0	9	54 ± 34	3	16 ± 11	5	21 ± 29
Irritation	1	12 ± 0	5	44 ± 34	1	6 ± 0	5	28 ± 31
Anxiety	0		4	54 ± 43	0	–	3	22 ± 32
Tenseness and aggressiveness	1	10 ± 0	4	57 ± 39	2	14 ± 5	2	49 ± 66
Confusion and disorientation	1	2 ± 0	7	58 ± 31	0	–	8	13 ± 9
Change of inner perception	2	16 ± 4	10	66 ± 30	5	19 ± 9	9	61 ± 37
Change of outer perception	0		8	53 ± 28	0	–	4	41 ± 36
Hallucinations	0		6	64 ± 29	0	–	5	39 ± 37
Strange thoughts, ideas, moods	0		7	51 ± 40	1	13 ± 0	3	43 ± 50
Nausea	0		5	25 ± 17	3	27 ± 7	6	11 ± 8
Headache	2	23 ± 8	6	63 ± 28	5	33 ± 33	5	36 ± 29
Difficulties in breathing	0		6	30 ± 33	2	18 ± 17	4	22 ± 23
Heart problems (tachycardia)	0		6	48 ± 37	0	–	1	97 ± 0
Digestive problems	0		5	25 ± 19	3	13 ± 12	4	8 ± 6
Dry mouth	5	21 ± 20	12	76 ± 28	8	31 ± 22	10	51 ± 37
Vertigo	3	6 ± 2	11	51 ± 35	5	12 ± 10	9	34 ± 33
Vomiting	0		0		4	–	2	–
Orthostatic disorder	0		0		1	–	2	–

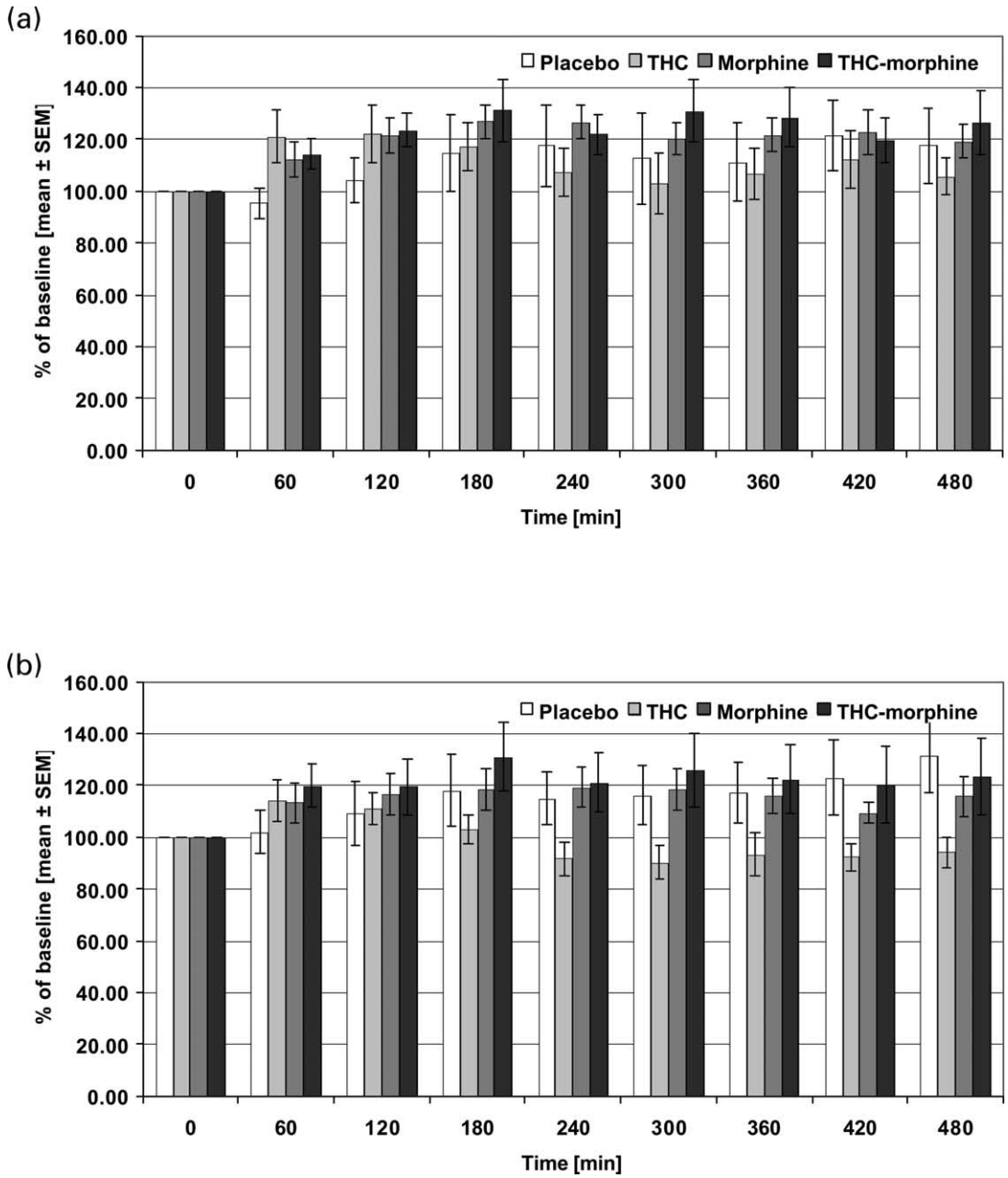

Fig. 3. Pain detection of THC, morphine, THC-morphine, and placebo after (a) single and (b) repeated transcutaneous electrical stimulation; values $>100\%$ of baseline (mean ± SEM) indicate analgesia, values $<100\%$ hyperalgesia. The effect of morphine was statistically significant versus placebo.

As can be seen in Fig. 7 morphine undergoes like THC an extensive metabolism resulting in the formation of the two glucuronides M-6-G and M-3-G. Morphine plasma levels of 0–11.2 ± 1.8 ng/ml were measured after 30 mg of oral morphine, with a peak at 60 min. M-6-G plasma concentrations were 10.5 ± 5.2–97.5 ± 15.1 ng/ml, mostly peaking at 120 min. M-3-G plasma levels were 98.7 ± 25.5–707.9 ± 67.3 ng/ml, with peaks from 60 to 120 min. After administration of THC-morphine the morphine, M-6-G, and M-3-G levels were 0–36.3 ± 24.4, 13.2 ± 5.7–143.2 ± 12.3 and 129.5 ± 16.3–561.8 ± 46.5 ng/ml, respectively. Morphine and metabolites were not detectable in any of the baseline samples.

4. Discussion

The multimodel, well established experimental pain test battery (Petersen-Felix et al., 1998; Enggaard et al., 2001; Luginbuhl et al., 2001) was used to cover different types of pain. On one hand the more superficial pain in the electrical stimulation and in the heat test, and on the other hand

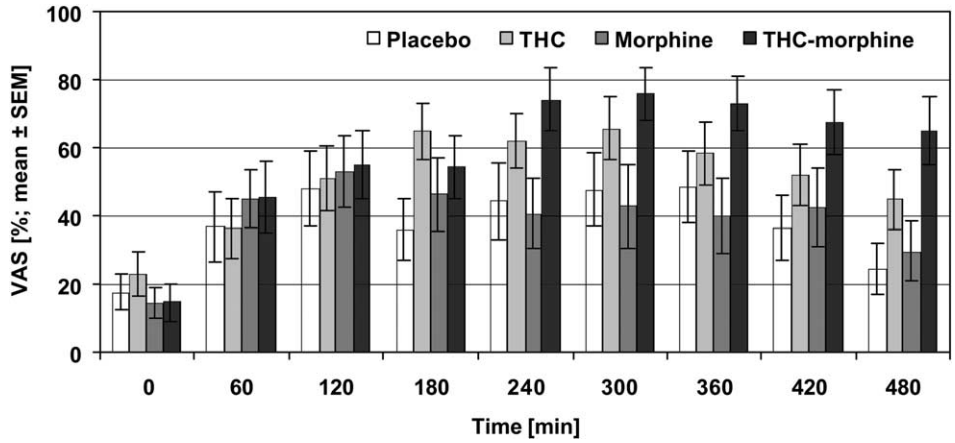

Fig. 4. Side effect 'sleepiness' measured by visual analog scales (VAS %, mean ± SEM) after THC, morphine, THC-morphine, and placebo.

the more deep pain in the pressure and cold test. Although from a pharmacokinetic point of view not ideal, THC encapsulated in sesame oil was used, as this oral formulation is registered in some countries. The 8-h study period enabled to register also effects at timepoints where mainly the metabolites were present in the plasma.

THC did not produce analgesia to pressure stimulation. Oddly, it seems to antagonize morphine analgesia. This could be the result of a hyperalgesic effect of THC that has not been detected with THC alone, but appeared when THC was combined with morphine.

In the heat test (pain tolerance threshold) THC produced a significant hyperalgesia. One problem in this test was the temperature limit of 52°C. There were some volunteers who had already a baseline of 52°C for the pain tolerance threshold. A similar hyperalgesic effect of THC on thermal pain was also reported in habitual marihuana users participating in a pain study carried out with Cannabis cigarettes (Clark et al., 1981). We recently found heat pain to be inadequate for detecting opioid induced analgesia (Luginbuhl et al., 2001). Hyperalgesia after THC was also observed with the cold test. Interestingly, the combination with morphine totally reversed this effect to an analgesic effect, which was comparable to that of morphine. The cold test is well-established to measure opioid-induced analgesia, but it is unclear whether it is also suited for non-opioid analgesics. Jones et al. showed the opiate sensitivity of the cold test but also the apparent insensitivity of the model for non-steroidal anti-inflammatory drugs (Jones et al., 1988). We assume that hyperalgesia measured in the cold test was due to an outlier which also caused the wide variability.

Our findings indicate that the analgesic effect of THC and morphine, which was in all our experiments gender-independent, is much depending on the pain model used. Luginbuhl et al. (2001) also reported that the experimental pain profile differed in substances like alfentanil, xenon, and nitrous oxide. This illustrates the benefit of a multimodel stimulation in the investigation of the analgesic properties of new drugs. None of the experimental pain tests used in this study produces inflammation or tissue damage. Different animal studies have shown an increased analgesic effect of THC in models of inflammatory pain (Pertwee, 2001). It has previously been reported that in rats

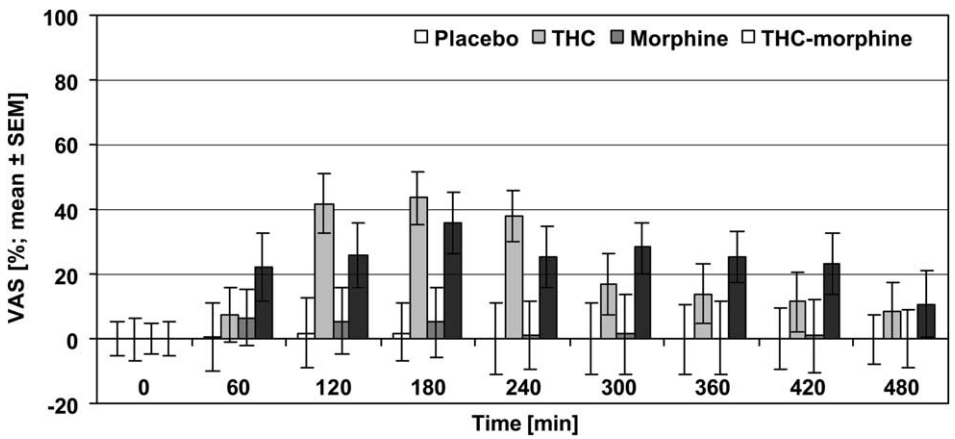

Fig. 5. Side effect 'change of inner perception' (VAS %, mean ± SEM) after THC, morphine, THC-morphine, and placebo.

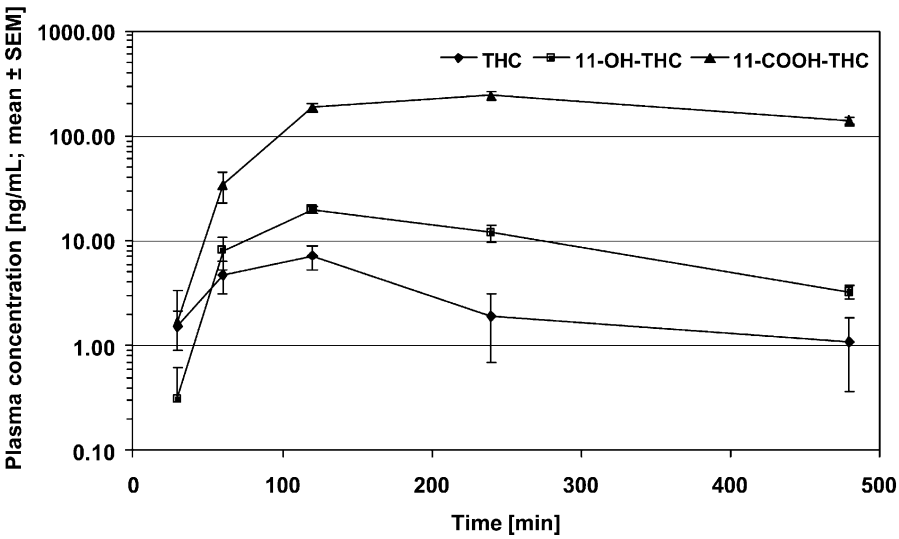

Fig. 6. Plasma profiles (ng/ml, mean ± SEM) of THC, 11-OH-THC and 11-COOH-THC after 20 mg oral THC.

cannabinoid CB_1 receptors are upregulated in chronic neuropathic pain and therefore could lead to an increased analgesic effect of THC in chronic pain (Siegling et al., 2001). In a retrospective study with patients suffering from chronic pain of different origins, three out of six patients could reduce their pain to a satisfactory level with a dose of THC of 5–20 mg/day, whereas the other three patients had to stop the medication due to lack of analgesia or intolerable side-effects (Elsner et al., 2001). Therefore, we cannot rule out that THC would have an analgesic effect after induction of inflammation, tissue or nerve damage.

The side-effects of THC were common and normally not severe. There was one volunteer out of 12 who reported disliking very much the psychotropic effects of THC. We assume that a strong aversion to the psychotropic effect leads to a discomfort and therefore possibly also to increased sensitivity to pain. This phenomenon could be observed in the ice cold immersion test where one subject showed a nice correlation between hyperalgesia and anxiety. Von Graffenried et al. (1978) found anxiety (and also other psychological factors, like mood) to be a factor that might be responsible for the unreliable results obtained in experimentally induced pain in man especially for mild analgesics. If anxiety towards pain tests played a role even when not using psychotropic drugs, anxiety feelings produced by the psychotropic THC could have an additional influence on the outcomes of pain tests. Many of the psychotropic side-effects of THC (e.g. euphoria, hallucinations, confusion) were lowered by combining with morphine. On the other hand THC was also influencing the side-effects of morphine. Nausea and vomiting was decreased in the combination session compared to the morphine session. This could be due to the antiemetic effect of THC (Gralla, 1999; Soderpalm et al., 2001; Tramèr et al.,

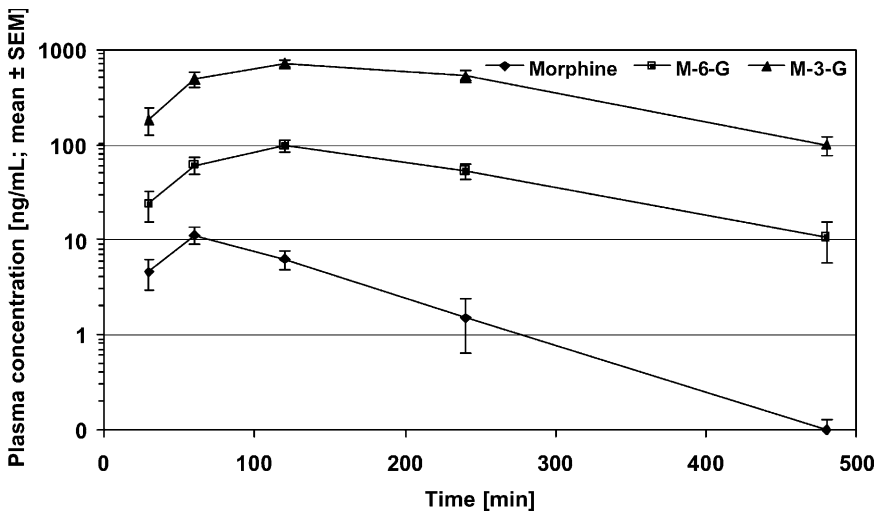

Fig. 7. Plasma profiles (ng/ml, mean ± SEM) of morphine, M-6-G and M-3-G after 30 mg oral morphine.

2001). The reduction of this common side-effect of opioids would be a great benefit in the therapy of chronic pain. The typical psychotropic side-effects of THC made the true blinding of the study impossible.

Although THC is almost completely absorbed (90–95%) after oral administration (Wall et al., 1983; Unimed Pharmaceuticals, 2001) the plasma profiles after 20 mg THC are characterized by very low levels of THC and high concentrations of the two main metabolites 11-OH-THC and 11-COOH-THC. The levels were similar to those measured in an earlier study after administration of oral THC (Brenneisen et al., 1996). Due to the combined effects of extensive first pass hepatic metabolism, pre-systemic elimination in the gut, and high lipid solubility (volume of distribution, $V = 10$ l/kg), only 10–20% of a dose is reaching the systemic circulation (Wall et al., 1983; Unimed Pharmaceuticals, 2001; Brenneisen, 2002). The metabolites are detectable already 30 min post drug indicating the rapid liver first pass metabolism. The microsomal hydroxylation is catalyzed by cytochrome P450 isoenzymes (Harvey, 1999). 11-OH-THC is psychoactive whereas the dominating metabolite 11-COOH-THC is inactive. In glucuronidated form the latter is the main urinary excretion product of THC (Harvey 1999). A correlation between the THC plasma levels and the pharmacological profiles could only be observed related to the side-effects. It is not known whether 11-OH-THC has analgesic properties, too. It should be tested if another application form of THC with a better bioavailability, for example a pulmonally administered aerosol, could increase the analgesic effect of THC. By avoiding or at least reducing the first pass effect and thus the formation of the psychotropic 11-OH-THC the unpleasant side-effects of THC could be decreased. The plasma profiles after the administration of 30 mg morphine showed only low levels of morphine, but high (M-6-G) to very high (M-3-G) concentrations of its metabolites. Like THC, biotransformation by first pass metabolism starts within min after administration. M-3-G is the dominating urinary excretion product of morphine (Gyr et al., 2000), but only M-6-G exhibits analgesic effects (Buetler et al., 2000).

In conclusion, in this study oral THC did not significantly reduce experimentally-induced pain in healthy subjects. Some analgesic effects were only observed when combining THC with morphine. Psychotropic and somatic side-effects were common, but not severe. The hypothesis that the analgesic effectiveness of THC is increased by using alternative application forms resulting in a better bioavailability deserves further investigation.

Acknowledgements

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VI. Paper pharmacokinetic study

Development and Pharmacokinetic Characterization of Pulmonal and Intravenous Delta-9-Tetrahydrocannabinol (THC) in Humans

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ABSTRACT: The aim of the present study was to develop a physiologically compatible inhalation solution of delta-9-tetrahydrocannabinol (THC), and to compare the pharmacokinetic and analgesic properties of pulmonal THC versus pulmonal placebo and intravenous (iv) THC, respectively. Eight healthy volunteers were included in this randomized, double-blind, crossover study. The aqueous THC formulations were prepared by using a solubilization technique. iv THC (0.053 mg/kg body weight), pulmonal THC (0.053 mg/kg), or a placebo inhalation solution was administered as single dose. At defined time points, blood samples were collected, and somatic and psychotropic side effects as well as vital functions monitored. An ice water immersion test was performed to measure analgesia. Using a pressure-driven nebulizer, the pulmonal administration of the THC liquid aerosol resulted in high THC peak plasma levels within minutes. The bioavailability of the pulmonal THC was $28.7 \pm 8.2\%$ (mean $\pm$ SEM). The side effects observed after pulmonal THC were coughing and slight irritation of the upper respiratory tract, very mild psychotropic symptoms, and headache. The side effects after iv THC were much more prominent. Neither pulmonal nor iv THC significantly reduced experimentally induced pain. J Pharm Sci 93:1176–1184, 2004

Keywords: delta-9-tetrahydrocannabinol; pulmonary aerosol; injection; pharmacokinetics; analgesia

INTRODUCTION

Numerous indications for cannabis preparations and delta-9-tetrahydrocannabinol (THC) have been postulated, with marked differences in the available supporting data. For applications such as nausea and vomiting associated with cancer chemotherapy, anorexia, and cachexia in HIV/AIDS, and spasticity in multiple sclerosis and spinal cord injury, there is strong evidence for medical benefits.[1–4] Relatively well-confirmed effects were described related to painful conditions, especially neurogenic pain, movement disorders, asthma, and glaucoma.[1] In folk medicine, cannabis is widely used to relieve pain of different origins, such as back pain, headache, and migraine.[5] Few human trials have been conducted so far and the outcomes were equivocal.[6] Fifteen to twenty milligrams of oral THC reduced cancer pain significantly, with 20 mg of THC corresponding to 120 mg of oral codeine.[7,8] Intravenous (iv) THC did not affect pain tolerance in dental surgical pain.[9] Analgesia could not be

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confirmed in a previous pain study with healthy subjects using oral THC (dronabinol, Marinol®) and experimental pain models.[10] Extensive first-pass metabolism by the liver was observed leading to early and high THC metabolite plasma levels. Additionally, the THC plasma peak concentrations showed a high interindividual variability between 30 and 120 min.[10] The bioavailability of orally administered THC is known to be low (6–20%) and to depend on the vehicle and co-ingested food.[11] The peak plasma levels, occurring at 1–5 h after administration, show a strong, also vehicle- and food-dependent variability.[11] After eating cannabis cookies, the bioavailability of THC was 6%,[12] whereas when using THC dissolved in sesame oil in soft gelatin capsules, it was 11 (women) to 19% (men).[13]

These factors make it very difficult to dose oral THC. There is a need for alternative application forms with better pharmacokinetic properties. Ohlsson et al.[12] studied the pharmacokinetic behavior of THC and its clinical effects after iv administration, oral ingestion of cannabis cookies, and smoking cannabis cigarettes. Plasma levels after smoking and iv injection were similar, but low and irregular after ingestion. Peak plasma levels after smoking occurred rapidly and the bioavailability was found to be much higher (18–50%) than after oral (6–20%) administration.[11,12] For a rapid onset of action, the United States Institute of Medicine recommended the development of reliable, and safe THC delivery systems for clinical trials with cannabinoid drugs for symptom management.[14] To the best of our knowledge, there are neither pharmacokinetic data of pulmonally administered THC in humans, except for smoked cannabis, nor data from cannabis-naïve subjects. Therefore, the aim of the present study was to develop and validate *in vitro* and *in vivo* a physiologically tolerable inhalation solution that could be administered with a commercially available nebulizer. In addition, this new application form should be easy to handle, lead to a higher bioavailability as well as early peak plasma levels of THC, and consequently show a rapid onset of action.

EXPERIMENTAL

Materials

The clinical test compound THC (dronabinol) was supplied by THC Pharm GmbH (Frankfurt am Main, Germany). Cremophor® RH 40 was provided from BASF AG (Ludwigshafen, Germany); all other chemicals were of pharmaceutical quality obtained by the pharmacy of the University Hospital of Bern. THC and THC-d_3 used for plasma analysis were obtained from Lipomed (Arlesheim, Switzerland), and (±)-11-hydroxy-Δ^9-THC (11-OH-THC), (±)-11-hydroxy-Δ^9-THC-d_3 (11-OH-THC-d_3), (±)-11-nor-9-carboxy-Δ^9-THC (11-COOH-THC), and (±)-11-nor-9-carboxy-Δ^9-THC-d_3 (11-COOH-THC-d_3) were from Radian (Austin, TX). All solvents were of high-performance liquid chromatography (HPLC) grade and purchased either from Merck (Basel, Switzerland) or Fluka Chemie (Buchs, Switzerland). Bacterial β-glucuronidase (*Escherichia coli*, type IX-A) and *N*,*O*-bis(trimethylsilyl) trifluoracetamide (BSTFA) containing 1% trimethylsilyl chloride (TMCS) were obtained from Sigma-Aldrich (Buchs, Switzerland) and Fluka Chemie, respectively. The solid phase extraction columns (Bakerbond SPE octadecyl cartridges) were purchased from Stehelin (Basel, Switzerland). Roche OnTrak TesTstiks (Roche Diagnostics, Rotkreuz, Switzerland) with a cut-off of 50 ng/mL were used for urine cannabis testing.

Subjects and Study Design

Eight healthy, cannabis-naïve, nonsmoking volunteers (four women, aged 26–35 years, body weight 60 ± 8 kg; four men, 27–50 years, 80 ± 5 kg) were accepted for this randomized, placebo-controlled, double-blind, crossover study which was performed at the Clinical Investigation Unit of the University Hospital of Bern. The subjects were informed about the risks of the study, gave their written informed consent, and were paid for participating. Exclusion criteria were past or existing drug abuse (including alcohol and prescription drugs; cannabis urine test before each session), pregnancy (urine test before first session), positive past history of any psychiatric disorders, and lung diseases. Each subject had to pass lung function tests including vital capacity and forced expiratory volume in 1 s. The subjects were not allowed to take analgesics, alcohol, and caffeinated beverages 48 h before and during the study and were asked to refrain from driving up to 24 h after the end of the study. The study was approved by the Regional Ethics Committee, the Swiss Agency for Therapeutic Products (Swissmedic), and the Swiss Federal Office for Public Health. In the first and second session, each

subject received randomly and double-blinded either the THC (0.053 mg/kg body weight) or the placebo inhalation aerosol. In the third session, THC was administered iv (0.053 mg/kg body weight) over a time period of 2 min. The between-session washout phases were at least 7 days. To familiarize the subjects with the pain test and visual analog scales (VAS), each session began with a training phase, during which the subjects performed a pain test and a 5-min inhalation training with the placebo aerosol. This was followed by recording the baseline of vital functions, side effect scores (VAS), and pain test. After administration of the THC and placebo preparations, vital functions and side effects were recorded and ice water pain determined at 20, 40, 60, 120, 240, and 480 min. Blood (5 mL per time point, corresponding to 45 mL per session, and 135 mL per study) was collected in all three sessions through a peripheral venous catheter from a forearm vein at baseline, 5, 10, 20, 40, 60, 120, 240, and 480 min after administration of the test medications. The heparinized blood samples were centrifuged and the plasma instantly deep-frozen and stored at −20°C until analysis.

Preparation and Validation of the Test Medications

The THC inhalation solution consisted of 0.3% (w/v) of THC, 5.0% (w/v) Cremophor® RH 40, 1.0% (v/v) benzyl alcohol, 0.05% (w/v) sodium ascorbate, and 84.2 mM sodium phosphate buffer (pH 7.4). THC and Cremophor® RH 40 were heated in a water bath at 63°C for 10 min. Two-third of the phosphate buffer, also heated at 63°C, was then incorporated in the mixture by shaking. After cooling to room temperature, benzyl alcohol, sodium ascorbate, and the remaining phosphate buffer were added to the mixture. The clear, yellowish solution was then sonicated for 30 s and finally filtrated through a 0.22-μm filter under aseptic conditions. The placebo inhalation solution was prepared like the THC solution. The THC content and the stability of THC in the inhalation solution, stored at 4°C and protected from light, was controlled by HPLC with diode array detection (HPLC-DAD). The THC content had to be within a range of ±5% of the initial value. Osmolality, viscosity, pH, and sterility were measured according to the standards of the European Pharmacopeia.[15] For the *in vitro* validation of the nebulizer system and the liquid aerosol, the pressure-driven PARI® Master apparatus (Labhardt, Basel, Switzerland) was connected to the tubing followed by the interrupter and the PARI® LC-Plus nebulizer equipped with an inspiratory valve. The nebulizer itself was connected to a PARI® filter set containing a filter pad collecting the aerosol. The filter set was then connected to a 3-L calibration hand pump (3-L Calibrated Syringe; Sensor Medics Corporation, Yorba Linda, CA). Samples of 2 and 3 mL ($n = 3$ each) were nebulized. The inhalation was simulated manually with the hand pump (velocity 1 pull/10 s). The aerosol absorbed on the filter pad was then extracted with ethanol, lyophilized, redissolved in ethanol, and analyzed by HPLC-DAD. The particle size distribution was determined by using a Malvern Mastersizer X equipped with a 100-mm lens and Malvern Software, Malvern, UK (using the algorithm for volume distribution, polydisperse aerosol, and the 2QAA-model representing water in air). To minimize light scattering, the room was darkened during the measurements. Temperature and humidity were kept constantly at 23°C and 40%, respectively. For the sample analysis, the inhalation solution was nebulized continuously into the laser beam and continuously removed by a vacuum cleaner. The obscuration was held on a value of approximately 10–30%. The particle size distribution was measured in the vehicle ($n = 10$) and in the THC liquid aerosol ($n = 5$). The injection solution consisted of 0.1% (w/v) of THC, 1.5% (w/v) Tween® 80, 5.0% (v/v) ethanol absolute, 0.1% (w/v) sodium ascorbate, and sodium chloride solution (0.9%).[16] Sodium ascorbate was added to prevent the oxidation of THC to cannabinol. THC was dissolved in ethanol and Tween® 80, then added to the sodium ascorbate dissolved in 1 mL of the sodium chloride solution. The remaining sodium chloride solution was finally added to the mixture. The clear, yellowish solution was then sonicated for 30 s and filtrated through a 0.22-μm filter under aseptic conditions.

Inhalation Procedure

The pressure-driven inhalation device PARI® Master and the PARI® LC-plus nebulizer with interrupter were used. The subjects were instructed to inhale deeply with a breath frequency of 1 breath per 10 s waiting for 3–5 s before expiration. The subjects were instructed to continue until all the inhalation solution had been inhaled. Inhalation time and any residue left in the nebulizer compartment were measured.

Pain Test

A standardized 2-min ice water test (ice cold immersion test) was used as model for acute pain.[17–19] The right hand was immersed in ice-saturated water ($1.6 \pm 0.04^{\circ}C$) and if pain was considered as intolerable before 2 min had elapsed, the subject could withdraw the hand. Perceived pain intensity was rated continuously with an electronically controlled VAS system and recorded on a computer. Peak pain, area under the pain intensity-time curve, and mean pain were determined. If the hand was withdrawn before the end of 2 min, pain intensity was considered to be maximal until the end of the 2-min period (for calculation of the area under the curve).

Monitoring of Side Effects

A VAS was used to asses psychological and somatic side effects, such as sedation, euphoria, anxiety, nausea, vertigo, headache, irritation of airways, etc. The volunteers were instructed to report how they felt at the moment of answering the VAS questionnaire. On the 10-cm VAS scale, 0 cm (0%) represented "not at all," 10 cm (100%) represented "very strong." Hemoglobin oxygen saturation (pulse oximetry), blood pressure, and heart rate were recorded by using an HP 78352C patient monitoring system from Hewlett Packard.

Statistical Analysis

The Wilcoxon matched-pairs signed-rank test for nonparametric data was used for comparison of the side effects in the pulmonary application sessions. $p < 0.05$ was considered as significant. No statistical comparison was made with the results from the iv session because this THC application was not blinded. Analyses were performed in STATA, version 8.1 for MacOS X (STATA Corp., College Station, TX).

Analysis of Plasma Samples

Plasma concentrations of THC and its metabolites 11-OH-THC and 11-COOH-THC were determined by gas chromatography/mass spectrometry. Extraction of the 0.5-mL plasma aliquots was performed automatically by using an ASPEC XL (Automatic Sample Preparation with Extraction Columns) system equipped with a Dilutor 402 (Gilson, Villiers Le Bel, France) and applying the method of Moeller et al.[20] Hydrolyzation, derivatization, and gas chromatography/mass spectrometry analysis were performed according to the method of Feng et al.[21] The method was linear in the following calibrated ranges: from 0.4 to 20 ng/mL for THC in the lower concentration levels, from 20 to 300 ng/mL for THC in the higher concentration levels, and from 0.4 to 100 ng/mL for the two metabolites 11-OH-THC and 11-COOH-THC. Samples exceeding the linearity range were diluted with blank plasma, re-extracted, and again analyzed. The limit of quantification for THC and its metabolites was 0.4 ng/mL plasma.

Pharmacokinetic Calculations

Plasma concentrations versus time were used to calculate pharmacokinetic parameters, including plasma peak concentrations (C_{max}), time to reach peak plasma concentrations (t_{max}), and area under the concentration-time curve (AUC). Based on a noncompartmental model, all pharmacokinetic parameters were assessed by use of standard calculation procedures performed by the TopFit (version 2.0) computer software.[22] AUC from time 0 to infinity ($AUC_{0-\infty}$) or the time corresponding to the last measurable concentration (AUC_{0-x}) was calculated by numeric integration using the linear trapezoidal rule. Values for C_0 (extrapolated) were determined by linear regression of the logarithmically transformed concentration values back to the time point 0.

RESULTS

The results of the quality assurance of the test medications, which allowed their clinical use, are listed in Table 1. Figures 1 and 2 show the plasma profiles of THC and the two metabolites 11-OH-THC and 11-COOH-THC after pulmonal and iv administration, respectively. None of the baseline samples showed measurable concentrations of THC or THC metabolites. The mean plasma level of pulmonal THC after 10 min was 18.7 ± 7.4 ng/mL (mean $\pm$ SEM) with a mean duration of the inhalation procedure of 23 ± 3 min. The peak plasma levels of 18.9 ± 5.0 ng/mL were measured at 20 min (Fig. 1). Then, the plasma concentrations decreased rapidly. Peak plasma levels of the two main metabolites 11-OH-THC and 11-COOH-THC were 1.4 ± 0.3 ng/mL occurring at 40 min and 10.0 ± 2.9 ng/mL at 120 min, respectively. The plasma levels 5 min after the iv injection of

Table 1. *In Vitro* Validation and Quality Assurance of the Test Medications

Test	Inhalation Solution	Injection Solution
Stability	3 months	3 weeks
Osmolality	550 mOsm/kg	321 mOsm/kg
Viscosity	1.478 mPas	Not determined
pH value	7.40	7.40
Output rate	$63.5 \pm 4.4\%$ (mean $\pm$ SD)	Not determined
Particle size distribution	3.8 ± 0.32 μm (median $\pm$ SD)	Not determined
Sterility	Not determined	Passed

THC (0.053 mg/kg body weight) ranged from 81.6 to 640.6 ng/mL (271.5 ± 61.1 ng/mL; Fig. 2). After that, the plasma levels decreased rapidly. Peak plasma levels of 11-OH-THC and 11-COOH-THC were 9.1 ± 0.8 ng/mL occurring at 5 or 10 and 36.7 ± 3.8 ng/mL occurring at 60 min, respectively. The ratio of the AUC_{0-480} of THC to the AUC_{0-480} of its psychoactive metabolite 11-OH-THC was 4.4 to 1 and 6.6 to 1 after pulmonal and iv THC, respectively. Tables 2 and 3 summarize the pharmacokinetic parameters for pulmonal and iv THC. The approximate half-lives for iv and pulmonal THC were 73 and 46 min, respectively.

The observed psychological and somatic side effects are depicted in Table 4 and Figure 3. After pulmonal THC, the symptoms irritation of the throat and upper respiratory tract, and coughing were highly significant compared with placebo. These side effects were reversible within 30 min of finishing inhalation. In contrast to iv THC, the psychotropic effects after pulmonal THC were usually very mild. A significant difference versus pulmonal placebo was observed for pulmonal THC concerning euphoria, confusion and disorientation, and change of inner perception. Blood pressure was not changed by THC, whereas both pulmonal and iv THC increased heart rate significantly as compared with placebo (data not shown).

As after oral THC,[10] pulmonal THC produced hyperalgesia in the ice water pain test, an effect which was significant versus pulmonal placebo only after 20 min (Table 5).

DISCUSSION

It was possible to develop an aqueous inhalation solution of the very hydrophobic THC. The output rate of the nebulizer device was sufficient to deliver the required dose of THC within an inhalation time of 20–25 min. The resulting droplet size should allow the aerosolized THC to reach the lower compartments of the lung, thus enabling a high absorption rate. The quality assurance of the pulmonal and iv formulation showed good stability and physiological compatibility. The pulmonal application of nebulized THC, therefore, seems to be a promising mode for the clinical use of THC. The pulmonal bioavailability of $28.5 \pm 23.1\%$ (0.4–60.6%) was higher than after oral administration, where the bioavailability was found to be 5–20%.[11–13] Some volunteers even

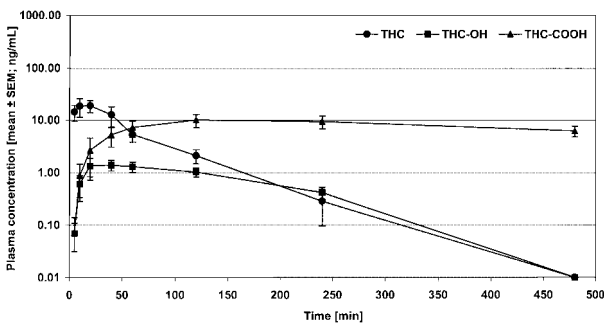

Figure 1. Plasma concentrations (mean $\pm$ SEM; $n = 8$) of THC and its main metabolites 11-OH-THC and 11-COOH-THC after pulmonal THC.

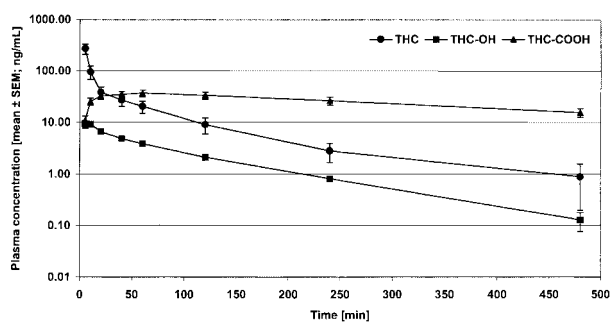

Figure 2. Plasma concentrations (mean $\pm$ SEM; $n = 8$) of THC and its main metabolites 11-OH-THC and 11-COOH-THC after iv THC.

Table 2. Pharmacokinetic Parameters of Pulmonal Versus iv THC

Subject		Pulmonal THC				iv THC	
No.	Gender	Dose (mg)	$AUC_{0-\infty}$ ($ng \cdot min \cdot mL^{-1}$)	Bioavailability F (%)	Elimination Rate Constant λ_z ($\cdot 10^{-2}$)	Dose (mg)	$AUC_{0-\infty}$ ($ng \cdot min \cdot mL^{-1}$)
1	F	3.71	2528	38.8	1.600	3.30	5792
2	M	2.98	694	28.5	1.500	4.20	3437
3	M	4.08	2607	59.4	1.090	4.24	4559
4	M	4.56	1257	21.4	1.710	4.53	5827
5	F	2.34	68	0.4	3.890	2.40	20103
6	F	2.97	528	15.3	1.570	2.92	3406
7	F	3.29	367	3.7	0.823	3.50	10506
8	M	4.03	1581	60.5	0.225	4.00	2592
Mean ± SD			1203 ± 969	28.5 ± 23.1	1.550 ± 1.070		7028 ± 5829

F, female; M, male.

showed a bioavailability of >40%. A study comparing the bioavailability of oral and pulmonal THC in individual volunteers would lead to more conclusive results. Most of the subjects reached plasma levels comparable to those of iv THC at 10 and 20 min. Peak plasma levels of THC were observed before the end of the inhalation procedure.

Regarding the plasma concentrations of the THC metabolites 11-OH-THC and 11-COOH-THC, similar patterns for pulmonal and iv THC were observed. The THC to 11-OH-THC-ratios found in the present study for iv THC, and in an earlier study[10] for oral THC, confirm the findings reported by Wall et al.[13] for iv THC. The significantly lower formation of the psychoactive 11-OH-THC after pulmonal THC, due to the absence of first-pass metabolism, results in remarkably less intensive psychotropic side effects compared with oral THC. This is an important fact regarding the development of future THC application forms.

The plasma concentration-time plot of the iv administration showed first a distribution phase with a very rapid decrease of the THC plasma levels followed by the elimination phase with a much longer terminal plasma elimination half-life. This pattern is compatible with two-compartment elimination kinetics described previously by Wall et al.[13] and Huestis.[11]

The placebo aerosol was very well tolerated indicating a good tolerability of the vehicle with the adjuvants used for solubilization and stabilization of the formulation. Nevertheless, irritation of the airways and coughing after pulmonal THC was observed for all subjects, meaning that THC itself caused these adverse effects ($p = 0.01$). Coughing

Table 3. Pharmacokinetic Parameters of iv THC

Subject		iv THC		
No.	Gender	Distribution Volume V_{ss}/kg Body Weight (Steady State) (L/kg)	Clearance CL/kg Body Weight ($mL/min \cdot kg$)	Elimination Rate Constant λ_z ($\cdot 10^{-2}$)
1	F	0.847	8.14	0.578
2	M	0.598	15.44	1.370
3	M	0.403	12.08	1.400
4	M	0.300	9.03	1.500
5	F	0.324	3.41	0.566
6	F	0.668	15.30	1.320
7	F	0.431	5.37	0.813
8	M	1.120	20.26	1.280
Mean ± SD		0.586 ± 0.285	11.13 ± 5.69	1.100 ± 0.390

F, female; M, male.

Table 4. Psychological and Somatic Side Effects (VAS) after Pulmonal THC and Placebo and iv THC

	Median of Maximum Values on VAS			
Symptom on VAS	iv THC	Pulmonal THC	Pulmonal Placebo	p Value[a] (Pulmonal THC vs. Pulmonal Placebo)
Sleepiness	89	64	22.5	0.12
Euphoria	62.5	20.5	0	0.02
Irritation	25	2	0	0.05
Anxiety	26.5	0	0	0.45
Tenseness and aggressiveness	18.5	1	0	0.45
Confusion and disorientation	80	2	0	0.03
Change of inner perception	85.5	9.5	0	0.03
Change of outer perception	72.5	0	0	0.09
Hallucinations	35	0	0	0.16
Strange thoughts, ideas, moods	34	0	0	0.32
Nausea	25	8	0	0.05
Headache	43	16.5	0	0.11
Difficulties in breathing	27.5	8.5	0	0.03
Irritation of the throat, coughing	0	75	2	0.01
Irritation of the upper respiratory tract	1.5	79.5	0	0.01
Heart problems (tachycardia)	34.5	0	0	0.16
Digestive problems	7.5	0	0	0.93
Dry mouth	100	3	3	0.48
Vertigo	76	30.5	0	0.03

[a]Wilcoxon matched-pairs signed-rank test.

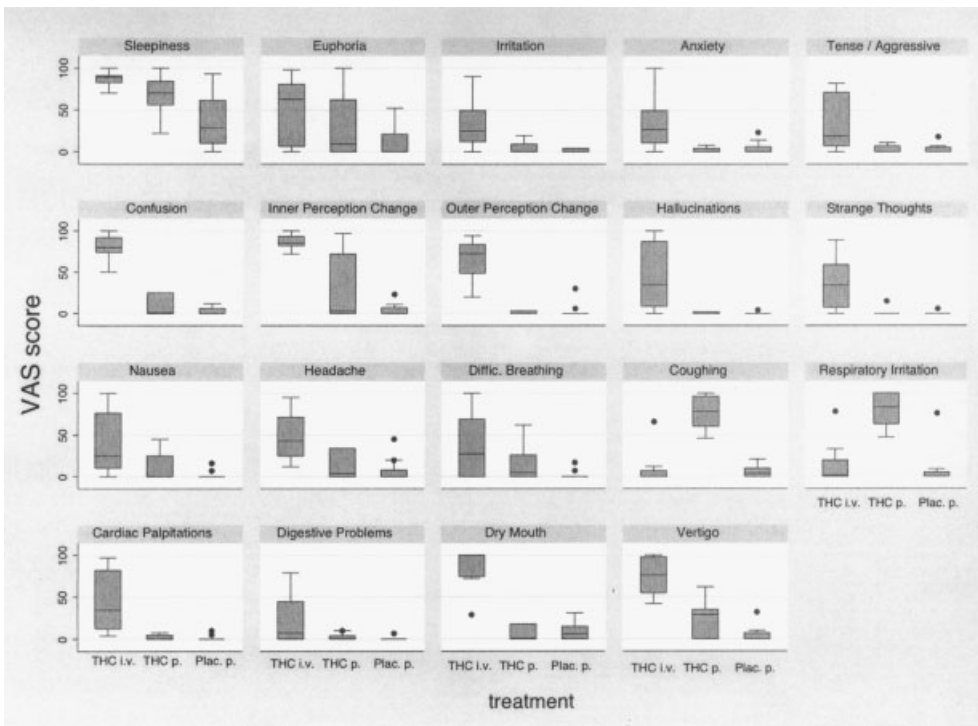

Figure 3. Psychological and somatic side effects after pulmonal and iv THC and pulmonal placebo. Box and whisker plots according to VAS showing median, interquartile range, lower and upper adjacent values, and outside values.

Table 5. Pain Tolerance in the Ice Cold Immersion Test after Pulmonal THC and Placebo

Time (min)	Median AUC Pain Test (Difference from Baseline)		p Value[a] (Pulmonal THC vs. Pulmonal Placebo)
	Pulmonal THC	Pulmonal Placebo	
20	127	26	0.03
40	54	34	0.21
60	129	80	0.53
120	170	62	0.12
240	160	127	0.89
480	235	99	0.67

[a]Wilcoxon matched-pairs signed-rank test.

impaired the inhalation procedure, and therefore, most likely also the interindividually most variable bioavailability, which would probably be higher with a less irritating formulation of THC. The irritations were reversible within a short time after the end of inhalation indicating no lasting damage to the mucosa. This particular effect of THC was also demonstrated by Tashkin et al.[23] Because the micellar formulation used in this study did not prevent mucosal irritation, other techniques should be tested, for example the use of liposomes or microencapsulation. A higher mean C_{max} and very rapid increase in concentration in the central nervous system were responsible for the more pronounced adverse effects of iv THC, which were mainly of a psychotropic nature. THC did not reduce pain in the ice water test after pulmonal administration. This confirms the ice water test results obtained in our previous study with oral THC. As postulated before,[10] this indicates that the low oral bioavailability of THC is not responsible for the lack of analgesia. It is assumed that the ice water test is not the right model to determine an analgesic effect of THC.

In conclusion, the pulmonal administration of a liquid THC aerosol leads to rapid and high plasma levels of THC, with a metabolic pattern similar to that of iv THC. Although the bioavailability was much higher than after oral THC, no significant analgesic effect was measured with an acute pain test. Because appropriate experimental chronic pain models are currently not available, the analgesic effect of pulmonal THC should be further tested in pain patients. In addition, other solubilization techniques should be evaluated to improve the physiological tolerability of pulmonal THC aerosols.

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