The effect of excipients on pharmacokinetic parameters of parenteral drugs

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Summary

In the pharmaceutical industry, the main goal of early phase *in vivo* studies is to assess pharmacokinetic properties of a compound in laboratory animals. These data provide a basis for selecting and optimizing drug candidates. However, formulation scientists face considerable challenges in finding intravenous preparations for first animal experiments. A common problem is the solubilization of lipophilic and sparingly water-soluble compounds. The search for suitable delivery vehicles often takes place under little compound availability, incomplete physicochemical property characterization, and time constraints. In addition, many experiments have recently generated distinct evidence about the impact of formulation vehicles on the drug pharmacokinetics by affecting transporters, metabolic enzymes, and distribution processes. Consequently, drug-excipient interactions are important to consider in the development of parenteral formulations intended for the proper evaluation of animal pharmacokinetics *in vivo*. Gaining a better understanding of potential interactions between drug and formulation in preclinical settings may play a crucial role in clinical and commercial phases of development as well.

So far, little is known about drug-excipient interactions occurring in blood, especially following iv administration of low dosed compounds (<50 ng/mL in blood) including e.g. highly active drug substances, biomarkers, PET ligands, and microdoses.

The **purpose** of the current work was to examine the potential of excipients commonly used in formulations to modify the blood distribution and protein binding of low dosed compounds under *in vitro* and *in vivo* conditions. Drug candidates in development at Novartis were used as model compounds and chosen based on different physicochemical and pharmacokinetic properties such as aqueous solubility (poor: COM1/COM2; good: COM3), lipophilicity (low: COM4; high: COM2), membrane permeability (low: COM5; high: COM3), and blood cell/plasma distribution (mainly in cells: COM3; mainly in plasma: COM4). Selected excipients comprised one cosolvent (polyethylene glycol 200, PEG 200), one complexing agent (hydroxypropyl-β-cyclodextrin, HP-β-CyD), and three non-ionic surfactants (Cremophor EL, CEL; Solutol HS 15, Solutol; D-α-tocopheryl polyethylene glycol 1000 succinate, TPGS), most of them present in commercially available intravenous formulations. TPGS, which is used orally but not as an intravenous excipient, was chosen due to its chemical structure and intrinsic properties, particularly its benzyl ring and potential modulation of transporter/enzyme activities.

Preliminary tests *in vitro* showed that selected excipients except for TPGS were non-hemolytic at 0.5% which is consistent with data reported in the literature. TPGS at 0.5% induced marked hemolysis after longer contact time (> 1h) in various species (mouse, rat, dog, and human), whereas TPGS at 0.1% showed no hemolysis under same conditions. Nevertheless, TPGS (0.5%) was used in the non-hemolytic time range for further investigations. The concentration of all excipients was set at 0.5% in test systems which is within the relevant range following intravenous dosing in animals.

In vitro, CEL, HP-β-CyD, Solutol, and TPGS influenced clearly the plasma protein binding and the distribution between blood cells and plasma of model compounds in mice (COM2) or rats (COM1, COM3, COM4, COM5). The addition of TPGS to incubations increased the distributed fraction of COM1 and COM2 in plasma with a concomitant decrease of drug unbound in plasma. Formulating COM4 in CEL and COM5 in Solutol lowered the protein binding, and the higher drug fraction unbound in plasma was associated with enhanced partitioning into blood cells. The presence of HP-β-CyD reduced both the uptake of COM3 into blood cells and the binding to plasma proteins.

To assess the correlation between the *in vitro* findings and the ***in vivo*** situation, pharmacokinetics and tissue distribution were determined up to 1 h (within PET scan times) after an intravenous bolus injection of model compounds in formulations based on excipients or none (control) to animals, using in each case the excipient with the most pronounced interactions detected *in vitro*. Injection preparations contained the excipient to yield estimated blood concentrations of about 0.5%, similar to those used in the *in vitro* experiments. COM2 formulated in TPGS caused a higher accumulation of parent drug and metabolites in plasma without affecting tissue levels in mice. Administering COM3 in HP-β-CyD altered the disposition of COM3 characterized by a lower binding to plasma proteins, decreased drug levels in the systemic circulation and skin, and a higher amount of unchanged drug in the urine. COM4 formulated in CEL resulted in a higher drug fraction unbound in plasma which had no impact on the pharmacokinetics and tissue distribution. The use of Solutol for COM5 application in rats was associated with decreased protein binding, longer persistence in the circulation, and higher concentrations in muscle and skin. Although TPGS induced a slight shift in the pharmacokinetic parameters of COM1 in rats, the compound turned out to be an inappropriate model compound due to its very rapid metabolism and elimination under *in vivo* conditions.

These *in vitro* and *in vivo* **findings demonstrated** that commonly used excipients have a substantial potential for drug-excipient interactions in blood by altering protein binding and blood cell/plasma distribution which can influence the tissue distribution and elimination within the first hour after dosing. As a result, the formulation vehicle can be an important determinant for the disposition of low dosed compounds administered intravenously in animals. Moreover, results indicate a direct correlation of the excipient effect under *in vitro* and *in vivo* conditions. Therefore, blood distribution and plasma protein binding data generated *in vitro* seem to be appropriate to reveal potential drug-excipient interactions, thereby providing helpful information to improve the rational approach and strategy in the development of parenteral formulations at the preclinical stage. A better insight into the contribution of excipients to drug pharmacokinetics suggests also new possibilities of targeting different blood compartments and tissues by selecting the appropriate excipient. Such investigations should be considered to develop formulations suitable for intravenous administration of PET ligands where sub-therapeutic doses and short scanning times are used.

Abbreviations

AGP	α1-acid glycoprotein
AUC	Area under the drug concentration-time curve
BCPR	Ratio of concentration in blood cells to that in plasma, no units
BPR	Ratio of concentration in blood to that in plasma, no units
C_0	Initial plasma concentration at time zero
C_B	Concentration of drug in blood
C_{BC}	Concentration of drug in blood cells
CEL	Cremophor EL
C_P	Concentration of drug in plasma
EtOH	Ethanol
F_P	Drug fraction distributed in plasma, %
fu	Fraction of unbound to total drug concentrations in plasma, %
$f_{unchanged}$	AUC ratio of parent drug to that of total radioactivity, %
Glu	5% aqueous solution of glucose
H	Hematocrit
HDL	High density lipoprotein
HP-β-CyD	Hydroxypropyl-β-cyclodextrin
im	Intramuscular
iv	Intravenous
k	Rate constant, h^{-1}
K_P	Distribution ratio of drug between tissue and blood/plasma, no units
LC-RID	Liquid chromatography-reverse isotope dilution
LDL	Low density lipoprotein
LOQ	Level of quantification
LSC	Liquid scintillation counting
nd	Not determined
PEG 200	Polyethylene glycol 200
PET	Positron emission tomography
SD	Standard deviation
Solutol	Solutol HS 15
TPGS	D-α-tocopheryl polyethylene glycol 1000 succinate
$t_{1/2}$	Half-life, h
V_0	Volume of distribution based on initial drug concentration in plasma, L
VLDL	Very low density lipoprotein
ρ	Ratio of concentration in blood cells to that unbound in plasma, no units

1 Introduction

1.1 The physiology of blood

Blood is composed of cellular elements suspended in the plasma, an aqueous fluid in which solids are dissolved. Table 1-1 summarizes the main blood constitution of different laboratory animal species and humans. The normal range can vary, depending mainly on genetic and environmental factors and methods handling.

Table 1-1 Normative data for laboratory animals and humans

	Mouse [1,2]	**Rat** [1,3,4]	**Human** [5]
Sex	Male	Male	Male
Strain	OF1	Wistar	
Body weight (kg)	0.030	0.250	70
Whole blood (ml/100 g)	7.2 (6.3-8.0)	7.2 ± 0.2	7.1 ± 0.6
Plasma (ml/100 g)	3.2	3.9 ± 0.1	4.4 ± 0.5
Total plasma proteins (g/100 mL)	5.4 ± 0.2	5.7 ± 0.5	7.5 ± 0.4
Albumin (% plasma proteins)	61 ± 1	48 ± 3	62 ± 3
α_1 globulin (% plasma proteins)	12 ± 1	17 ± 2	4 ± 1
α_2 globulin (% plasma proteins)	(α globulin)	10 ± 2	9 ± 1
β_1 globulin (% plasma proteins)	20 ± 1	19 ± 1	11 ± 2
β_2 globulin (% plasma proteins)	(β globulin)	(β globulin)	(β globulin)
γ globulin (% plasma proteins)	7 ± 1	6 ± 1	15 ± 2
Blood cells			
Hematocrit (%)	43 ± 3	46 ± 2	44 ± 2
Red blood cells (x10^6 cells/µL)	9 ± 1	7 ± 1	5 ± 1
White cells (x10^3 cells/µL)	4 ± 2	6 ± 2	7 ± 1
Platelets (x10^6 cells/µL)	1.3 ± 0.4	1.2 ± 0.2	0.3 ± 0.1

1.1.1 The blood cells

The different specialized cells found in blood are white blood cells (leukocytes), red blood cells (erythrocytes) and platelets (thrombocytes). Of these, the erythrocytes are the most numerous and compose about one-half of the circulating blood volume. By carrying hemoglobin in the circulation, the red blood cells supply O_2 to tissues and remove CO_2. Leukocytes are classified as granulocytes (further classification in neutrophils, eosinophils, and basophils), lymphocytes, and monocytes. Acting together, these cells provide the body with a powerful defense against tumors, viral, bacterial, and parasitic infections. Compared to the other blood cells, the platelets are much smaller and aid in hemostasis by their primary function in blood clotting. Furthermore, blood cells can play a key role in binding and transporting of drugs in the circulation, thereby contributing to their pharmacokinetic and pharmacological characteristics (6,7).

1.1.2 Plasma

The plasma, the liquid portion of the blood, is a complex fluid composed of water (approximately 90%) and a large number of ions, inorganic molecules, and organic molecules in solution. These dissolved substances, primarily proteins, are in transit to

various parts of the body or aid in the transport of other substances. The plasma proteins consist of albumin, globulin, and fibrinogen fractions, which can be separated by electrophoresis. Electrophoretic separation followed by immunoprecipitation (immunoelectrophoresis) results in a further division of the proteins. If whole blood is allowed to clot and the clot is removed, the remaining fluid is called serum and has essentially the same composition as plasma except for the removed fibrinogen and few clotting factors (II, V, and VIII). Table 1-2 lists the main protein fractions with their main characteristics. The table also indicates that a large number of drugs associate with proteins within the bloodstream. Albumin is the major drug-binding plasma protein (8) followed by alpha 1-acid glycoprotein as the next important one (9). In recent years, studies have shown, that lipoproteins are also substantially involved in the binding/transport of drugs in the blood compartment (10). So far, γ-globulins play only a marginal role in plasma binding of drugs.

Table 1-2 Proteins in human plasma

Protein fraction		**Physiological Function**	**Binding characteristics**	
Electro-phoresis	Immuno-electrophoresis		Endogenous entities	Drugs
Albumin	Prealbumin Albumin	Binding and carrier protein, osmotic regulator	Hormones, amino acids, steroids, vitamins, fatty acids	Mainly acidic, but also basic and neutral compounds
α_1 globulin	α1-acid glycoprotein	Uncertain (acute phase protein)		Mainly basic and neutral compounds
	α_1-lipoprotein ("high density lipoproteins")	Transporter	Lipids	Lipoproteins: mainly lipophilic neutral and basic compounds
α_2 globulin	Ceruloplasmin	Transporter	Copper	
	α_2-Macroglobulin	Enzyme inhibitor	Serum endoproteases	
	α_2-Haptoglobin	Binding and carrier protein	Cell-free hemoglobin	
β globulin	Transferrin	Transporter	Iron	
	β-lipoprotein ("low density lipoproteins")	Transporter	Lipids (mainly cholesterol)	Lipoproteins: mainly lipophilic neutral and basic compounds
	Fibrinogen	Precursor to fibrin in hemostasis		
γ globulin	IgG, IgA, IgM, IgE	Humoral immunity (antibodies/immunoglobulins)	Antigen	Few basic compounds

1.2 In vitro methods to investigate blood binding parameters

The investigation of the partitioning of a drug in the blood compartment is essential in predicting its pharmacokinetic/-dynamic profile. In general, the unbound concentration of a drug in blood reflects more accurately pharmacological effects of the drug than its total concentration in blood (bound + unbound), because only the drug unbound to blood components is able to diffuse through the membranes and then reach the target organ (11). Furthermore, the binding to plasma proteins also relates to the volume of distribution and the clearance of the drug. For instance, many experimental and clinical studies have generated substantial evidence summarized by Akhlaghi (12), that the unbound fraction of cyclosporin in plasma correlates more closely with pharmacodynamic and pharmacokinetic characteristics of cyclosporin than its total blood concentration. Therefore, determination of extent and rate of blood/plasma distribution and plasma protein binding of a drug is important in both the discovery and clinical phases of drug development.

1.2.1 Blood distribution method

The rate and extent of blood/plasma distribution of drugs is determined *in vitro* in spiked whole blood. The experiments are performed under controlled physiological conditions (pH 7.4, 37°C, gently shaken) to reflect the *in vivo* situation over the entire clinically relevant concentration range of the drug. Time samples are taken and centrifuged. Subsequently, drug concentrations in blood and plasma are determined to calculate the time required to reach equilibrium. The extent of blood/plasma and blood cell/plasma distribution derives from measured concentrations in blood and plasma and can be expressed with distribution parameters like F_P, BPR, and BCPR. BPR depends on the hematocrit of the whole blood used in the determination, whereas BCPR is independent of the hematocrit value.

1.2.2 Protein binding methods

Various methods are available for the determination of free drug concentration and protein-drug binding fraction in plasma (13,14,15), including conventional separation methods summarized in Table 1-3. However, the routinely used methods like ultrafiltration or equilibrium dialysis are limited in the case of lipophilic drugs due to their nonspecific adsorption to ultrafiltration device or to the dialysis membrane. Along with a trend to more lipophilic compounds observed in the pharmaceutical industry in recent years (16), these adsorption problems are expected to increase. As a result, ongoing method modifications and new methods are needed to overcome these difficulties. Overall, the selection of the method of binding assay depends upon the aim of the study and the physicochemical properties of the particular test compound including its formulation.

The ratio of bound and total drug concentrations in plasma expresses the degree of drug binding to plasma proteins and ranges between values of 0 and 1. Based on these values, drugs can be classified into very highly bound (>0.95), highly bound (>0.90), poorly bound (<0.9), and little/not bound (<0.2).

Table 1-3 Conventional methods for determination of plasma protein binding

Method	Principle	Advantages	Disadvantages
Equilibrium dialysis (reference method)	Equilibrium establishment between two compartments separated by semipermeable membrane with defined molecular weight cutoffs	Physiological conditions, universal binding method	Sample dilution, volume shifts, Donnan effects, nonspecific adsorption, sieve effect, time consuming, unsuitable for unstable drugs
Ultrafiltration	Separation by filtration through a semipermeable membrane with defined molecular weight cutoffs accelerated by centrifugation or positive pressure (N2 gas, syringe)	Simply applicable, short analysis time, simple commercially available kits, no volume shifts, no dilution effects	Donnan effects, nonspecific adsorption, binding equilibrium changes during separation process, small amount for analysis, sieve effect
Ultracentrifugation	Separation by centrifugation at high speed in absence of a membrane	No membrane effects, "natural environment", no dilution problems, adoptable for lipophilic and high MW drugs, evaluation of lipoprotein binding	Time consuming, expensive equipment, false estimation of free fraction by physical phenomena (e.g. sedimentation, back diffusion), protein contamination of free drug layer
Gel filtration	Separation by size exclusion and affinity of column	Adoptable for lipophilic drugs, automatable, binding differences detectable (e.g. affinity)	Complex handling, time consuming

1.3 Characterization of drug candidates

Successful candidates in drug development must have proper physicochemical properties in addition to acceptable pharmacokinetics, efficacy, and safety profiles. As a result, a clear understanding of compound characteristics and their correlations are helpful to rank and sort out unsuitable compounds in drug research (17,18).

1.3.1 Physicochemical properties

The chemical structure of a drug candidate is used in both predicting the pharmacology and selecting formulation strategies. Table 1-4 shows physico-chemical parameters, which are critical for *in vivo* drug action.

The molecular weight (MW) indicates roughly the size of a chemical entity and is connected to its membrane permeability, namely to the intestinal and brain penetration (16,19).

LogP, the octanol-water partition coefficient, has been widely accepted as a measure of molecular lipophilicity. Lipophilicity affects both the pharmacokinetic and pharmacodynamic behavior of drug molecules (20,21). LogP considers the molecule in its neutral state (neutral substance or ionizable substance in its neutral form), whereas logD reflects the pH-dependent distribution coefficient, consequently taking the ionization of molecules into account. If logP and pKa of a compound are known, logD can be calculated at any pH (21).

The aqueous solubility (LogS) is closely related to drug stability, liberation, and absorption by passive diffusion, thereby playing a key role in its bioavailability (22). Causes for poor solubility are mainly excessive lipophilicity and crystal packing issue (23,24,25,26). The solubility of nonionic molecules is pH independent, while molecules with ionizable groups show pH dependent solubility. Acid drugs have higher solubility at pH higher than pKa and basic drugs at pH lower than pKa due to better solubility of ionic species as compared to the neutral species. The acid-basic character accounts also for crossing the blood-brain barrier (27).

The polar surface area (PSA) of a molecule is a useful parameter for predicting drug transport properties. PSA is the sum of the molecular surface (either van der Waals or solvent-accessible) that arises from polar atoms, usually N, O, N-H, and O-H atoms. Some scientists also include sulphur and phosphor and attached hydrogens as polar atoms. The PSA of a compound is also closely related to its hydrogen bond accepting and donating ability which can be responsible for interactions with active efflux pumps (28,29). PSA has been shown to correlate well with blood-brain distribution (27,30,31), intestinal absorption (32,33,34,35,36,37), and oral bioavailability (38) of compounds.

Table 1-4 Physicochemical parameters

Parameter	Description	Predictor	Optimal value
MW	Molecular weight	Size, Permeability	< 500 < 450 (BBP)
LogP	Logarithm of the octanol-water partition coefficient	Lipophilicity, Permeability	< 5
LogS	Logarithm of the aqueous solubility	Hydrophilicity	> 20 µg/mL
pKa	Negative logarithm of the acid-base dissociation constant	Acid-base character	Acids >4 and bases <10 (BBP)
PSA	Polar surface area	Permeability, H-bonding capability	< 140 Å < 80 Å (BBP)

BBP: blood brain penetration

1.3.2 Pharmacokinetic parameters

The pharmacokinetic profile is crucial for the clinical success of drug candidates and their development into marketable drugs. Therefore, today the contribution of pharmacokinetic investigations to the selection and optimization of promising drug candidates is well recognized. The four most important parameters are clearance, volume of distribution, elimination half-life, and bioavailability, which is the fraction of drug absorbed as such into the systemic circulation (100% per definition for drugs given intravenously).

Drug clearance (CL) expresses the rate or efficiency of drug removal from the systemic circulation and is estimated as the ratio of dose to AUC following intravenous administration of the drug:

$$CL = \frac{Dose}{AUC}$$

AUC is the total area under the curve that describes the concentration of drug in blood or plasma as a function of time. AUC represents the drug exposure and is calculated by the trapezoidal rule.

The volume of distribution (V_0) relates the amount of drug in the body to the concentration of drug in the blood or plasma, depending upon the fluid measured. This volume does not necessarily refer to an identifiable physiological volume, but merely to the fluid volume that would be required to contain all of the drug in the body at the same concentration as in the blood or plasma:

$$V_0 = \frac{Dose_{iv}}{C_o}$$

C_0 represents the blood or plasma concentration at time zero and is determined by extrapolation to zero time of the linear plot of concentration vs. time in semilogarithmic scale.

The half-life ($t_{1/2}$) is the time it takes for the blood or plasma concentration or the amount of drug in the body to be reduced by 50%:

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k},$$

where k is the elimination rate constant, which can be calculated by the slope of the best-fit line to a semilogarithmic plot of the concentration over time. The relationship of $t_{1/2}$ to both clearance and volume of distribution is given by:

$$k = \frac{CL}{V}$$

1.3.3 New trends in characterizing drug candidates

Before conducting clinical trials in humans, preclinical testing is carried out to discover the pharmacology, ADME (adsorption, distribution, metabolism, and excretion), and toxicology of a new drug candidate (39). Appropriate pharmacokinetics and a good balance between drug efficacy and safety contribute mainly to an efficient and effective drug development. However, these factors are the major hurdles in development which primarily cause increased costs and failure rate of candidates. Thus, pharmaceutical industry needs new concepts able to speed and improve activities and decision-making in drug development (40,41). In this context, microdosing, biomarkers, and PET ligands can help to prioritize resources and optimize drug selection in development. In many cases, these approaches deal with compound concentrations ranging from sub-therapeutic to low pharmacological levels, and thus information obtained from these techniques must reflect correctly the conditions at therapeutic doses, including interactions with macromolecules like enzymes, transporters, and proteins. In the end, a successful integration requires a profound understanding of strengths and limitations of these new concepts.

The administration of a low dosed (microdosed) drug candidate to humans was proposed to obtain human pharmacokinetic data before conducting Phase I trial (42). A microdose is one-hundredth of the proposed pharmacological dose determined from animal and/or *in vitro* models, or a dose up to 100 µg, whichever is the smaller (43). Human microdosing uses labeled agents administered mostly intravenously, and their fate *in vivo* is recorded by positron emission tomography combined with accelerator mass spectrometry or nuclear magnetic resonance (43,44). With this new

strategy of microdosing, drug information regarding human kinetics will be available along with preclinical data and can be useful for the acceptance or rejection of a candidate at an early stage.

A biomarker is an indicator of a normal biological or pathophysiological process or a therapeutic response (45). Biomarkers help to select the most sensitive drugs in all phases of drug development (46) by providing data of pharmacological response, dosing regimen, and risk-benefit assessment. Therefore, efforts are moving rapidly forward to achieve strong predictive biomarkers which could be used for diagnostic and therapeutical purposes (47).

PET tracers labeled with short-lived radionuclides (e.g. ^{11}C, ^{18}F, ^{124}I) are used as molecular probes of physiology and pathophysiology in animals and humans. These labeled compounds are administered mostly intravenously at 600 MBq to humans which corresponds to 6-20 nmol (3-10 µg assuming a MW of 500) (48). To achieve the same imaging quality in animals, roughly the same total amount of radiopharmaceutical must be given to animals as to a human subject (49).

1.4 Strategies and administration of intravenous formulations

In the pharmaceutical industry, formulation scientists have faced growing challenges in recent years as a result of new drug candidates characterized as being more lipophilic, hydrophobic, and water-insoluble, particularly candidates originated from leads associated with combinatorial chemistry and high-throughput screening (16,24). In addition, timelines and resources are very limited to develop an optimized formulation and thus the search for a suitable dosing vehicle intended for activities in preclinical research represents a challenging task for the formulators (50). Ideally, it is best to select and use solubilizers that would maximize the solubility of the compound and could be applied for all preclinical settings. Moreover, the solubilizing agents should not influence the intrinsic pharmacokinetic characteristics of the compound being evaluated (except the interaction is well understood), which would lead to misinterpretation of the pharmacological response (51). Strategies for solubilization of intravenous drugs are summarized in Table 1-5 and well exemplified by the formulation approaches for the anticancer agent Paclitaxel (52).

Usually, the first step is to check the solubility of the compound in an aqueous dosing vehicle at physiological pH and osmolarity. If the target concentration cannot be achieved with this approach and the drug molecule is ionizable, adjustment of the pH to non-physiological values can be suitable to increase water solubility (pKa must be sufficiently away from the formulation pH). Non-electrolytes are insensitive to pH modification. The next approach most frequently tried is the addition of water-miscible organic solvents (cosolvents) and the use of surfactants or complexing agents. To reach the required dose, combination of these methods is often used. Dispersal systems are other techniques, but they may be difficult, costly, and time-consuming due to biological and technical complexity, e.g. liposomes (53).

Table 1-5 Intravenous formulation approaches

Approach	Examples	Administered in commercial products[a]	Potential drawbacks
Aqueous solution at physiological osmolarity and pH / or with pH adjustment	NaCl 0.9% (w/v), Glucose 5% (w/v) Strong acids/bases (HCl, NaOH), Buffers (tartarte, phosphate)	pH 2-12 (bolus), pH 2-10 (infusion) → preferred range pH 4-9	Precipitation Pain
Cosolvents	Propylene glycol	≤ 68% (bolus), ≤ 6% (infusion)	Precipitation
	Ethanol	≤ 20% (bolus), ≤ 10% (infusion)	Irritation/Pain
	Polyethylene glycol 300	≤ 50% (bolus)	Hemolysis
	Polyethylene glycol 400	≤ 9% (bolus)	Impact on PK profile
Surfactants	Cremophor EL	≤ 10% (infusion)	dito
	Tween 80	≤ 0.4% (bolus), ≤ 2% (infusion)	
	Solutol HS 15	50%	
Complexing agents	Hydroxypropyl-β-cyclodextrin	20% (infusion)	dito
Dispersal systems:			Impact on PK profile
Emulsion[b]/Microemulsion[c]	Water with 10-20% oil (fatty acids + lecithin + glycerol)		Sustained release
Liposomes	Water with phospholipids (5-20 mg/mL) + isotonicifier + buffer ± cholesterol		Instability
Nanosuspension[d]	Water with stabilizer	not yet marketed[e]	Slow dissolution

[a](54), [b](55), [c](56), [d](57), [e](58)

For compounds administered intravenously to animals, the dose volume, viscosity of injection material, speed of injection, and species are important factors to consider in addition to formulation properties including additives, solubility, and stability (Table 1-6) (59). A compound can be given over a short period of ≤1 min (bolus injection), 5-10 min (slow injection), and longer time period (intravenous infusion). Rapid injections require the dose to be compatible with blood and not too viscous, and the rate of injection is suggested not to exceed 3 mL/min for rodents. Depending on study objectives and compound solubility in an acceptable formulation, a larger volume may be needed to be given to animals to accomplish requirements. Regarding the formulation, aqueous solutions or simple systems containing cosolvent, surfactant, or complexing agent are recommended for animal investigations at early stage in development due to easy handling and characterization. For excipient selection, consideration should be given for toxic and biological effects, interferences with the drug compound, and suitability for clinical use (Table 1-5). Injectable excipients preferred for dosing in animals are: ethanol, propylene glycol, low molecular weight polyethylene glycols, Cremophor EL, Tween 80, and cyclodextrins.

Table 1-6 Dose volumes and rates for intravenous administration(59)

Species	Bolus injection		Slow injection		Infusion		
	Volume (mL/kg)	Rate (mL/min)	Volume (mL/kg)	Rate (mL/min)	Time (h)	Volume (mL/kg/d)	Rat (mL/kg/h)
Mouse	5	3	max. 25	3	4		
					24	96	4
Rat	5	3	max. 20	3	4	20	5
					24	60	2.5

1.5 Effect of excipients on pharmacokinetic parameters in blood

Over the last years, more attention has been paid to the extensive investigation of formulation vehicles as biologically and pharmacologically active compounds. The main stages in which pharmaceutical excipients can interact and hence may modulate the properties of an administered drug-agent are transporter, enzyme, and distribution process in the systemic circulation (e.g. plasma protein binding). The effect of excipients on transporter activity has been studied intensively, namely for P-glycoprotein (60,61,62,63,64,65,66,67,68), multidrug resistance-associated protein (69,70) and peptide transporter (71). It is interesting to say that particularly nonionic surfactants effectively inhibit transporters. In contrast, up to this day little is known about drug-excipient interactions at the level of cytochrome-mediated metabolism (63,72,73,74) and blood distribution (see below). The biological and pharmacological properties of excipients with a focus on the central blood compartment will be reviewed in the following paragraphs.

1.5.1 Cremophor EL

The amphiphilic polyethoxylated castor oil derivative Cremophor EL (CEL) is one of the most frequently used surface-active formulation ingredients in parenteral dosage forms. As early as 1977 lipoprotein alterations were observed in patients receiving miconazole therapy (75) which was caused only by CEL, both *in vitro* and *in vivo* (76,77,78). Extended studies revealed later on that CEL has a destructive effect on HDL resulting in a shift of the electrophoretic and density gradient HDL to LDL (79,80,81,82). Furthermore, several hydrophobic anti-tumor agents, tin etiopurpurin (83,84), C8KC (85) and Taxol (81,82), showed strong affinity for these lipoprotein dissociation products inducing changes in plasma protein binding, potentially affecting pharmacokinetics.

Various animal studies demonstrated (85,86,87,88,89,90,91,110) that CEL modifies the pharmacokinetic behavior of drugs after intravenous administration, like paclitaxel (Taxol), C8KC, and cyclosporin. The most common observation was a substantial increase in the area under the plasma concentration-time curve and in peak plasma concentration of studied agent with a reduction in the clearance, as was first described for paclitaxel in a mouse model (91). The drug-CEL interactions were supposed to be caused not only by altered protein binding characteristics (82), but also by altered hepatobiliary secretion (92) and endogenous P-glycoprotein-mediated biliary excretion (93). However, the very small volume of distribution of CEL, approximately equal to the volume of the central blood compartment, suggests that the observed interference occurs in the central blood compartment. This hypothesis was confirmed by studies recently published (94,95). The main finding was a profound alteration of cellular partitioning and blood/plasma concentration ratio of paclitaxel in a CEL concentration-dependent manner as a result of an entrapment of the compound into micelles formed by CEL (96). Consequently, the free drug fraction available for distribution was reduced. This effect was also observed in the absence of plasma proteins, pointing at contributing factors other than altered protein binding and increased affinity of paclitaxel for CEL-induced lipoprotein degradation products (81,82).

For the purpose of finding out a potential paclitaxel delivery vehicle with an ideal profile, the investigation of several delivery vehicles based on the chemical structures of CEL and Tween 80 led to alteration of blood distribution of paclitaxel in presence of all tested vehicles (97). Different formulation approaches such as liposomes and poloxamer-micelles affected the pharmacokinetics of paclitaxel in mice as compared to the CEL-containing formulation (98). In contrast, paclitaxel administered in a solvent-free formulation in a clinical study showed a higher efficacy combined with reduced adverse effects compared to the drug delivered in a solution of CEL (99).

Overall, numerous investigations have shown that CEL can play a pivotal role in the pharmacological behavior of the formulated drugs. In addition, several drug-drug interactions are reported on agents administered intravenously in conjunction with CEL-containing formulation of other compounds, namely paclitaxel (100,101,102,103), cyclosporin (104,105,106,107), and valspodar (108,109). Most likely, the presence of CEL in drug formulations contributes to the observed pharmacokinetic interactions. Indeed, recent experiments revealed a substantial increase of plasma concentrations of cyclosporin after an additional injection of another drug preparation containing CEL (110).

1.5.2 Cyclodextrins

Cyclodextrins are ring-shaped oligosaccharides with a hydrophilic exterior and a hydrophobic interior (111). The interior cavity is capable of forming water-soluble complexes with many drugs. Due to the rapid release of a drug from the complex after administration *in vivo*, it is assumed that drug-cyclodextrin complexes do not affect the drug pharmacokinetics (112). However, if the drug is slowly or incompletely released from the complex, drug dosing as complexes in cyclodextrin can be critical.

The binding of drugs to plasma proteins was influenced *in vitro* in the presence of β-cyclodextrin (113) and hydroxypropyl-β-cyclodextrin (HP-β-CyD) (114,115). The intravenous administration of flurbiprofen in HP-β-CyD led to transient higher tissue concentrations in rats (114). Alterations in tissue distribution were also found for other drugs injected as cyclodextrin complexes either free in solution (116,117) or included into liposomes (118). Following iv dosing in HP-β-CyD, a higher amount of carbamazepine appeared in the urine compared to oral preparations (122). A similar trend was observed in dogs treated iv with either dexamethasone formulated in HP-β-CyD or as its phosphate prodrug (123). In addition, cyclodextrins interacts with endogenous lipids such as lipoproteins (119,120) and cholesterol (113,121).

1.5.3 Tween 80

As mentioned above for CEL, lipoprotein alteration induced by Tween 80 was observed (80). However, this effect was not confirmed in a further study (85). In patients receiving Tween 80 co-administered with etoposide, an increase of the volume of distribution and the clearance of doxorubicin was detected due to reduced plasma concentrations of doxorubicin during the early phase of the concentration-time profile (124). Lately, changes in the blood/plasma ratio of paclitaxel were described in the presence of Tween 80 and other solubilizers structurally related to Tween 80 (97). More recently, it was shown that Tween 80 has a concentration-dependent influence on the normal binding of docetaxel to serum proteins leading to changes in pharmacokinetics of docetaxel *in vivo* (125) although Tween 80 is

degradated rapidly by esterases in plasma (126). The mechanistic basis for altered plasma binding of docetaxel in the presence of Tween 80 still needs to be clarified.

1.5.4 Other excipients

To date, little is reported in the literature about the impact of Solutol HS 15 and Poloxamer 188 on blood distribution of drugs. An interference between Solutol HS 15 and the co-administered ketochlorin photosensitizer C8KC was suggested by Woodburn (127). The similar half-lives of Solutol HS 15 and the sensitizer found in mice indicate the correlation of the persistence of C8KC in plasma with that of the vehicle. Further, recent plasma protein binding interaction studies demonstrated an apparent increase in the unbound fraction of propranolol in combination with Poloxamer 188 (128). Also the administration of compounds formulated in mixed micelles can alter the protein binding (129). Most notably compounds binding with high affinity but low capacity to α_1-acid glycoprotein showed free fractions increased by 50 to 85%. Moreover, blood protein interactions can occur with dosing vehicles like liposomes (130), thereby affecting maybe the fate of co-administered drugs in blood and body (131).

1.5.5 Nanoparticles

Methyl methacrylate nanoparticles of 130 nm in size suspended in different concentrations (0.1-5%) of Tween 80 or poloxamine 908 exhibited prolonged circulation time with altered tissue concentrations as compared to uncoated nanoparticles (132). Extended blood circulation time was also found for polystyrene nanoparticels (40-137 nm) coated with poloxamer 407 (133).

1.6 Objectives and specific aims

The main purpose of this thesis was to investigate *in vitro* drug-excipient interactions in blood and to assess the implications of the *in vitro* findings both for the *in vivo* situation and the formulation strategy. Compounds in drug development at Novartis were chosen as model substances and dosed at concentrations ranging from sub-therapeutic to low pharmacological levels. Excipients commonly used in formulations were selected, including CEL, HP-β-CyD, Solutol, PEG 200, and TPGS. The following specific aims of the thesis were:

1. To collect and use available compound information, including physicochemical properties and pharmacokinetics, to select appropriate model substances with as many different properties as possible
2. To determine the hemolytic activity of selected excipients to rule out any changes of blood distribution caused by hemolysis
3. To explore *in vitro* possible effects of selected excipients in the blood, with special emphasis on the blood distribution and plasma protein binding of model compounds
4. To identify the pharmacokinetic profile and tissue distribution of model compounds following single intravenous dosing in the presence and absence of selected excipients
5. To compare and relate pharmacokinetic outcomes to the *in vitro* findings, thereby assessing the impact of *in vitro* data for the *in vivo* situation and evaluating the *in vitro-in vivo* correlation
6. To generate criteria for optimizing delivery vehicle selection in drug research that allow reducing drug-excipient interactions and leading to more rational and selective drug formulations
7. To propose an intravenous formulation strategy based on the data generated by this research project to provide better candidate-tailored formulations in drug development

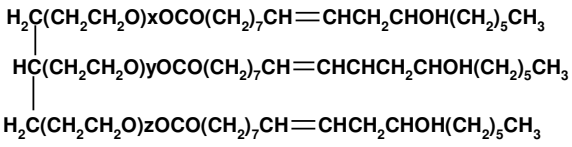
2 Selection and experimental procedure

2.1 Excipients and model compounds

Investigations involved five excipients along with five pharmacologically active compounds exhibiting different properties.

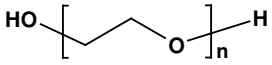
The excipients CEL, HP-β-CyD, Solutol, and PEG 200 were selected based upon their common use in intravenous formulations and their diversity of molecular structure and solubilization technique (Figure 2-1 and Table 2-1). CEL and Solutol are surface-active agents which increase the drug solubility by incorporation of the drug into a micellar structure. Whereas CEL exhibit a highly variable composition with the major hydrophobic component (~87%) identified as oxyethylated triglycerides of ricinoleic acid (Figure 2-1), Solutol consists of ~70% lipophilic polyglycol mono- and di-esters of 12-hydroxystearic acid and ~30% hydrophilic polyethylene glycol. HP-β-CyD is a cyclic (α-1,4)-linked oligosaccharide containing seven α-D-gluco-pyranose units (Figure 2-1) which form a relatively hydrophobic central cavity and a hydrophilic outer surface. The inclusion of a drug within the inner core of the complexing agent and the interaction of the outer core with water render the complex soluble. PEG 200 is often used as a cosolvent for improving solubility of preclinical compounds by interrupting the hydrogen structure of water (e.g. water exclusion) and lowering the dielectric constant of the solution. Although TPGS is exclusively known in oral formulations, it was chosen due to its chemical structure (benzyl ring) and drug interaction potential at the level of active transporters and metabolizing enzyme systems.

Cremophor EL

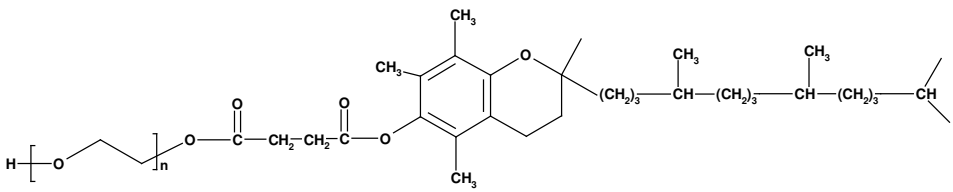


primary constituent with x + y + z ~35

Polyethylene glycol 200

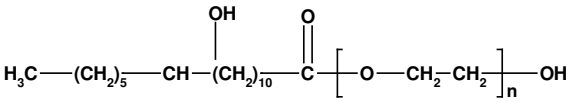


Vitamin E TPGS

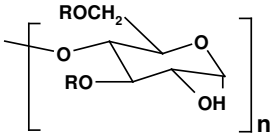


n=20-22

Solutol HS 15



Hydroxypropyl-β-cyclodextrin



glucopyranose with R=CH_2CH_2OH or H and n=7

Figure 2-1 Chemical structures of selected excipients

Table 2-1 Properties of selected excipients

Excipient name	Type	Solubilization approach	Use in iv formulation	Biological activity
Cremophor EL (polyoxyethylene castor oil derivatives) MW ~3000 CMC ≥0.09 mg/mL	Non-ionic surfactant	Micelles	Yes (developmental & commercial)	• Dyslipidaemia • Inhibition of active transporters
Hydroxypropyl-β-cyclodextrin MW ~1600	Oligomeric substance	Complexation	Yes (developmental & commercial)	• Lipid interactions
Solutol HS 15 (polyethyleneglycol 660 12-hydroxystearate) MW 960 CMC ≥0.2 mg/mL	Non-ionic surfactant	Micelles	Yes (developmental & commercial)	• Dyslipidaemia • Inhibition of active transporters • Inhibition of cyto-chrome enzymes
Polyethylene glycol 200 MW ~200	Oligomeric substance	Cosolvent (change of solution polarity)	Yes (developmental & commercial*)	
Vitamin E TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate) MW ~1513 CMC ≥0.2 mg/mL	Non-ionic surfactant	Micelles	No (oral use: developmental & commercial)	• Inhibition of active transporters • Inhibition of cyto-chrome enzymes

CMC: Critical micelle concentration, **MW**: Molecular weight, *: Higher molecular weight PEGs such as PEG 300 and 400

Drug candidates in development at Novartis were chosen as model compounds regarding aqueous solubility, lipophilicity, membrane permeability, and blood cell/plasma distribution (Figure 2-2 and Table 2-2). COM2 and COM1 (base) are lipophilic and poorly water-soluble PET ligands which are used in sub-therapeutic doses, and COM2 distributes equally between plasma and whole blood. COM3 is much better water-soluble and is mainly located in the cellular fraction in blood. In contrast, COM4 with a low lipophilicity penetrates hardly into blood cells and distributes poorly into tissues. COM5 is a bigger molecule characterized by a high polar surface area, many H-bond acceptors, and a very low volume of distribution similar to that obtained for COM4.

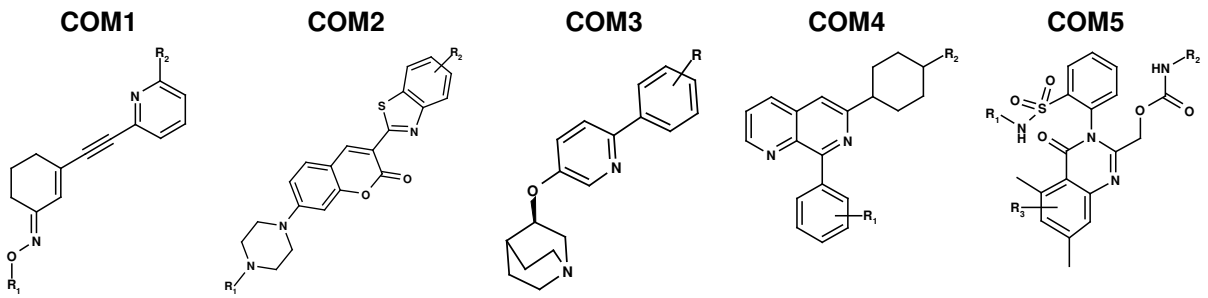


Figure 2-2 Chemical structures of model compounds

Table 2-2 Properties of model compounds

Physicochemical (PC) and pharmacokinetic (PK) data available at the time of selecting model compounds for investigating drug-excipient interactions in blood.

	COM1 PET ligand	**COM2** PET ligand	**COM3** NCE	**COM4** NCE	**COM5** NCE
PC properties					
MW (g/mol)	240	410	295	<400	533
LogD 6.8	3.4	4.5	2.3	1.7	3.7
pKa	3.8	5.7	4.1, 8.7	3.2, 4.6	10.6
H_2O solubility, pH 6.8 (mg/L)	20	<2.5	4000	<500	100
PSA ($Å^2$)	35	50	25	63	166
H-bond acceptors	3	5	3	4	12
H-bond donors	0	0	0	1	3
PK properties					
Species		Mouse	Rat	Rat	Rat
In vitro					
Fraction in plasma (%)		45	20	~100	80
Free in plasma (%)			12	2.4	11
In vivo					
Matrix		Blood	Blood	Plasma	Plasma
$t_{1/2}$ (h)		1.1	1.1	10	0.4
CL (mL/min/kg)		10	142	0.4	7.2
V_{ss} (L/kg)		14	10	0.3	0.3
$f_{unchanged}$ (%)		49	14	98	94
Main selection criteria	• Active principle • H_2O sol. (base)	• Active principle • H_2O sol. • Lipophilicity	• Blood distribution	• Blood distribution • V_{ss}	• MW • Polarity • V_{ss}

$f_{unchanged}$: Fraction of unchanged drug based on AUC ratio of parent drug and total radioactivity, **LogD**: Logarithm of octanol-water distribution coefficient, **MW**: Molecular weight, **NCE**: New chemical entity, **pKa**: Negative logarithm of dissociation constant, **PSA**: Polar surface area, **CL**: Drug clearance, **$t_{1/2}$**: Main elimination half-life, **V_{ss}**: Volume of distribution under steady-state conditions

2.2 Experimental setup

The effect of excipients on pharmacokinetic parameters was examined in rats except for COM2 which was investigated in mice due to available animal models appropriate to analyze drug target interactions if required. Model compounds were used in their clinically relevant blood range as follows: sub-therapeutic (<5 ng/mL) for COM1 and COM2, low-therapeutic (5-50 ng/mL) for COM3, and therapeutic (>50 ng/mL) for COM4 and COM5 because of low distribution volumes to assure detectable tissue concentrations. To allow *in vitro* and *in vivo* study comparisons, the amount of excipient in blood was set at ~0.5% which is within the range after an iv bolus injection in mice and rats.

Blood distribution and protein binding studies were done *in vitro* using model compounds in the appropriate concentration range with and without selected excipients fixed at 0.5% in the test system. Since COM4 is almost completely located in the plasma fraction in blood (F_P ~100%) independent of the concentration (10-10'000 ng/mL) (149), the concentration of COM4 was kept constant (100 ng/mL), whereas the excipient amount was varied between 0.01-1%. Compounds formulated as excipient-free solution in glucose 5% or saline served as reference. The excipient with the most prominent effect compared to the reference was selected for the *in vivo*

study where animals received intravenously a single dose as a control formulation or solution containing the selected excipient. Control formulation were based on glucose 5% (COM1, COM3), saline (COM4, COM5), or blank plasma (COM2). To assure a fast and complete solubility of COM3 and COM5 in the control formulation, convenient excipients were added with *in vitro* binding parameters similar to those obtained for the *in vitro* reference. The concentration of model compounds in blood, plasma, and tissue were measured until 1 h after iv administration, thereby including the scanning time of PET ligands. Moreover, it is assumed if excipient-induced changes occur they should be detectable in this time period.

3 Materials and methods

3.1 Chemicals

COM1 and COM2 were supplied by the Neuroscience Research Department of Novartis (Basel, Switzerland). COM3 was obtained from the Novartis Institutes for BioMedical Research (Basel, Switzerland). ^{3}H-radiolabeled COM1 (specific activity 11780 MBq/mg, >99%), COM2 (specific activity 2320 MBq/mg, >98%), and COM3 (base, specific activity 31.1 MBq/mg, >98%) were provided by the Isotope Laboratories of Novartis (Basel, Switzerland). ^{14}C-radiolabeled COM3 used for investigation of renal excretion (2·HCl salt, specific activity 5.87 MBq/mg, >98%), COM4 (specific activity 5.85 MBq/mg, >98%), and COM5 (specific activity 3.3 MBq/mg, >98%) were provided by the Isotope Laboratories of Novartis (Basel, Switzerland).

The excipients, purchased by the Pharmaceutical and Analytical Development Department of Novartis (Basel, Switzerland), were: Cremophor EL (CEL; BASF), hydroxypropyl-β-cyclodextrin (HP-β-CyD; CERESTAR USA Inc.), polyethylene glycol 200 (PEG 200; Fluka), Solutol HS 15 (Solutol; BASF), and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS; Eastman). All other chemicals and reagents were of analytical grade or will be described separately in the methods section.

3.2 Blood and plasma sources

Fresh blood was obtained from healthy male species (n≥3) as follows: mice (albino OF1, Charles River Laboratories, L'Arbresle, France), rats (Wistar HAN IGS, Charles River Laboratories, Sulzfeld, Germany), dogs (Marshall beagles, Marshall Farm, NY, USA and Harlan France SARL, Gannat, France), and humans (drug-free blood donors, Blutspendezentrum SRK Basel, Switzerland). Pooled plasma (n≥3) was defrosted from storage at -20°C. Lithium heparin was used as an anticoagulant for all species.

3.3 In vitro studies

Test compounds in the *in vitro* samples excluding protein binding samples of COM2 were quantified by LSC due to no major degradation (>95%) under investigated conditions (146,147,148,149,150). Protein binding samples of COM2 were quantified by LC-RID due to instability after longer incubation (>2 h) and very low fraction unbound in plasma (<2%).

3.3.1 Preparation of test solutions

For *in vitro* blood distribution and protein binding studies, test solutions were prepared by dissolving the radiolabeled test compound in ethanol. Ethanol was evaporated and the residue was reconstituted in the appropriate formulation, namely excipient-free solution as control (saline or glucose 5%) and solutions containing CEL/EtOH 65:35 (v/v), CEL, HP-β-CyD, Solutol, PEG 200, and TPGS. Final compound concentrations in blood or plasma were: 0.06-6 ng/mL (COM1), 0.01-100 ng/mL (COM2), 5-500 ng/mL (COM3), 100 ng/mL (COM4), and

10-300 ng/mL (COM5). Excipient concentrations were kept constant at 0.5% in the test system except for COM1 (0.5-5% due to no alterations at 0.5%) and COM4 (0.01-1% due to its plasma fraction, see 2.2).

3.3.2 Hemolytic activity

The hemolytic activity of the excipients was assayed using a spectrophotometric method. CEL/EtOH 65:35 (v/v), EtOH, HP-β-CyD, PEG 200, Solutol, and TPGS were added to the appropriate volume of freshly prepared heparinised whole blood to obtain a final excipient concentration of 0.5%. To avoid hemolysis due to higher concentrations during the adding, the blood was partially centrifuged and the excipient was pipetted in the cell-free layer. By tapping the test tubes, the samples were immediately mixed. Two control tubes were prepared, one for spontaneous hemolysis (pure blood used as the reaction blank) and another for 100% hemolysis (total cell lysis induced by sodium dodecyl sulfate at a final blood concentration of 1%). Samples were incubated at 37°C. At certain points of time, aliquots were removed and centrifuged for 10 min at 3100 x *g* (37°C) to obtain plasma. The absorbance of hemoglobin in the supernatant (dilution with water 1:200) was measured at 405 nm (Emax precision microplate reader, Bucher Biotech, Basel, Switzerland). The degree of hemolysis due to the excipient activity was calculated according to

$$Hemoylsis(\%) = \frac{A_e - A_0}{A_{100} - A_0} \times 100$$

where A_e is the absorbance of hemoglobin in the supernatant after incubation with excipient, A_0 is the absorbance of hemoglobin in the supernatant of the reaction blank, and A_{100} is the absorbance of hemoglobin in the supernatant after total cell lysis with sodium dodecyl sulfate. Hemolytic activity was considered to have started when mean values were greater than 2% of hemolysis.

3.3.3 Blood distribution

Freshly prepared heparinised blood was used, and experiments were performed in triplicate both in the presence and absence of excipients. The hematocrit was determined using a hematocrit centrifuge and a hematocrit reader (Haemofuge Heraeus Sepatech, Germany). In order to reduce hemolysis, blood aliquots (1 mL) were partially centrifuged (500 x *g* for 2 min) before adding the test solution in the cell-free layer, followed by mixing immediately. Samples were incubated at 37°C. Time aliquots (1 mL) were removed and prepared for measuring radioactivity of the test compound in whole blood before centrifugation and in plasma after centrifugation for 10 min at 3100 x *g* (37°C) by LSC.

The fraction of the test compound in plasma (F_P) was calculated according to

$$F_P(\%) = \frac{C_P \times (1 - H)}{C_B} \times 100$$

where C_B and C_P are the drug concentration in blood and plasma respectively, and H is the hematocrit value. The concentration in blood cells (C_{BC}) was calculated as follows:

$$C_{BC} = \frac{C_B - C_P \times (1 - H)}{H}$$

and used for calculations of blood cell to plasma concentration ratio (BCPR: C_{BC}/C_P) and blood cell to unbound in plasma concentration ratio (ρ: $C_{BC}/(C_P*fu)$). fu is the drug fraction unbound in plasma determined by the appropriate protein binding method for each compound.

3.3.4 Plasma protein binding

Control experiments in phosphate buffered saline (PBS, Gibco, Paisley, Scotland) were carried out to assess the suitability of the methods described below for each test compound in the following order: ultrafiltration > dialysis > ultracentrifugation, with ultrafiltration being the first procedure. Control experiments indicated that ultrafiltration is a suitable method for COM1, COM3, COM4, and COM5 (free-permeation >0.75, recovery >85%) and ultracentrifugation for COM2 (no sedimentation after 6-h centrifugation, recovery >85%; ultrafiltration and dialysis showed insufficient recovery and free-permeation). Therefore, protein binding was determined by the ultrafiltration technique (COM1, COM3, COM4, and COM5) or the ultracentrifugation technique (COM2).

Ultrafiltration

Samples of spiked plasma were incubated at 37°C until binding equilibrium. Aliquots of 1 mL were introduced in prewarmed (37°C) Centrifree micropartition tubes (Amicon Inc., Beverly, MA, USA) and centrifuged for 10 min at 2000 x *g* (37°C). For the determination of the unbound drug fraction in plasma, concentrations of the test compound in ultrafiltrate and plasma were measured. The unbound fraction in plasma (fu) was calculated as follows: $fu(\%)=(C_{UF}/C_P)x100$, where C_{UF} and C_P are the drug concentration in ultrafiltrate and in plasma, respectively.

Equilibrium dialysis

Test solution was added to plasma followed by mixing. Dialysis was carried out with 150 µL of this sample against an equal volume of phosphate-buffered saline (pH 7.2) in a 96-well micro-equilibrium dialysis block (HTDialysis LLC, Gales Ferry, CT, USA). Dialysis membranes with a 12000-14000 molecular weight cut-off were soaked in phosphate buffered saline (pH 7.2) before use. After establishment of the equilibrium, buffer solution aliquots, containing only unbound drug, and plasma aliquots, containing both bound and unbound drug, were analyzed for the test compound. The ratio of drug concentrations measured in the buffer and plasma after dialysis was taken as an estimate of unbound drug fraction in plasma.

Ultracentrifugation

Samples of spiked plasma were incubated at 37°C until binding equilibrium. Aliquots of 1 mL were transferred to polycarbonate centrifuge tubes (Beckman) and either centrifuged in a TLA 100.2 rotor in Beckman TL 100 centrifuge (200000 x *g*, 6 h, 37°C) or incubated for 6 h (37°C). After centrifugation, samples were separated into three layers according to density. A 80-µL aliquot of the middle layer (protein-free part/plasma water) was taken and analyzed for the test compound, representing the unbound concentration in plasma (C_U). Total plasma concentration (C_P) was

determined in incubated samples. The unbound drug fraction in plasma was calculated using C_U/C_P.

Determination of major binding protein

The affinity of test compounds to different plasma proteins was determined using the appropriate method for each compound. Purified human plasma proteins were dissolved in phosphate buffered saline (PBS, Gibco, Paisley, Scotland) at physiological concentrations as follows: albumin 40 g/L (≥96%, Sigma), α-acid glycoprotein 1 g/L (from Cohn Fraction VI, 99%, Sigma), γ-globulins 12 g/L (from Cohn Fraction II and III, Sigma), high density lipoprotein 3.9 g/L (>95%, Calbiochem), low density lipoprotein 3.6 g/L (>95%, Calbiochem), and very low density lipoprotein 1.3 g/L (>95%, Calbiochem). Test solution was added to protein solutions to obtain a compound concentration of 10 ng/mL (COM1, COM2) or 1000 ng/mL (COM3, COM4, COM5). After incubation at 37°C, separation of bound and unbound compound was achieved according methods. Ultrafiltration was performed by centrifugation for 10 min for samples containing albumin and γ-globulins and for 2 min for all other samples.

3.3.5 Determination of protein concentration

Protein concentration was measured by the method of Bradford (Coomassie blue protein assay) at 595 nm by using a Bio-Rad protein assay (Bio-Rad Laboratories, München, Germany). The protein concentration was determined by using a calibration curve that was established with known concentrations of human serum albumin (≥96%, Sigma) ranging from 0 to 0.5 mg/mL. 10-µL aliquots of plasma (1:200 dilution) and plasma water were pipetted into microtiter plate wells. 200 µL dye reagent were added, and samples were mixed. After 1-h incubation at room temperature, absorbance was measured.

3.4 In vivo studies

Samples collected after intravenous administration of COM1, COM2, and COM3 were assayed for radioactivity by LSC and parent drug by LC-RID. COM4 and COM5 were quantified in all *in vivo* samples only by radioactivity measurements (LSC) since the radioactivity of both radiolabeled compounds reflects well the parent drug due to no major degradation at 1 h after intravenous administration in rats (151,152).

3.4.1 Experimental animals

Male Wistar rats (~250 g) and male OF1 mice (~30 g) were obtained from Charles River (Sulzfeld, Germany). All animals were housed in standard cages in a controlled environment maintained on an automatic 12-h lighting cycle at a temperature of 22°C according to institutional guidelines. The animals were given a standard chow and water *ad libitum*. The animals were used after having been starved overnight.

3.4.2 Drug administration and sample collection

All dosing solutions were prepared within 1 h prior to injection and stored at room temperature until use. Administration was performed by a single bolus injection into the femoral vein after animals had been lightly anesthetized by isoflurane (Forene®). Rats received [^{3}H]COM1 at 4 µg/kg as solution (1 mL/kg) in glucose 5% containing

ethanol 1% (v/v) or TPGS 20% (w/v). Mice were injected a dose of 400 ng/kg of [^{3}H]COM2 formulated as solution (5 mL/kg) in blank plasma (obtained by centrifugation of freshly drawn mouse blood) or in glucose 5% containing TPGS 10% (w/v). An iv dose of 1 µmol/kg radiolabeled COM3 (^{3}H: 300 µg/kg, ^{14}C: 370 µg/kg) in EtOH/PEG200/Glu 5:5:90 (v/v/v) or 40% HP-β-CyD (w/v) was injected to rats (1 mL/kg). [^{14}C]COM4 was administered at 400 µg/kg in saline or 17% CEL (v/v) to rats (2 mL/kg). [^{14}C]COM5 at 1 mg/kg in saline containing either ethanol 10% (v/v) or 17% Solutol (w/v) were injected to rats (2 mL/kg).

Using these injection preparations, excipient concentrations in blood may be estimated as about 0.3% (COM1), 0.5% (COM3, COM4, COM5), and 0.7% (COM2) in animals (~70 mL blood/kg). These concentrations were similar to those used in the *in vitro* experiments.

Samples were collected after drug administration at 0.08, 0.25, and 0.5 h for COM1 and at 0.08, 0.25, 0.5, and 1 h for COM2, COM3, COM4, and COM5. Animals (n=3 per time point) were sacrificed by isoflurane inhalation for sample collection. Blood samples were collected from the vena cava and transferred into tubes containing heparin (heparin-Na, B.Braun) as anticoagulant. Plasma samples were obtained by immediate centrifugation of blood samples at 3000 x *g* for 10 min. Tissues were excised, blotted dry, and weighed. Collected tissue comprised lung, heart, liver, kidney, fat, muscle, skin, and brain for COM1, COM2, COM3 and lung, muscle, and skin for COM4 and brain, muscle, and skin for COM5. All samples were immediately frozen and stored at -20°C until analysis. Tissue samples were homogenized before quantification.

3.4.3 Bladder catheterization and urine collection

The experiment was performed *in situ* under anesthetized rats. Animals (n=3/formulation) received im injections of ketamine hydrochloride at a dose of 50 mg/kg (100 mg/mL, 0.5 mL/kg) and are positioned on an isothermal heating pad prewarmed at 38°C. The abdomen was opened through a mid-line incision. A polyethylene tubing (Clay-Adams PE-50) was inserted into the dome of the bladder and held in place with a purse string suture. The formulation was injected into the surgically exposed femoral vein, and urine was collected at 0.5, 1, 1.5, and 2 h after dosing. All samples were frozen and stored at -20°C until analysis.

3.4.4 Ex vivo protein binding

Ex vivo protein binding was determined for COM1, COM3, COM4, and COM5 according to the *in vitro* procedure. Briefly, remaining plasma samples of each time point were pooled, and the unbound drug concentration in plasma was quantified using the ultrafiltration technique (see 3.3.4). After centrifugation, plasma and ultrafiltrate samples were assayed for radioactivity by LSC and parent drug by LC-RID.

3.5 Measurement of the radioactivity

Aliquots of blood, plasma, urine (25-50 µL) and homogenates (250 µL) were introduced into counting vials and solubilized in Biolute-S (Zinsser Analytic). Samples obtained from *in vivo* studies containing tritium-labeled drug were dried, and the residue was reconstituted in water before solubilization. To the blood samples,

hydrogen peroxide 30% was additionally added, and vials were gently swirled for several seconds and let stand for 30 min. After adjusting pH >7 by addition of hydrochloric acid 2 N, the vials were filled with scintillation cocktail (Irgasafe Plus, Zinsser Analytic), kept in the dark for 16 h, and measured in a Tri-Carb liquid scintillation spectrometer Model A2200 (Packard).

3.6 Determination of parent drug

^{3}H-radiolabeled COM1, COM2, and COM3 were determined by a liquid chromatography-reverse isotope dilution method (LC-RID). A sample aliquot (100-500 µL) and 200 µL water containing 5 µg (COM1, COM3) or 2 µg (COM2) non-radiolabeled test compound as internal standard was added to a glass tube. After further addition of 1 mL water, 100 µL Titrisol buffer (pH 4: COM1, COM2; pH 7: COM3), and 4 mL diethyl ether (COM1, COM2) or tert-butylmethylether (COM3), tubes were shaken for 30 min and centrifuged (3300 *x g* for 10 min). The organic layer was collected in another glass tube and evaporated in a vacuum centrifuge (Univapo 150H, UniEquip, Martinsried, Germany). The residue was taken up in 250 µL of mobile phase-water (80:20, v/v) and 75 µL n-hexane, and the mixture was transferred in an auto sampler glass vial. After centrifugation (13000 x *g* for 2 min), the hexane layer was discarded, and 200 µL of the remainder was injected into the HPLC system equipped with a Supelcosil LC-18 column (5 µm, 4.6 mm x 150 mm) for COM1 or Waters XTerra RP 8 column (5 µm, 3.9 x 150 mm) for COM2 and COM3. The column temperature was 40°C, and the absorbance was detected at a wavelength of 312 nm (COM1), 441 nm (COM2), or 261 nm (COM3). The mobile phase (isocratic gradient) consisted of ammonium acetate 10 mM-acetonitrile (45:55, COM1; 50:50, COM2) or ammonium acetate 10 mM-triethylamine 0.1% in acetonitrile (58:42, COM3) and was pumped at a rate of 1.0 mL/min. The peak corresponding to the unchanged compound was collected in a polyethylene vial by a fraction collector (Pharmacia LKB SuperFrac) and analyzed for radioactivity. Concentrations of the test compound in samples were calculated from the ratio of the amount of radioactivity in the eluted fraction and the area of the absorbance of the non-radiolabeled test compound used as internal standard.

3.7 Data analysis

Total radioactivity concentrations, expressed as ng-eq/mL or ng-eq/g, were estimated by dividing the radioactivity concentration in samples by the specific radioactivity of administered test compound using Microsoft Excel. Concentrations of parent drug were determined by the principle of reverse isotope dilution using following equation in Microsoft Excel

$$\frac{A_{AS}}{A_{IS}} = \frac{A_{AD}}{A_{ID}}$$

where A_{AS} is the amount of analyte in the sample (unknown, to be determined), A_{IS} is the amount of internal standard added to the sample, A_{AD} is the amount of analyte detected, and A_{ID} is the amount of internal standard detected. A_{AD} was calculated using R/(SRxS) where R is the amount of radioactivity determined in the peak fraction, SR is the specific radioactivity, and S is the slope. The amount of internal standard detected was calculated as A_{ID}=Area/RF-A_{AD} where RF is the response factor (Area/ng). The level of quantification (LOQ) was set to 75 dpm. LOQs of

radioactivity and test compound in blood, plasma, urine, and tissues were calculated by dividing 75 dpm by the specific radioactivity of the administered test compound and by the sample amount. P values were calculated with a two-sample t-test in Microsoft Excel assuming unequal variances. The level of significance was set at $P < 0.05$.

3.8 Pharmacokinetic analysis

Pharmacokinetic parameters were estimated as follows: The area under the drug concentration-time curve (AUC) was determined by the linear trapezoidal rule using the mean data points. The half-life ($t_{1/2}$) was calculated using ln2/k, where k is the rate constant. k was estimated by the slope of the regression line plotted through the three final data points of the semilogarithmic AUC, taking into consideration a square of correlation coefficient of RSQ >0.90. Volume of distribution (V_0) was calculated by dividing the dose by the concentration at time zero (C_0). C_0 was obtained by extrapolation to zero time of the concentration-time plot in semilogarithmic scale.

4 Results and discussions

4.1 Hemolytic activity of excipients

In vitro results

CEL/EtOH 65:35, EtOH, HP-β-CyD, PEG 200, and Solutol did not induce hemolysis in dog and human blood at 0.5% and a contact time of 4 h (data not shown). In contrast, TPGS at 0.5% incubated with blood of various species caused hemolysis in a time-dependent manner (Figure 4-1). Erythrocytes from rat and human were more sensitive than those of mouse and dog, indicated by cell lysis at shorter contact times. Reducing the TPGS concentration from 0.5% to 0.1% induced no hemolysis in all four species in the investigated time range (data not shown).

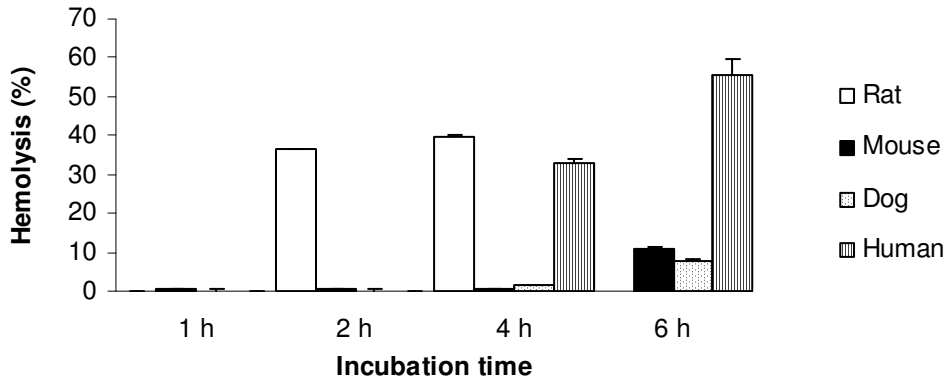


Figure 4-1 Effect of incubation time on the hemolytic activity of TPGS

Induced hemolysis by 0.5% TPGS in blood of various species (n=3, mean ± SD). Hemolysis in rat blood after 6-h incubation was not determined.

Discussion

Except for TPGS, all tested excipients (CEL, EtOH, HP-β-CyD, Solutol, and PEG 200) were non-hemolytic which is consistent with data reported in the literature (134,135,136,137,138) and the fact that they are widely used in commercially available parenteralia (54). TPGS at 0.5% exhibited marked hemolysis after longer contact time (>1 h), whereas TPGS at 0.1% showed no hemolysis under equal incubation conditions. The detected hemolysis might possibly result not mainly from TPGS but from metabolites, namely α-tocopheryl succinate and polyethylene glycols, both being able to destruct erythrocytes (134,139,140). This phenomena could contribute to the extensively delayed onset of hemolysis. For the investigations, TPGS at 0.5% was used in the non-hemolytic time range.

4.2 Impact of the hematocrit on blood partition parameters

In vitro results

Whole blood derived from three species was incubated with COM2 (100 ng/mL) at varying hematocrit values. Concentrations of COM2 in blood and plasma were measured at equilibrium, and partition parameters calculated from these data are summarized in Table 4-1. Concentrations in blood, plasma, and blood cells remained unaffected by the hematocrit value (0.40-0.60). The partition parameter BPR was also similar over the investigated hematocrit range, whereas BCPR changed slightly and F_P distinctly, both decreasing by increasing the hematocrit from 0.40 to 0.60.

Table 4-1 Effect of hematocrit on the *in vitro* blood distribution of COM2

Blood cell concentrations and partition parameters (F_P, BPR, and BCPR) derived from [^{3}H]COM2 concentrations measured in blood and plasma using same blood pools at different hematocrit values (n=3, mean ± SD).

Species	Hematocrit	Concentration (ng/mL)			F_P	BPR	BCPR
		Blood	Plasma	Blood cells	(%)		
Mouse	0.40				50 ± 1	1.21 ± 0.03	1.52 ± 0.07
	0.45				46 ± 2	1.20 ± 0.06	1.44 ± 0.13
	0.50	101 ± 3	87 ± 4	116 ± 8	43 ± 0	1.16 ± 0.01	1.32 ± 0.01
	0.55				40 ± 1	1.13 ± 0.04	1.23 ± 0.06
	0.60				36 ± 1	1.12 ± 0.03	1.19 ± 0.05
Dog	0.40				48 ± 2	1.26 ± 0.06	1.66 ± 0.14
	0.45				44 ± 1	1.25 ± 0.04	1.57 ± 0.09
	0.50	109 ± 4	90 ± 2	131 ± 15	41 ± 1	1.25 ± 0.03	1.51 ± 0.05
	0.55				38 ± 1	1.19 ± 0.03	1.35 ± 0.06
	0.60				35 ± 1	1.13 ± 0.02	1.22 ± 0.03
Human	0.40				70 ± 3	0.86 ± 0.03	0.66 ± 0.08
	0.45				65 ± 2	0.84 ± 0.03	0.65 ± 0.07
	0.50	101 ± 5	125 ± 10	78 ± 6	63 ± 2	0.80 ± 0.03	0.60 ± 0.06
	0.55				55 ± 2	0.82 ± 0.03	0.68 ± 0.06
	0.60				55 ± 1	0.73 ± 0.02	0.55 ± 0.03

Discussion

The *in vitro* method for investigating distribution of drugs in blood commonly uses whole blood freshly prepared and pooled. Drug concentrations in blood and plasma are determined. Based on these data, further partition parameters, including C_{BC}, F_P, BPR, and BCPR, can be estimated, but they are partially dependent on the hematocrit. Therefore, it is important to know how the hematocrit affects these parameters, thereby providing useful information for comparing results. With this in mind, present experiments were performed over the entire physiological hematocrit range in blood pools of three different species (mouse, dog, and human). COM2 was used as test compound due to sufficient availability.

The rank order of hematocrit influences was F_P > BCPR > BPR > $C_B \approx C_P \approx C_{BC}$ with most pronounced changes for F_P and none for $C_B/C_P/C_{BC}$. Parameters calculated from concentrations measured in samples decreased constantly with increasing the hematocrit (0.40-0.60), which was most distinct for F_P. But within a hematocrit variation of 0.05 none of the parameters was dependent on the

hematocrit. Consequently, blood partition data obtained from *in vitro* experiments with similar hematocrits are consistent and can be compared together. For data comparison across studies, hematocrit adjusting to values of previous studies is suggested taking into consideration a difference of ≤0.05 between the lowest and highest value.

4.3 Major binding proteins of model compounds

In vitro results

Figure 4-2 illustrates the qualitative binding of model compounds to isolated proteins compared to the total fraction bound in plasma. The following ranking was obtained with regard to decreasing order of protein binding:

COM1: albumin > α1-acid glycoprotein > γ-globulins ≈ lipoproteins;

COM2: albumin > lipoproteins > γ-globulins >> α1-acid glycoprotein;

COM3: α1-acid glycoprotein > albumin > γ-globulins >> lipoproteins;

COM4: albumin > α1-acid glycoprotein >> γ-globulins ≈ lipoproteins;

COM5: albumin ≈ α1-acid glycoprotein >> γ-globulins ≈ lipoproteins.

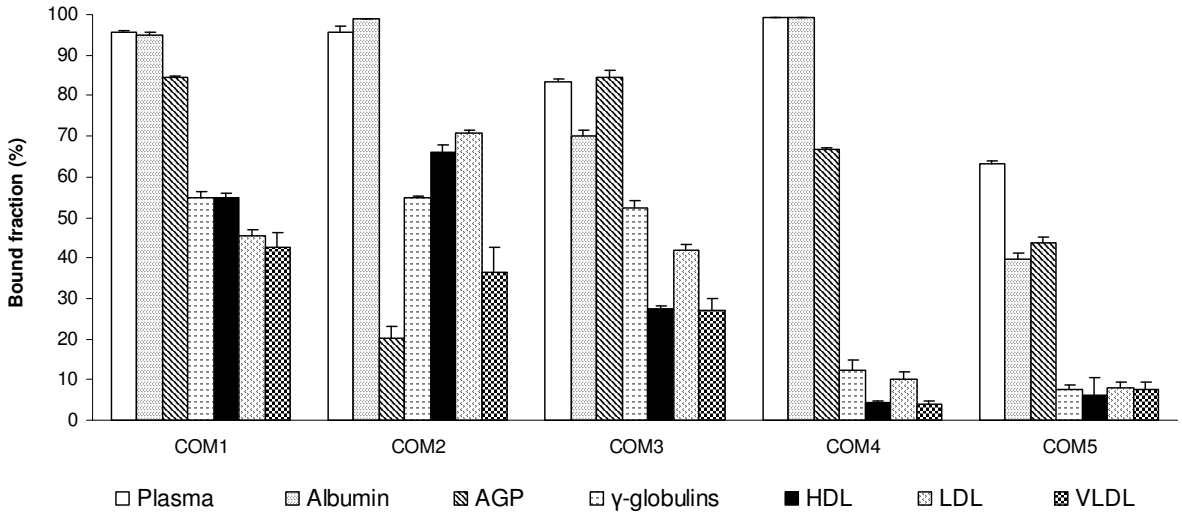


Figure 4-2 Qualitative differences in protein binding patterns of model compounds *in vitro*

Total protein-bound fraction of compounds in human plasma compared to the qualitative extent of compound binding to various isolated human proteins (albumin, AGP, γ-globulins, and lipoproteins such as HDL, LDL, and VLDL). Each bar represents mean ± SD (n=3).

Discussion

In vitro experiments showed the binding of model compounds with different degrees to the three major drug-binding proteins in plasma (albumin, α1-acid glycoprotein, lipoproteins). A high binding to albumin (A) and α1-acid glycoprotein (AGP) was found for COM1 and COM4 (A>AGP), COM5 (A≈AGP), and COM3 (A<AGP). In contrast, COM2 was highly bound to albumin and lipoproteins.

4.4 The impact of Vitamin E TPGS on COM1 in rat

In vitro results

The equilibrium of COM1 between plasma and blood cells was reached within few minutes (<5 min, data not shown), and the fraction of COM1 distributed in plasma (~75%) was independent of initial blood concentrations of COM1 (0.06-6 ng/mL) and of excipients at 0.5% (Table 4-2). Enhancing the excipient concentration to 5% in blood resulted in decreased COM1 in blood cells, especially in the presence of TPGS (Table 4-2). In plasma, COM1 was highly protein bound with high contribution of albumin and α1-acid glycoprotein in binding (Figure 4-2), and a lower free fraction was found for COM1 in TPGS at 5% (Table 4-2). TPGS was taken for the *in vivo* study due to the most pronounced changes observed in the *in vitro* experiments.

Table 4-2 Effect of excipients on blood distribution and protein binding of COM1 *in vitro*

Partition parameters of [^{3}H]COM1 at 0.06-6 ng/mL obtained at equilibrium after incubation with and without excipients in rat blood (pH 7.4, H 0.46 ± 0.03, n ≥3, mean ± SD).

Excipient		F_P	BPR	BCPR	fu	ρ
	(%)	(%)			(%)	
None		72.8 ± 2.2	0.71 ± 0.02	0.41 ± 0.04	4.1 ± 0.2	9.9 ± 1
HP-β-CyD	0.5	79.8 ± 1.7	0.65 ± 0.01	0.27 ± 0.02	nd	nd
	5	88.2 ± 2.4	0.63 ± 0.02	0.17 ± 0.04	nd	nd
Solutol	0.5	70.2 ± 2.4	0.73 ± 0.01	0.43 ± 0.01	nd	nd
	5	87.8 ± 2.3	0.64 ± 0.01	0.18 ± 0.02	nd	nd
TPGS	0.5	80.2 ± 2.4	0.64 ± 0.01	0.26 ± 0.01	3.9 ± 0.7	6.6 ± 0.3
	5	95.6 ± 2.0	0.63 ± 0.01	0.08 ± 0.03	1.7 ± 0.3	5.3 ± 1.5

In vivo results

In contrast to the control group where a dose of 4 µg/kg was applied, COM1 was administered in the TPGS group at 2.7 µg/kg due to little compound availability. Data presented within this section are normalized to a dose of 1 µg/kg.

Independent of the formulation, plasma levels declined very rapidly with an apparent half-life of 0.17 h, and COM1 was rapidly metabolized (<30% unchanged COM1 in plasma at 0.08 h post-dose). Metabolites were fast eliminated from body, indicated by a low recovery of administered COM1 shortly after dosing (<30% of administered dose recovered at 0.08 h post-dose).

Upon intravenous administration of COM1 as a TPGS-containing solution, lower total plasma concentrations combined with an increase of COM1 unbound in plasma by 50% were found in contrast to the control group (Table 4-3, Figure 4-3). However, free drug concentrations in plasma did not differ between both groups, resulting in similar AUCs (Table 4-3). This is in line with identical tissue concentrations observed in both groups (data not shown) since only the unbound drug is supposed to pass across membranes. Furthermore, COM1 in TPGS led to a lower fraction of parent drug in plasma ($f_{unchanged}$, Table 4-3) and higher metabolite-related concentrations in plasma (2-fold increase, Figure 4-3), indicating alterations in the kinetic profile of metabolites.

Table 4-3 Comparative plasma kinetics of COM1 with and without TPGS

Plasma concentrations and pharmacokinetic parameters of [^{3}H]COM1 administered intravenously in formulations based on glucose 5% (control) or TPGS 20% to rats (LOQ=2 pg/mL, n=3, mean ± SD). Data are normalized to a dose of 1 µg/kg. $AUC(u)_{0.08\text{-}0.5h}$ relates to area under unbound drug plasma concentration-time curve.

Time	Control	TPGS	Percentage of
(h)	COM1 in plasma (pg/mL)		control value
0.08	184 ± 77	110 ± 6	60
0.25	59 ± 13	39 ± 6	66
0.5	32 ± 11	20 ± 6	62
C_0 (pg/mL)	*215*	*132*	
$AUC_{0.08\text{-}0.5h}$ (pg·mL^{-1}·h)	*32*	*20*	
$AUC(u)_{0.08\text{-}0.5h}$ (pg·mL^{-1}·h)	*0.67*	*0.76*	
$t_{1/2}$ (h)	*0.17*	*0.17*	
$f_{unchanged}$ (%)	*18.7*	*5.5*	
fu (%)	*2.1 ± 0.1*	*3.8 ± 0.3*	

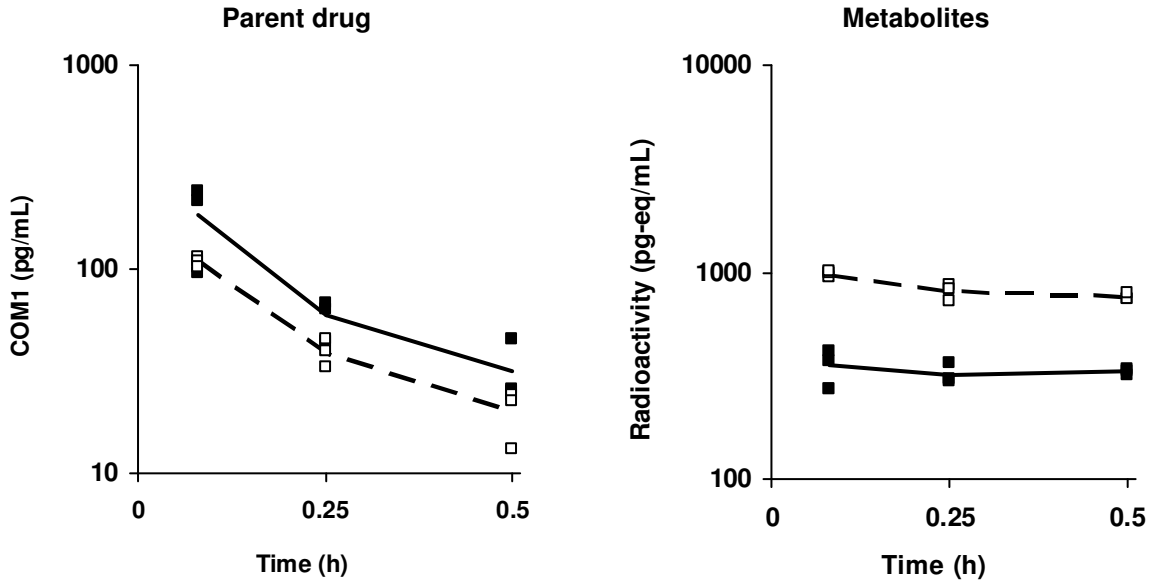


Figure 4-3 Influence of TPGS on the systemic exposure of COM1 and metabolites

Plasma concentration-time profiles of parent drug and metabolite-related radioactivity of [^{3}H]COM1 after intravenous administration to rats. COM1 was injected in glucose 5% (closed symbols, black line) or TPGS 20% (open symbols, dashed line). Data shown are normalized to a dose of 1 µg/kg, and symbols represent single values and lines mean values (n=3). Values of metabolite-related radioactivity were obtained by subtracting concentrations of parent drug from concentrations of total radioactivity.

Discussion

Drug-excipient interaction studies showed no direct correlation between the *in vitro* results and the *in vivo* situation in rats. Upon intravenous administration of COM1 in TPGS, slightly lower plasma concentrations and binding to plasma proteins were observed in animals as compared to those received COM1 in a TPGS-free solution. In contrast to these *in vivo* findings, COM1 displayed *in vitro* no alterations in the presence of TPGS at 0.5%, whereas a higher TPGS concentration (5%) led to

enhanced distribution into plasma and a higher fraction bound in plasma. Furthermore, very rapid metabolism and elimination of COM1 under *in vivo* conditions contributed to a pharmacokinetic profile inappropriate for studying drug-excipient interactions.

4.5 The impact of Vitamin E TPGS on COM2 in mouse

In vitro results

To assess whether the ultracentrifugation time could be shortened for minimizing the degradation of COM2 in plasma (147), blank plasma samples were centrifuged, and time aliquots were analyzed for total protein concentrations in plasma and plasma water. After 4-h centrifugation, protein levels in the plasma water section were below 0.05 ng/mL corresponding to ~0.1% of total plasma proteins adequate to assure a sufficient separation of plasma water and proteins. Therefore, samples were collected after 4-h centrifugation for analysis.

COM2 (0.01-100 ng/mL) distributed almost equally between whole blood and plasma (F_P ~45%) and was very highly bound to plasma proteins (>98%, mainly to albumin and lipoproteins) (Figure 4-2). The plasma fraction (63%) was enhanced for COM2 in TPGS at the beginning (<1-h incubation) followed by equalization to excipient-free incubations (Table 4-4, Figure 4-4). The presence of TPGS reduced also the free fraction in plasma by 30% (Table 4-4). Finally, TPGS was selected for the *in vivo* study because of the most pronounced drug-excipient interactions detected *in vitro*.

Table 4-4 Effect of excipients on blood distribution and protein binding of COM2 *in vitro*

Partition parameters of [^{3}H]COM2 at 0.1 ng/mL obtained at equilibrium after incubation with and without excipients (0.5%) in mouse blood (pH 7.3, H 0.45, n≥3, mean ± SD).

Excipient	Time (h)	F_P (%)	BPR	BCPR	fu (%)	ρ
None	0.08	40.6 ± 0.7	1.36 ± 0.02	1.79 ± 0.05	1.76 ± 0.03	102 ± 3
	1	44.8 ± 2.4	1.23 ± 0.07	1.51 ± 0.15		86 ± 8
CEL/EtOH, 65:35(v/v)	0.08	45.5 ± 1.4	1.21 ± 0.04	1.46 ± 0.09	nd	nd
	1	43.8 ± 0.3	1.25 ± 0.01	1.57 ± 0.02		nd
HP-β-CyD	0.08	47.2 ± 1.4	1.17 ± 0.09	1.38 ± 0.19	nd	nd
	1	49.4 ± 1.6	1.11 ± 0.04	1.25 ± 0.08		nd
Solutol	0.08	46.8 ± 1.1	1.18 ± 0.03	1.39 ± 0.06	nd	nd
	1	47.2 ± 1.6	1.17 ± 0.04	1.37 ± 0.09		nd
PEG 200	0.08	42.5 ± 1.3	1.29 ± 0.04	1.65 ± 0.09	nd	nd
	1	41.8 ± 2.0	1.32 ± 0.06	1.71 ± 0.14		nd
TPGS	0.08	62.9 ± 2.3	0.87 ± 0.03	0.72 ± 0.07	1.22 ± 0.12	58 ± 7
	1	53.3 ± 0.7	1.03 ± 0.01	1.07 ± 0.03		88 ± 3

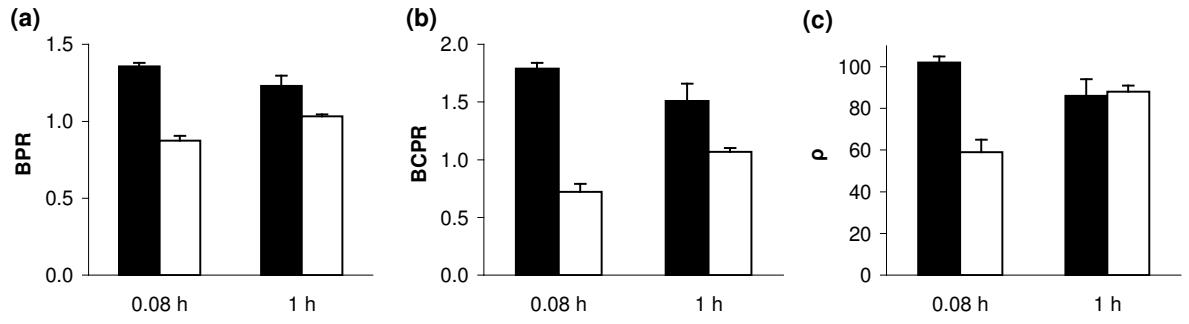


Figure 4-4 TPGS-mediated alteration of COM2 distribution in blood

Blood-plasma (a), blood cell-plasma (b), and blood cell-unbound in plasma (c) concentration ratios of [^{3}H]COM2 at 0.1 ng/mL in the presence (white bars) and absence (black bars) of TGPS (0.5%) after 0.08-h and 1-h incubation in mouse blood (pH 7.3, H 0.45, n≥3, mean ± SD).

In vivo results

In a previous study (153), the disposition kinetics of COM2 was evaluated in mice after iv administration in a solution containing HP-β-CyD 10% (5 mL/kg). To enable a comparison of former data with those in the current investigation for internal purposes only, COM2 was injected at a volume of 5 mL/kg dissolved in plasma or TPGS 10%. The blood concentration of TPGS from this injection preparation was estimated as ~0.7% assuming a blood volume of 72 mL/kg. Thus, the amount of TPGS in blood was higher *in vivo* compared to the amount used *in vitro* (0.5%).

The administration of COM2 as TPGS-containing solution at the same dosage caused approximately 2-fold higher plasma concentrations as compared to COM2 formulated in plasma (control) (Table 4-5, Figure 4-5). These findings are in line with data obtained in blood distribution studies *in vitro*, where partitioning into blood cells was reduced in the presence of TPGS, resulting in a higher concentration in plasma at the same total blood concentration. Furthermore, *in vivo* a higher plasma exposure to metabolites and a slower elimination of metabolites from the systemic circulation were found for COM2 in TPGS, indicated by 4-fold increased AUC and $t_{1/2}$ of metabolite-related radioactivity in plasma (Table 4-6). Determination of unbound COM2 in plasma could not be performed on samples from mice due to concentrations below LOQ (3 pg/mL).

No differences in tissue concentrations were observed between both groups, although COM2 in TPGS resulted in a decrease of V_0 (Table 4-5) and K_P (Table 4-7), both suggesting altered tissue distribution and being in line with higher drug accumulation in the circulation. Because the free drug fraction in plasma generally reflects more accurately distribution processes due to the ability of unbound drugs to pass through membranes and then reach the target organ, the free drug fraction determined *in vitro* was considered. Calculated tissue-unbound in plasma concentration ratios, $K_P(u)$, were reduced in the TPGS group only within the first minutes after drug administration (Table 4-7).

Table 4-5 Comparative plasma kinetics of COM2 with and without TPGS

Plasma concentrations (a) and pharmacokinetic parameters (b) of [^{3}H]COM2 after iv dosing at 400 ng/kg in formulations based on blank plasma (control) or TPGS to mice (LOQ=3 pg/mL, n=3, mean ± SD). *,** significantly different from the control at P<0.05 and 0.01, respectively.

(a)

Time (h)	COM2 in plasma (pg/mL)		Percentage of control value
	Control	TPGS	
0.08	104 ± 11	247 ± 23**	238
0.25	81 ± 12	159 ± 16**	196
0.5	59 ± 2	124 ± 17*	210
1	34 ± 7	80 ± 17*	235

(b)

Parameter	Unit	Formulation	
		Blank plasma	TPGS 10%
Body weight	kg	0.028	0.027
Dose	ng	11.0	10.7
C_0	pg/mL	111	237
$t_{½}$	h	0.57	0.61
V_0	L/kg	3.6	1.7
$AUC_{0.08\text{-}1h}$	pg·mL^{-1}·h	56	121
$Dose/AUC_{0.08\text{-}1h}$	mL/h	196	89
$f_{unchanged}$	%	54	35

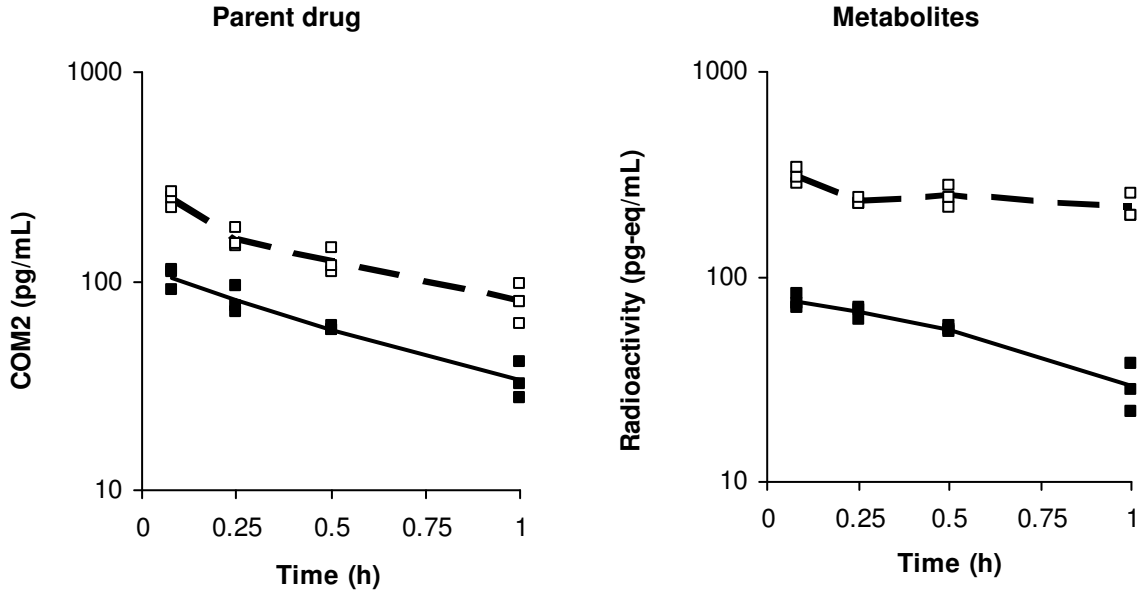


Figure 4-5 Influence of TPGS on the systemic exposure of COM2 and metabolites

Plasma concentration-time profiles of parent drug and metabolite-related radioactivity of [^{3}H]COM2 after intravenous administration to mice at 400 ng/kg formulated in blank plasma (closed symbols, black line) or as a solution containing TPGS 10% (open symbols, dashed line). Symbols represent single values and lines mean values (n=3). Values of metabolite-related radioactivity were obtained by subtracting concentrations of parent drug from concentrations of total radioactivity.

Table 4-6 Comparative plasma kinetics of metabolites of COM2 with and without TPGS

Plasma concentrations and pharmacokinetic parameters of metabolite-related radioactivity after iv administration of [^{3}H]COM2 (400 ng/kg) in blank plasma (control) or TPGS 10% to mice (n=3, mean ± SD). Values of metabolite-related radioactivity were obtained by subtracting concentrations of parent drug from concentrations of total radioactivity. ** significantly different from the control at P<0.01.

Time	Control	TPGS	Percentage of
(h)	Metabolites in plasma (pg-eq/mL)		control value
0.08	75 ± 6	307 ± 28**	408
0.25	67 ± 4	233 ± 9**	346
0.5	56 ± 2	246 ± 30**	441
1	29 ± 8	218 ± 31**	744
$AUC_{0.08\text{-}1h}$ ($pg{\cdot}mL^{-1}{\cdot}h$)	*49*	*222*	*453*
$t_{1/2}$ (h)	*0.6*	*2.4*	*395*

Table 4-7 Comparison of tissue distribution of COM2 with and without TPGS

Tissue concentrations and tissue-plasma concentration ratios (K_P) of [^{3}H]COM2 administered intravenously at 400 ng/kg in blank plasma (control) or 10% TPGS solution to mice (n=3). K_P(u) relates to the free compound concentration in plasma based on the free fraction determined *in vitro*. Values represent mean ± SD.

Time	Tissue	Concentration (pg/g)		K_P		K_P(u)	
(h)		Control	TPGS	Control	TPGS	Control	TPGS
0.08	Lung	1808 ± 263	1861 ± 64	17.3 ± 0.7	7.6 ± 0.5	981 ± 39	621 ± 42
	Heart	750 ± 111	699 ± 33	7.2 ± 0.3	2.8 ± 0.1	407 ± 17	233 ± 11
	Liver	2188 ± 355	2314 ± 56	20.9 ± 1.2	9.4 ± 0.9	1186 ± 69	773 ± 72
	Kidney	1761 ± 390	1735 ± 84	16.7 ± 2.2	7.1 ± 0.7	951 ± 124	580 ± 59
	Fat	232 ± 94	238 ± 83	2.2 ± 0.9	1 ± 0.3	127 ± 49	79 ± 25
	Muscle	166 ± 40	190 ± 82	1.6 ± 0.6	0.8 ± 0.3	92 ± 32	62 ± 22
	Brain	1095 ± 70	1038 ± 113	10.5 ± 0.6	4.2 ± 0.1	598 ± 35	345 ± 9
0.25	Lung	1206 ± 258	1277 ± 213	15 ± 2.6	8 ± 0.5	850 ± 148	657 ± 43
	Heart	464 ± 31	473 ± 19	5.8 ± 0.6	3 ± 0.2	330 ± 37	246 ± 20
	Liver	1447 ± 203	1626 ± 122	18 ± 1.9	10.3 ± 1.3	1024 ± 109	846 ± 109
	Kidney	1176 ± 93	1161 ± 83	14.7 ± 1.9	7.4 ± 1	837 ± 108	605 ± 86
	Fat	650 ± 169	1109 ± 321	8.1 ± 1.9	7.1 ± 2.4	458 ± 108	581 ± 198
	Muscle	215 ± 13	214 ± 28	2.7 ± 0.5	1.4 ± 0.1	155 ± 28	111 ± 12
	Brain	1199 ± 143	1180 ± 81	15.2 ± 3.4	7.5 ± 1.2	863 ± 194	616 ± 100
0.5	Lung	755 ± 76	923 ± 40	12.9 ± 1.5	7.5 ± 0.6	732 ± 83	615 ± 53
	Heart	296 ± 34	354 ± 53	5 ± 0.6	2.9 ± 0.4	287 ± 34	234 ± 29
	Liver	960 ± 91	1138 ± 58	16.3 ± 1.5	9.3 ± 1.2	929 ± 88	760 ± 100
	Kidney	814 ± 102	987 ± 120	13.9 ± 1.9	8 ± 0.4	789 ± 110	654 ± 34
	Fat	863 ± 227	1079 ± 444	14.7 ± 3.7	8.9 ± 5.1	834 ± 209	732 ± 416
	Muscle	181 ± 12	170 ± 13	3.1 ± 0.2	1.4 ± 0.2	175 ± 11	113 ± 13
	Brain	1086 ± 108	1215 ± 147	18 ± 1.8	9.8 ± 0.8	1050 ± 77	805 ± 69
1	Lung	439 ± 60	688 ± 187	13.3 ± 3.2	8.6 ± 0.5	758 ± 179	702 ± 43
	Heart	186 ± 28	220 ± 72	5.7 ± 1.5	2.7 ± 0.4	322 ± 87	222 ± 31
	Liver	581 ± 43	867 ± 140	17.6 ± 2.3	11 ± 1.5	998 ± 132	903 ± 123
	Kidney	481 ± 37	740 ± 89	14.5 ± 1.9	9.4 ± 0.9	825 ± 105	771 ± 78
	Fat	551 ± 143	504 ± 260	17.3 ± 7.7	7.1 ± 5.4	984 ± 439	584 ± 442
	Muscle	91 ± 12	119 ± 37	2.8 ± 0.8	1.5 ± 0.3	159 ± 43	121 ± 25
	Brain	731 ± 88	1073 ± 161	22 ± 2.6	13.6 ± 1.1	1249 ± 147	1114 ± 93

Discussion

Results signified the ability of the excipient TPGS to modify the blood distribution of COM2 in mouse under *in vitro* and *in vivo* conditions in a similar manner. Plasma concentrations of COM2 and metabolites were significantly increased, and the free fraction of drug in plasma (*in vitro*) decreased. Concentrations in tissues were independent of the formulation, whereas distribution ratios of drug in tissue to drug unbound in plasma were lower within the first minutes after dosing COM2 in TPGS. Overall, the altered pharmacokinetic profile of COM2 in plasma suggests drug inclusion in excipient-micelles and/or promoted protein binding by excipient in plasma.

The altered disposition of COM2 and metabolites in plasma is likely caused by the ability of TPGS to form micelles (141). Drug trapping by micelles in blood can be responsible for increased total plasma concentrations and decreased unbound fraction in plasma, thereby influencing drug accumulation in plasma and blood cells (94). Changes in the free fraction could also be caused by altered protein binding. There are different suggested mechanisms by which formulation vehicles can influence the free fraction of compounds, such as vehicle-compound interactions (association and/or micellar encapsulation) and vehicle-protein interactions. The interaction either promotes or blocks the binding of the compound in plasma. Most likely, different interacting processes contribute to the effective free fraction (125).

The alteration in tissue distribution at the beginning may be induced by changes of the free drug fraction in the presence of TPGS and exists only for few minutes probably due to the excipient degradation in blood. The phenomenon found after some minutes post-dose is reported in the literature for Paclitaxel formulated in Cremophor EL (94). The main characteristics are disproportionally increased plasma concentrations accompanied by unchanged tissue levels and tissue distribution processes.

4.6 The impact of hydroxypropyl-β-cyclodextrin on COM3 in rat

In vitro results

COM3 was predominantly located in the cellular fraction (80%) and was moderately bound to plasma proteins with high binding to α1-glycoprotein and albumin in a concentration-independent manner (Table 4-8, Figure 4-2). The drug partitioning into blood cells and the fraction bound to proteins were markedly reduced in incubations containing HP-β-CyD, consequently lowering both the blood-plasma and blood cell-unbound in plasma concentration ratios (Table 4-8, Figure 4-6). Whereas HP-β-CyD decreased the protein binding suggesting more COM3 available for uptake into cells, higher plasma levels associated with reduced concentrations in cells were observed for COM3 in HP-β-CyD. In conclusion, HP-β-CyD was selected for the *in vivo* study because of the most pronounced drug-excipient interactions detected *in vitro*.

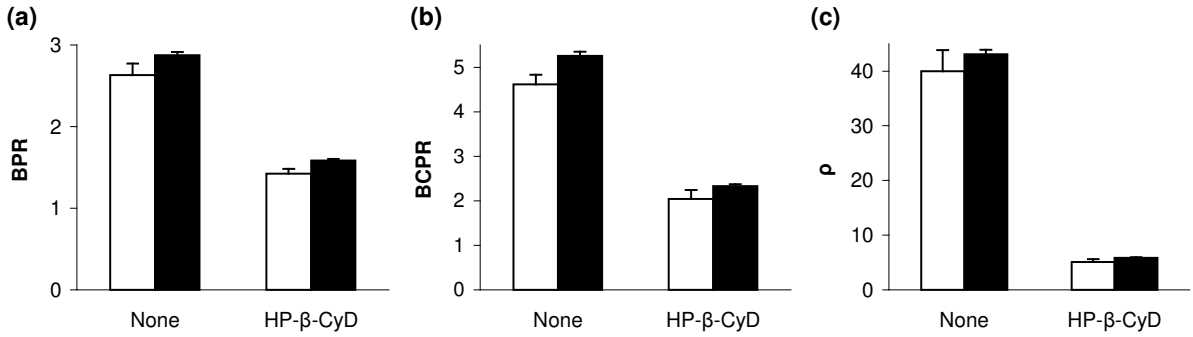


Figure 4-6 HP-β-CyD-mediated alteration of COM3 distribution in blood

Blood-plasma (a), blood cell-plasma (b), and blood cell-unbound in plasma (c) concentration ratios of [^{3}H]COM3 at 5 (white bars) and 500 ng/mL (black bars) in the presence and absence of HP-β-CyD (0.5%) after incubation in rat blood (pH 7.6, H 0.44, n≥3, mean ± SD).

Table 4-8 Effect of excipients on blood distribution and protein binding of COM3 *in vitro*

Partition parameters of [^{3}H]COM3 obtained at equilibrium after incubation with and without excipients (0.5%) in rat blood (pH 7.6, H 0.44, n≥3, mean ± SD).

Excipient	COM3 (ng/mL)	F_P (%)	BPR	BCPR	fu (%)	ρ
None	5	20.9 ± 1.5	2.6 ± 0.1	4.6 ± 0.2	12.1 ± 0.9	39.9 ± 3.9
	500	19.5 ± 0.3	2.9 ± 0.0	5.3 ± 0.1	12.2 ± 0.2	43.1 ± 0.8
CEL/EtOH,	5	19.4 ± 1.4	2.9 ± 0.2	5.6 ± 0.2	nd	nd
65:35 (v/v)	500	17.5 ± 0.3	3.2 ± 0.1	6.0 ± 0.1	nd	nd
HP-β-CyD	5	38.5 ± 2.2	1.4 ± 0.1	2.1 ± 0.2	40.1 ± 1.8	5.1 ± 0.5
	500	35.3 ± 0.4	1.6 ± 0.0	2.3 ± 0.0	39.6 ± 0.7	5.9 ± 0.1
Solutol	5	17.4 ± 1.4	3.2 ± 0.3	6.1 ± 0.4	nd	nd
	500	16.1 ± 0.3	3.5 ± 0.1	6.7 ± 0.1	nd	nd
PEG 200	5	19.7 ± 2.0	2.9 ± 0.3	5.1 ± 0.6	nd	nd
	500	18.6 ± 0.5	3.0 ± 0.1	5.6 ± 0.2	nd	nd
EtOH/PEG200/Glu,	5	22.7 ± 1.8	2.5 ± 0.2	4.4 ± 0.4	12.6 ± 0.1	34.6 ± 3.6
5:5:90 (v/v/v)	500	20.0 ± 0.4	2.8 ± 0.1	5.1 ± 0.1	11.8 ± 0.2	43.2 ± 1.0
TPGS	5	24.9 ± 1.8	2.3 ± 0.2	4.0 ± 0.2	nd	nd
	500	23.7 ± 0.3	2.4 ± 0.0	4.1 ± 0.1	nd	nd

In vivo results

Since COM3 dissolved in EtOH/PEG200/Glu (5:5:90, v/v/v) showed similar distribution kinetics *in vitro* as compared to glucose 5% without additives (Table 4-8), the control formulation consisted of EtOH/PEG200/Glu. This assured a sufficient solubility of COM3.

Dosing COM3 in a HP-β-CyD-containing formulation resulted in decreased protein binding and blood cell partitioning as compared to the control group (Table 4-9). However, these changes were transient and most pronounced after 5 min post-dosing followed by a decline. At 60 min after drug administration, differences in blood pharmacokinetics could not be longer detected between both groups anymore (Table 4-9). In contrast to the *in vitro* findings, a lower plasma distribution was found at 5 min after applying COM3 in HP-β-CyD, resulting in slightly enhanced blood-plasma and blood cell-plasma concentration ratios (Table 4-9, Figure 4-7).

The bolus injection of COM3 in HP-β-CyD led to an earlier decrease of kidney and lung concentrations and to a later decrease of skin, liver, and fat concentrations, compared to tissue levels obtained after administration of COM3 alone (Table 4-11). The calculation of tissue-blood concentration ratios supported the lower distribution of COM3 formulated in HP-β-CyD (Table 4-11), especially at 1 h post-dose (Figure 4-8).

As a result of the difference in plasma pharmacokinetic parameters (Table 4-10) and tissue levels between both groups, the renal elimination of COM3 was examined in addition. More COM3 appeared in the urine of rats from the HP-β-CyD containing formulation (Table 4-12, Figure 4-9), especially within the first 30 minutes after drug administration (36-fold higher amount of unchanged COM3 in urine compared to the control group).

Table 4-9 Comparison of blood and plasma levels of COM3 with and without HP-β-CyD

[^{3}H]COM3 concentrations in the systemic circulation (a) and partition parameters derived from these concentrations (b) after iv administration to rats at 300 µg/kg formulated in HP-β-CyD or in a cyclodextrin-free solution (n=3, mean ± SD, LOQ=0.08 ng/mL, assuming a hematocrit of 0.46[145] for calculations). *,** significantly different from the control at P<0.05 and 0.01, respectively.

(a)

Formulation	Time (h)	Drug concentration (ng/mL)	
		Blood	Plasma
EtOH/PEG200/Glu, 5:5:90	0.08	43.1 ± 6.8	17.5 ± 2.4
(control)	0.5	17.9 ± 4.0	6.1 ± 1.9
	1	9.7 ± 0.9	4.1 ± 0.5
HP-β-CyD 40%	0.08	32.7 ± 1.6	10.4 ± 1.1*
	0.25	26.3 ± 2.2	8.4 ± 0.9
	0.5	18.0 ± 0.8	5.9 ± 0.1
	1	10.4 ± 0.6	3.5 ± 0.2

(b)

Formulation	Time (h)	fu (%)	F_P (%)	BPR	BCPR	ρ
EtOH/PEG200/Glu,	0.08		22.5 ± 1.5	2.5 ± 0.2	4.2 ± 0.4	55.9 ± 4.8
5:5:90	0.5	7.6 ± 0.8	18.7 ± 1.9	3.0 ± 0.3	5.4 ± 0.7	70.9 ± 8.9
(control)	1		23.3 ± 1.2	2.4 ± 0.1	4.0 ± 0.3	53.3 ± 3.7
HP-β-CyD 40%	0.08	24.9 ± 1.2*	17.4 ± 1.1	3.2 ± 0.2**	5.8 ± 0.4	23.3 ± 1.7
	0.25	17.7 ± 1.3	17.5 ± 0.6	3.1 ± 0.1	5.8 ± 0.2	32.5 ± 1.3
	0.5	14.1 ± 0.6*	18.0 ± 0.7	3.1 ± 0.1	5.6 ± 0.3	39.7 ± 1.8
	1	7.8 ± 0.5	18.8 ± 1.2	2.9 ± 0.2*	5.3 ± 0.4	68.1 ± 5.2

Table 4-10 Comparative pharmacokinetics of COM3 with and without HP-β-CyD

Pharmacokinetic parameters of [^{3}H]COM3 administered intravenously in EtOH/PEG 200/Glu (control group) or in HP-β-CyD 40% to rats (n=3).

Parameter	Unit	Control group		HP-β-CyD group	
		Blood	Plasma	Blood	Plasma
Body weight	kg	0.233		0.221	
Dose	µg	70		66	
C_0	ng/mL	46	17	35	11
V_0	L/kg	6.6	17.4	8.5	27.1
$AUC_{0.08\text{-}1h}$	$ng \cdot mL^{-1} \cdot h$	19.7	7.5	17.7	5.8
$f_{unchanged}$	%	28	10	28	9

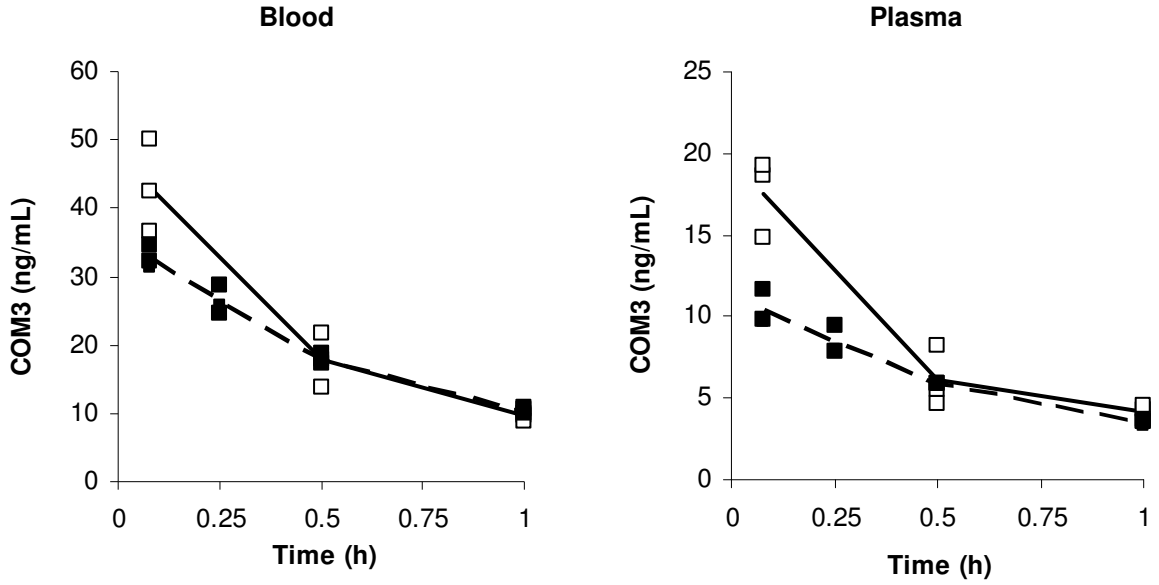


Figure 4-7 Influence of HP-β-CyD on the systemic exposure of COM3

Blood and plasma concentration-time profiles of [^{3}H]COM3 after intravenous administration to rats at 300 µg/mL formulated in EtOH/PEG 200/Glu (open symbols, black line) and as a solution containing HP-β-CyD (closed symbols, dotted line). Symbols represent single values and lines mean values (n=3).

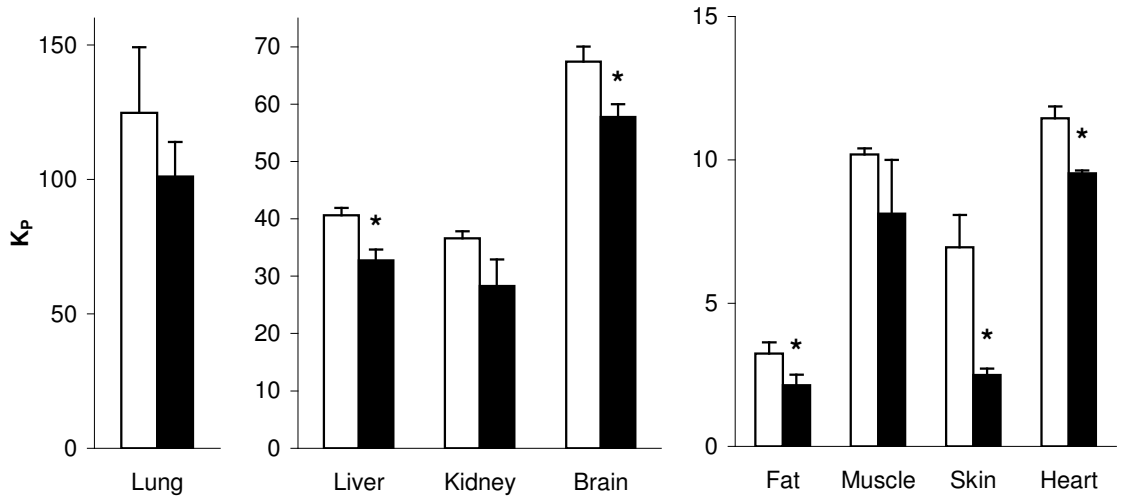


Figure 4-8 Influence of HP-β-CyD on tissue distribution of COM3

Tissue to blood concentration ratio (K_P) at 1 h after an iv injection of COM3 in EtOH/PEG 200/Glu (white bars) or in HP-β-CyD (black bars) in rats (dose: 300 µg/kg, n=3, mean ± SD). * significantly different from EtOH/PEG 200/Glu at P<0.05.

Table 4-11 Comparison of tissue distribution of COM3 with and without HP-β-CyD

Tissue concentrations and tissue-blood concentration ratios (K_P) of [^{3}H]COM3 after bolus injection to rats at 300 µg/kg in EtOH/PEG 200/Glu (control) or HP-β-CyD (n=3, mean ± SD). * significantly different from the control at P<0.05.

Time (h)	Tissue	Concentration (ng/mL)		Percentage of control value	K_P	
		Control	HP-β-CyD		Control	HP-β-CyD
0.08	Liver	1412 ± 225	1058 ± 211	75	32.8 ± 0.1	32.6 ± 7.5
	Kidney	1809 ± 191	1406 ± 72*	78	42.4 ± 5.0	43.1 ± 2.4
	Fat	128 ± 29	85 ± 18	67	3.1 ± 1.1	2.6 ± 0.6
	Heart	597 ± 108	563 ± 55	94	13.9 ± 1.1	17.2 ± 1.3*
	Lung	6179 ± 809	4595 ± 192*	74	144.1 ± 11.0	140.7 ± 6
	Muscle	123 ± 25	142 ± 12	115	3.0 ± 1.1	4.4 ± 0.3
	Skin	144 ± 62	74 ± 15	51	3.6 ± 2.0	2.2 ± 0.3
	Brain	1164 ± 189	951 ± 41	82	27.1 ± 1.9	29.1 ± 2.1
0.5	Liver	588 ± 149	617 ± 60	105	32.9 ± 5.3	34.5 ± 4.2
	Kidney	587 ± 163	479 ± 55	82	32.7 ± 5.1	26.7 ± 2.9
	Fat	57 ± 10	46 ± 6	81	3.2 ± 0.2	2.6 ± 0.4
	Heart	178 ± 46	187 ± 23	105	9.9 ± 1.0	10.4 ± 0.8
	Lung	2003 ± 599	2376 ± 129	119	110.8 ± 15.6	132.4 ± 7.6
	Muscle	155 ± 63	130 ± 12	84	8.4 ± 1.6	7.2 ± 0.5
	Skin	94 ± 11	40 ± 9*	42	5.4 ± 0.6	2.2 ± 0.6*
	Brain	905 ± 100	766 ± 49	85	51.5 ± 6.0	42.8 ± 4.4
1	Liver	394 ± 29	340 ± 6	86	40.7 ± 1.2	32.8 ± 1.9*
	Kidney	357 ± 44	293 ± 39	82	36.6 ± 1.2	28.3 ± 4.7
	Fat	31 ± 1	22 ± 5	72	3.2 ± 0.4	2.1 ± 0.4*
	Heart	111 ± 13	99 ± 6	89	11.4 ± 0.4	9.5 ± 0.1*
	Lung	1197 ± 128	1053 ± 178	88	124.7 ± 24.6	101.1 ± 12.8
	Muscle	99 ± 9	85 ± 23	86	10.2 ± 0.2	8.1 ± 1.9
	Skin	68 ± 16	25 ± 3*	37	6.9 ± 1.1	2.5 ± 0.2*
	Brain	653 ± 35	599 ± 10	92	67.4 ± 2.7	57.8 ± 2.2*

Table 4-12 Comparison of COM3 excretion in urine with and without HP-β-CyD

[^{14}C]COM3 (a) and its cumulative amount (b) appeared in the urine of rats following iv dosing at 370 µg/kg in EtOH/PEG 200/Glu (control) or HP-β-CyD 40% (mean ± SD, n=2: control, n=3: HP-β-CyD). *,** significantly different from the control at P<0.05 and 0.01, respectively.

(a)

Time (h)	COM3 in urine (ng)		$f_{unchanged}$ (%)	
	Control	HP-β-CyD	Control	HP-β-CyD
0.5	123 ± 10	4440 ± 594**	2.5 ± 0.2	44.8 ± 8.2*
1	240 ± 129	762 ± 191*	3.4 ± 0.8	13 ± 4.1
1.5	85 ± 19	325 ± 150	2.5 ± 0.8	7.3 ± 2.8
2	43 ± 11	174 ± 89	2 ± 0.8	4.6 ± 2.1

(b)

Time (h)	Cumulative amount of COM3 in urine			
	ng		%dose	
	Control	HP-β-CyD	Control	HP-β-CyD
0.5	123 ± 10	4440 ± 594**	0.13 ± 0.01	4.6 ± 0.9**
1	363 ± 139	5202 ± 693**	0.39 ± 0.14	5.4 ± 1.1**
1.5	449 ± 159	5527 ± 799**	0.48 ± 0.16	5.7 ± 1.2**
2	491 ± 169	5702 ± 857**	0.53 ± 0.17	5.9 ± 1.2**

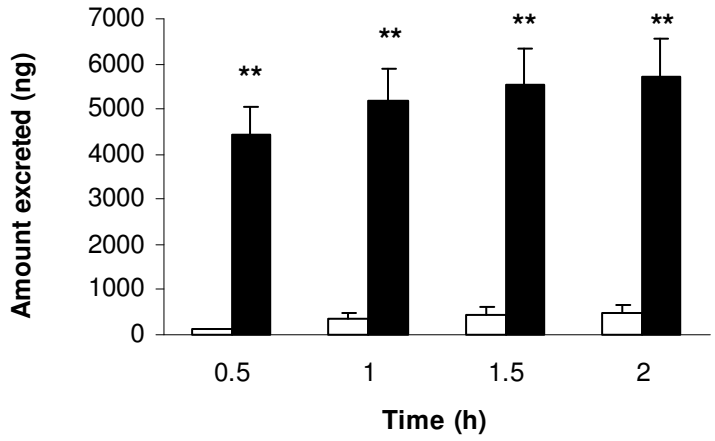


Figure 4-9 Effect of HP-β-CyD on the renal elimination of COM3

Cumulative amount of [^{14}C]COM3 appeared in urine after iv administration of 370 µg/kg either in EtOH/PEG 200/Glu (white bars) or as a solution in HP-β-CyD (black bars). Data are expressed as mean ± SD (n=2: EtOH/PEG 200/Glu, n=3: HP-β-CyD). ** significantly different from the EtOH/PEG 200/Glu at P<0.01.

Discussion

In vitro and *in vivo* experiments demonstrated a strong influence of the excipient HP-β-CyD on the disposition kinetics of COM3 in rats. There was a significant decrease in the plasma protein binding and in plasma and tissue concentrations of COM3 formulated in HP-β-CyD. Changes in the systemic circulation were transient (≤0.5 h post-dose) and most distinct shortly after dosing. Alterations in tissue

concentrations and tissue-blood distribution ratios were tissue dependent and most pronounced for skin. The amount of unchanged COM3 eliminated in the urine was significantly increased following drug administration in HP-β-CyD. This alteration in the pharmacokinetic behavior of COM3 is most likely due to the elimination of COM3 included in cyclodextrin complexes.

It is known (142) that HP-β-CyD forms drug/cyclodextrin complexes in biological fluids, is unable to cross biological membranes, and is rapidly eliminated renally. Even though the release of drugs from drug/cyclodextrin complexes is expected to be fast (within seconds) (112) drug pharmacokinetics can be modified in the presence of cyclodextrins (143). Given that COM3 is included in complexes in the systemic circulation, less COM3 is available for distribution, which could have led to the lower protein binding and cell/tissue partitioning of COM3 formulated in HP-β-CyD, compared to the cyclodextrin-free solution of COM3. Lower ρ-values of COM3 in HP-β-CyD support the possible existence of long-lasting complexes *in vivo* and indicate that unbound plasma concentrations obtained from ultrafiltration (30000 MW cut-off) are probably the sum of free and cyclodextrin-associated drug ($MW_{HP\text{-}\beta\text{-}CyD}$ ~1500). Furthermore, blood partition parameters altered till 30 min post-dose indicate a slow dissociation of COM3 from complexes. After total drug release, COM3 formulated in HP-β-CyD seemed to assimilate the pharmacokinetic behavior in blood obtained in the control group (60 min post-dose).

The marked increase of unchanged COM3 in the urine occurred mainly in the first 30 min following drug administration in HP-β-CyD which is consistent with the decreased plasma levels right after dosing in the HP-β-CyD group. Similar observations are reported for carbamazepine and dexamethasone where the renal excretion of unchanged drug applied intravenously in HP-β-CyD was increased most likely due to the renal clearance of the formulation vehicle (112). The faster elimination of COM3 associated to HP-β-CyD in contrast to COM3 alone might have attributed to less COM3 available for tissue distribution even after completed drug release from the vehicle, being in line with decreased tissue levels and K_P values at 60 min post-dose.

4.7 The impact of Cremophor EL on COM4 in rat

In vitro results

In excipient-free incubations, COM4 was highly bound to plasma proteins (>97% bound, predominantly to albumin) and penetrated hardly into blood cells (>95% located in plasma) (Tables 4-13 and 4-14, Figure 4-2). The free fraction in plasma and the partitioning into blood cells of COM4 were enhanced in the presence of CEL, HP-β-CyD, and Solutol. The protein binding decreased in an excipient concentration-dependent manner (Table 4-13, Figure 4-10). At excipient concentrations below 0.1%, the protein binding was not altered. At and above excipient concentrations of 0.1%, the free fraction was markedly elevated with a maximal effect at 1% for all three formulations. The addition of these excipients to incubations resulted to higher blood-plasma and blood cell-plasma concentration ratios, both indicating increased uptake of COM4 into blood cells (Table 4-14). CEL was selected for the *in vivo* study because of the most pronounced drug-excipient interactions detected *in vitro*.

Table 4-13 Effect of excipients on the *in vitro* protein binding of COM4

Protein binding of [^{14}C]COM4 at 100 ng/mL in the presence and absence of excipients in rat plasma using the ultrafiltration technique (pH 7.4, n=3, mean ± SD).

Excipient	(%)	fu (%)	Percentage of control value
None		2.4 ± 0.2	
CEL	0.01	2.3 ± 0.1	96
	0.1	3.1 ± 0.1	131
	0.5	11.1 ± 0.3	464
	1	26.0 ± 0.3	1089
HP-β-CyD	0.01	2.6 ± 0.2	109
	0.1	4.3 ± 0.2	179
	0.5	9.8 ± 0.1	412
	1	14.1 ± 1.1	592
Solutol	0.01	2.3 ± 0.2	97
	0.1	3.2 ± 0.2	134
	0.5	9.7 ± 0.2	407
	1	15.2 ± 0.3	638
PEG 200	0.5	2.4 ± 0.1	99
TPGS	0.5	2 ± 0.1	85

Table 4-14 Effect of excipients on the *in vitro* partition parameters of COM4 in blood

Plasma fraction (F_P) and distribution ratios (BPR, BCPR, ρ) of [^{14}C]COM4 at 100 ng/mL in rat blood obtained at equilibrium after incubations with and without excipients at 0.5 and 1% (pH 7.5, H 0.44, 37°C, n≥3, mean ± SD).

Excipient	(%)	F_P (%)	BPR	BCPR	ρ
None		95.9 ± 2.0	0.58 ± 0.01	0.05 ± 0.03	2.1 ± 0.6
CEL	0.5	73.9 ± 1.6	0.75 ± 0.02	0.43 ± 0.06	3.9 + 0.5
	1	65.6 ± 1.7	0.85 ± 0.02	0.67 ± 0.05	2.6 + 0.2
HP-β-CyD	0.5	90.0 ± 1.3	0.62 ± 0.01	0.14 ± 0.02	1.5 ± 0.2
	1	81.9 ± 1.4	0.68 ± 0.01	0.28 ± 0.03	2.0 ± 0.2
Solutol	0.5	78.6 ± 1.5	0.71 ± 0.01	0.35 ± 0.03	3.6 ± 0.3
	1	74.9 ± 1.4	0.75 ± 0.01	0.43 ± 0.03	2.8 ± 0.3

In vivo results

Dosing COM4 in a CEL-containing formulation resulted in a decrease of the protein binding as compared to the control group (Table 4-15, Figure 4-10). This effect was time-dependent and most pronounced at 5 min after drug administration followed by a decline. The increased fraction of COM4 unbound in plasma did not affect the drug levels in the systemic circulation, resulting in similar pharmacokinetic parameters in both groups (Table 4-15). Identical tissue concentrations were observed independently of the formulation (Table 4-16).

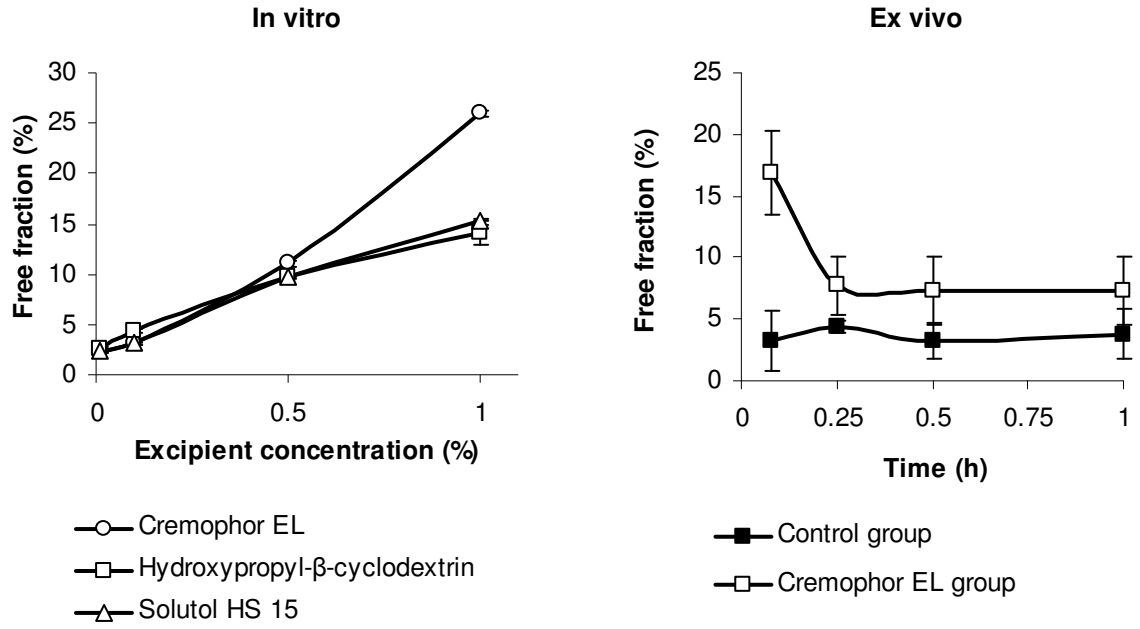


Figure 4-10 Excipient-mediated alterations of COM4 protein binding in plasma

Left: Unbound fraction of [^{14}C]COM4 (100 ng/mL) incubated with different excipient concentrations in rat plasma (mean ± SD, n=3). **Right:** Unbound fraction of compound-related radioactivity in plasma after intravenous administration of [^{14}C]COM4 at 400 µg/kg formulated in buffer (control group: closed symbols) or in CEL (open symbols). Symbols represent mean ± SD (n=3).

Table 4-15 Comparative blood and plasma kinetics of COM4 with and without CEL

Compound-related radioactivity concentrations in the systemic circulation (a) and pharmacokinetic parameters (b) after iv administration of [^{14}C]COM4 at 400 µg/kg formulated in buffer or in CEL to rats (n=3, mean ± SD, LOQ=4.3 ng-eq/mL, assuming a hematocrit of 0.46[145] for calculations). * significantly different from the control at P<0.05.

(a)

Formulation	Time (h)	Concentration (ng-eq/mL)		Unbound in plasma (%)
		Blood	Plasma	
Phosphate buffered saline (control)	0.08	1555 ± 299	2483 ± 382	3.8 + 1.5
	0.25	1192 ± 119	2073 ± 238	
	0.5	1377 ± 67	2388 ± 153	
	1	1248 ± 68	2105 ± 172	
Cremophor EL 17%	0.08	1471 ± 115	2426 ± 186	16.9 ± 3.4*
	0.25	1316 ± 119	2180 ± 237	7.7 ± 2.4
	0.5	1216 ± 155	2084 ± 237	7.4 ± 2.7
	1	1315 ± 337	2210 ± 607	7.4 ± 2.8

(b)

Parameter	Unit	Control group		Cremophor group	
		Blood	Plasma	Blood	Plasma
Body weight	kg	0.201		0.199	
Dose	µg	81		80	
C_0	ng/mL	1427	2373	1385	2296
V_0	L/kg	0.28	0.17	0.29	0.17
$AUC_{0.08\text{-}1h}$	ng·mL^{-1}·h	1211	2068	1186	1998

Table 4-16 Comparison of tissue distribution of COM4 with and without CEL

Tissue concentrations and tissue-blood concentration ratios of radioactivity of [^{14}C]COM4 after bolus injection to rats at 400 µg/kg formulated either in buffer (control group) or in CEL (n=3, mean ± SD).

Time	Tissue	Drug concentration (ng-eq/g)		Tissue-blood distribution ratio	
		Control group	Cremophor group	Control group	Cremophor group
0.08 h	Lung	737 + 78	768 + 17	0.48 + 0.04	0.52 + 0.03
	Muscle	222 + 6	243 + 12	0.15 + 0.02	0.17 + 0.02
	Skin	276 + 63	254 + 46	0.19 + 0.08	0.17 + 0.02
0.25 h	Lung	654 + 44	710 + 51	0.55 + 0.02	0.54 + 0.01
	Muscle	209 + 5	211 + 6	0.18 + 0.02	0.16 + 0.02
	Skin	363 + 70	395 + 41	0.30 + 0.04	0.30 + 0.04
0.5 h	Lung	695 + 46	676 + 40	0.50 + 0.01	0.56 + 0.04
	Muscle	202 + 4	204 + 1	0.15 + 0	0.17 + 0.02
	Skin	408 + 39	372 + 25	0.30 + 0.04	0.31 + 0.04
1 h	Lung	628 + 19	629 + 78	0.50 + 0.01	0.59 + 0.13
	Muscle	212 + 10	196 + 13	0.17 + 0.01	0.19 + 0.08
	Skin	409 + 24	391 + 45	0.33 + 0.01	0.37 + 0.09

Discussion

CEL lowered the binding of COM4 to plasma proteins *in vitro*, and the higher free drug fraction was associated with enhanced partitioning into blood cells as compared to the excipient-free incubations. Similar increases of unbound drug levels in plasma were observed after bolus injection of COM4 in CEL. In contrast to the *in vitro* findings, CEL induced no changes in the *in vivo* blood/plasma distribution of COM4, and no alterations in the tissue distribution were detected as well.

Even though the plasma protein binding of COM4 is influenced by CEL in an analogous manner under *in vitro* and *in vivo* conditions, a direct translation of the altered protein binding into blood and plasma concentrations is lacking *in vivo*. Different to the *in vitro* investigations, the *in vivo* situation involves not only distribution but also elimination processes which can initiate shifts in the systemic drug exposure. Besides this, the effective excipient concentration achieved *in vivo* in the circulation is usually unknown and can quite differ from concentrations applied *in vitro*. Although excipient concentrations can be theoretically estimated using blood volume, dose, and application volume, values are fairly arbitrary because excipient levels can decline fast in the body due to dilution (144) and degradation (126) within the blood compartment.

4.8 The impact of Solutol HS 15 on COM5 in rat

In vitro results

The distribution of COM5 between whole blood and plasma was slightly concentration-dependent in rat regardless of incubations with or without excipients (Table 4-17), and the equilibrium conditions were rapidly reached for all solutions (<5 min) except for CEL (equilibrium after 1-h incubation, data not shown). The binding of COM5 to plasma proteins was moderate (<90%) with a high binding to albumin and α1-acid glycoprotein (Figure 4-2), and the bound fraction was reduced by the addition of CEL and Solutol. Furthermore, both excipients caused alterations in the blood-plasma and blood cell-plasma concentration ratios, characterized by

enhanced drug partitioning into blood cells (Table 4-17, Figure 4-11). The higher cell uptake is consistent with decreased drug binding to plasma proteins in the presence of CEL and Solutol. These effects were more prominent for COM5 with Solutol than with CEL. Thus, Solutol was selected for the *in vivo* study.

Table 4-17 Effect of excipients on blood distribution and protein binding of COM5 *in vitro*

Partition parameters of [^{14}C]COM5 obtained at equilibrium after incubation with and without excipients (0.5%) in rat blood (pH 7.5, H 0.45, n≥3, mean ± SD).

Excipient	COM5 (ng/mL)	F_P (%)	BPR	BCPR	fu (%)	ρ
None	10	85.7 ± 4.0	0.64 ± 0.03	0.21 ± 0.07	16.2 ± 1.1	1.4 ± 0.3
	300	76.8 ± 1.6	0.72 ± 0.02	0.37 ± 0.03	11.9 ± 0.2	3.1 ± 0.2
Ethanol	10	81.2 ± 0.1	0.66 ± 0.05	0.23 ± 0.11	11.4 ± 0.4	2.2 ± 0.7
	300	77.9 ± 0.9	0.72 ± 0.01	0.36 ± 0.02	11.6 ± 0.2	3.0 ± 0.2
CEL/EtOH,	10	66.0 ± 2.2	0.83 ± 0.03	0.63 ± 0.06	22.7 ± 1.8	2.8 ± 0.3
65:35 (v/v)	300	57.7 ± 1.2	0.95 ± 0.02	0.90 ± 0.04	23.7 ± 0.4	3.8 ± 0.2
HP-β-CyD	10	80.3 ± 2.7	0.69 ± 0.02	0.30 ± 0.05	nd	nd
	300	75.4 ± 1.8	0.73 ± 0.02	0.40 ± 0.04	nd	nd
Solutol	10	55.0 ± 0.5	0.97 ± 0.06	1.00 ± 0.02	28.1 ± 1.0	3.3 ± 0.5
	300	48.7 ± 0.9	1.13 ± 0.02	1.29 ± 0.04	27.7 ± 0.4	4.3 ± 0.4
PEG 200	10	76.9 ± 2.3	0.72 ± 0.02	0.37 ± 0.05	nd	nd
	300	73.6 ± 1.3	0.75 ± 0.01	0.44 ± 0.03	nd	nd
TPGS	10	83.2 ± 1.3	0.63 ± 0.05	0.18 ± 0.12	nd	nd
	300	78.5 ± 1.8	0.70 ± 0.02	0.33 ± 0.04	nd	nd

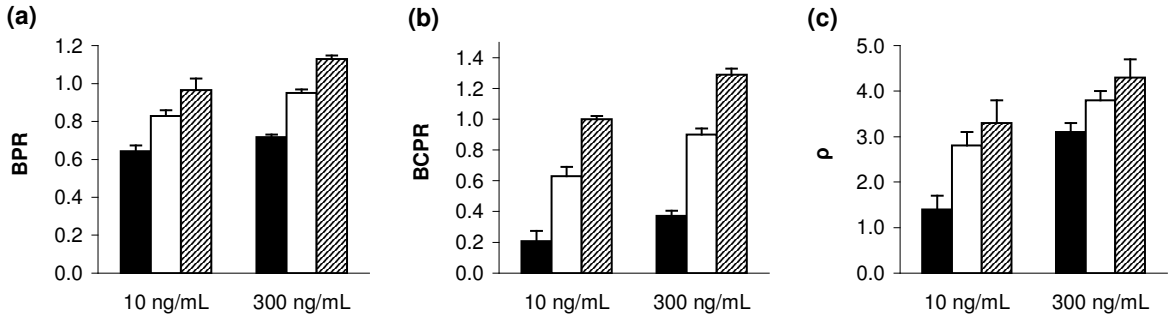


Figure 4-11 Excipient-mediated alterations of COM5 distribution in blood by CEL and Solutol

Blood-plasma (a), blood cell-plasma (b), and blood cell-unbound in plasma (c) concentration ratios of [^{14}C]COM5 (10-300 ng/mL) without excipients (black bars) and in the presence of Cremophor EL (white bars) and Solutol HS 15 (hatched bars) at 0.5% in rat blood (pH 7.5, H 0.45, n≥3, mean ± SD).

In vivo results

In vitro studies demonstrated that the addition of ethanol to incubations had no impact on the blood partition of COM5 (Table 4-17). Therefore, COM5 was dissolved in ethanol 10% for the administration in the control group, thereby assuring sufficient solubility of the compound

Within the first 15 min post-dose, higher blood concentrations accompanied by similar plasma concentrations were observed for COM5 in Solutol compared to concentrations for COM5 in ethanol 10% (control group), thereby increasing

concentration ratios of blood-plasma and blood cell-plasma in the Solutol group (Table 4-18). Subsequently, similar ratios were obtained in both groups with a concomitant slower decline of circulation concentrations in animals treated with Solutol. These changes in the Solutol group are in line with a reduced rate constant that resulted in a 2-fold higher half-life compared to that in the control group (Table 4-19). At 1 h post-dose, concentrations of COM5 in the systemic circulation were 300% higher than those obtained after dosing the compound in the control formulation (Table 4-18, Figure 4-12). The dose/AUC ratio was also decreased for COM5 in Solutol (Table 4-19). In agreement with the *in vitro* findings, COM5 formulated in Solutol led to a permanent lower protein binding up to 1 h post-dose in addition to enhanced uptake into blood cells right after dosing (higher blood-plasma concentration ratio) (Table 4-18).

Table 4-18 Comparison of blood and plasma levels of COM5 with and without Solutol

Concentrations of compound-related radioactivity in the systemic circulation (a) and partition parameters (b) derived from these concentrations after iv administration of [^{14}C]COM5 at 1 mg/kg formulated in Solutol or in a Solutol-free solution (control group) to rats (n=3, mean ± SD, LOQ=8 ng-eq/mL, assuming a hematocrit of 0.46[145] for calculations). *,** significantly different from the control group at P<0.05 and 0.01, respectively.

(a)

	Time (h)	Drug concentration (ng-eq/mL)		Percentage of control value
		Control group	Solutol group	
Blood	0.08	1308 ± 178	1853 ± 155*	142
	0.25	895 ± 195	1243 ± 250	139
	0.5	615 ± 193	976 ± 115	159
	1	194 ± 24	585 ± 113*	301
Plasma	0.08	2592 ± 558	2720 ± 266	105
	0.25	1584 ± 168	1895 ± 337	120
	0.5	1014 ± 385	1526 ± 112	150
	1	311 ± 47	900 ± 165*	289

(b)

Formulation	Time (h)	fu (%)	F_P (%)	BPR	BCPR	ρ
Control (ethanol 10%)	0.08	8.4 ± 1.4	106.2 ± 9.3	0.51 ± 0.04	-0.06 ± 0.1	-0.7 ± 1.1
	0.25		97.5 ± 14.8	0.56 ± 0.08	0.05 ± 0.17	1.9 ± 0.5
	0.5		87.6 ± 12.5	0.62 ± 0.09	0.18 ± 0.19	4.2 ± 2.2
	1		86.2 ± 2.5	0.63 ± 0.02	0.19 ± 0.04	1.9 ± 0.4
Solutol 17%	0.08	15.0 ± 2.3**	79.2 ± 1.8	0.68 ± 0.02**	0.31 ± 0.03	2.2 ± 0.2
	0.25		82.5 ± 1.8	0.65 ± 0.01	0.25 ± 0.03	1.9 ± 0.2
	0.5		84.7 ± 5.1	0.64 ± 0.04	0.21 ± 0.08	1.4 ± 0.5
	1		83.2 ± 1.2	0.65 ± 0.01	0.24 ± 0.02	1.2 ± 0.1

Independent of the formulation, brain levels ranged in concentrations corresponding to the vascular contamination, indicating no significant penetration of COM5 into brain (Table 4-20). A later increase in muscle and skin concentrations was detected for COM5 administered in Solutol (Table 4-20). The muscle concentration-time profile showed an analogous pattern to that in the systemic circulation with a longer persistence going along with higher concentrations at later post-dose times in the Solutol group (Table 4-21, Figure 4-12). However, similar muscle-plasma concentration ratios between both groups pointed that Solutol did not affect the

muscle distribution pattern. In contrast, the higher skin concentrations of COM5 formulated in Solutol were associated with altered K_P values (0.25 and 1 h post-dose) and same half-life compared to the control group (Tables 4-20 and 4-21).

Table 4-19 Comparative pharmacokinetics of COM5 with and without Solutol

Pharmacokinetic parameters of compound-related radioactivity after intravenous administration of [^{14}C]COM5 in ethanol 10% (control group) or in Solutol 17% to rats (n=3).

Parameter	Unit	Control group		Solutol group	
		Blood	Plasma	Blood	Plasma
Body weight	kg	0.214		0.212	
Dose	µg	214		212	
C_0	ng/mL	1562	2998	1840	2749
V_0	L/kg	0.64	0.33	0.54	0.36
$AUC_{0.08\text{-}1h}$	$ng \cdot mL^{-1} \cdot h$	578	1011	931	1426
$t_{1/2}$	h	0.33	0.32	0.69	0.69
$Dose/AUC_{0.08\text{-}1h}$	mL/h	369	211	228	149

Table 4-20 Comparison of tissue distribution of COM5 with and without Solutol

Tissue concentrations and tissue-plasma concentration ratios of radioactivity of [^{14}C]COM5 after bolus injection at 1 mg/kg to rats in ethanol 10% (control) or Solutol 17% (n=3, mean ± SD).

Time	Tissue	Concentration (ng/g)		Percentage of control value	Tissue-plasma ratio	
		Control	Solutol		Control	Solutol
0.08 h	Muscle	400 + 53	476 + 69	119	0.16 ± 0.02	0.18 ± 0.04
	Skin	248 + 26	240 + 48	97	0.10 ± 0.03	0.09 ± 0.03
	Brain	27 + 3	40 + 2	150	0.01 ± 0	0.01 ± 0
0.25 h	Muscle	311 + 43	424 + 21*	137	0.20 ± 0.03	0.23 ± 0.04
	Skin	243 + 15	413 + 62*	170	0.15 ± 0.01	0.22 ± 0.02*
	Brain	16 + 2	26 + 4	161	0.01 ± 0	0.01 ± 0
0.5 h	Muscle	228 + 25	333 + 15*	146	0.25 ± 0.09	0.22 ± 0.01
	Skin	235 + 33	384 + 11*	163	0.25 ± 0.08	0.25 ± 0.01
	Brain	10 + 2	18 + 3	188	0.01 ± 0	0.01 ± 0
1 h	Muscle	57 + 2	174 + 30*	307	0.19 ± 0.03	0.19 ± 0
	Skin	152 + 11	246 + 46	162	0.50 ± 0.11	0.28 ± 0.04
	Brain	—[a]	11 + 1	nd	0.01 ± 0	0.01 ± 0

a: below LOQ, *:significantly different from the control at P<0.05

Table 4-21 Comparison of muscle and skin kinetics of COM5 with and without Solutol

Pharmacokinetic parameters of compound-related radioactivity in tissues after intravenous administration of [^{14}C]COM5 at 1 mg/kg formulated in ethanol 10% (control group) or Solutol 17% to rats (n=3).

Parameter	Unit	Muscle		Skin	
		Control group	Solutol group	Control group	Solutol group
$AUC_{0.08\text{-}1h}$	$ng \cdot mL^{-1} \cdot h$	199	298	198	313
C_0	ng/g	533	548	nd	nd
$t_{1/2}$	h	0.30	0.58	1.04	0.97

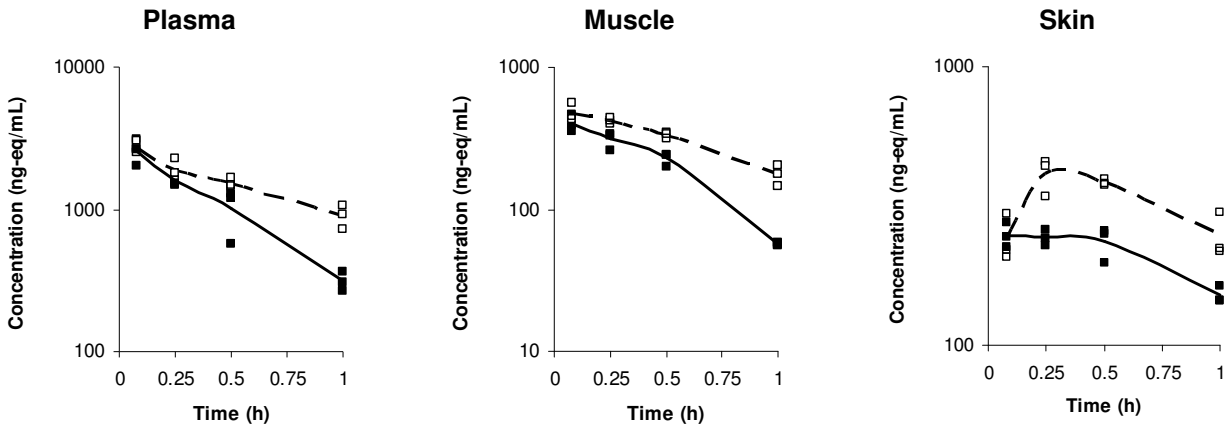


Figure 4-12 Influence of Solutol on plasma, muscle, and skin profiles of COM5

Concentration-time courses of compound-related radioactivity in plasma, muscle, and skin after intravenous administration of [^{14}C]COM5 at 1 mg/kg formulated in ethanol 10% (closed symbols, black line) and as a solution containing Solutol 17% (open symbols, dotted line). Symbols represent single values and lines mean values (n=3).

Discussion

In vitro and *in vivo* experiments showed that Solutol significantly modulated the disposition profile of COM5 in rats by drug-excipient interactions in blood. COM5 formulated in Solutol decreased both the drug distribution into plasma (transient *in vivo*) and the binding to plasma proteins (prolonged *in vivo*). Furthermore, animals treated with Solutol showed a later concentration increase in the circulation and tissues (muscle, skin) (≥0.25 h post-dose).

Altered drug levels were related to prolonged half-lives in the circulation and muscle. The longer blood persistence is consistent with the reduced clearance (dose/AUC) which presumes a lower elimination of COM5 in the presence of Solutol. In contrast to the muscle concentration-time profile similar to that in the circulation, the skin profile exhibited no changes in the half-life, potentially due to a lower vascularization in skin compared to that in muscle. However, the skin-plasma concentration ratio was affected by the Solutol formulation, whereas no changes in K_P values were observed for muscle. This altered pharmacokinetic profile might relate to a lower clearance of COM5 in the presence of Solutol.

Previous investigations in mice revealed an interaction between Solutol and a co-administered ketochlorine photosensitizer (C8KC) in plasma (127). Protein binding experiments indicated the formation of either a binary drug-excipient complex or a ternary complex, involving drug, excipient, and plasma components. This effect was correlated to similar half-lives of Solutol and C8KC in plasma, suggesting the persistence of C8KC in the circulation associated with that of the excipient. Likewise, half-lives in the same range were also observed for C8KC and the excipient Cremophor EL, going along with prolonged C8KC persistence in plasma and tissues in mice (85).

5 General discussion and conclusions

In vitro and *in vivo* experiments were carried out to investigate the potential of five excipients commonly used in formulations to modify the pharmacokinetics of co-administered model compounds at concentrations ranging from sub-therapeutic to pharmacological levels. Conducted studies focused mainly on the impact of excipients in the blood compartment, since such data have rarely been published up to now, especially for intravenously low dosed compounds including highly active drug substances, biomarkers, PET ligands, and microdoses.

PEG 200, CEL, HP-β-CyD, and Solutol were chosen as excipients because of their use in intravenous formulations and their high solubilizing ability related to different molecular structures and solubilization principles by either a direct solvent effect or by formation of micelles or complexes. TPGS, known in oral formulations, completed the set of selected excipients and is an interesting functional excipient in terms of its chemical properties (benzyl ring) and ability to alter metabolism and/or transporter activities, thereby potentially influencing cellular distribution.

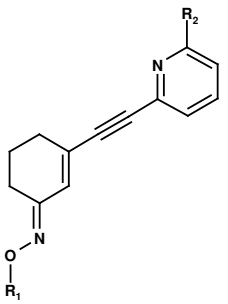
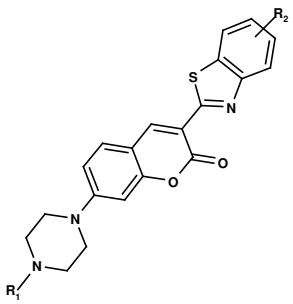
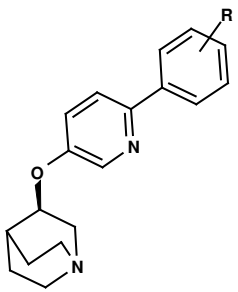
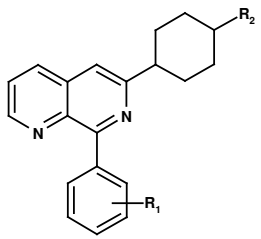
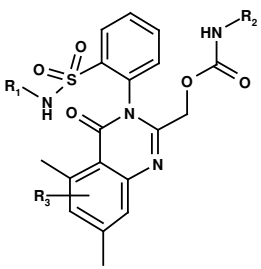
Five drug candidates in development at Novartis were selected as model compounds, exhibiting different physicochemical and pharmacokinetic properties (Table 5-1). The two PET ligands COM1 (base) and COM2 are lipophilic and poorly water-soluble. COM3 is characterized by its predominant location in the cellular fraction in blood, whereas COM4 hardly penetrates into blood cells. COM2 distributes equally between plasma and whole blood. COM5 is a molecule with a high polar surface area and a low volume of distribution similar to that of COM4. Moreover, COM2, COM1, and COM4 are very highly protein bound (>95%), and COM3 and COM5 are moderately protein bound (<90%). In addition, binding studies using isolated plasma proteins showed that model compounds bind with different affinity to the three major drug-binding proteins in plasma (albumin, α1-acid glycoprotein, and lipoproteins).

Model compounds were used in low doses to achieve blood concentrations of <5 ng/mL for COM1 and COM2 and low-therapeutic levels for COM3 (5-50 ng/mL). Due to the very low volume of distribution, COM4 and COM5 were investigated at normal pharmacological concentrations (>50 ng/mL in blood) to guarantee detectable tissue levels.

In order to analyze the *in vitro-in vivo* correlation, the excipient was set at an excipient-blood ratio of 1:200. This concentration was found to be non-hemolytic *in vitro* for selected excipients except TPGS which induced hemolysis after a longer contact time. To rule out any changes caused by cell lysis, TPGS investigations were carried out in the non-hemolytic range. The excipient concentration of 0.5% also correlates to the normal dosing range estimated for an intravenous bolus injection in mice and rats, assuming a blood volume of ~70 mL/kg and an injection volume of <3 mL/kg. However, in humans according to the literature (54), excipient concentrations above 0.1% in blood are regarded as high and generally only obtained following intravenous infusions. Considering marketed injectable formulations for example, the amount of excipient in blood is up to 0.15% HP-β-CyD (Sporanox) and 0.3-0.5% CEL (Vumon and Taxol respectively).

Table 5-1 Overview of model compounds

Physicochemical (PC) and pharmacokinetic (PK) properties of model compounds selected for investigating drug-excipient interactions in blood

Compound	**COM1** PET ligand	**COM2** PET ligand	**COM3** NCE	**COM4** NCE	**COM5** NCE
Chemical structure					
PC properties					
MW (g/mol)	240	410	295	<400	533
LogD 6.8	3.4	4.5	2.3	1.7	3.7
pKa	3.8	5.7	4.1, 8.7	3.2, 4.6	10.6
H_20 solubility, pH 6.8 (mg/L)	20	<2.5	4000	<500	100
PSA ($Å^2$)	35	50	25	63	166
H-bond acceptors	3	5	3	4	12
H-bond donors	0	0	0	1	3
Critical value(s)	Solubility (base)	Solubility			MW, PSA, H-bond acc.
PK parameters					
Species	Rat	Mouse	Rat	Rat	Rat
In vitro:					
Fraction in plasma (%)	75	45	20	~100	80
Free in plasma (%)	2.1	1.8	12	2.4	11
Major binding protein	Albumin > AGP	Albumin > LP	AGP > Albumin	Albumin > AGP	Albumin ≈ AGP
In vivo:					
Matrix	Plasma	Blood	Blood	Plasma	Plasma
$t_{1/2}$ (h)	0.08	1.1	1.1	10	0.4
CL (mL/min/kg)	202	10	142	0.4	7.2
V_{ss} (L/kg)	11	14	10	0.3	0.3
$f_{unchanged}$ (%)	1.4	49	14	98	94

AGP: α1-acid glycoprotein, **CL**: Drug clearance, $\mathbf{f_{unchanged}}$: Fraction of unchanged drug based on AUC ratio of parent drug and total radioactivity, **LogD**: Logarithm of octanol-water distribution coefficient, **LP**: Lipoproteins, **MW**: Molecular weight, **NCE**: New chemical entity, **pKa**: Negative logarithm of dissociation constant, **PSA**: Polar surface area, $\mathbf{t_{1/2}}$: Main elimination half-life, $\mathbf{V_{ss}}$: Volume of distribution under steady-state conditions

In trying to evaluate the potential of excipients to affect partition parameters in blood, selected excipients were screened *in vitro* for interactions with the model compounds using a blood distribution method and protein binding assays (ultrafiltration/ultracentrifugation). The most interacting excipients were subsequently taken forward for *in vivo* studies in animals given a single iv dose to ascertain whether there was a change in pharmacokinetics, and whether an *in vitro-in vivo* correlation existed.

Amongst the excipients tested, the excipient PEG 200 was not active as an interacting agent, whereas the others were more or less active (Table 5-2). However, there was no apparent relationship between the nature of interactions and the compound properties.

TPGS was found *in vitro* to enhance the plasma fraction of COM1 and COM2 in blood, and higher plasma concentrations were associated with a decrease of compound unbound in plasma. A significant increase in plasma concentrations of parent drug (2-fold) and metabolites (4-fold) were observed following intravenous administration of COM2 in mice, and $t_{1/2}$ of COM2 remained unchanged, whereas $t_{1/2}$ of metabolites was 4-fold higher. While TPGS led to similar trends of COM1-related metabolites after injection in rats, values determined for parent drug were inconsistent with those obtained *in vitro*. A very extensive metabolism coupled to fast elimination was detected for COM1 which led to a stop in additional investigations to understand the *in vitro-in vivo* discrepancy.

The use of CEL and Solutol in rats reduced *in vitro* the binding of COM4 and COM5 to plasma proteins, and the higher free drug fraction was accompanied by enhanced partitioning into blood cells. Both excipients led to 2-fold higher unbound plasma concentrations following iv administration of COM4 formulated in CEL and COM5 in a Solutol-containing solution. Systemic and tissue levels of COM4 remained unaffected. In contrast, dosing COM5 in Solutol resulted in an earlier increase of blood-plasma concentration ratios, indicating enhanced uptake into blood cells. Later on, COM5 concentrations in blood and plasma were significantly enhanced to a similar extent (2-fold) yielding ratios equalized to control values. The systemic accumulation was correlated to higher $t_{1/2}$ (2-fold). The AUC of COM5 in muscle and skin increased going along with a raise of $t_{1/2}$ in muscle and none in skin.

In vitro, HP-β-CyD increased the amount of COM3 distributed into rat plasma and lowered the binding to plasma proteins. Concentration ratios were only about 1/2 for blood-plasma and 1/8 for blood cell-unbound in plasma as compared to reference values. At earlier time points after iv dosing, concentrations of COM3 in blood and plasma were lower in the presence of HP-β-CyD (~2/3 of control), whereas over 30-fold higher levels of COM3 in urine were found. Subsequently, tissue concentrations and tissue-blood concentration ratios of COM3 with HP-β-CyD were significantly decreased.

Table 5-2 Qualitative impact of excipients on pharmacokinetic parameters

Excipient-compound interactions obtained after *in vitro* incubations and after intravenous administration in animals using excipients at ~0.5% in blood and compounds at a dose of either ~1 nmol/kg (COM1, COM2) or ~1 µmol/kg (COM3, COM4, COM5). Arrows indicate excipient-induced changes compared to the excipient-free group (↔: no changes, ↑: increase, ↓: decrease).

Compound	Excipient	Species	Changes		Comments
			In vitro	*In vivo*	
COM1	Vit. E TPGS	Rat	↑ Accumulation in plasma ↓ Partitioning into blood cells ↑ Plasma protein binding	↓ Plasma protein binding ↓ $AUC_{0.08\text{-}0.5h}$ ↓ C_0 ↔ $t_{1/2}$ ↔ Tissue distribution	• No changes *in vitro* at ≤0.5% TPGS • Similar AUCs of drug free in plasma • Increase in AUC of metabolites • Inappropriate model compound due to rapid metabolism/elimination
COM2	Vit. E TPGS	Mice	↑ Accumulation in plasma ↓ Partitioning into blood cells ↑ Plasma protein binding	↑ $AUC_{0.08\text{-}1h}$ ↑ C_0 ↔ $t_{1/2}$ ↔ Tissue distribution	• ↑ AUC and ↑ $t_{1/2}$ of metabolites • *Ex vivo* protein binding not available due to values <LOQ
COM3	HP-β-cyclodextrin	Rat	↑ Accumulation in plasma ↓ Partitioning into blood cells ↓ Plasma protein binding	↓ Plasma protein binding ↓ $AUC_{0.08\text{-}1h}$ ↓ C_0 ↔ $t_{1/2}$ ↓ Skin concentrations ↑ Urinary excretion	
COM4	Cremophor EL	Rat	↓ Accumulation in plasma ↑ Partitioning into blood cells ↓ Plasma protein binding	↓ Plasma protein binding ↔ $AUC_{0.08\text{-}1h}$ ↔ C_0 ↔ $t_{1/2}$ ↔ Tissue distribution	
COM5	Solutol HS 15	Rat	↓ Accumulation in plasma ↑ Partitioning into blood cells ↓ Plasma protein binding	↓ Plasma protein binding ↑ $AUC_{0.08\text{-}1h}$ ↔ C_0 ↑ $t_{1/2}$ ↑ Muscle/skin concentrations	

AUC: Area under the plasma drug concentration-time curve, **C_0**: Initial plasma concentration at time zero, **LOQ**: Limit of quantification, **$t_{1/2}$**: Half-life in plasma using the last three data points

These results clearly show the role of excipients as a modulator of the protein binding and cellular partitioning of compounds in blood which can impact the overall drug disposition. Excipient-induced changes in the free fraction and cell uptake of drugs within the blood compartment are explained by various mechanisms (Figure 5-1). An excipient is able to influence the fraction of a co-administered drug in plasma and blood cells by direct drug interactions and/or interferences with blood components such as proteins and cell constituents, particularly membranes. Consequently, bound and free concentrations in plasma and cellular concentrations may be shifted, potentially contributing to a “new” steady state. Altered plasma concentrations can be the result of changes in drug protein binding, drug adsorption to excipients, and/or drug trapping into excipient micelles or complexes. Whichever effect dominates, more drug can be free or bound, thereby accounting for the amount of drug available for cellular partitioning. Excipients could also vary the uptake into blood cells due to alterations in membrane structure and/or drug presentation, both mechanisms either facilitating or hindering drug transport via membranes. In the end, most likely different interacting processes contribute to the final effect depending on the force and nature of each single interaction.

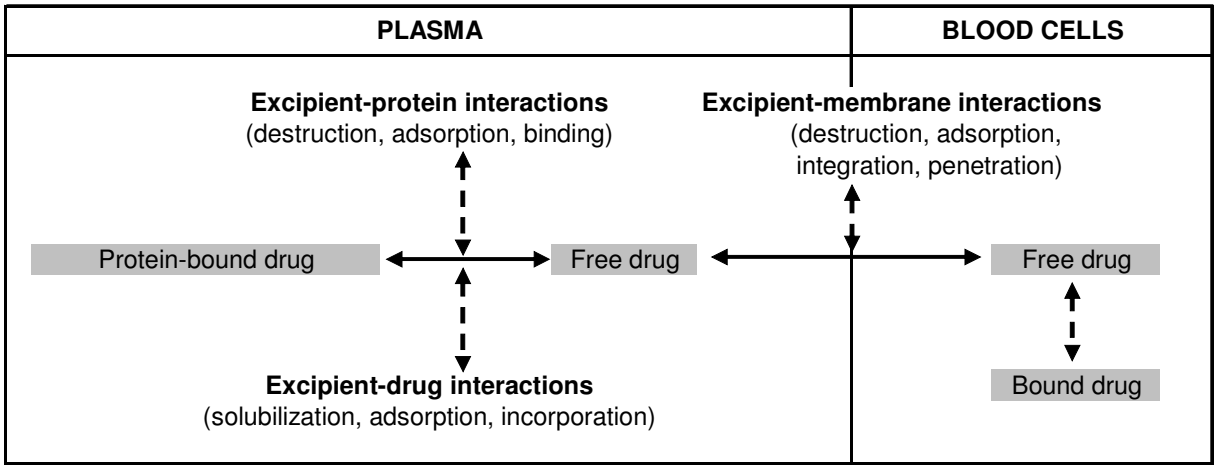


Figure 5-1 Mechanisms underlying alterations in drug partition parameters in blood

The presence of excipients in blood may influence drug levels in plasma (protein-bound and free fraction) and blood cells by interacting with the drug or blood components, thereby either blocking or promoting the protein binding and/or the drug available for cellular partitioning.

Analyzing the *in vitro* with the *in vivo* situation, the effect of selected excipients on the protein binding showed a good *in vitro-in vivo* correlation, whereas systemic concentrations after iv administration in animals could often not be predicted well from *in vitro* data. In contrast to *in vitro* assays reflecting stationary conditions, the *in vivo* situation entails processes running in an open and dynamic system, thereby involving not only distribution but also elimination. This can initiate shifts in the systemic drug exposure and be responsible for the absence of a direct correlation to blood/plasma concentrations and thus to pharmacokinetic parameters as well. Apart from this, the effective excipient concentration achieved *in vivo* in the circulation is usually unknown and may differ from concentrations applied *in vitro*. Although excipient concentrations can be theoretically estimated using blood volume, dose, and application volume, values are fairly arbitrary because excipient levels decline most likely fast in the body due to dilution and degradation within the blood

compartment. This suggests that if alterations in blood partitioning occur following administration *in vivo,* they should be best detectable at times shortly post-dose.

Even though differences between the *in vitro* and *in vivo* results were found, the *in vitro* test can serve as a straightforward tool for rapid detection of excipient-drug interactions in blood while requiring little amounts of drug. As a result, the determination of *in vitro* blood partition parameters is proposed as a rough estimator of a possible potential of excipients to contribute to pharmacokinetics of parenteral drugs. In addition to the blood-to-plasma distribution ratio, the protein binding provides useful information to elucidate excipient-induced alterations and allows the calculation of the blood cell-to-free in plasma distribution ratio. These data help not only to optimize the proper evaluation of pharmacokinetics in animals, but also to enable a more rational approach in the development of formulations for drug candidates. Therefore, the *in vitro* investigation of blood distribution and protein binding with and without excipient is recommended at a very early preclinical stage in cases where animal pharmacokinetics of lower dosed compounds (e.g. PET ligands) must be examined from excipient-containing solutions (e.g. surfactants, complexing agents). The influence assessment of excipients may also be a promising formulation approach for iv solutions of non-ionizable, lipophilic, and nonpolar molecules which are very challenging to formulate appropriately due to poor water solubility (e.g. paclitaxel, docetaxel, cyclosporin).

Figure 5-2 illustrates a possible formulation procedure that integrates data generated by *in vitro* investigations in blood. Cosolvents, complexing agents, surfactants, and combinations of these excipients are frequently used to dissolve a compound for intravenous administration in animals. Method(s) are chosen considering available compound characteristics such as physicochemical and pharmacokinetic properties, doses, and clinical objectives. If an appropriate formulation in terms of solubility and stability is found, blood distribution and protein binding studies are performed both in the presence and absence (reference) of excipient(s) using *in vitro* assays. Protein binding studies should mainly be considered if the extent of binding can be appropriately determined by ultrafiltration, dialysis, or ultracentrifugation, all three conventional and simple methods. Next, partition parameters (F_P, fu, BPR, BCPR, and ρ) are evaluated and compared to those obtained for the reference formulation representing an excipient-free solution (e.g. glucose 5% or saline including pH adjustment, buffer, blank plasma). Formulations are ranked in order of changes relative to the reference. As a result, a formulation can be modified, adapted, or selected for intravenous administration in animals. In cases where the *in vivo* administration requires the addition of excipient(s) to the reference formulation to solubilize the compound, the formulation must be tested *in vitro*. The formulation is an acceptable *in vivo* reference if the *in vitro* parameters are similar to those of the excipient-free solution, i.e. the *in vitro* reference. The outcome of the *in vivo* study triggers formulation optimization to minimize or abolish excipient effects, further investigation to better interpret data, or formulation acceptance for *in vivo* dosing and formulation development. Finally, it should be possible to exclude or at least be aware of a potential impact of excipient(s) on the drug disposition. Data can also be applied to identify both the activity of a certain excipient with different compounds and the excipient sensitivity of compounds with similar properties.

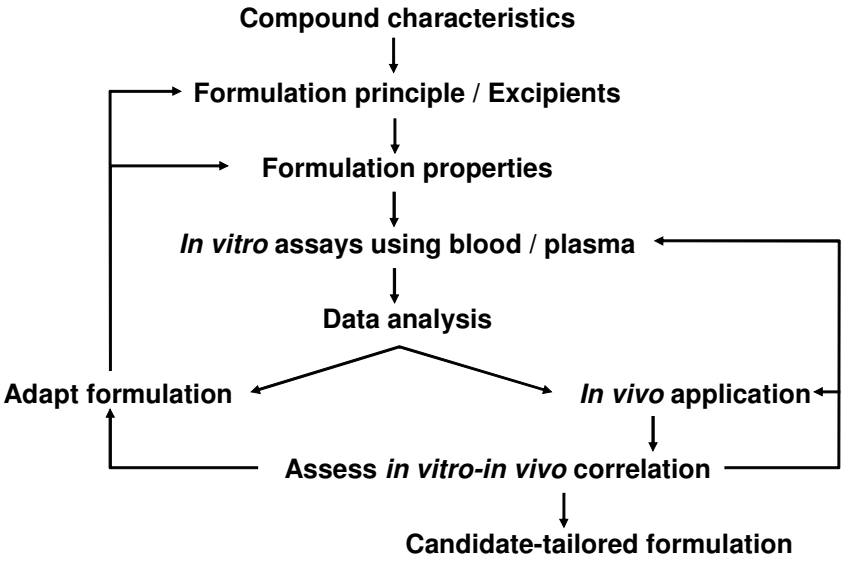


Figure 5-2 Suggested flow chart for an iv formulation development strategy

In conclusion, substantial drug-excipient interactions in animals have been identified for different excipients commonly used in drug formulation and pharmacologically active compounds with diverse properties. These findings support the assumption that certain excipients are not “inert” but are “functional” excipients and are able to alter the disposition of co-administered drugs. Such data need special attention since excipient-related changes may occur with similar or other drug/excipient formulations. Identifying excipient-drug interactions and understanding how excipients affect the drug behavior will assist in the pharmacokinetic characterization and the formulation development/optimization of drug candidates in development. Furthermore, a better knowledge of the relationship between drug, excipient, and blood constituents suggests new approaches in drug development in terms of controlling the pharmacokinetic profile by the right choice of excipient and its amount. This may enable to better anticipate the target of different blood and body compartments without extensive animal and human tests, potentially resulting in modulated pharmacological responses.

6 Outlook

The results of this work demonstrated the ability of commonly used injectable excipients, especially CEL, HP-β-CyD, and SHS, to influence pharmacokinetic parameters in blood, consequently contributing to altered drug disposition following iv dosing. However, to increase the knowledge of drug-excipient interactions, additional studies are required to confirm the above findings with further drug/excipient formulations, and with an emphasis on the role of excipients *per se* in pharmacokinetics of parenteral drugs.

- The excipients were tested *in vitro* and *in vivo* at a fixed concentration of 0.5% in blood, being within the normal range for animal injections and higher for clinical ones.

  Investigations by varying the excipient amount would give insight into the concentration-effect relationship and potency of excipients. Examination of lower excipient levels should also give an indication about the relevance and impact of excipient-induced alterations on applications in humans.

- No direct correlation between the interacting excipient and the compound properties were found, and no trends could be apparently observed in changes regarding the excipient characteristics.

  Continued investigations are necessary to elucidate whether observed phenomena are associated with certain drugs or more by some drug classes characterized by specific physicochemical properties. Focusing on excipients, it would be of interest to explore more in detail the frequency and significance of interactions and the excipient preference in terms of particular changes.

- The emphasis throughout the current work was placed on providing an insight into excipient-triggered effects and their *in vitro-in vivo* correlation. According to the literature, little is known about mechanisms underlying these alterations taking into account *in vitro-in vivo* investigations (94,110,112).

  To explain findings and improve excipient characterization, further investigations should focus on the mechanistic basis for the role of excipients in protein binding, blood/plasma partitioning, and cellular distribution using *in vitro* assays appropriate to understand the observed effects. In addition, modification of *in vitro* approaches could facilitate correlation of effects and mechanisms determined *in vitro* with *in vivo* situations, increase the understanding of *in vitro-in vivo* relationships, and enable extrapolation of *in vitro* results to *in vivo* pharmacokinetics. A better *in vitro-in vivo* correlation could also reduce *in vivo* studies and may contribute to more efficient drug development.

- There are drug-drug interactions reported in the literature where drugs were administered intravenously in conjunction with excipient-containing formulation of other drugs (e.g. valspodar formulated in CEL) (108,109). Most likely, the presence of excipient(s) in drug formulations contributed to observed pharmacokinetic interactions.

The research into the potential of excipients to induce kinetic alterations of agents administered intravenously in conjunction with excipient-containing formulation of other compounds should lead to a deeper insight into drug-drug interactions. These data may provide helpful information relevant for safety issues, dosage planning, and treatment optimization in clinical drug monitoring.

- Further studies could focus on the potential clinical significance of drug-excipient interactions observed in animals.

Finally, ongoing efforts for a proper understanding of excipients as biologically and pharmacologically active compounds will allow a better control of designing formulations with optimal characteristics for a better drug therapy.

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8 Appendix

Raw data of COM1 in rat

In vitro blood distribution of [^{3}H]COM1 using blood pools with different hematocrits: Total radioactivity concentration determined in blood and plasma						
Excipient		**Nominal COM1 in blood**	**H**	**Incubation time**	**Actual values** (pg-eq/mL, mean ±SD, n=3*)	
	% in blood	(pg/mL)		(min)	**Blood**	**Plasma**
None (Glu)		60	0.48	0	53 ± 1	76 ± 1
				5	53 ± 1	75 ± 0
				10	52 ± 1	74 ± 1
				20	52 ± 0	74 ± 1
		6000		0	5335 ± 80	7835 ± 121
				5	5637 ± 116	7784 ± 103
				10	5576 ± 40	7598 ± 114
				20	5502 ± 76	7502 ± 98
HP-β-CyD	0.5	60	0.48	0	62 ± 1	97 ± 0
				5	62 ± 1	96 ± 1
				10	62 ± 1	94 ± 1
				20	62 ± 0	93 ± 1
		6000		0	6564 ± 21	10052 ± 31
				5	6550 ± 30	10057 ± 214
				10	6434 ± 85	9896 ± 66
				20	6280 ± 213	9918 ± 80
	5	60	0.45	0	64 ± 0	102 ± 1
				5	65 ± 1	102 ± 1
				10	63 ± 1	102 ± 1
				20	64 ± 1	101 ± 1
		6000		0	6516 ± 132	10660 ± 26
				5	6598 ± 273	10762 ± 382
				10	6475 ± 288	10454 ± 163
				20	6653 ± 79	10482 ± 130
SHS	0.5	60	0.48	0	60 ± 2	86 ± 2
				5	60 ± 0	94 ± 19
				10	60 ± 1	81 ± 1
				20	60 ± 0	81 ± 1
		6000		0	6265 ± 40	8845 ± 102
				5	6240 ± 92	8562 ± 44
				10	6127 ± 110	8432 ± 35
				20	6104 ± 112	8109 ± 122
	5	60	0.45	0	59 ± 1	93 ± 1
				5	58 ± 1	92 ± 0
				10	56 ± 1	91 ± 1
				20	58 ± 1	91 ± 1
		6000		0	6166 ± 65	9908 ± 44
				5	6180 ± 162	9607 ± 123
				10	6230 ± 99	9529 ± 119
				20	6189 ± 104	9489 ± 211

***In vitro* blood distribution of [^{3}H]COM1 continued**						
Excipient		**Nominal COM1 in blood**	**H**	**Incubation time**	**Actual values** (pg-eq/mL, mean ±SD, n=3*)	
	% in blood	(pg/mL)		(min)	**Blood**	**Plasma**
TPGS	0.5	60	0.48	0	59 ± 1	92 ± 1
				5	58 ± 1	90 ± 1
				10	58 ± 1	88 ± 2
				20	58 ± 0	87 ± 1
		6000		0	5494 ± 102	8949 ± 87
				5	5518 ± 124	8549 ± 165
				10	5552 ± 74	8387 ± 101
				20	5431 ± 23	8286 ± 71
	5	60	0.40	0	50 ± 1	81 ± 1
				5	50 ± 1	80 ± 1
				10	50 ± 0	79 ± 1
				20	50 ± 1	72 ± 1
		6000		0	5042 ± 20	8175 ± 148
				5	5014 ± 34	7907 ± 136
				10	5102 ± 59	7952 ± 77
				20	5069 ± 68	7204 ± 35

*: Triplicate analyses were conducted on one aliquot for each time point

***In vitro* protein binding of [^{3}H]COM1 using the same plasma pool: Total radioactivity concentration determined in plasma and ultrafiltrate samples**				
Excipient		**Nominal COM1**	**Actual values** (pg-eq/mL)	
	% in plasma	(pg/mL)	Plasma*	Ultrafiltrate
None (Glu)		60	65	2.6
				2.6
				2.6
		6000	6302	270
				268
				272
TPGS	0.5	60	62	2.7
				2.1
				2.9
		6000	5708	171
				230
				133
	5	60	67	1.2
				1.3
				1.1
		6000	5886	187
				103
				68

*: Mean of duplicate determination of plasma spiked with different COM1 stock solutions

Tissue and plasma levels of parent drug and total radioactivity after iv administration of [^{3}H]COM1 (4 µg/kg) in the control group

Time (h)	Tissue	Individual concentration values					
		COM1 (pg/g)			Radioactivity (pg-eq/g)		
0.08	Plasma*	866	381	958	2529	1871	2052
	Lung	2012	1440	1867	3431	2224	2538
	Heart	2366	973	1619	2593	1490	1808
	Liver	1069	384	724	11805	9501	10699
	Kidney	2263	995	1692	6350	5770	5281
	Fat	2803	1982	4753	2638	2213	4859
	Muscle	1405	811	1234	1312	882	1172
	Skin	433	225	296	937	524	583
	Brain	6229	4525	4712	5431	4004	4053
0.25	Plasma*	275	257	178	1719	1482	1366
	Lung	399	607	518	1491	1378	1374
	Heart	517	645	285	1034	1023	778
	Liver	274	511	238	7904	6502	6920
	Kidney	557	664	321	5270	5046	5425
	Fat	6508	5897	4206	5953	5307	4112
	Muscle	565	574	306	659	686	463
	Skin	228	213	219	581	702	570
	Brain	3407	4085	3014	3033	3552	2719
0.5	Plasma*	179	103	101	1525	1442	1379
	Lung	622	367	335	1365	1089	1030
	Heart	310	211	214	684	684	661
	Liver	138	71	100	7090	5263	4993
	Kidney	345	220	202	4924	4585	5053
	Fat	8008	4790	4248	6586	4071	3649
	Muscle	270	159	166	409	349	342
	Skin	199	64	104	559	449	480
	Brain	2676	2419	2123	2310	2180	1953

*: pg/mL and pg-eq/mL

Tissue and plasma levels of parent drug and total radioactivity after iv administration of [^{3}H]COM1 (4 µg/kg) in the TPGS group

Time (h)	Tissue	Individual concentration values					
		COM1 (pg/g)			Radioactivity (pg-eq/g)		
0.08	Plasma*	312	297	278	2842	2939	3006
	Lung	1370	1173	1560	2364	2057	2272
	Heart	1190	971	912	1584	1262	1291
	Liver	449	234	337	10459	8898	10720
	Kidney	1012	768	806	5047	4767	4829
	Fat	2182	1485	1860	2712	2380	1703
	Muscle	845	726	1132	880	720	1046
	Skin	100	45	53	665	351	343
	Brain	4072	2765	3083	3816	2353	2709
0.25	Plasma*	89	107	121	2040	2440	2368
	Lung	531	688	546	1343	1488	1356
	Heart	351	471	454	737	864	851
	Liver	104	205	170	5354	6770	5184
	Kidney	321	435	446	6931	4972	6603
	Fat	1352	3916	1578	1456	4764	2035
	Muscle	355	649	588	532	789	675
	Skin	71	72	37	563	518	342
	Brain	1811	2327	1955	1648	2017	1841
0.5	Plasma*	35	65	61	2027	2054	2186
	Lung	214	195	319	1172	1067	1115
	Heart	167	207	250	539	566	650
	Liver	75	70	117	4225	4417	4314
	Kidney	197	209	215	6923	6364	5600
	Fat	3406	2067	2295	3071	1625	2734
	Muscle	191	226	256	343	394	368
	Skin	120	52	41	515	389	398
	Brain	1516	1332	1541	1413	1295	1480

*: pg/mL and pg-eq/mL

***Ex vivo* protein binding of [^{3}H]COM1 in the control and TPGS group: Parent drug concentration determined in plasma and ultrafiltrate**

	Dose (µg/kg)	Time (h)	Individual values (pg/mL)		
			Plasma*	Ultrafiltrate	
Control group	4	0.08	794	16.9	16.9
		0.25	249	5.5	5.1
		0.50	137	2.7	2.6
TPGS group	2.7	0.08	331	12.7	12.0
		0.25	120	4.1	4.4
		0.50	60	2.5	2.4

*: Mean of duplicate determination of plasma pooled for each time point

Raw data of COM2 in mouse

***In vitro* blood distribution of [^{3}H]COM2 using blood pools with different hematocrits: Total radioactivity concentration determined in blood and plasma**					
Excipient	**Nominal COM2 in blood** (pg/mL)	**H**	**Incubation time** (min)	**Actual values** (pg-eq/mL, mean ±SD, n=3*) **Blood**	**Plasma**
None** (Glu)	100	0.45	5	115 ± 2	85 ± 3
			60	115 ± 5	93 ± 4
			240	112 ± 6	101 ± 2
CEL/EtOH, 65:35	100	0.45	5	104 ± 5	86 ± 4
			60	105 ± 2	83 ± 1
			240	102 ± 1	87 ± 6
	100'000	0.46	5	103797 ± 1430	83684 ± 1446
			60	102979 ± 1547	81784 ± 896
			240	105912 ± 2594	80887 ± 998
HP-β-CyD	100	0.45	5	103 ± 5	88 ± 2
			60	104 ± 5	94 ± 1
			240	99 ± 2	94 ± 7
	100'000	0.43	5	105176 ± 1165	92396 ± 1519
			60	104609 ± 1238	95791 ± 668
			240	107331 ± 880	96454 ± 1780
SHS	100	0.45	5	101 ± 1	86 ± 1
			60	99 ± 4	85 ± 1
			240	105 ± 3	84 ± 3
	100'000	0.44	5	118080 ± 891	99140 ± 1915
			60	116422 ± 2822	93052 ± 1190
			240	116363 ± 890	89790 ± 1735
PEG 200	100	0.45	5	124 ± 4	96 ± 1
			60	125 ± 6	95 ± 2
			240	122 ± 2	103 ± 5
	100'000	0.46	5	112001 ± 3923	87146 ± 1273
			60	112161 ± 1189	89801 ± 1646
			240	110680 ± 652	90561 ± 1883
TPGS	100	0.45	5	101 ± 2	115 ± 2
			60	104 ± 2	101 ± 1
			240	101 ± 3	95 ± 3
	100'000	0.43	5	100911 ± 1443	106192 ± 992
			60	100608 ± 3207	88758 ± 1037
			240	100236 ± 4088	81755 ± 810

*: Triplicate analyses were conducted on one aliquot for each time point, **: similar to previous study

***In vitro* protein binding of [^{3}H]COM2 at 10 ng/mL using the same plasma pool: Parent drug concentration determined in plasma and ultracentrifugate**			
Excipient		**Actual values** (pg/mL)	
	% in plasma	Plasma	Ultracentrifugate
None (plasma)		9649	166
		9278	164
		9162	164
		9292	164
TPGS	0.5	8624	116
		8651	124
		8722	103
		8739	99

Tissue and plasma levels of parent drug and total radioactivity after iv administration of [^{3}H]COM2 (0.4 µg/kg) in the control group

Time (h)	Tissue	Individual concentration values					
		COM2 (pg/g)			Radioactivity (pg-eq/g)		
0.08	Plasma*	110	112	91	181	194	164
	Lung	1964	1955	1504	2450	2199	1704
	Heart	796	830	623	1074	950	741
	Liver	2398	2387	1778	3112	3022	2360
	Kidney	2083	1871	1327	2276	2024	1511
	Fat	140**	327	230	58**	540	603
	Muscle	124	170	203	160	218	234
	Skin	0**	35**	10**	14**	76**	32**
	Brain	1155	1112	1018	1097	1086	1046
0.25	Plasma*	94	71	77	164	141	139
	Lung	1344	908	1365	1648	1849	1759
	Heart	495	465	433	622	593	580
	Liver	1579	1214	1549	2218	2161	2381
	Kidney	1194	1075	1259	1468	1445	1518
	Fat	714	459	778	1103	977	1218
	Muscle	201	217	227	251	268	258
	Skin	113**	16**	59**	160**	73**	114**
	Brain	1060	1191	1345	1064	1150	1287
0.5	Plasma*	60	58	58	119	113	111
	Lung	720	703	842	1192	1444	1350
	Heart	295	262	331	454	447	447
	Liver	978	861	1040	1918	2348	2129
	Kidney	757	753	932	1017	1303	1336
	Fat	1014	602	973	1836	1164	2203
	Muscle	187	167	189	242	252	231
	Skin	87**	16**	18**	97**	129**	104**
	Brain	1209	1006	1044	1160	1003	1044
1	Plasma*	32	41	28	60	79	49
	Lung	369	478	468	1465	1274	1171
	Heart	154	199	205	347	377	364
	Liver	591	618	534	2077	1736	1383
	Kidney	471	522	451	1088	1020	846
	Fat	496	444	713	1066	1458	1253
	Muscle	103	80	91	171	169	146
	Skin	0**	38**	48**	138**	123**	122**
	Brain	673	832	688	826	902	806

*: pg/mL and pg-eq/mL, **: value below LOQ

Tissue and plasma levels of parent drug and total radioactivity after iv administration of [^{3}H]COM2 (0.4 µg/kg) in the TPGS group

Time (h)	Tissue	Individual concentration values					
		COM2 (pg/g)			Radioactivity (pg-eq/g)		
0.08	Plasma*	223	248	268	506	549	606
	Lung	1789	1913	1880	2036	2137	2116
	Heart	661	711	724	766	890	924
	Liver	2331	2252	2360	2593	2585	2656
	Kidney	1697	1831	1677	1815	2014	1839
	Fat	246	150**	317	362	366	740
	Muscle	108	190	272	144	246	330
	Skin	0**	20**	60**	39**	36**	77**
	Brain	913	1069	1133	904	1024	1080
0.25	Plasma*	178	148	151	403	377	394
	Lung	1522	1175	1133	1820	1412	1436
	Heart	489	478	452	593	597	578
	Liver	1636	1743	1500	2243	2293	2130
	Kidney	1112	1114	1256	1387	1415	1513
	Fat	932	915	1480	1742	1392	2239
	Muscle	237	222	183	290	288	283
	Skin	23**	0**	38**	144**	140**	96**
	Brain	1088	1244	1207	1004	1150	1166
0.5	Plasma*	111	118	143	330	359	421
	Lung	890	911	968	1292	1728	1599
	Heart	292	385	384	420	630	570
	Liver	1086	1200	1128	1901	2342	2002
	Kidney	864	996	1103	1124	1584	1452
	Fat	1393	—***	765	1492	—***	1576
	Muscle	156	181	173	232	273	269
	Skin	44**	73**	114**	106**	131**	192**
	Brain	1048	1272	1325	1003	1298	1237
1	Plasma*	79	97	63	333	296	264
	Lung	669	885	512	1672	1588	1769
	Heart	231	285	143	519	483	423
	Liver	774	1028	798	2073	1946	2394
	Kidney	726	835	659	1431	1342	1374
	Fat	—***	320	688	—***	1036	1712
	Muscle	143	137	76	210	240	221
	Skin	30**	147**	52**	158**	220**	194**
	Brain	1141	1188	889	1204	1202	1070

*: pg/mL and pg-eq/mL; **: value below LOQ, ***: no fat dissectible

Raw data of COM3 in rat

In vitro blood distribution of [^{3}H]COM3 using the same blood pool (H 0.44): Total radioactivity concentration determined in blood and plasma				
Excipient	**Nominal COM3 in blood** (ng/mL)	**Incubation time** (min)	**Actual values** (ng-eq/mL, mean ±SD, n=3*) **Blood**	**Plasma**
None (Glu)	5	5	4.6 ± 0.3	1.7 ± 0
		30	4.6 ± 0.2	1.7 ± 0.1
		60	4.7 ± 0.4	1.7 ± 0.1
	500	5	511 ± 2	179 ± 0
		30	507 ± 6	178 ± 2
		60	512 ± 9	176 ± 2
CEL/EtOH, 65:35	5	5	4.9 ± 0.4	1.8 ± 0.1
		30	4.9 ± 0.3	1.7 ± 0
		60	5.1 ± 0.2	1.7 ± 0
	500	5	516 ± 6	161 ± 2
		30	508 ± 2	160 ± 1
		60	508 ± 9	156 ± 1
HP-β-CyD	5	5	5.3 ± 0.2	3.5 ± 0.1
		30	5.2 ± 0.4	3.5 ± 0.1
		60	5.1 ± 0	3.6 ± 0.1
	500	5	516 ± 5	324 ± 2
		30	510 ± 2	320 ± 5
		60	512 ± 4	326 ± 2
SHS	5	5	4.9 ± 0.2	1.4 ± 0
		30	4.7 ± 0.1	1.5 ± 0
		60	4.9 ± 0.3	1.6 ± 0
	500	5	498 ± 10	143 ± 0
		30	506 ± 14	143 ± 3
		60	498 ± 1	144 ± 1
PEG 200	5	5	4.9 ± 0.4	1.7 ± 0.1
		30	5.1 ± 0.1	1.7 ± 0.1
		60	4.8 ± 0.1	1.8 ± 0.1
	500	5	520 ± 7	169 ± 1
		30	510 ± 9	170 ± 2
		60	518 ± 6	176 ± 3
EtOH/PEG200/Glu, 5:5:90	5	5	4.6 ± 0.3	1.8 ± 0.1
		30	4.3 ± 0.2	1.8 ± 0.1
		60	4.4 ± 0.1	1.8 ± 0.1
	500	5	535 ± 10	188 ± 1
		30	535 ± 10	194 ± 2
		60	538 ± 8	192 ± 1
TPGS	5	5	5.2 ± 0.3	2.3 ± 0
		30	5.1 ± 0	2.2 ± 0.1
		60	5.1 ± 0	2.4 ± 0.3
	500	5	546 ± 3	231 ± 3
		30	542 ± 6	231 ± 2
		60	551 ± 5	231 ± 2

*: Triplicate analyses were conducted on one aliquot for each time point

***In vitro* protein binding of [^{3}H]COM3 using the same plasma pool:**

Excipient	**Nominal COM3 in plasma** (ng/mL)	**Actual values** (ng-eq/mL)	
		Plasma*	Ultrafiltrate
None	5	5.8	0.67
(Glu)			0.76
			0.68
	500	512	63
			63
			62
HP-β-CyD	5	5.4	2.2
			2.1
			2.2
	500	538	214
			216
			209
EtOH/PEG200/Glu 5%, 5:5:90	5	5.2	0.66
			0.67
			0.65
	500	552	66
			66
			64

*: Mean of duplicate determination of plasma spiked with different COM3 stock solutions

***Ex vivo* protein binding of [^{3}H]COM3 in the control and HP-β-CyD group: Parent drug concentration determined in plasma & ultrafiltrate**

	Time (h)	**Individual values (pg/mL)**		
		Plasma*	Ultrafiltrate	
Control group	0.08	16.8	1.1	1.3
	0.5	4.6	0.4	
	1	3.5	0.3	
HP-β-CyD group	0.08	9.7	2.5	2.3
	0.5	5.4	0.8	0.7
	1	3.4	0.2	0.3

*: Mean of duplicate determination of plasma pooled for each time point

Circulation and tissue levels of parent drug and total radioactivity after iv administration of [^{3}H]COM3 (0.3 mg/kg) in the control group

Time (h)	Tissue	Individual concentration values					
		COM3 (ng/g)			Radioactivity (ng-eq/g)		
0.08	Blood*	39	42	57	60	63	81
	Plasma*	15	19	19	38	40	41
	Liver	1196	1394	1645	2097	2517	2767
	Kidney	1760	1646	2019	2354	2286	2677
	Fat	152	137	95	193	176	97
	Heart	536	534	722	668	676	927
	Lung	5712	5711	7113	8073	7897	9688
	Muscle	152	112	105	161	112	98
	Skin	209	139	85	216	136	86
	Brain	1048	1062	1382	1323	1334	1736
0.5	Blood*	20	13	18	75	85	83
	Plasma*	8	5	6	89	115	104
	Liver	644	419	700	2619	2716	2654
	Kidney	671	400	692	1162	843	1144
	Fat	66	45	59	89	80	83
	Heart	210	125	199	296	201	278
	Lung	2425	1317	2267	3403	1811	2947
	Muscle	222	98	144	282	144	185
	Skin	103	81	99	131	119	164
	Brain	1002	803	909	1260	996	1164
1	Blood*	11	11	10	80	70	60
	Plasma*	4	5	4	105	99	74
	Liver	383	427	373	1866	2152	2030
	Kidney	345	405	320	707	758	659
	Fat	30	31	33	49	52	53
	Heart	106	126	102	161	188	157
	Lung	1158	1092	1340	1680	1539	1817
	Muscle	96	109	92	154	167	149
	Skin	55	85	63	118	148	129
	Brain	647	691	622	811	875	775

*: pg/mL and pg-eq/mL

Circulation and tissue levels of parent drug and total radioactivity after iv administration of [^{3}H]COM3 (0.3 mg/kg) in the HP-β-CyD group							
Time	**Tissue**	**Individual concentration values**					
(h)		COM3 (ng/g)			Radioactivity (ng-eq/g)		
0.08	Blood*	34	31	32	53	52	48
	Plasma*	12	10	10	29	32	28
	Liver	850	1052	1271	1181	1665	1901
	Kidney	1413	1330	1474	1770	1611	1778
	Fat	68	85	103	70	99	121
	Heart	592	500	598	719	593	725
	Lung	4669	4377	4739	6069	5352	5939
	Muscle	151	147	129	159	157	129
	Skin	84	64	nd**	66	49	nd**
	Brain	954	990	908	1125	1165	1087
0.5	Blood*	19	18	17	74	70	82
	Plasma*	6	6	6	77	75	96
	Liver	617	558	677	2110	2107	2159
	Kidney	524	417	496	743	652	810
	Fat	47	39	51	59	51	68
	Heart	212	178	169	276	233	222
	Lung	2355	2513	2258	2931	3032	2853
	Muscle	137	137	116	172	169	143
	Skin	32	37	50	52	65	135
	Brain	733	743	822	895	864	958
1	Blood*	11	10	10	65	69	71
	Plasma*	4	3	4	77	87	92
	Liver	344	nd**	336	1765	nd**	1597
	Kidney	300	251	328	515	454	617
	Fat	28	19	20	41	43	44
	Heart	106	98	94	143	153	143
	Lung	1257	926	976	1582	1205	1237
	Muscle	98	98	59	133	161	84
	Skin	29	27	23	49	110	68
	Brain	606	604	588	734	726	697

*: pg/mL and pg-eq/mL, **: not determined due to glas vial broken by homogenization

In situ **bladder catheterization of [^{14}C]COM3 in the control and HP-β-CyD group:**										
	Time	**Urinary concentration**						**Urine excreted**		
	(h)	COM3 (ng/mL)			Radioactivity (ng-eq/mL)			(g)		
Control group*	0.5	190	331		7226	13857		0.685	0.350	
	1	721	339		17998	12039		0.460	0.439	
	1.5	88	151		2855	7869		1.130	0.477	
	2	64	134		2435	9293		0.790	0.261	
HP-β-CyD group	0.5	1911	5216	4903	3523	13028	12248	2.635	0.851	0.784
	1	7900	524	2965	44618	5086	27031	0.119	1.068	0.265
	1.5	4209	899	1268	40724	18439	19080	0.111	0.188	0.267
	2	1993	184	597	31856	7994	11100	0.124	0.412	0.335

*: Rat N°3 died during in situ experiment

Raw data of COM4 in rat

***In vitro* blood distribution of [^{14}C]COM4 at 100 ng/mL using the same blood pool (H 0.44): Total radioactivity concentration determined in blood and plasma**				
Excipient		**Incubation time**	**Actual values** (ng-eq/mL, mean ±SD, n=3*)	
	% in blood	(min)	Blood	Plasma
None (saline)	0.5	5	98 ± 2	169 ± 0
		30	98 ± 1	167 ± 1
		60	98 ± 2	168 ± 3
CEL	0.5	5	99 ± 0	147 ± 0
		30	99 ± 2	135 ± 2
		60	98 ± 1	128 ± 1
	1	5	103 ± 1	139 ± 1
		30	104 ± 0	125 ± 1
		60	104 ± 2	120 ± 2
HP-β-CyD	0.5	5	125 ± 2	202 ± 1
		30	125 ± 1	201 ± 3
		60	124 ± 0	199 ± 3
	1	5	116 ± 2	171 ± 3
		30	117 ± 3	171 ± 2
		60	117 ± 2	170 ± 3
SHS	0.5	5	110 ± 2	165 ± 2
		30	110 ± 1	155 ± 1
		60	110 ± 1	153 ± 3
	1	5	112 ± 1	150 ± 2
		30	112 ± 3	150 ± 1
		60	112 ± 0	148 ± 2

*: Triplicate analyses were conducted on one aliquot for each time point

***In vitro* protein binding of [^{14}C]COM4 at 100 ng/mL using the same plasma pool: Total radioactivity concentration determined in plasma and ultrafiltrate**			
Excipient		**Actual values** (pg-eq/mL)	
	% in plasma	Plasma*	Ultrafiltrate
None (saline)		97	2.5 2.3 2.2
CEL	0.01	105	2.5 2.4 2.3
	0.1	117	3.6 3.8 3.5
	0.5	102	11.5 10.9 11.3
	1	97	25.4 25.0 24.9
HP-β-CyD	0.01	99	2.7 2.5 2.4
	0.1	102	4.3 4.7 4.3
	0.5	134	13.1 13.3 13.1
	1	112	17.2 15.1 15.0
SHS	0.01	99	2.5 2.2 2.2
	0.1	101	3.4 3.1 3.3
	0.5	116	11.5 11.1 11.2
	1	112	17.1 17.4 16.7
PEG 200		100	2.2 2.4 2.4
TPGS		114	2.3 2.4 2.2

*: Mean of duplicate determination of plasma spiked with different COM4 stock solutions

Circulation and tissue levels of total radioactivity after iv administration of [^{14}C]COM4

Time (h)	Tissue	Individual concentration values (ng-eq/g)					
		Control group			CEL group		
0.08	Blood*	1258	1855	1551	1340	1515	1556
	Plasma*	2047	2761	2640	2211	2548	2517
	Plasma water*	123	50	49	287	477	478
	Lung	659	815	737	754	763	788
	Muscle	216	228	222	251	230	250
	Skin	348	229	253	229	227	307
0.25	Blood*	1306	1202	1069	1350	1183	1414
	Plasma*	2343	1983	1893	2357	1910	2273
	Plasma water*	92	99	83	118	179	201
	Lung	697	657	608	737	651	741
	Muscle	206	215	205	204	212	216
	Skin	443	317	330	351	402	432
0.5	Blood*	1398	1431	1303	1394	1112	1142
	Plasma*	2382	2543	2238	2357	1969	1926
	Plasma water*	63	124	46	106	151	191
	Lung	710	731	644	720	666	641
	Muscle	202	206	197	204	203	204
	Skin	363	426	436	384	389	343
1	Blood*	1322	1189	1233	1329	732	1301
	Plasma*	2297	1967	2049	2361	1192	2059
	Plasma water*	35	101	99	99	106	186
	Lung	649	618	616	674	539	674
	Muscle	218	200	217	187	212	191
	Skin	424	381	421	425	340	407

*: ng-eq/mL

Raw data of COM5

In vitro blood distribution of [^{14}C]COM5 using blood pools with the same H 0.44: Total radioactivity concentration determined in blood and plasma				
Excipient	**Nominal COM5 in blood** (ng/mL)	**Incubation time** (min)	**Actual values** (ng-eq/mL, mean ±SD, n=3*) **Blood**	**Plasma**
None (saline)	10	5	7.3 ± 0.6	11.5 ± 0.2
		30	7.8 ± 0.2	11.8 ± 0.2
		60	7.6 ± 0.7	11.8 ± 0.7
	300	5	278 ± 2	386 ± 4
		30	277 ± 0	391 ± 4
		60	275 ± 7	384 ± 2
EtOH	10	5	9.8 ± 0.6	13.8 ± 0.5
		30	9.8 ± 0.6	13.5 ± 0.5
		60	9.2 ± 0.6	13.9 ± 0.1
	300	5	286 ± 2	404 ± 3
		30	287 ± 6	400 ± 4
		60	284 ± 5	395 ± 3
CEL/EtOH, 65:35	10	5	8.8 ± 0.3	12.9 ± 0.6
		30	9.1 ± 0.5	12.8 ± 0.3
		60	9.2 ± 0.3	11 ± 0.3
		120	9.8 ± 0.3	10.9 ± 0.3
	1	5	257 ± 4	322 ± 3
		30	259 ± 3	313 ± 5
		60	255 ± 5	267 ± 2
		120	281 ± 3	279 ± 1
HP-β-CyD	0.5	5	9.3 ± 0.5	14.3 ± 0.3
		30	9.5 ± 0.3	13.9 ± 0.3
		60	9.6 ± 0.2	14.1 ± 0.7
	1	5	293 ± 1	412 ± 10
		30	297 ± 6	414 ± 6
		60	296 ± 6	405 ± 1
SHS	0.5	5	10.6 ± 0.4	12.5 ± 0.4
		30	10.7 ± 0.4	10.8 ± 0.3
		60	10.8 ± 0.5	11.2 ± 0.3
	1	5	256 ± 1	257 ± 1
		30	257 ± 2	247 ± 4
		60	260 ± 3	230 ± 1
PEG 200	0.5	5	10.1 ± 0.3	14.1 ± 0.3
		30	9.9 ± 0.8	14.5 ± 0.2
		60	10.2 ± 0.3	14.2 ± 0.2
	1	5	304 ± 7	402 ± 3
		30	304 ± 8	413 ± 4
		60	307 ± 2	410 ± 4
TPGS	0.5	5	8.8 ± 0.3	14.6 ± 0.4
		30	9.1 ± 0.6	14.5 ± 0.3
		60	8.9 ± 0.2	13.3 ± 0.3
	1	5	308 ± 5	448 ± 3
		30	312 ± 7	446 ± 7
		60	311 ± 3	390 ± 22

*: Triplicate analyses were conducted on one aliquot for each time point

***In vitro* protein binding of [^{14}C]COM5 using the same plasma pool: Total radioactivity concentration determined in plasma and ultrafiltrate**			
Excipient	**Nominal COM5 in plasma** (ng/mL)	**Actual values** (ng-eq/mL)	
		Plasma*	Ultrafiltrate
None (saline)	10	8.6	1.3 1.5
	300	292	35 35 34
EtOH	10	10.8	1.3 1.2
	300	295	35 35 34
CEL/EtOH, 65:35	10	10.4	2.5 2.4 2.1
	300	269	65 64 63
SHS	10	10.7	3.0 2.9 3.1
	300	268	76 74 73

*: Mean of duplicate determination of plasma spiked with different COM5 stock solutions

Circulation and tissue levels of total radioactivity after iv dosing of [^{14}C]COM5 (1 mg/kg)

Time (h)	Tissue	Individual concentration values (ng-eq/g)					
		Control group			SHS group		
0.08	Blood*	1109	1450	1365	1694	2005	1859
	Plasma*	2009	3120	2647	2486	3010	2665
	Muscle	355	458	387	555	426	448
	Skin	275	224	245	296	207	219
	Brain	24	29	28	41	38	41
0.25	Blood*	712	873	1100	1519	1179	1033
	Plasma*	1508	1468	1777	2265	1813	1606
	Muscle	262	341	330	423	445	404
	Skin	229	242	259	456	441	342
	Brain	14	16	18	29	27	21
0.5	Blood*	642	409	792	1096	867	965
	Plasma*	1194	572	1275	1655	1455	1467
	Muscle	244	199	241	342	341	315
	Skin	252	198	256	397	382	374
	Brain	11	7**	11	22	15	18
1	Blood*	172	192	219	688	603	464
	Plasma*	269	304	361	1055	919	727
	Muscle	59	55	57	203	176	144
	Skin	164	146	145	299	217	223
	Brain	3**	3**	3**	12	11	9**

*: ng-eq/mL, **: value below LOQ

***Ex vivo* protein binding of [^{14}C]COM5 in the control and SHS group: Total radioactivity concentration determined in plasma & ultrafiltrate**

	Time (h)	Individual values (pg-eq/mL)		
		Plasma*	Ultrafiltrate	
Control group	0.08	2465	217	221
	0.25	1587	123	126
	0.5	1031	69	67
	1	305	31	31
SHS group	0.08	2679	378	372
	0.25	1914	245	
	0.5	1524	230	230
	1	864	167	

*: Mean of duplicate determination of plasma pooled for each time point

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10 Curriculum Vitae

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2003-2005	PhD student, University of Basel Laboratory work conducted at Novartis Pharma AG, Basel

During my PhD thesis, I attended following lectures and courses given by the following lecturers:

Prof. Dr. H. Leuenberger, PD Dr. G.Imanidis, Prof. Dr. J. Drewe, Prof. Dr. J. Seelig, PD Dr. H. Heerklotz, PD Dr. A. Seelig, Dr. S. Rogers