Clinical Safety of Metamizole

Inauguraldissertation

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

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aus Trub, Bern

Basel, 2016

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

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Basel, den 23.2.2016

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Dekan

„Erfahrungen sind Maßarbeit. Sie passen nur dem, der sie macht."

Carlo Levi

Danksagung

Diese Dissertation wurde von Oktober 2012 bis Februar 2016 am Universitätsspital Basel auf der Abteilung Klinische Pharmakologie und Toxikologie verfasst und durch Prof. Dr. med. Dr. phil. Stephan Krähenbühl, Dr. phil. Alexandra Rätz Bravo und PD Dr. med. Manuel Haschke betreut. Meine vielseitigen Projekte ermöglichten mir die Zusammenarbeit mit einer Vielzahl von unterschiedlichen Leuten. Ich habe dies sehr geschätzt und bin dankbar für die vielen spannenden, lehrreichen und heiteren Begegnungen.

Prof. Dr. med. Dr. phil. Stephan Krähenbühl, vielen herzlichen Dank für die Chance, diese Dissertation auf der Klinischen Pharmakologie und Toxikologie schreiben zu können. Herzlichen Dank für das Vertrauen, die Unterstützung und dein voranbringender Optimismus.

Dr. phil. Alexandra Rätz Bravo, du warst die erste Ansprechperson insbesondere zu Beginn meiner Dissertation. Herzlichen Dank für deine grosse Unterstützung. Deine Arbeitsweise und dein Führungsstil nehme ich als Vorbild mit. Danke für die schöne Zeit im Grazienzimmer.

PD Dr. med. Manuel Haschke, unser gemeinsames Projekt war das grösste „Baby" mit dementsprechenden Herausforderungen. Herzlichen Dank für die grosszügig bemessene Zeit, bei denen du Fragen beantwortest hast und mich auf wichtige Punkte aufmerksam gemacht hast, sowohl inhaltlich wie auch planerisch. Herzlichen Dank für dein Vertrauen.

PD Dr. med. Anne Barbara Taegtmeyer, du hast die Leitung eines Projektes übernommen, was sich für mich als Glücksfall herausgestellt hat. Herzlichen Dank für deinen Einsatz und deine Ermutigungen. Ich habe viel von dir gelernt.

Andrea Stoller, Claudia Bläsi und Luisa Baselgia, ihr wart meine unentbehrliche Stütze insbesondere bei der Durchführung der klinischen Studie. Darüber hinaus habe ich eure Freundschaft geniessen dürfen. Ein herzliches Dankeschön für die schöne Zeit mit euch.

Dres. med. Eva Liakoni und Felix Hammann, ihr habt mich sowohl bei der Koala wie auch der Frosch-Studie begleitet. Danke für die vielen wertvollen Inputs. Es war sehr bereichernd, mit euch zusammenzuarbeiten.

Barbara Zimmermanns, Deborah Rudin und Annette Harings-Kaim, ich werde unsere Meinungsaustausche bezüglich Zeitpunkts des Mittagessens und Öffnung des Fensters vermissen. Herzlichen Dank für die schöne Zeit im Grazienzimmer.

Evelyne Rudin, ボンボヤージュ. では、また.

Labfolk, thank you for the warm hospitality in your area: Bea, Franzi, Gerda, David, Dino, Riccardo, Benji, Francois, Jamal and Urs. You made my lab trips to exciting and entertaining experiences. Thanks in particular Dr. Jamal Bouitbir and Dr. Urs Duthaler for your comprehensive support that caused you quite a headache. I am very grateful for it.

Psychopharmacology Research Group, danke für die unterhaltsamen Pausen und Mittagessen.

Alexandra Tramonti, Hala Hassna und Raife Ibrahimova, ihr habt einen wichtigen Teil zu den jeweiligen Projekten beigetragen. Danke für euren grossen Einsatz und die tolle Zeit mit euch.

Anna Rickli, wir haben das Abenteuer PhD gemeinsam begonnen und ich bin sehr froh, diesen Weg mit dir geteilt haben zu dürfen. Danke für deine Freundschaft.

Meiner Familie, insbesondere meinen Eltern Peter und Ursula Blaser-Schweizer, gilt ein Dank, der über diese Zeilen weit hinausgeht. Danke!

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Summary

Metamizole is an old analgesic agent. Its use is highly controversial due to its capacity to induce agranulocytosis, a rapid and severe fall of neutrophil granulocytes with risk of fatal outcome. Despite of this debate and withdrawal from the market in several countries, the use of metamizole increased in the last ten years in Switzerland as well as in Germany. The goal of this PhD-thesis project was therefore to improve our understanding of the clinical toxicity of metamizole. The work should contribute to a better understanding of the risk-benefit profile and promote the safe use of metamizole.

In a first approach, we descriptively analyzed worldwide pharmacovigilance data concerning adverse effects related to metamizole. The main objective was to characterize spontaneously reported cases of hematological adverse drug reactions associated with metamizole as suspected drug regarding appearance, course, and severity of the reactions. The worldwide case safety reports were selected from the WHO Global Individual Case Safety Report Database (VigibaseTM), and the national reports from the National Pharmacovigilance Database from Swissmedic. This allowed a comparison of reported hematological adverse drug reactions on a national and international level. A total of 1417 international and 77 Swiss reports were analyzed. Around 52% of the international and 33% of the Swiss metamizole-associated hematological ADR occurred within a latency time of ≤7 days. More women were reported. The annual number of hematological reports and those with fatal outcomes increased over the last years parallel to metamizole sales figures. The minimal incidence rate of agranulocytosis was 0.46-1.63 cases per million person-days of use (2006-2012) estimated via sales figures and number of reports. Female sex, old age, pancytopenia, and co-medication with methotrexate were striking characteristics of the 7 Swiss fatal cases. Early detection of myelotoxicity and avoidance of other myelotoxic substances like methotrexate (also at an immunosuppressive dose) are important measures for preventing fatalities.

In a second study, we retrospectively performed a case-control study of metamizole associated leucopenias. We focused on the search of risk factors for the development of metamizole-induced white blood cell disorders. Fifty-seven cases and 139 controls were identified. Of the 57 cases, 32 were post-operative (post-OP) which were compared to age-,

sex- and ward-matched post-OP controls (n= 64). The remaining cases (n= 25) were compared to sex-matched, non-post-OP controls (n= 75). The number of patients with a positive allergy history was higher among post-OP cases than controls (p= 0.0015) as was the number with previous leucopenic episodes (p= 0.03). The prevalence of diagnosed hepatitis C infection was 7% among all cases compared to 1% among all controls (p= 0.01). The use of concomitant cytostatic agents (even at immunosuppressive doses) was significantly higher among non-post-OP cases than controls (p= 0.007), with a trend to this distribution among post-OP patients. We concluded that a history of allergies, leucopenic episodes, hepatitis C infection and concomitant cytostatic agents are possible risk factors leucopenia associated with metamizole use.

The third project, a clinical study about the renal safety of metamizole, dealt with a possible advantage of metamizole. The aim of this study was to examine the effects of metamizole on renal function (inulin clearance and urinary excretion of sodium and of the prostacyclin metabolite 6-keto-PGF1α in healthy, salt-depleted volunteers) in comparison with the non-specific COX-inhibitor naproxen. If it could be shown that metamizole does not have negative effects on renal function, its use in patients with impaired renal function who cannot be treated with NSAIDs would be supported.

After single and repetitive dosing, neither metamizole nor naproxen had a significant effect on inulin clearance or sodium excretion. After repetitive dosing, there was a trend for decreased sodium excretion after naproxen but not after metamizole. Both metamizole and naproxen inhibited renal 6-keto-PGF1α excretion starting 2 hours after ingestion and lasting the entire dosing period during repetitive dosing, suggesting that metamizole inhibits renal prostaglandin synthesis similarly to naproxen. Therefore, renal excretion of 6-keto-PGF1α may not be the ideal marker to differentiate between the renal adverse effects of NSAIDs and metamizole. Nevertheless, in healthy, sodium-depleted subjects, metamizole had no significant effect on inulin clearance or renal sodium excretion, whereas there was a trend to a decreased sodium excretion after repetitive naproxen dosing. Further studies in more susceptible individuals have to be conducted in order to answer the question whether the effect of metamizole on renal function is different compared to NSAIDs.

Abbreviations

4-AA	4-amino-antipyrine
4-AAA	4-acetyl-amino-antipyrine
4-FAA	4-formyl-amino-antipyrine
4-MAA	4-methyl-amino-antipyrine
ADR	adverse drug reaction
AUC	area under the concentration–time curve
Cmax	peak plasma concentration
CNS	central nervous system
COX	cyclooxygenase
CYP	cytochrome P450
G-CSF	granulocyte colony-stimulating factor
GFR	glomerular filtration rate
IAAAS	International Agranulocytosis and Aplastic Anemia Study
IC50	half maximal inhibitory concentration
LLOQ	lower limit of quantification
NSAIDs	non-steroidal anti-inflammatory drugs
PGE_2	prostaglandin E_2
ROS	reactive oxygen species
T1/2	half-life

1. Introduction

1.1. Historical background*: A long story*

Almost 100 years of metamizole's controversial history can already be narrated. The story begins in Germany, where metamizole was first introduced by the company Hoechst in the 1920s [1]. But already in the early 1930s, metamizole was identified as a cause for severe blood dyscrasias, particularly agranulocytosis, together with other structural related pyrazolones including amidopyrine [2]. But several years have come and gone before the antipyretic analgesic has fallen increasingly into disgrace in the 1970s. This has led to withdrawal or banning in some countries (for example Australia 1965, Norway 1976, USA 1977) [3]. The question how frequent metamizole induces agranulocytosis remained unclear but is essential for a proper evaluation of the risk benefit profile. In the 1980s, a population-based case-control study, the International Agranulocytosis and Aplastic Anemia Study (IAAAS), tried to unveil this question for analgesic drug use [4]. The study analyzed 221 cases of agranulocytosis and 1425 hospital controls from centers in Israel, Spain, Germany, Italy, Hungary, Bulgaria and Sweden. The estimated excess risk for any exposure was 1.1 per million users within one week, but with striking local differences. The wide regional variability was linked with different utilization patterns of metamizole. Regions with a common use of metamizole (for example Israel and Hungary) showed lower risks, whereas regions in which metamizole is not commonly used showed higher rates (Germany and Spain). Subsequently, the hypothesis of a genetic predisposition was born [5]. But the study design and data analysis has been criticized substantially [6, 7]. Nevertheless, the low incidence found in the IAAAS somehow rehabilitated the reputation of metamizole to a certain degree. In Sweden for example, where the drug has been withdrawn in 1974 after a Swedish publication [8] with an estimated risk of 1 in 3000 patients, the results of the IAAAS led to reintroduction of metamizole in 1995, but it was withdrawn again in 1999 [9]. The ups and downs fit well with the sometimes quite emotional discussion regarding metamizole. Many publications were published dealing with the safety profile of this drug, not only in professional journals. Headings like "Dipyrone (Metamizole) Use in the United States: A Lethal Tango?" or "Dipyrone: A drug no one needs" demonstrate the hard front of the opponents [10, 11]. But this is only one side of the story. In other countries, metamizole

remained on the market, sometimes even as over the counter medication (for example Poland). In Switzerland, metamizole was subjected to prescription control in 1992 [3]. Today, the official indications in the Swiss product informations are severe pain or severe fever, which do not respond to other measures [12]. After some years of rather cautious use of metamizole, the Swiss sale figures started to rise again in the 2000s [13]. The same trend was observed in Germany [14]. The renaissance of the analgesic was paralleled by an increased awareness of the safety risks of treatment alternatives, namely non-steroidal anti-inflammatory drugs (NSAIDs). Especially the cardio-vascular risk of NSAIDs has increasingly been recognized in addition to the well-known gastrointestinal and renal risk [15]. Metamizole seems to gain supporters again.

1.2. Drug properties: *What it's like*

1.2.1. *Mechanism of action*

Metamizole is one of the examples of an old drug with uncertain mechanism of action. Known are its analgesic, spasmolytic, and antipyretic effects [16]. The mechanisms of those actions have different origins.

Spasmolytic effect

The mode of action regarding the spasmolytic properties of metamizole has not been investigated in detail. The decrease of the excitability of peripheral smooth muscle is assumed to rise from a reduced increase of intracellular Ca^{2+} concentration by synthesis of inositol phosphate. The synthesis of inositol phosphate depends on phospholipase C which may be directly inhibited by metamizole or indirectly via inhibition of G protein-coupled receptors activating phospholipase C [17].

Antipyretic effect

The antipyretic effect was first thought to be mediated by inhibition of prostaglandin E_2 (PGE_2) synthesis via cyclooxygenase (COX)-inhibition, analogous to the antipyretic effects of NSAIDs [18, 19]. The inhibition of PGE_2 production in the CNS which is responsible for the antipyretic effect, is thought to take place in the median preoptic nucleus located in the hypothalamus [20]. Inhibition of peripheral COX has actually been shown for metamizole in

several studies, but the reported half maximal inhibitory concentrations (IC50) for COX-1 inhibition shows high variability ranging from 2.6 µmol/L to >400 µmol/L (in comparison IC50 for COX-1 for ibuprofen 12-42 µmol/L, and for naproxen 0.3-24 µmol/L) [21-24]. Similarly, the reported IC50 for COX-2 inhibition by metamizole show high variability ranging from 4.7 µmol/L to >400 µmol/L [21, 22, 25]. Additionally, there is evidence for a PGE_2 independent mechanism contributing to the antipyretic effects [26, 27]. In 2014, Malvar and colleagues identified the metabolites 4-methylaminoantipyrine (4-MAA) and 4-aminoantipyrine (4-AA) as the metabolites responsible for reduced Lipopolysaccharides (LPS)-induced fever and the reduced PGE_2 increase in plasma, cerebrospinal fluid and hypothalamus. But only 4-MAA inhibited also PGE_2-independet fever induced by Tsv (Tityus serrulatus venom). Apart of the antipyretic effects, 4-MAA shows hypothermic effects. An explanation for the mechanism of the hypothermic effect of 4-MAA is still missing [28].

To sum up, the antipyretic action of metamizole is based on central effects, partially by COX-dependent effects via PGE_2 inhibition, but also by PGE_2-independent reactions by unknown mechanisms. In contrast to NSAIDs, metamizole has hypothermic effects at higher doses via unknown mechanisms.

Analgesic effect

Analogous to the antipyretic mechanism, the analgesic mechanism of metamizole is still a matter of ongoing investigations. As already mentioned, metamizole seems to inhibit COX with high and variable IC50 values, but possibly with a tendency to a preferred COX-2 inhibition [21, 23]. Moreover, the inhibition of COX was described to be different from the direct interaction with the active center of the COX enzyme known from NSAIDs. Pierre and colleagues suggested an indirect COX-inhibition by sequestering radicals which COX needs for the catalytic activity and by the reduction of the oxidative state of COX [23]. Apart from the discussed COX-inhibition, other mechanisms are considered to explain the differences to NSAIDs. In 2012, Rogosch and colleagues identified two novel bioactive metabolites of dipyrone. The novel metabolites derived from metabolites already known, namely the arachidonoyl amides of 4-MAA and 4-AA. They were extracted from the CNS and were positively tested for cannabis receptor binding (CB_1 and CB_2) as well as COX inhibition [25]. Due to the association of metamizole with the endocannabinoid system, Crunfli and colleagues investigated in 2015 cannabinoid CB_1 receptors mediated effects of dipyrone. They have examined mice treated with metamizole regarding known effects of

cannabinoids, including antinociceptive and cataleptic effects, hypolocomotion and hypothermia. Their results indicated involvement of the endocannabinoid system in the analgesic and cataleptic effect of metamizole and also of hypolocomotion, but not of hypothermic effects of metamizole. They hypothesized an indirect modulation of primarily CB_1 receptors by providing additional arachidonic acid as substrate for endocannabinoid synthesis or other related molecules by inhibition of COX and fatty acid amide hydrolase (FAAH) [29]. An effect via endogenous opioids has been suggested before [30, 31]. But the complexity of suggested mechanisms of actions is not at all finished here. The peripheral antinociceptive effect was also associated with COX-independent activation of ATP-sensitive K^+-channels [32]. The central analgesic effect was additionally linked with glutamatergic mechanisms, inhibition of neurokinin-1 (NK1) mediated responses, and inhibition of the protein kinase C-dependent pathway [33]. Lastly, also the descending serotonergic and noradrenergic system and appertaining spinal receptor subtypes ($5\text{-}HT_{2a}$, $5\text{-}HT_3$, $5\text{-}HT_7$ - serotonergic, α1, α2, β-adrenergic receptors) seem to be involved in the antinociceptive effect of metamizole [34].

In summary, there is evidence for both, peripheral and central analgesic effects of metamizole. What strikes are the parallels of the proposed mechanisms with the suggested mechanisms of paracetamol. Paracetamol as well interacts with cannabinoid receptors [35]. Paracetamol also was shown to form an arachidonic acid-conjugated metabolite in the spinal cord and brain of mice by fatty acid amide hydrolase (FAAH) which acts as an agonist of the potent pain receptor TRPV1 (transient receptor potential cation channel subfamily V member 1) and inhibits purified COX-1 and COX-2 in vitro [36]. In the end, the revelation of the mechanisms of action of old drugs is of great interest for the development of new analgesics [37].

1.2.2. *Pharmacokinetics*

Metamizole is a prodrug since it is immediately non-enzymatically hydrolyzed into the active metabolite 4-MAA in the gastrointestinal tract after oral administration but also directly after parenteral administration (Figure 1). Therefore, no parent substance appears in blood and urine. The bioavailability of this primary metabolite is high (85% for tablets, 89% for drops, 54% for suppositories, 87% for intramuscular injection) [16]. Peak plasma

concentrations (Cmax) of 4-MAA are reached within 1.4 to 2 hours [38]. No significant difference in drug exposure (area under the concentration–time curve (AUC) and Cmax) has been shown between fasting and non-fasting conditions [39].

None of the major metabolites of metamizole showed a high plasma protein binding (4-MAA 57.6%, 4-AA 47.9%, 4-FAA 17.8%, 4-AAA 14.2%) [40]. 4-MAA has a low volume of distribution (0.5 L/kg) without extensive tissue binding, matching to the hydrophilic properties of 4-MAA [16]. Actually, metamizole was developed as improved water-soluble form of the analgesic and antipyretic drug aminopyrine [41].

The quickly formed active metabolite 4-MAA is further demethylated, possibly in the liver, to the likewise active 4-AA. Cytochrome (CYP) P450 enzymes are suggested to be involved, but the specific CYPs have not yet been identified. There is indirect evidence for CYP 1A2 due to correlation between CYP 1A2 activity and aminopyrine metabolism, which is metabolized in the same way and to the same metabolites like metamizole [42, 43]. Alternatively, either directly from 4-MAA or via 4-AA, the inactive metabolite 4-formyl-amino-antipyrine (4-FAA) is formed via oxidation, probably also mediated by CYPs. The last major metabolite rises from 4-AA which is acetylated to the inactive 4-acetyl-amino-antipyrine (4-AAA). This step is performed by the hepatic polymorphic *N*-acetyl-transferase 2, leading to marked varying levels of 4-AA (active) and 4-AAA (inactive) in slow and fast acetylators, respectively [44]. 4-MAA is the first metabolite reaching Cmax, but is also the first metabolite that falls below the detection limit. Both 4-AA and 4-FAA follow next to reach Cmax. The two metabolites are also the two main metabolites found in the urine [16]. Up to a dose of 1500 mg, the pharmacokinetics of 4-MAA is linear whereas higher doses show a deviation from linearity, but this was considered to be marginal for the usually applied dose range [38]. The four main metabolites mentioned above account together for approximately 70% of a dose [12]. Four minor additional metabolites have previously been described and new active metabolites in the CNS were discovered in 2012 [12, 25, 38]. The metabolites are primarily excreted in the urine, with a higher renal elimination after intravenous compared to oral application [45]. The half-life (T1/2) of 4-MAA after oral application of 1000 mg ranges between 2.5 and 3.5 hours [16]. Regarding the T1/2 of the other active metabolite 4-AA, it has to be distinguished between slow and fast metabolizers regarding *N*-acetyl-transferase 2. Slow metabolizers showed a longer T1/2 between 5.5 and

8.1 hours due to slower conversion into the *N*-acetyl-metabolite [16, 44]. Fast metabolizers have a T1/2 of about 3.8 hours [44]. Total body clearance for 4-MAA was found to be approximately 10 L/h with a renal clearance of about 0.24 L/h [16, 38].

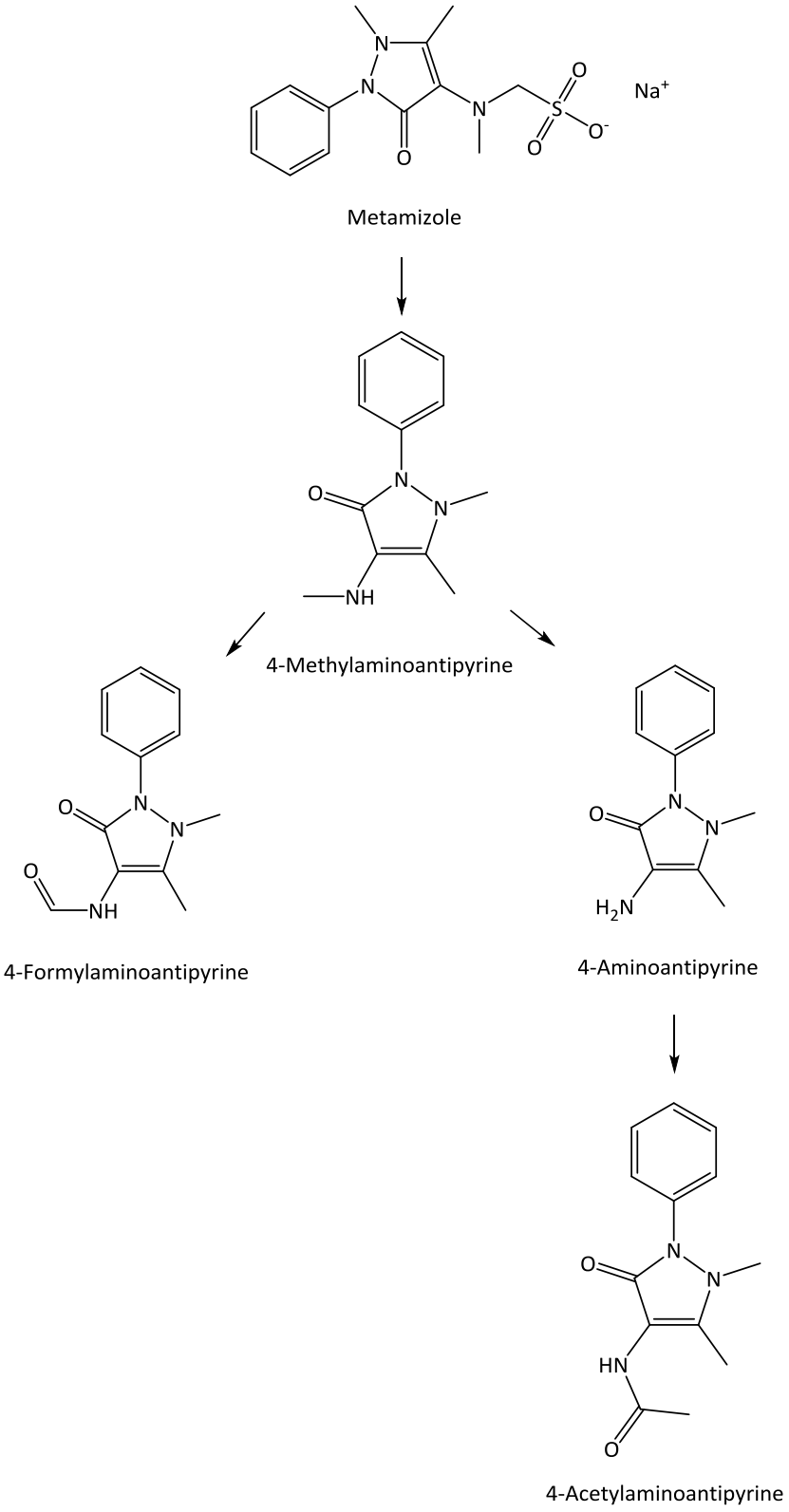

Figure 1 Metamizole metabolism

1.3. Hematological Safety: *The big problem*

1.3.1. *Definitions and incidence*

As a well-known fact, metamizole can induce agranulocytosis, a rapid and severe fall of neutrophil granulocytes with risk of fatal outcome. The definitions of blood cell disorders associated with metamizole are listed in the Table 1.

Table 1 Definitions of blood cell disorders associated with metamizole [46, 47]

	Definition
Leucopenia	Leucocyte count below 3.0 x 10^9/L
Neutropenia	Neutrophil granulocyte count below 1.5 x 10^9/L
Severe Neutropenia	Neutrophil granulocyte count below 0.5 x 10^9/L
Agranulocytosis	Neutrophil granulocyte count below 0.5 x 10^9/L with abrupt onset and clinical signs (fever, severe asthenia, buccopharynegeal and/or perineal ulcers)
Pancytopenia	Abnormal levels of all blood cell lines (anemia with neutropenia and thrombocytopenia)
Aplastic Anemia	Pancytopenia induced by a hypoplasia of the bone marrow

Generally, the term agranulocytosis is understood as severe neutropenia with decreased neutrophil granulocytes below 0.5 x 10^9/L [48, 49]. The laboratory findings are accompanied with characteristic symptoms and signs, including an abrupt onset of fever, asthenia and buccopharynegeal or perineal ulcers [46]. Drug-induced agranulocytosis is a serious adverse event due to possible complications including severe sepsis associated with infections and septic shock. Although the mortality rate decreased over the last years, it is still in the range of 5% [50]. In about 70% of cases of drug-induced agranulocytosis an association with a drug can be found [51]. The first step in case of a potential drug induced agranulocytosis is therefore the withdrawal of all suspected drugs [49]. Other therapeutic options are intravenous application of broad-spectrum antibiotics in febrile patients [52] and granulocyte colony-stimulating factor (G-CSF) in patients with severe neutropenia and manifest or suspected infections [53-55]. But the evidence regarding the efficacy of G-CSF in patients with drug-induced agranulocytosis (outside of the oncology setting) is disputable [56]. The question about the frequency of this blood disorder is also controversial. As already mentioned in the historical background section (chapter 1.1), the published incidence rates vary markedly, as shown in Table 2.

Table 2 Published incidence rates of metamizole-induced agranulocytosis (or aplastic anemia)

Year	Country / Study name	Study design	Incidence rate	Ref.
1979	Greece	Retrospective epidemiological study (1 year, National Statistical Service of Greece)	1:133'000	[57]
1986	IAAAS/ Europe and Israel	Population-based case-control study (221 agranulocytosis cases, 1425 hospital controls)	1.1 cases per million users per week 1:20'000 users/year	[4] [58]
1973	Sweden	Pharmacovigilance data analysis Estimations, based on the reporting frequencies Calculated from reported numbers	 1:3000 users 1:10'000 users	[8]
1981	Germany	Calculation of German Bundesgesundheitsamt (BGA)	1:20'000 users/year	[58]
2002	Sweden	Pharmacovigilance data analysis, sales data	1:1439 cases per prescription	[59]
2002	Poland	Retrospective case study (5 years, approximately 15 million people), sales data	0.2 cases per million person-days*	[60]
2004	Poland	Prospective case study (1 year, approximately 15 million people), sales data	0.25 cases per million person-days* (aplastic anemia)	[61]
2005	Spain	Case-control surveillance study (78.73×10^6 person-years)	0.56 cases per million inhabitants per year	[62]
2010	Poland	Prospective 12-month surveillance study	0.16 cases per million person-days*	[63]
2015	Germany	Case-control surveillance study	0.96 cases per million per year*	[14]

IAAS International Agranulocytosis and Aplastic Anemia Study

*potentially exposed patients (data from sale figures or statutory health insurance data divided per DDD (defined daily dose))

1.3.2. *Mechanisms of Toxicity*

Immune-mediated toxicity

Another unsolved question is the mechanism of metamizole-induced agranulocytosis. The mechanism is idiosyncratic, since the adverse reaction develops with therapeutic doses and is a rare event. Idiosyncratic toxicities can be immune-mediated or non-immune-mediated (metabolic) [64]. A schematic overview of possible mechanisms is shown in Figure 2. Arguments for an immune-mediated toxicity are described cases with a toxicity following only one dose, the lack of a distinct dose-dependency, or aggravated reactions after a rechallenge. In case of immunological drug induced agranulocytosis, the drug, or more likely a reactive metabolite, may act as hapten (a hapten is a small molecule that can provoke an immune response only when attached to a large carrier) that binds to the neutrophil membrane glycoproteins. Due to this binding, the immune system identifies the changed structure of the membrane no longer as endogenous structure and antibodies or T-cells directed against the altered structure of the membrane are produced. This mechanism has been shown for the thyreostatic drug propylthiouracil, the antimalarial drug amodiaquin, and the antiarrhythmic drug flecainide [65-69]. The production of autoantibodies against neutrophils, without binding of the drug or a metabolite to the neutrophil plasma membrane, has also been suggested [65]. For aminopyrine, which is closely structurally related to metamizole and is converted to the same metabolites, drug-dependent antibodies that lead to destruction of mature neutrophils have been demonstrated in vitro and in vivo [70, 71]. In transfusion experiments, Swiss investigators administered two subjects aminopyrine (rumor has it that it was a self-experiment) and injected 300 mL blood from a patient with aminopyrine-induced agranulocytosis. They were able to induce in both subjects a distinct fall of neutrophils (from 5000/mm^3 to 800/ mm^3 and from 8400/mm^3 to 1700/mm^3 within 40 min). The transfusion of blood of a healthy person in the recipients showed no decrease of leucocytes as well as orally administered aminopyrine one week after the transfusion [71]. Thus, drug-dependent antibodies are able to destroy mature neutrophils in the periphery. But typically, the bone marrow of patients with metamizole-associated agranulocytosis shows either too few cells or a halt in the maturation of granulopoietic precursors (maturation arrest). Due to the invasive character of a bone-marrow puncture, an examination is often omitted. But although the bone marrow of a

metamizole-induced agranulocytosis is not uniformly described, the maturation arrest on the level of myelocytes or promyelocytes is considered as typical finding for a toxic drug-induced bone marrow damage [72, 73]. Precursors can therefore be affected as well [48, 74]. Interestingly, the lack of granulocyte precursors concerns cells back to the promyelocyte stage, which is the stage when neutrophil precursors start to synthesize myeloperoxidase [75]. This suggests a role of reactive oxygen species (ROS) and the production of reactive metabolites as a mechanism of drug-induced agranulocytosis [76, 77].

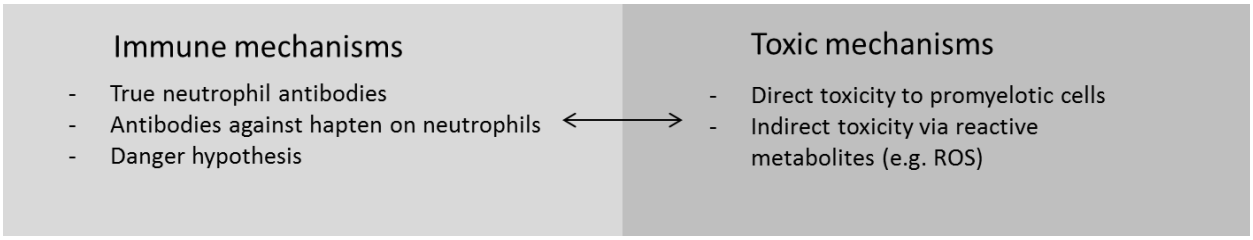

Figure 2 Schematic overview of possible mechanisms of the hematologic toxicity

Non-immune-mediated (metabolic) toxicity

The toxicity associated with ROS formation is traditionally classified as non-immune-mediated or metabolic toxicity. Hints for metabolic toxicity include the fact that all three blood cell lines can be affected and the lack of accompanying immunological features in patients with metamizole-induced agranulocytosis (for example eosinophilia, rash or fever). In case of drug-induced agranulocytosis, metabolic toxicity is usually understood as a damage of the myeloid progenitor cells in the bone marrow without direct involvement of immunological pathways. Either the drug itself or a reactive metabolite damages the cells. Regarding myelotoxicity, the cytotoxic effect of anticancer drugs is a good example of neutropenia caused by a dose-dependent or intrinsic mechanism. Non-chemotherapeutic drug-induced agranulocytosis is an idiosyncratic drug reaction which occurs at a therapeutic dosage and is therefore supposed to be unpredictable [78]. Regarding metamizole, there are some studies indicating dose-dependent toxicity to promyelotic cells. In vitro experiments with HL-60 promyelocytes showed that metamizole and 4-MAA induce apoptosis, but only at supratherapeutic concentrations (concentrations above 100 µM, therapeutic levels reach approximately 50 µM) [79].

Teamwork between immune-mediated and non-immune-mediated toxicity

Meanwhile, the distinction between immune-mediated and metabolic mechanisms is increasingly questioned due to new insights into the regulation of myelopoesis and neutrophil maturation as well as new concepts of immunological pathways, including the pharmacological interaction (PI) hypothesis and the danger hypothesis [64, 80, 81]. Especially the danger hypothesis may help to understand the question, how a reactive metabolite can initiate an immune response and therefore enable the combination of metabolic and immune-mediated toxicity [75].

According to the already introduced current idea, generation of ROS could lead to hapten formation. Via NADPH oxidase and myeloperoxidase, neutrophils generate ROS which are needed to oxidize or kill drugs or pathogens. Apart of neutrophils and myeloid progenitor cells starting at the promyelocyte stage, significant quantities of myeloperoxidase are also present in monocytes and macrophages [82]. One of the formed ROS is hypochlorus acid that is considered to be a major factor for oxidizing drugs into reactive metabolites. Such reactive metabolite formed can bind covalently to cellular molecules, which creates a complex that may serve as a hapten and thereby favoring T-cell mediated immune reactions [64]. The formation of a very reactive metabolite by hypochlorus acid has been demonstrarted for aminopyrine [83]. Another possibility of how a reactive metabolite can induce or modulate immune reactions is provided by the danger hypothesis. Matzinger and colleagues described 1994 the idea that organisms can differentiate between harmless and dangerous, similar to the immunological paradigm of self and non-self (as implied by the hapten hypothesis) [81]. The two hypotheses are not mutually exclusive. According to the hapten hypothesis, the complex of reactive metabolite bound to a protein (hapten) is taken up and processed by antigen-presenting cells and finally presented to helper T cells, which recognize it as non-self (signal one). According to the danger hypothesis, the drug or reactive metabolite can cause cell damage, leading to a release of “danger signals” that upregulates costimulatory factors for the antigen-presenting cell (signal two). Both signals, signal one and two, are needed for the initiation of a strong T-cell proliferation [75]. In contrast to the classical immune approach, not cells of the immune system are the main stimulus for the initiation of an immune response, but endogenous signals from damaged tissues [81]. Without these second signals, there would be immune tolerance [84]. The question, which kind of danger signals are formed, is unclear. Preliminary data suggest that activation of

inflammasomes could be one mechanism, with production of inflammatory cytokines (IL-1β and IL-18) which may act as danger signals [85]. Additional factors associated with cell damage including infections, may increase the risk for such reactions [86].

To sum up, reactive metabolites seem to play a crucial role in metamizole-induced agranulocytosis via direct (metabolic) toxicity and/or via stimulating immune-mediated destruction of granulocyte precursors in the bone marrow [64].

1.3.3. *Risk factors and genetic susceptibilities*

Since metamizole-induced agranulocytosis is considered to be idiosyncratic and is therefore not dose-dependent, investigations trying to find risk factors for the development of this disorder were performed. Female sex has been suggested in several studies due to a higher proportion of affected women [59, 60, 87]. Likewise, drug-induced agranulocytosis has been associated with higher age [88, 89]. Both risk factors where assumed to be eventually exposure-related, but clarifying data was missing. An increased risk with increasing duration of treatment has been discussed as well [14, 62]. But in the systematic review of Andersohn and colleagues about non-chemotherapeutic drug-induced agranulocytosis, metamizole showed a median drug exposure of only two days, emphasizing the existing risk even in short-time therapies [49]. A higher dose, in addition to longer therapy durations, was also supposed to be a possible risk factor [62]. This hypothesis is not supported by the fact that metamizole-induced agranulocytosis is not associated with cases of overdoses. A review of cases of metamizole overdoses found mainly mild gastrointestinal complications but not bone marrow damage [90]. Moreover, there are case reports of kidney toxicity without hematotoxicity after metamizole overdose [91, 92].

Concomitant drugs, especially such with a known risk for agranulocytosis, have also been investigated as possible risk factors [59, 62], but no clear implications were reported. Regarding underlying diseases, especially allergies and viral infections were considered as potential risk factors [59, 93, 94].

The investigation of a possible genetic predisposition is still in its infancy. In 1996, Vlahov and colleagues published a paper about genetic risk factors of metamizole-induced agranulocytosis [5]. They examined five patients with metamizole-induced agranulocytosis and four patients with agranulocytosis independent of metamizole and compared them to a

reference group of 180 healthy subjects. In the collective of the nine agranulocytosis patients, more were carriers of the human lymphocyte antigen 24 (HLA24) antigen and less of the DQA1*0501 allele compared to the controls (11% versus 57%). Regarding the metamizole-associated agranulocytosis patients, the HLA24-B7 haplotype was found with a higher frequency than in the metamizole-independent cases and controls. Additionally, all five patients with metamizole-associated agranulocytosis were found to have a HLA-DQwl antigen and in four of five also a HLA2 antigen, while in the control group only 56% were carriers of these genes. The number of cases in this study is very small, but the study has shown relevant differences regarding human lymphocyte antigen HLA allele frequencies. In 2015, a new study about the genetic determinants of metamizole metabolism which modify the risk of developing anaphylaxis has been published [95]. Their results support the hypothesis of genetically determined metabolic variability as a risk factor for developing anaphylaxis. Slow acetylators showed an increased risk of developing selective hypersensitivity, especially anaphylaxis. But there are no hints for a higher risk for agranulocytosis in patients with slow acetylation [5]. In summary, little is known about the genetic predisposition, leaving a big potential for further investigations.

1.4. Renal Safety: *A possible advantage*

Apart from the discussion about the risks of metamizole, benefits of the substance quickly get forgotten. One benefit of metamizole compared to NSAIDs is its supposed better renal tolerability. The available data basis for this hypothesis, however, is poor. It is still not entirely clear, whether metamizole is a valuable alternative to NSAIDs in patients with low intravascular pressure (stimulated renin-angiotensin-aldosterone system), where prostaglandins are crucial for the maintenance of renal perfusion. This concerns patients with diseases such as heart failure, liver cirrhosis with ascites, chronic renal insufficiency or nephrotic syndrome [96]. Of course, this is related to the unclear mechanism of action. The question, whether metamizole inhibits renal prostaglandin synthesis has to be answered as well as the question, whether metamizole shows an acute effect on renal function in sensitive patients. Regarding renal complications, mainly case reports, case series and reviews about acute kidney injury (AKI) or acute interstitial nephritis (AIN) are described in the literature [91, 97, 98]. The absolute number of cases reported, however, is small. Berruti

et al. [98] described the differences between the clinical presentations of acute interstitial nephritis after metamizole intake compared with NSAIDs. The shortened time course between the metamizole administration and the clinical presentation (24 hours to a few days for metamizole versus several months up to 1 year for NSAIDs), as well as the lack of significant proteinuria in the metamizole cases compared with a NSAID-induced acute interstitial nephritis have been discussed. Finally, the accuracy of the diagnosis of the metamizole-induced acute interstitial nephritis can be questioned.

Overall, clinical experience suggests better renal tolerability of metamizole possibly due to less potent renal COX-inhibition compared to classical NSAIDs. If this could be confirmed, metamizole would be a valuable alternative for treatment of painful conditions in patients with impaired renal function.

2. Aims of the thesis: *Remaining questions*

As apparent from the introduction, many questions remain around the old substance metamizole. Considering the increasing use of metamizole, the demand for more information about metamizole has gained relevance. The goal of this PhD-thesis project was to improve our understanding of the clinical toxicity of metamizole. The planned work should contribute to a better understanding of the risk-benefit profile and promote the safe use of metamizole. To reach this goal, the PhD thesis project has been divided into the following three subprojects:

- Descriptive analysis of pharmacovigilance data concerning adverse effects related to metamizole
- Retrospective case control-study of metamizole-associated leucopenia
- Clinical study investigating the renal safety of metamizole in salt-depleted healthy volunteers

The main objective of the pharmacovigilance analysis was to characterize spontaneously reported cases of hematological adverse drug reactions with metamizole as suspected drug regarding appearance, course, and severity of the reactions. The case safety reports were either selected from the WHO Global Individual Case Safety Report Database (Vigibase^{TM}) or from the National Pharmacovigilance Database Swissmedic. This investigation allowed a comparison of reported hematological adverse drug reactions on a national and international level.

After the characterization of pharmacovigilance data cases, the retrospective case-control study focused on the search of risk factors for the development of metamizole-induced white blood cell disorders. In addition of the detailed characterization of patients with metamizole-induced agranuloyctosis managed at the University Hospital Basel, the comparison with a control group should allow more valid identification of risk factors.

The clinical study about the renal safety of metamizole, however, dealt with a possible advantage of metamizole. The aim of this study was to examine the effects of metamizole on renal function in comparison with the non-specific COX-inhibitor naproxen. If it could be shown that metamizole does not have negative effects on renal function, it would support the use of metamizole in patients with impaired renal function that cannot be treated with NSAIDs.

3. Results

Before immersing into the three main projects, let's start with a case. The case shows impressively the brisance of the topic and makes it easy to understand that a once experienced negative event constraints to legitimate caution.

3.1. Fatale Agranulozytose nach Metamizol-Reexposition

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Praxis (Bern 1994). 2015 Jan 28;104(3):151-4.

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Fatale Agranulozytose nach Metamizol-Reexposition

Fatal Agranulocytosis after Re-Exposure to Metamizole

Anamnese und Befunde

Ein 63-jähriger Patient wurde Mitte Mai 2014 notfallmässig aus der Altersiedlung aufgrund seit dem Vorabend bestehenden Fiebers und respiratorischer Verschlechterung hospitalisiert. Als Grunderkrankungen lagen ein beginnendes dementielles Syndrom bei chronischem Alkoholkonsum, eine Kardiopathie (am ehesten äthylotoxisch) und ein Typ-2-Diabetes mellitus vor. Aufgrund von Schmerzen bei bekannter Polyneuropathie war zehn Tage zuvor eine Metamizol-Therapie mit Novalgin® 500 mg dreimal täglich peroral gestartet worden. Die weitere Eintrittsmedikation bestand aus Pantoprazol 40 mg/d, Torasemid 10 mg/d, Levocetirizin 5 mg/d, Atenolol 10 mg/d, Escitalopram 10 mg/d, Magnesium 5 mmol/d. Auf der Notfallstation zeigte sich der Patient febril bis 38,7°C, kaltschweissig, hypoton (BD 109/80 mmHg), tachykard (Herzfrequenz 120/min) und dyspnoisch mit einem Sauerstoffbedarf von 15 l/min. Klinisch fielen diffuse Rasselgeräusche über allen Lungenfeldern auf bei sonst unauffälligem Status. Im Notfalllabor imponierte eine Agranulozytose (Neutrophile $0{,}001\times10^9$/l [Normbereich $1{,}3–6{,}7\times10^9$/l]), Leukozyten $0{,}19\times10^9$/l [3,5–10,0], erhöhte Entzündungswerte (CRP 280 mg/l [<10 mg/l], pCT 12 ng/ml [<0,1 ng/ml]) sowie ein erhöhtes Kreatinin (125 µmol/l [49–79 µmol/l]). Die Thrombozytenzahl war zudem leicht reduziert (125×10^9/l [150–450]), das Hämoglobin jedoch im Normbereich.

Bei zunehmender Verschlechterung und klinischem Bild eines schweren septischen Schocks erfolgte die Verlegung auf die medizinische Intensivstation, wo der Patient aufgrund respiratorischer Erschöpfung intubiert werden musste. Da unter Volumensubstitution und Noradrenalingabe der Kreislauf nicht ausreichend stabilisiert werden konnte, erfolgte die zusätzliche Gabe von Adrenalin. Bei echokardiographischem Nachweis einer schwer eingeschränkten Pumpfunktion bei bekannter äthyltoxischer Kardiopathie begannen wir zudem mit Dobutamin. Bei zunächst unklarem Infektfokus erfolgte eine Breitspektrumantibiotika-Therapie mit Meropenem. Des Weiteren erhielt der Patient Infektprophylaxen mit Aciclovir, Fluconazol und Trimethoprim/Sulfamethoxazol. Computertomografisch zeigten sich bilaterale Infiltrate. Bei vorbestehenden perinealen Fisteln erfolgte eine prophylaktische Seton-Drainageneinlage, bildmorphologisch ergab sich jedoch kein Hinweis für eine Abszessformation.

Ungefähr 18 Monate zuvor hatte der Patient nach einer Halswirbelkörperfraktur bereits auf Metamizol mit einer Agranulozytose (ohne Beteiligung anderer Zelllinien) reagiert, die sich nach Absetzen des Medikaments und Behandlung mit Granulozyten-Kolonien stimulierenden Faktoren (G-CSF) erholte. Die Neutropenie präsentierte sich damals in einer Routine-Blutbildkontrolle zwölf Tage nach Metamizol-Beginn und lag bei $1{,}123\times10^9$/l [Normbereich $1{,}3–6{,}7\times10^9$/l] und fiel auf einen Nadir von $0{,}021\times10^9$/l sieben Tage später. Unter der damaligen Neutropenie hatte er perianale Fisteln mit Abszessbildung entwickelt, die chirurgisch angegangen werden mussten. Als andere mögliche medikamentöse Auslöser der damaligen Agranulozytose kamen auch Quetiapin und Piperacillin/Tazobactam infrage.

Differenzialdiagnostische Gedanken

Eine wiederholte Agranulozytose nach Metamizol-Reexposition, wie in diesem Fall, machte eine Metamizol-induzierte Agranulozytose zur wahrscheinlichsten Diagnose. Gemäss WHO-Kausalitätskriterien für unerwünschte Arzneimittelwirkungen (UAW) war Metamizol als sicherer Auslöser der Agranulozytose zu betrachten [1].

Weitere Abklärungsschritte und Verlauf

Im Verlauf konnten als Erreger Streptokokken der Gruppe A in den Blut-

Im Artikel verwendete Abkürzungen:

BD	Blutdruck
CRP	C-reaktives Protein
G-CSF	Granulozyten-Kolonien stimulierender Faktor
pCT	Procalcitonin
UAW	Unerwünschte Arzneimittelwirkung

DOI 10.1024/1661-8157/a001916

kulturen wie auch im Trachealsekret nachgewiesen werden und die antibiotische Therapie resistenzgerecht auf Clindamycin und Ceftriaxon umgestellt werden. Unter Behandlung mit G-CSF kam es nur zu einer geringfügigen Stimulation der Proliferation der Granulozyten. Trotz Ausbau der Vasoaktiva, zusätzlicher Hydrocortisongabe und Applikation von Immunglobulinen verlief die Sepsis weiter fulminant. Es kam zu ausgeprägten Perfusionsstörungen der Extremitäten sowie anurischem Nierenversagen. Nach Stopp der sedierenden Medikamente zeigte sich der Patient weiterhin komatös, sodass auch von einer zerebralen Beeinträchtigung durch die Sepsis ausgegangen worden ist. Es wurden mehrere Gespräche mit den Angehörigen geführt und man entschied sich, gemäss mutmasslichem Patientenwillen und infauster Prognose, auf eine Fortführung der lebensverlängernden Massnahmen zu verzichten. Der Patient verstarb am fünften Hospitalisationstag.

Beide Metamizol-induzierten Agranulozytose-Episoden wurden gemäss Bundesgesetz innerhalb von 15 Tagen als schwerwiegende unerwünschte Arzneimittelwirkungen in anonymer Form beim Schweizerischen Heilmittelinstitut (Swissmedic) gemeldet.

Diagnose

Fataler septischer Schock mit *Streptococcus pyogenes* und Multiorganversagen bei Metamizol-induzierter Agranulozytose

Kommentar

Im vorliegenden Fall kam es nach erneuter Metamizol-Exposition zu einer schweren Agranulozytose mit fatalen Konsequenzen. Der Fall unterstreicht, wie ernst eine medikamentös induzierte Agranulozytose in der Vorgeschichte zu nehmen ist. Trotz Vermerk «Dauerhafter Verzicht auf Novalgin®» im Austrittsbericht kam es zu einer erneuten Exposition. Ein Grund hierfür könnte die Tatsache sein, dass drei verdächtige Medikamente als Auslöser bei der ersten Episode infrage kamen. Eine Metamizol-Rechallenge bei Notwendigkeit einer Schmerzlinderung bei einem Patienten mit erhöhtem Alkoholkonsum wurde vielleicht deswegen trotzdem als vertretbar eingestuft. Zudem könnte das demenzielle Syndrom einen ungünstigen Einfluss auf die Mitteilungsverantwortung des Patienten ausgeübt haben oder gar seine Erinnerung an die vorausgegangene Agranulozytose verunmöglicht haben. Weiter sind UAW, die im Spital auftreten, für Patienten oft weniger einschneidend und somit auch weniger präsent. Eine Art «Kontraindizierte Medikamente-Pass» (analog zu einem Allergie-Pass), der immer auf sich getragen werden muss, könnte als Massnahme zur Verhinderung solcher Ereignisse erwogen werden. Dieser Fall unterstreicht die Wichtigkeit einer lückenlosen Kommunikation der verschiedenen Mitspieler im Gesundheitswesen.

Mechanismus

Der Mechanismus der Metamizol-induzierten Agranulozytose ist leider aktuell nur unzureichend aufgeklärt [2]. Metamizol wird jedoch häufiger eingesetzt [3], weshalb ein Anstieg in der Gesamtfallzahl von Metamizol-induzierten Agranulozytosen in der Schweiz und in Deutschland zu erwarten ist.

Zwei pathogenetische Ursachen werden vermutet [2]. Es gibt Hinweise für eine immunologische Pathogenese, aber auch für eine direkte toxische Schädigung der Vorläuferzellen im Knochenmark. Bei der immunvermittelten Agranulozytose handelt es sich um eine allergisch bedingte Reaktion, bei der das Pharmakon oder einer seiner Metaboliten als Hapten oder Prohapten fungiert und eine humorale oder zelluläre Immunreaktion auslöst [4]. Gemäss dem p-i(pharmakologische Interaktion)-Konzept kann eine Immunantwort auch über direkte Interaktion von Arzneistoffen oder Metaboliten mit dem T-Zell-Rezeptor erfolgen [5].

Bei dem direkt knochenmarktoxischen Mechanismus handelt es sich in der Regel um einen schleichenden toxischen Prozess. Dieser ist meist zeit- wie auch dosisabhängig. Für eine Zeitabhängigkeit spricht die Tatsache, dass eine Agranulozytose nicht in Fällen von akuter Toxizität (z.B. in Rahmen einer einmaligen Überdosierung) beobachtet wird [6]. Nach längerer Medikamenteneinnahme kann es zu einer toxischen Schädigung der Vorläuferzellen im Knochenmark kommen [7]. Metaboliten können an Kernmaterial oder an zytoplasmatische Proteine binden und dadurch eine direkte Toxizität an den Myeloid-Vorläuferzellen im Blut oder Knochenmark ausüben [2]. Es können auch andere Blutzelllinien betroffen sein. Immer wieder werden Fälle von Metamizol-induzierten Bizytopenien (wie in diesem Fall) oder Panzytopenien berichtet [8].

Epidemiologie

Metamizol gehört zu jenen Medikamenten, die am häufigsten mit Agranulozytosen assoziiert sind [9,10]. Eine Studie aus Spanien errechnete eine Odds Ratio, eine Agranulozytose unter Metamizol zu entwickeln, von 26 im Vergleich zu nicht-exponierten Kontrollen [10]. Die Inzidenz der Metamizol-induzierten Agranulozytose wird unterschiedlich berichtet und eingeschätzt und variiert zwischen 1:1439 Verschreibungen bis zu 1:1 Mio. Anwender pro Woche [11]. Eine geografische und/oder genetische Prädisposition scheint vorzuliegen [12]. Die Mortalität lag in früheren Jahren bei bis zu 29%, wird aber heute zwischen 5 und 7% geschätzt [11]. Eine Bi- oder Panzytopenie ist mit einer erhöhten Mortalität assoziiert [8]. Dabei muss zwischen den beiden Krankheitsbildern Agranulozytose und aplastische Anämie unterschieden werden. Eine Agranulozytose, die ausschliesslich die Neutrophilen betrifft, ist meist reversibel nach Absetzen der Verursacher, während eine aplastische Anämie, die zu einer Panzytopenie mit hypozellulärem Knochenmark führt,

Key messages

- Die Metamizol-induzierte Agranulozytose ist eine schwerwiegende unerwünschte Arzneimittelwirkung.
- Bei hospitalisierten Patienten, die einen Neutrophilen-Abfall oder Agranulozytose unter Metamizol entwickeln, muss das Metamizol sofort abgesetzt werden. Prophylaktische Antibiotika bei fehlendem Fieber oder Infektzeichen sind nicht routinemässig indiziert. Sobald jedoch Infektzeichen auftreten, sollte unverzüglich eine Breitspektrumantibiotika-Therapie begonnen werden, ohne die Abklärungen abzuwarten.
- Ambulante Patienten müssen instruiert werden, Metamizol bei Fieber und/oder Halsschmerzen abzusetzen und sich notfallmässig beim Arzt vorzustellen.
- Eine Reexposition nach stattgefundener Agranulozytose, bei der Metamizol verdächtigt war, muss vermieden werden, da ein schwerwiegender Verlauf wahrscheinlich ist.

ohne entsprechende Behandlung meist nicht reversibel ist. Aufgrund der geographischen Variation in der Inzidenz und des immer noch erhöhten Risikos, an einer Agranulozytose zu sterben, ist Metamizol in mehreren Ländern nicht zugelassen, wie z.B. den USA, Grossbritannien und Schweden [8,13].

Es liegen in der Literatur unseres Wissens bisher nur zwei Fälle zu einer wiederholten Agranulozytose nach einer Reexposition mit Metamizol vor. In beiden Fällen trat eine erneute Neutropenie auf [12]. Zwischen 1969 und Januar 2013 ist weltweit 920 Mal eine mit Metamizol assoziierte Agranulozytose bei der WHO gemeldet worden (Vigisearch-Suche) [14]. Bis zur Datenzusammenschau (7.7.2014) hat sich die weltweit gemeldete Anzahl Metamizol-assoziierter Agranulozytosen auf 1042 erhöht, wovon 68 Fälle aus der Schweiz stammen. Unter den Fällen, die zwischen 1969–2013 gemeldet wurden, entwickelte sich in 23 Fällen die Agranulozytose nach einer Reexposition [14]. In zwei Reexpositionsfällen wurde eine Panzytopenie berichtet und in einem Fall eine hypozelluläre Knochenmarkhistologie. Insgesamt sind drei Patienten gestorben, zwei erlitten anhaltende Beschwerden und in fünf Fällen war der Outcome nicht bekannt. Die Mortalität nach einer Reexposition scheint daher in etwa zwischen 13 und 17% zu liegen.

Management

Es liegen keine formalen Leitlinien zur Behandlung einer Metamizol-induzierten Agranulozytose vor. Eine Agranulozytose bleibt asymptomatisch bis zum Auftreten einer infektiösen Komplikation. Gemäss Fachinformation wird keine routinemässige Überwachung des Blutbildes unter Metamizol-Therapie empfohlen (im Gegensatz zu Clozapin). Eine gute Übersicht zur Behandlung Medikament-induzierter Agranulozytosen ist im Uptodate® zu finden [15]. Als erste Massnahme sollten in jedem Fall Metamizol und, wenn vorhanden, andere verdächtige Medikamente abgesetzt werden. Durch Absetzen der verdächtigen Medikamente ist die Agranulozytose meist reversibel. In der Regel erholt sich die Neutrophilenzahl innerhalb von ein bis drei Wochen ohne G-CSF-Behandlung [15]. Zudem sollte eine antibiotische Therapie sowie eine G-CSF-Behandlung in Betracht gezogen werden. Allergologische Abklärungen nach Abklingen der Reaktion sind zurzeit nicht in der Routineklinik etabliert. Allerdings laufen hierzu klinische Studien.

Zusammenfassung

Wir stellen den Fall eines 63-jährigen Mannes vor, der einen schweren septischen Schock mit letalem Ausgang auf Basis einer Agranulozytose entwickelte. Aufgrund des zeitlichen Zusammenhangs, der Verbesserung nach Dechallenge in der Vorgeschichte und aktuellem Rechallenge ist der Kausalzusammenhang für eine erneute Metamizol-induzierte Agranulozytose sicher. Wir diskutieren die Wichtigkeit, Patienten mit einer verdächtigten Metamizol-induzierten Agranulozytose nicht erneut zu exponieren und Patienten sowie behandelnde Ärzte lückenlos zu informieren.

Schlüsselwörter: Agranulozytose – Metamizol – Septischer Schock

Abstract

We present the case of a 63 year old man who died of severe septic shock in the setting of agranulocytosis induced by dipyrone (metamizole). The patient had previously developed agranulocytosis after dipyrone exposure 18 months prior to this. The case illustrates the seriousness of dipyrone-induced agranulocytosis, highlights the risks associated with re-exposure and underlines the need for excellent communication between treating physicians and their patients. The possible underlying mechanisms, epidemiology and management of dipyrone-induced agranulocytosis are discussed.

Key words: agranulozytosis – metamizole – septic shock

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Interessenskonflikt: Die Autoren erklären, dass kein Interessenskonflikt besteht.

Manuskript eingereicht: 11.7.2014, revidierte Fassung angenommen: 10.9.2014

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After the entrance with a single case (truly a worst case scenario), the pharmacovigilance data analysis should supply data of a large case collective. The characterization of these cases was our priority.

3.2. Hematological Safety of Metamizole: Retrospective Analysis of WHO and Swiss Spontaneous Safety Reports

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Eur J Clin Pharmacol. 2015 Feb;71(2):209-17

Eur J Clin Pharmacol
DOI 10.1007/s00228-014-1781-z

PHARMACOEPIDEMIOLOGY AND PRESCRIPTION

Hematological safety of metamizole: retrospective analysis of WHO and Swiss spontaneous safety reports

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Received: 29 April 2014 / Accepted: 4 November 2014

Abstract
Purpose Since the 1970s, the use of metamizole is controversial due to the risk of agranulocytosis. The aim of this study was to analyze individual case safety reports (ICSRs) of metamizole-associated hematological adverse drug reactions (ADRs).
Methods International and Swiss metamizole-associated ICSR concerning selected hematological ADR were retrieved from VigiBase™, the World Health Organization Global Database of ICSR, and the Swiss Pharmacovigilance Database. We evaluated demographic data, co-medication, drug administration information, dose and duration of metamizole treatment, as well as the latency time of ADR, their course, and severity. The subgroup analysis of Swiss reports allowed us to analyze cases with fatal outcome more in depth and to estimate a rough minimal incidence rate.
Results A total of 1417 international and 77 Swiss reports were analyzed. Around 52 % of the international and 33 % of the Swiss metamizole-associated hematological ADR occurred within a latency time of ≤7 days. More women were affected. The annual number of hematological reports and those with fatal outcome increased over the last years parallel to metamizole sales figures. In Switzerland, the minimal incidence rate of agranulocytosis was 0.46–1.63 cases per million person-days of use (2006–2012). Female sex, old age, pancytopenia, and co-medication with methotrexate were striking characteristics of the seven Swiss fatal cases.
Conclusions Metamizole-associated hematological ADR remain frequently reported. This is underscored by increasing annual reporting rates, which mainly reflect growing metamizole use. Early detection of myelotoxicity and avoidance of other myelotoxic substances such as methotrexate are important measures for preventing fatalities.

Keywords Metamizole (dipyrone) · Agranulocytosis · Hematological safety · Pharmacovigilance

Introduction

Metamizole, also known as dipyrone, is an old antipyretic and analgesic drug with a controversial history. Although metamizole has poor anti-inflammatory effects, it is often, probably improperly, classified as a non-steroidal anti-inflammatory drug (NSAID). The pharmacological mechanism of action is still not entirely understood. Inhibition of cyclooxygenase (COX) enzymes by metamizole has been demonstrated, but the corresponding IC50 values show a high variability between 2.55 and >400 μmol/L (Ibuprofen: IC50 values COX-1 12–42 μmol/L, Naproxen: IC50 value COX-1 0.3–24 μmol/L) [1–4]. Other mechanisms of action have been proposed, including stimulation of endogenous cannabinoid receptors [5].

For years, the safety profile, in particular the risk of blood disorders including agranulocytosis, has been controversially discussed. Estimated incidences for agranulocytosis range from 1:3000 users a year to 1:1,000,000 users a week [6, 7].

Electronic supplementary material The online version of this article (doi:10.1007/s00228-014-1781-z) contains supplementary material, which is available to authorized users.

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Published online: 18 November 2014

Due to the risk of agranulocytosis, metamizole was banned or withdrawn from the market in many countries (e.g. Great Britain, USA, Sweden, Japan, and Australia), while it is currently on the market in other countries and parts of the world (Switzerland, Germany, France, Spain, Latin and South America, Far East, and Africa) [8, 9].

Supporters of metamizole emphasize the advantages of metamizole over classical NSAIDs, especially the better gastrointestinal and renal tolerability [10]. A meta-analysis of several epidemiologic studies of serious adverse effects associated with different analgesics demonstrated that the absolute risk of mortality associated with metamizole is 25 deaths per 100 million users, which is in the same range as for paracetamol but much lower than for NSAIDs [11].

As the prescription rate of metamizole in Switzerland is increasing and since we had received several reports of metamizole-associated hematological adverse reactions, we decided to analyze the international and Swiss hematological pharmacovigilance data of this drug.

Methods

Study design and selection of individual case safety reports

This retrospective descriptive study is based on selected individual case safety reports (ICSRs) from the World Health Organization (WHO) global database VigiBase™ and, for a more detailed subgroup analysis, from the Pharmacovigilance database of the Swiss Drug Authority Swissmedic. We included only ICSR with metamizole as suspected or interacting drug associated with adverse drug reaction (ADR) terms corresponding to the WHO adverse reaction terminology (WHO-ART) "preferred terms" agranulocytosis, granulocytopenia, leucopenia, white blood cell abnormal not otherwise specified, pancytopenia, anemia aplastic, aplasia bone marrow, and marrow depression.

Recording of individual hematological case safety reports

International data

We received the coded data elements of the international metamizole-associated ICSR from the WHO global database VigiBase™ as a Microsoft Excel file. Since the international ICSR did not contain a narrative, verification of coding, adding codes or recoding could not be performed. The following data were analyzed: demographic data (age at ADR onset and gender), route of administration, daily dose, duration of treatment, cumulative dose, reported ADR and their causality assessments, latency time, duration of ADR, number of suspected drugs, and total number of drugs. Using the available coded dosage information, therapy and ADR dates, we calculated the daily dose, cumulative dose, duration of treatment, latency time, and duration of the ADR. The latency time was defined as the period between the metamizole start date and the date of ADR onset. For all international metamizole-associated hematological ICSR, we analyzed the annual reporting rate, reporting rate for fatal outcome, and cumulative reporting rates stratified per country. ICSR with unlikely or not assessable causality assessment or with a negative latency time (ADR occurred before metamizole administration) were excluded. If more than one metamizole preparation was listed in a ICSR, we did not use the data concerning the metamizole causality assessment, start and stop date of metamizole treatment, outcome of reported ADR, dechallenge and rechallenge information, or route of administration for the final analysis due to difficulties in handling the various data.

Swiss data

We received coded data elements of the Swiss metamizole-associated ICSR from the pharmacovigilance database of Swissmedic accompanied by the original ICSR with the case narrative in a portable document file. The case narrative contained more detailed information about the course of each ADR and allowed us to analyze co-medication and cases with fatal outcome more in depth. Additionally, we could estimate a rough minimal incidence rate. We reviewed the information of the case narratives and, in case of discrepancies between the narrative and the coded information, we recoded according to the narrative. The WHO-ART codes for reported ADRs were first verified with the narrative according to the definitions for blood dyscrasias [12, 13]. Afterwards, they were recorded as follows: If in an ICSR, a patient had received neutropenia ("preferred term") with fever (preferred term), we recoded the "included term" febrile neutropenia which belongs to the preferred term agranulocytosis. We recoded the terms only, if neutrophil counts were in accordance with the definition of agranulocytosis [12, 13]. In cases with fatal outcome, we recorded the day of death as the stop date of the ADR. In cases without ADR stopping date, we added the date, if it was obvious from the reported laboratory values or if it was mentioned in the narrative. General statements of ADR courses, for example "the hemogram improved within three weeks" without an exact reported stopping date, were recorded as 21 days. Therapy options such as administration of granulocyte-colony stimulating factor (G-CSF), antibiotics, transfer of the patient to a hospital's intensive care unit, or withdrawal of the drug were also recorded.

For the calculation of the daily and cumulative dose, we coded or recoded the data according to the narrative as follows: If possible, we calculated the daily and the cumulative doses according to the coded dosage information. If

metamizole was prescribed as an "as required" medication and metamizole intake and dosage regimen could be assured, we interpreted metamizole as being dosed maximally (4 g per day). If the intake was uncertain or unknown, we did not calculate the daily or cumulative dose for this ICSR. Cumulative drug doses reported as circa 60 g were recorded as exactly 60 g. A single administration of metamizole was recorded as therapy duration of 1 day. Other possible coding terms for time specification such as week(s), month(s), or year(s) were counted as 7, 30, and 365 days, respectively. When only the month and year (MM.YYYY) of the start or stop date was reported, we counted the recorded month as a full month (30 days). Coded time terms such as day(s), short term, and long term without more specific explanations were not used for the calculation of daily or cumulative dose or therapy duration. For co-suspected drugs, we coded and analyzed generic names and the corresponding ATC codes. In addition to the annual reporting rate of metamizole-associated hematological ICSR, we estimated a rough minimal incidence for agranulocytosis using annual sales figures of all Swiss metamizole preparations. The Swiss annual sales figures were obtained from the respective marked authorization holders. They were first expressed as defined daily dose (DDD) and were pooled afterwards.

Statistical analysis

Descriptive analysis was performed using Microsoft Office Excel (2010) and SPSS for Windows software (version 21). For variables with normally distributed numeric values, the arithmetic mean and standard deviation were calculated. For variables without normally distributed values, median and range were determined.

Results

Between June 22, 1968, and January 25, 2013, 1475 ICSR concerning metamizole-associated hematological ADR were transmitted to Vigibase™. After excluding ICSR according to the defined exclusion criteria, 1417 international ICSR remained for analysis. Between 1991 and December 31, 2012, the National Pharmacovigilance Center at Swissmedic received 85 ICSR of metamizole-associated hematological ADR. After the exclusion of ICSR using the previously defined exclusion criteria, 77 ICSR remained for the subgroup analysis.

Table 1 summarizes the findings of the international and Swiss data. Concerning ADR terms, agranulocytosis was listed most often in both datasets in almost one third of the reports. This was followed by leucopenia in one fifth and granulocytopenia in approximately 10 % of the reports. The other terms concerned adverse drug reactions with a decrease in all blood cell lines.

In both datasets, around two thirds of reports concerned women and the median age was almost 60 years. When stratified for age, there were no apparent differences between international and Swiss cases, showing highest numbers in the age group of 70–79 years (18–19 %) and lowest numbers of ICSR in the age groups 0–9 years (1–2 %) and 10–19 years (3–6 %). For detailed information about the age categories, see supplemental Table 1. While 43 % (n=610) of the international cases were reported as serious, this number reached 90 % (n=69) for Swiss reports.

As shown in Table 2, the daily dose was within the recommended range in both datasets. The duration of treatment could be calculated for 765 international and 63 Swiss ICSR, resulting in a median duration of 8 days (range 1 day to 9 years) and 13 days (range 1 to 594 days), respectively. Among the 63 Swiss ICSR with known treatment duration, almost half of the patients (44 %, n=27) received metamizole for ≤7 days. As a consequence of the large range regarding treatment duration, the corresponding cumulative doses also showed a wide range. For 858 international ICSR, the latency time could be calculated, resulting in a median of 7 days until the diagnosis of the blood disorder, while the median latency time in the Swiss ICSR was 14 days. In 442 (52 %) international and 22 (33 %) Swiss hematological ICSR, the ADR onset was within the first week after start of treatment. This is shown in Fig. 1, which displays the latency time distribution for the international ICSR stratified by weekly periods. The latency time distribution of the Swiss reports was similar (data not shown). The median number of concomitant drugs per report was 3 and 4 in the international and Swiss dataset, respectively. Also, the median number of suspected drugs per report (including metamizole), was similar, namely 1 in the international and 2 in the Swiss dataset. In 436 (31 %) international ICSR, metamizole was reported as the only suspected drug, whereas in the Swiss dataset, this was the case in only 13 (17 %) reports. In Swiss reports, co-suspected drugs were 28 anti-invective agents followed by 23 nervous system drugs and 8 drugs for acid-related disorders (see supplemental Table 2 for further information).

In the Swiss reports, causality for metamizole was never judged as certain, unknown, or not assessable. In two thirds of the reports, the causality for metamizole was judged as possible and in one third as probable. A rechallenge was reported in 45 (3 %) international ICSR. In rechallenged patients, the ADR reoccurred in almost half of the cases (n=23). In 16 ICSR, the rechallenge outcome was unknown and in 6 ICSR, the rechallenge was negative. None of the patients was rechallenged in Switzerland.

In Switzerland, 31 (40 %) patients with metamizole-associated hematological toxicity received G-CSF and 33 (42 %) patients were treated with antibiotics. Patients with

Table 1 Characteristics of international (1968-01/2013) and Swiss individual case safety reports (1991–2012) of metamizole-associated hematological adverse drug reactions

Characteristics	Total international ICSR (n=1417)	Total subgroup of Swiss ICSR (n=77)
Reported hematological WHO-ART terms [number (%)]		
Agranulocytosis	920 (57)	47 (61)
Leucopenia	349 (21)	12 (16)
Granulocytopenia	166 (10)	9 (12)
Pancytopenia	120 (7)	8 (10)
Anemia aplastic	29 (2)	1 (1)
Marrow depression	26 (2)	NR
Aplasia bone marrow	18 (1)	NR
WBC abnormal NOS	1 (0)	NR
Female [number (%)]	876 (62)	51 (66)
Male [number (%)]	517 (36)	26 (34)
Age at ADR diagnosis [years; median (range)]	57 (1–96)	59 (9–96)
Fatal outcome [number (%)]	186 (13)	7 (9)
Total number of drugs per report [median (range)]	3 (1–53)	4 (1–19)
Number of suspected drug per report [median (range)]	1 (1–11)	2 (1–9)
Metamizole causality rating [number (%)]	1628 terms[a] (100)	77 terms (100)
Certain	104 (6)	–
Probable	393 (24)	19 (25)
Possible	503 (31)	58 (75)
Not assessable	252 (15)	–
Unknown	376 (23)	–

[a] More terms than cases due to more than one adverse drug reaction per report in some cases

NR not reported, ICSR individual case safety reports, NOS not otherwise specified, WBC with blood cell

G-CSF treatment had a mean duration of the ADR of 9 days (n=31), whereas the patients without explicitly mentioned G-CSF treatment showed a mean duration of 6.6 days (n=42). In four cases, therapy with G-CSF was explicitly omitted. These patients had a mean ADR duration of 9.5 days. The most often reported underlying diseases in the Swiss dataset were

Table 2 Metamizole administration information retrieved from international (1968-01/2013) and Swiss individual case safety reports (1991–2012) of metamizole-associated hematological adverse drug reactions

Characteristics	International ICSR (n=1417)	Subgroup Swiss ICSR (n=77)
Daily dose [mg; (SD or range)]	2865 (2201) (n=196)	2226 (1169) (n=57)
Oral	1700 (500–10,000) (n=174)	n.d.
Parenteral	4000 (500–12,000) (n=122)	n.d.
Cumulative dose [g; median (range)]	20 (0.5–3048) (n=296)	19.5 (0.5–1188) (n=56)
Oral	22,6 (0.5–3048) (n=174)	n.d.
Parenteral	18 (0.5–498) (n=122)	n.d.
Route of administration [number (%)]		
Oral	789 (60)	62 (81)
Parenteral	317 (24)	11 (14)
Duration treatment [days; median (range)]	8 (1–3303)	13 (1–594)
Latency time [days; median (range)][a]	7 (1–3305)	14 (1–594)

The maximal daily dose of metamizole is 4000 mg

[a] Time from the start of the metamizole therapy till the adverse drug reaction was diagnosed

ICSR individual case safety reports, n number, n.d. not differentiated

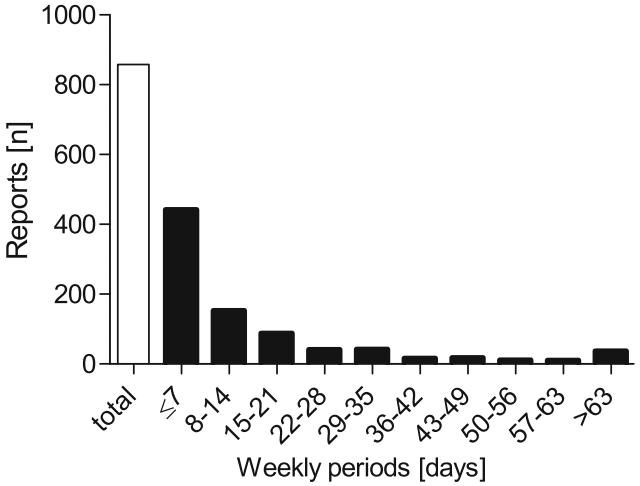

Fig. 1 Latency times stratified by weekly periods for metamizole-associated hematological adverse drug reactions among 858 reports reported to VigiBase™, the WHO Global Database of Individual Case Safety Reports (ICSRs), between 1968 and 01/2013

diseases of the circulatory system and injuries and diseases of the musculoskeletal system (see supplemental table 3 for more information).

Figure 2 shows the international annual reporting rate for metamizole-associated hematological ADR and the reporting rate of cases with fatal outcome. There was a first rise of the international reporting rate in 2008 (76 ICSR) followed by a remarkable increase in 2010 (219 ICSR). Most ICSR were submitted by Germany (44 %), followed by Spain (35 %), Switzerland (6 %), Italy (2 %), and Sweden (2 %). In Switzerland, the annual reporting rate was stable until 2005 with zero to two reports per year. Thereafter, the reporting rate increased remarkably (2006: 7 reports; 2007: 6 reports; 2008: 2 reports; 2009: 5 reports; 2010: 16 reports; 2011: 14 reports; 2012: 15 reports). In total, 186 (13 %) metamizole-associated hematological ICSR with fatal outcome have been reported to the WHO, the first in 1968 and 85 since 2008. In Switzerland, seven ICSR (9 %) with a fatal outcome were reported, the first in 2006, two cases in 2011 and four in 2012. The fatal Swiss cases are described in detail in supplementary Table 4. Six of the 7 cases were women. The metamizole dosage was in the therapeutic range in all cases and the median latency time until detection of the ADR was 14 days (range 4 to 594 days). In Table 3, characteristics of the seven Swiss cases with fatal outcome were compared with the 70 surviving cases. Among the cases with fatal outcome, there were more females, more patients with triple blood cell line injury (43 % versus 10 % among non-fatal cases), fatal cases were in median 11 years older, and in 4 of the 7 fatal cases, there was a co-treatment with methotrexate. Three of the four patients with methotrexate therapy received an immunosuppressive low dose regimen and two of them had received only one single dose of methotrexate. The proportion of certain characteristics such as sex, number of affected cell lines, and co-treatment with methotrexate were not statistically different between patients with fatal outcome and surviving patients.

Finally, the Swiss annual reporting rates of metamizole-associated agranulocytosis were compared with pooled Swiss sales figures of all metamizole preparations on the Swiss market. The sales figures were available from 2006–2012 and were converted to DDD. As shown in Fig. 3, the course of these two variables was comparable during the observation period, suggesting that the observed increase in ICSR is mainly the result of growing metamizole use. Using the sales figures, we could calculate minimal incidence rates. For agranulocytosis, this was 0.46–1.63 cases per million person-days.

Discussion

We evaluated data from the international pharmacovigilance database Vigibase™ and Swiss ICSR regarding metamizole-associated hematological ADR. In addition, the subgroup analysis of the more detailed Swiss ICSR allowed us to evaluate some specific issues such as comorbidities and co-suspected drugs as well as analysis of fatal cases. Furthermore, we could obtain a rough estimate of a minimal incidence rate of metamizole-associated agranulocytosis in Switzerland.

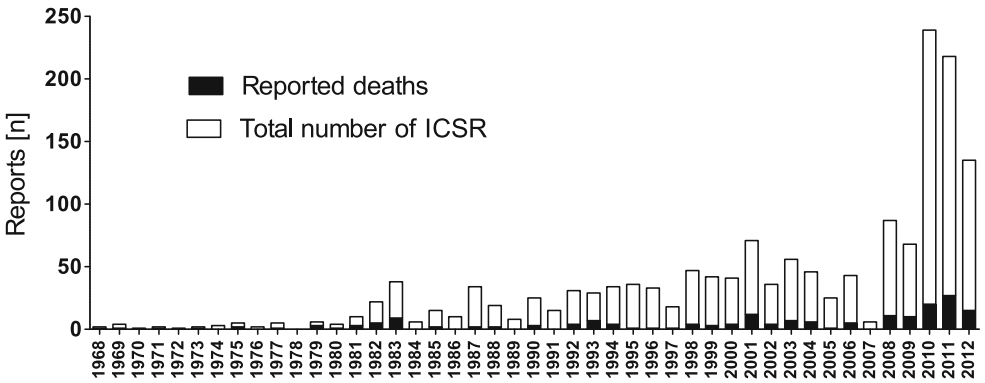

Fig. 2 International annual cumulative reporting rate of metamizole-associated hematological adverse drug reactions and reporting rate of cases with fatal outcome, retrieved from VigiBase™, the WHO Global Database of Individual Case Safety Reports (ICSR), between 1968 and 01/2013

Table 3 Comparison of fatal and non-fatal Swiss individual case safety reports of metamizole-associated hematological adverse drug reactions between 1991 and 2012 (n=77)

	Fatal	Non-fatal
Total number of cases	7	70
Number of females	6 (86 %)	45 (64 %)
Age (years), mean (SD)	66 (22)	55 (21)
Daily dose (mg/day), mean (SD)	2286 (958)	2881 (2221)
Cumulative dose (g), median (range)	21 (10.5–1188)	19.5 (0.5–252)
Cumulative number of treatment days, median (range)	14 (4–594)	13 (1–365)
White blood cell line affected, only	4 (57 %)	58 (83 %)
Two blood cell lines affected	0 (0 %)	5 (7 %)
All three blood cell lines affected	3 (43 %)	7 (10 %)
Number of cases with methotrexate as co-suspected drug	4 (57 %)	0 (0 %)

The maximal daily dose of metamizole is 4000 mg

The percentages of certain variables (sex, number of affected cell lines, co-treatment with methotrexate) were not statistically different between patients with fatal outcome and surviving patients

SD standard deviation

All age groups were affected by metamizole-associated hematological ADR, although more ICSR for elderly people (median age close to 60 years in both data sets) were reported. An increased incidence of hematological disorders in older patients has been reported in several population-based studies of drug-induced agranulocytosis [14, 15]. However, since no data about the exposure of specific age groups to metamizole are available, our data do not allow the conclusion that metamizole-associated hematological ADR occur more frequently in older patients. Similarly, the reporting rate for metamizole-associated hematological ADR was higher for women than men, both in the Swiss and the international ICSR dataset. This finding is in line with previous studies of drug-induced agranulocytosis and, more specifically, also with metamizole-associated agranulocytosis [8, 16, 17]. Again, our data support the results of these studies but do, due to a lack of specific exposure data, per se not allow the conclusion that metamizole-associated agranulocytosis is more frequent in females.

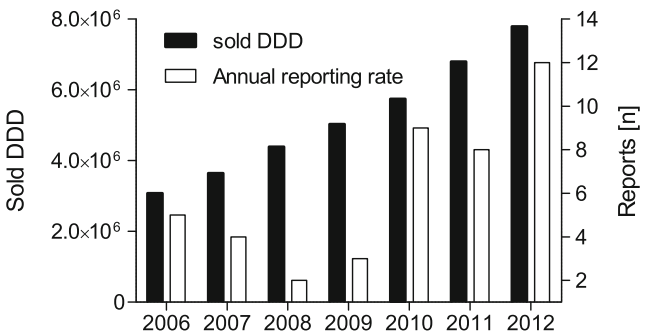

Fig. 3 Annual reporting rate of metamizole-associated agranulocytosis and the course of sold defined daily doses in Switzerland between 2006 and 2012

The average daily dose in reported cases of metamizole-associated hematological ADR was within the recommended range according to the product information. This finding argues against a typical dose-dependent toxicity of metamizole and favors toxicity associated with the presence of immunological or metabolic susceptibility factors in affected patients. Another important factor regarding the mechanism of toxicity is the latency time, although this parameter depends also on other factors than disease mechanism such as frequency of blood monitoring, severity of symptoms, and/or vigilance of doctors. Our data showed that in 33 % of the Swiss and in 52 % of the international ICSR, hematotoxicity appeared during the first week of treatment. In support of this finding, a systematic review including 980 drug-associated agranulocytosis case reports described a median metamizole exposure of only 2 days until hematological abnormalities appeared [18]. These findings are also in agreement with the case control study of Ibanez et al. [19]. Furthermore, 88 % of the Swiss ICSR and 96 % of the international hematological ADR occurred within 2 months of metamizole treatment. These findings agree well with a Swedish study, where 92 % of the cases occurred within the first 2 months [8]. Interestingly, these findings concerning metamizole are very similar to those reported for clozapine, where >80 % of patients with agranulocytosis were observed during the first 3 months of treatment [20]. Considering the lack of accompanying immunological features in most patients with metamizole-associated myelotoxicity, both a metabolic and an immunological mechanism are possible.

In several European studies, the mortality rate of drug-induced agranulocytosis decreased over the last years from 10 to 16 % to approximately 5 % [21]. This was considered to be a consequence of the therapy with G-CSF and with broad-spectrum antibiotics [8, 21]. Our data, however, do not support this assumption. We observed an increase of reported fatal cases in parallel with the increased reporting rate and use of metamizole in Switzerland, despite the option for G-CSF treatment. Since only 40 % of the Swiss patients were treated with G-CSF, this finding may be explained by a selection bias. It can be assumed that patients with more severe hematological ADR were treated preferentially with G-CSF.

The reporting rate of metamizole-associated blood dyscrasias with fatal outcome in the Swiss and international dataset increased over time, in particular in 2010 and 2011. The Swiss data suggest that the increased use of metamizole is the main reason for the increased number of reported fatal cases (see Fig. 3). In their review, Andrès et al. listed age >65 years, septicemia and/or shock, metabolic disorders such as renal failure, and a neutrophil count below 0.1 G/L as poor prognostic factors for drug-induced agranulocytosis [21]. These risk factors could also be identified in the Swiss cases with

fatal outcome. Five of the seven patients were over 65 years, and patients with a fatal outcome were 11 years older than those who recovered. In four of the seven fatal cases, septicemia was reported as cause of death and all fatal cases had neutrophil counts below 0.1 G/L. Furthermore, the proportion of patients in whom all three blood cell lines were affected was higher among patients with fatal outcome than in those who recovered.

Two additional potential risk factors for a fatal outcome identified in our study are female sex and co-treatment with methotrexate. In agreement with the overrepresentation of females in the entire dataset, six out of the seven patients with a fatal outcome were females. Importantly, methotrexate was considered as co-suspected drug in four out of seven fatal cases and in none of the non-fatal cases. Three of these patients were on low-dose (immunosuppressive) methotrexate, two of them were on long-term treatment, and one of them had received only a single dose. In addition, one patient with T-cell lymphoma had been treated with an oncological dose (single dose of 5500 mg) of methotrexate. In this patient, methotrexate appears to be the more likely reason for myelotoxicity than metamizole. Methotrexate-associated myelotoxicity is dose-dependent. Since methotrexate is mainly eliminated by the kidney, impaired renal function is associated with increased exposure and with an increased risk for myelotoxicity [22]. Unfortunately, no serum creatinine concentrations have been reported or could be retrieved in patients with low-dose methotrexate before the diagnosis of myelotoxicity. Since low-dose methotrexate is used as an immunosuppressant in autoimmune diseases such as rheumatoid arthritis and women are more likely to be affected by autoimmune diseases, this may at least partially explain the female predominance for fatalities in metamizole-associated myelotoxicity.

Between 2006 and 2012, the pooled annual Swiss sales figures of all metamizole preparations on the Swiss market increased constantly by about 800,000 DDD per year. The annual reporting rate for metamizole-associated agranulocytosis also increased remarkably between 2006 and 2012, but the absolute numbers remained small, with an annual reporting rate for all blood dyscrasias between 2 and 16 and for agranulocytosis between 2 and 12 reports per year. Overall, the annual reporting rates paralleled the sales figures and therefore probably the use of metamizole in Switzerland, although with annual fluctuations. These fluctuations can be explained by changes in the reporting rate for pharmacovigilance data. This is influenced by many different factors, in particular by underreporting and selective reporting [23].

In 2002, Hedenmalm et al. [8] reviewed all spontaneous reports of serious metamizole-associated blood dyscrasia in Sweden and estimated the incidence for agranulocytosis associated with metamizole to be 1 in 1439 prescriptions. Similarly, Baeckerstroem et al. [24] analyzed spontaneous reports of agranulocytosis and the use pattern of metamizole in Swedish inpatients and outpatients. They calculated the risk of metamizole-associated agranulocytosis to be approximately 1 in 31,000 metamizole-treated inpatients and 1 in 1400 outpatients. In our study, we were able to estimate a minimal incidence rate for metamizole-associated agranulocytosis in Switzerland using the annual reporting rates and annual metamizole sales figures expressed as DDD. The estimated incidence rate was 0.46–1.63 per million person-days, which was within the range of the incidence rate in the International Agranulocytosis and Aplastic Anemia Study (IAAA study) of 0.3–4.0 per million person-days [6, 8]. Our incidence rate is higher than reported in a Polish study with an incidence rate of 0.2 per million person-days [16]. A detailed comparison with the reported incidence rates of agranulocytosis in Sweden (at least 1 per 1439 prescriptions) is not possible due to different estimation methods and different units [8]. Taking into account the median treatment until agranulocytosis occurred (13 days in Swiss patients) it can be assumed that the incidence rate in Sweden is higher than in Switzerland. It has to be considered, however, that the Swiss incidence rate may be higher due to underreporting. It is well known that only approximately 6 % of all ADR are spontaneously reported to the pharmacovigilance systems [25].

Limitations

This study is based on spontaneously reported metamizole-associated ICSR. As stated above, underreporting and missing data in spontaneous reports are well-known problems in this type of studies. Reasons for missing data are unavailable information or the reporter did not consider certain facts as relevant. Furthermore, the standardized requirements for reporting ADR changed over the last decades, and the ICSR include progressively more information. In addition, ICSR derive from different sources (countries, National Pharmacovigilance Centers and companies) and were written and coded by different reporters with potentially different coding policies. Therefore, heterogeneity of ICSR must be taken into account when interpreting data from pharmacovigilance databases.

Conclusions

In patients treated with metamizole, we estimated an annual incidence rate for agranulocytosis of 0.46–1.63 per million person-days, which is within the range of the IAAA study [6]. This is a minimal risk, since underreporting is known to be substantial for pharmacovigilance data. Female sex, triple blood cell line dyscrasia, older age, and concomitant treatment with methotrexate were identified risk factors for fatal outcome. A large proportion of the Swiss (33 %) and the

international cases (52 %) occurred during the first 7 days of metamizole treatment, compatible with an immunologic or a toxic mechanism in predisposed patients. Taking into account the rapid onset of metamizole-associated agranulocytosis, close monitoring of the patient starting already in the first week of treatment is essential to detect the onset of myelotoxicity as early as possible. Information of the patients to self-identify the first symptoms of myelotoxicity, to immediately stop metamizole intake, and to consult a doctor may be crucial to identify affected patients as soon as possible. Metamizole prescribing should be conservative, and the prescriber should outweigh the risk for myelotoxicity against the adverse reactions of possible alternatives [11].

Acknowledgments The authors would like to thank Sara Hult, member of the analysis team of the Uppsala Monitoring Centre, the WHO Collaborating Centre for International Drug Monitoring, for providing the data and for answering our questions. We also would like to thank Guy Levy from the Vigilance Unit of Swissmedic for his collaboration. We thank also Sanofi-Aventis SA, Streuli Pharma AG, and Sintetica SA for providing the sales figures of their metamizole preparations in Switzerland. We thank also the Senglet-Foundation, the foundation for the promotion of young pharmaceutical researchers in Basel, for their financial support.

Author contribution Performed research: LB, AT, PE, ARB. Analyzed data: LB, AT, MH, SK, ARB. Wrote manuscript: LB, SK, ARB. All authors contributed to the review and final approval of the manuscript.

Conflict of interest The authors declare that there are no conflicts of interest regarding this work.

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Accompanying statement

The data for this work were obtained from the WHO Collaborating Centre for International Drug Monitoring, Uppsala, Sweden and from the Swiss health authority, Swissmedic, in Berne, Switzerland. Data from spontaneous reporting are inhomogeneous as a result of different

reporting policies worldwide and are vulnerable to underreporting and reporting bias. The information contained in this work is therefore not homogeneous, at least with respect to origin and also to likelihood that the pharmaceutical product caused the adverse reaction. The conclusions drawn based on these data do not necessarily represent the opinion of the World Health Organization or of Swissmedic.

3.2.1. *Supplementary material: Hematological Safety of Metamizole: Retrospective Analysis of WHO and Swiss Spontaneous Safety Reports*

Supplementary Table 1 Age categories retrieved from international and the subgroup of Swiss individual case safety reports of metamizole-associated hematological adverse drug reactions between 1968-01/2013 and 1991-2012, respectively

Age categories at ADR diagnosis [number (%)]	**Total international ICSR (n=1319)**	**Total Swiss ICSR (n=77)**
< 10 years	22 (2)	1 (1)
10 – 19 years	81 (6)	2 (3)
20 – 29 years	163 (12)	11 (14)
30 – 39 years	100 (8)	6 (8)
40 – 49 years	164 (12)	10 (13)
50 – 59 years	174 (13)	9 (12)
60 – 69 years	234 (18)	13 (17)
70 – 79 years	239 (18)	15 (19)
> 80 years	142 (11)	10 (13)

ADR adverse drug reaction, ICSR individual case safety reports, n number

Supplementary Table 2 Drug classes of co-suspected drugs retrieved from the subgroup of Swiss individual case safety reports of metamizole-associated hematological adverse drug reactions between 1991-2012

Drug classes of co-suspected drugs according to ATC codes [number]	**Total Swiss ICSR (n=77)**
Antiinfectives for systemic use	28
Nervous system	23
Cardiovascular system	14
Others	13
Antineoplastic agents and Immunomodulating agents	10
Alimentary tract and metabolism	10
Musculo – skeletal system	9

ICSR individual case safety reports, n number

Supplementary Table 3 Most prevalent underlying diseases expressed as ICD-10 codes among 65 Swiss metamizole-associated hematological individual case safety reports (ICSR) reported to Swissmedic between 1991-2012

Organ classes	Number (% of patients)	Diseases (number of patients)
Circulatory system	17 (26)	Hypertension (9), cerebral disease (2), coronary heart disease (2), pulmonary disease (2), others (2)
Injury and external causes	17 (26)	Fractures (11), allergies (3), concussions (3)
Musculoskeletal system	16 (25)	Osteoporosis (4), rheumatoid arthritis (4), others (6)
Endocrine and metabolic diseases	14 (22)	Diabetes (4), hypercholesterolemia (4), obesity (2), others (4)
Symptoms unclassified	11 (17)	Pain (8), others (3)
Digestive system	10 (15)	Not differentiated
Infections	10 (15)	Bacterial infections (3), bacterial infections unspecified (4), herpes zoster (1), viral encephalitis (1), hepatitis C infection (1)

Supplementary Table 4 Detailed information on the seven Swiss individual case safety reports of metamizole-associated hematologic adverse drug reactions between 1991 and 2012 with fatal outcome

Age sex	Reporting year	Indication metamizole	Underlying diseases	Reported preferred term	Metamizole causality assessment	Daily dose, treatment duration, route of administration	Latency time[b] [days]	Therapy	Co-suspected drugs	Concomitant drugs	Death cause	Time to death[a] [days]	Laboratory values
21 F	2006	Abdominal pain	Crohn's disease, bile stone with cholangitis or cholecystitis	Agranulocytosis	probable	1500 mg 7 days oral	10	GCSF, AB, W	Azathioprine Mesalazine Methotrexate (single dose of 25 mg) Paracetamol	Pantoprazole	Septicemia unspecified	10	Neutrophils 0.02 G/L
78 F	2011	NR	Atrial fibrillation and flutter, fracture of femur, rheumatoid arthritis	Pancytopenia, gastrointestinal hemorrhage	possible	1500 mg 14 days oral	14	GCSF, AB, W	Alendronate Esomeprazole Etodolac Methotrexate (10 mg/week) Prednisolone	Atenolol Calcium Colecalciferol Domperidone Flupentixol Lactitol Melitracene Morphine sulfate	Gastrointestinal hemorrhage unspecified	10	Leucocytes 0.3 G/L Thrombocytes 12 G/L Hemoglobin 43 g/L
79 M	2011	Pain, not specified	Multiple fractures of ribs	Agranulocytosis	possible	2000 mg 9 days oral	9	GCSF, AB, W	-	-	Bacterial pneumonia, respiratory failure	17	Leucocytes 0.4G/L
84 F	2012	Joint pain	Rheumatoid arthritis, hyperuricemia	Pancytopenia	possible	2000 mg 594 days oral	594	GCSF, AB, W	Allopurinol Methotrexate (7.5 mg/week) Moxifloxacin Spironolactone Torasemid	-	Multiple organ failure	5	Neutrophils 0.0 G/L Thrombocytes 66 G/L Hemoglobin 64 g/L

AB antibiotics, *F* female, *GCSF* granulocyte colony-stimulating factor, *M* male, *NR* not reported, *W* Withdrawal of metamizole

[a] Time from the diagnosis of the adverse drug reaction till death

[b] Time from the start of the metamizole therapy till the adverse drug reaction was diagnosed

Supplementary Table 4 (continued) Detailed information on the seven Swiss individual case safety reports of metamizole-associated hematologic adverse drug reactions between 1991 and 2012 with fatal outcome

Age sex	Reporting year	Indication metamizole	Underlying diseases	Reported preferred term	Metamizole causality assessment	Daily dose, treatment duration, route of administration	Latency time[b] [days]	Therapy	Co-suspected drugs	Concomitant drugs	Death cause	Time to death[a] [days]	Laboratory values
66 F	2012	Fever of unknown origin	Peripheral T-cell lymphoma, bacterial infection, viral encephalitis	Pancytopenia	possible	4000 mg 4 days intravenous	4	AB, W	Amoxicillin Acyclovir Ceftriaxone Dalteparin Esomeprazole Methotrexate (single dose of 5500 mg in preexisting pancytopenia)	Calcium folinate Escitalopram Glucarpidase Pantoprazole Prednisolone	Septicemia unspecified	13	Leucocytes 1.62 G/L Thrombocytes 64 G/L Hemoglobin 84 g/L
49 F	2012	Unspecified renal colic	Nephrolithiasis, hypertension, hypercholesterolemia	Agranulocytosis	probable	3500 mg 36 days oral	56	GCSF, AB, tonsillectomy, W	-	Atorvastatin Candesartan	Septicemia unspecified	3	Neutrophils 0.01 G/L
83 F	2012	Low back pain	Hypertension, fracture of pelvic ring, chronic lumbar pain	Agranulocytosis	possible	1500 mg 120 days oral	120	AB, W	-	Acetylsalicylic acid (100mg) Bisoprolol Calcium carbonate Colecalciferol Eprosartan Simvastatin	Septicemia due to gram-negative organism	5	Leucocytes 0.4 G/L

AB antibiotics, *F* female, *GCSF* granulocyte colony-stimulating factor, *M* male, *NR* not reported, *W* Withdrawal of metamizole

[a] Time from the diagnosis of the adverse drug reaction till death

[b] Time from the start of the metamizole therapy till the adverse drug reaction was diagnosed

With the intense preoccupation of cases, we gained a better comprehension of hematological adverse drug reactions under metamizole. But for some questions, the lack of a control group makes final conclusions impossible. We therefore decided to compile data of control patients in addition to characterized cases managed at the University Hospital Basel. The question of how to find an appropriate control group was the crucial point. Finally, we split our cases in post-operative and non-post-operative cases and searched corresponding controls.

3.3. Leucopenia associated with Metamizole: a Case-Control Study

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Submitted

Abstract

Purpose: The aim of this study was to identify possible risk factors for the development of leucopenia associated with metamizole use.

Methods: A retrospective case-control study was performed. Cases of metamizole-associated leucopenia managed at a single center (2005-2013) were characterized and compared to matched controls who took metamizole without developing complications.

Results: Fifty-seven cases and 139 controls were identified. Of the cases, 32 were post-operative (post-OP) and these were compared to age-, sex- and ward-matched post-OP controls (n= 64). The remaining cases (n= 25) were compared to sex-matched, non-post-OP controls (n= 75). The number of patients with a positive allergy history was higher among post-OP cases than controls (p= 0.0015) as was the number with previous leucopenic episodes (p= 0.03). The prevalence of diagnosed hepatitis C infection was 7% among all cases compared to 1% among all controls (p= 0.01). The use of concomitant cytostatic agents (even at immunosuppressive doses) was significantly higher among non-post-OP cases than controls (p= 0.007), with a trend to this distribution among post-OP patients.

Conclusions: A history of allergies, leucopenic episodes, hepatitis C infection and concomitant cytostatic agents are possible risk factors leucopenia associated with metamizole use.

Keywords: Metamizole (Dipyrone), Agranulocytosis, Leucopenia, Hematological Safety, Case-control study, Risk factors

Introduction

Metamizole (dipyrone) is an old antipyretic and analgesic drug. Its safety profile - in particular the risk of blood disorders including agranulocytosis – is controversial. This has led many countries (including the United Kingdom) to withdraw or withhold metamizole from the market [1, 2]. However, in other countries such as Switzerland and Germany, metamizole is still frequently used and has even gained market share in recent years [3, 4], most likely due to its lack of hepatotoxicity and minimal renal toxicity. Routine monitoring of blood counts while under metamizole therapy is not common practice and is not included in the drug label [5]. The mechanism by which metamizole causes blood disorders has not yet been fully elucidated. Available data suggest an immunological process as well as direct toxicity towards the progenitor cells in the bone marrow [6].

In the 1980s, a large population-based case-control study examined the risk of agranulocytosis or aplastic anemia under treatment with several drugs including metamizole [7]. The study found that the incidence of metamizole-induced agranulocytosis varied markedly between countries. Little is known, however about the underlying risk factors for developing blood cell disorders under metamizole. If strong risk factors are found, these could be used to identify patients in whom metamizole should be avoided. Conversely, patients who do not possess identified risk factors may benefit from metamizole's advantages over other analgesic agents such as paracetamol and non-steroidal antiinflammatory agents. Metamizole would therefore be applied in a safer, more targeted fashion.

A number of studies characterizing cases of metamizole-induced white blood cell disorders have been conducted [2-4], however as far as we know, no case-control studies have been performed. In the recently published study by Huber and colleagues, data of a prospective case-control surveillance study were used, but the presented results only contain data pertaining to the cases [4].

The aim of the present study was therefore to compare cases of metamizole-associated leucopenia with control patients to identify possible risk factors for developing this complication and therefore to improve our knowledge about metamizole`s risk-benefit profile.

Methods

This retrospective, descriptive case-control study examined cases of metamizole-associated leucopenia which were managed at the University Hospital in Basel, Switzerland, between April 2005 and August 2013. Cases were either post-operative (post-OP) or non-post-OP. In order to avoid confounding by selection, cases were compared to post-OP and non-post-OP controls. Post-OP cases were compared to age, sex, and ward matched post-OP control-patients who had received metamizole without complication between 2005 and 2013. Non-post-OP cases were compared to sex-matched control patients who had received metamizole for at least four weeks between 2001 and 2014 in primary care settings in the Basel area without complication. The study was approved by the local ethics committee "Ethikkommission Beider Basel" (protocol number EKBB 2013/130). As data were analyzed anonymously, no patient informed consent was required.

Selection and assessment of cases

The electronic medical records of the University Hospital Basel were screened for the keywords metamizole or Novalgin® in combination with agranulocytosis, neutropenia, or leucopenia. Additionally, the in-patient referrals to the hematology department which included the keyword Novalgin® were screened for metamizole-associated leucopenia. Cases with a strong temporal relationship between metamizole exposure and the development of laboratory-confirmed leucopenia (leucocyte count below 3.5 x 10^9/L) were included.

We evaluated demographic data such as age at the time of diagnosis of the adverse drug reaction (ADR) and body mass index (BMI), underlying diseases, history of immediate- and delayed-type hypersensitivity reactions ("allergy"), co-medication, drug administration information (dose, route, frequency) as well as duration of metamizole therapy, latency time of the ADR, laboratory findings and the outcome and treatment of the ADR for each case. Co-medication with drugs known to be associated with acute agranulocytosis (level 1 evidence according to Andersohn and colleagues) were taken into consideration when assessing ADR causality [8] and were named "potentially myelotoxic". The concomitant use of cytostatic agents (which cause dose-dependent myelotoxicity) such as cancer chemotherapeutic drugs or immunosuppressant-dose methotrexate (maximum 30 mg/week oral or subcutaneous) or azathioprine (maximum 5 mg/kg body weight per day) was also recorded. We assessed ADR causality according the Naranjo ADR probability scale which

includes assessment of other possible causes such as co-morbidities and co-medication [9]. Only cases with an at least “possible” causality assessment according the Naranjo scale were evaluated further.

Using the available dosage information, therapy and ADR dates, we calculated the daily dose, cumulative dose, duration of treatment, latency time and duration of the ADR. The latency time was calculated as the period between the metamizole start date and the date of ADR onset according to laboratory assessments as defined above. If metamizole was prescribed as an ‘as required’ medication and metamizole intake and dosage regimen were not known with confidence, we recorded the mean of the minimal daily dose (500mg) and the individual’s maximal prescribed daily dose (14 of 57 cases).

Full blood counts (hemoglobin, leucocyte- and platelet-counts) were recorded at the start date of the metamizole therapy, during metamizole therapy (controls) or when the leucocyte count reached its nadir (cases). Since metabolites of metamizole are mainly renally excreted and their accumulation in renal impairment might be a risk factor for the development of leucopenia, GFR estimated via the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [10] was assessed at the start of metamizole therapy and at the start of the ADR (cases) or before and during metamizole therapy (controls).

Selection and assessment of controls

Post-OP controls

The electronic medical records of the University Hospital Basel were screened for post-OP patients treated between 2005 – 2013 who received metamizole as post-OP analgesia and were discharged with a prescription for daily metamizole until resolution of pain. These patients were not known to develop any hematological complications. Two age- (±5 years), sex-, and ward-matched controls per post-OP case were randomly chosen from the list containing all possible controls (n= 21381) using a list randomizer provided by random.org [11]. We evaluated demographic data (sex, age at last intake, BMI), underlying diseases, history of allergy, co-medication, and if available, laboratory findings (full blood count and GFR) before and during metamizole therapy. Co-medication was evaluated in the same manner as for the cases. Cumulative metamizole dose and duration of treatment were not known because these patients were discharged home with a metamizole prescription.

Non-post-OP controls

Non-post-OP controls were selected from an outpatient setting to ensure selection of patients who had been regularly exposed to metamizole for at least 4 weeks without developing hematological complications. The minimal duration of metamizole intake in this non-post-OP control group was chosen according to the latency time data from our previous pharmacovigilance study [3]. In this study, 84% of the cases occurred within 28 days of metamizole treatment. General practitioners in the Basel area were asked to identify patients attending during one reporting month (May 2013) who had taken metamizole continuously for at least 28 days (at least 500 mg per day) and who had not developed any obvious blood disorders. Due to the rarity of such controls, it was only possible to sex-match them with the cases. Data as outlined above for cases and post-op controls, where applicable, were recorded from the medical records.

Using the available dosage information and therapy dates, we calculated the daily dose, cumulative dose, and duration of treatment of the corresponding episode of metamizole intake. If metamizole was prescribed as an 'as required' medication and metamizole intake and dosage regimen were not known with confidence, the same method as described above was used. If the amount of metamizole that the patient obtained was known (dispensing practices), this was used for the calculation of the cumulative and daily dose. When only the month and year of the start date was reported, we counted the recorded month as a full month (30 days). In cases where several episodes of more than 28 days of metamizole intake with breaks between the episodes took place, only the longest episode was evaluated. The same laboratory values as in the case group were recorded, but at the nearest available date before the start date of metamizole as well as the newest values under the metamizole therapy.

Statistical Analysis

Descriptive analyses were performed using Microsoft Office Excel (Version 2010, Redmond, Washington, USA) and GraphPad PRISM (Version 6, La Jolla, California, USA). For variables with normally distributed numerical values, the arithmetic mean and standard deviation were calculated. For variables without normally distributed values, median and range were determined. Significance of differences between the cases and controls were

assessed using the Chi-square test, Fisher exact test for small numbers, and Mann-Whitney-U test. Differences were considered as significant when $p \leq 0.05$.

Results

Fifty-seven cases of metamizole-associated leucopenia managed at the University Hospital Basel between April 2005 and August 2013 were included according to the defined inclusion criteria. Of these, 32 were post-OP and 25 non-post-OP. Sixty-four age, sex- and ward matched post-OP controls and 75 non-post-OP control patients from general practice settings were identified. In total 543 general practitioners were approached, 19 of whom provided 93 patients, from which 75 could be sex-matched to non-post-OP cases.

Patient and metamizole treatment characteristics

Table 1 summarizes the patient characteristics. The median age of the non-post-OP controls was higher than that of the non-post-OP cases. In keeping with the older age, the number of diagnoses and total number of co-medications per patient were also higher among those controls compared to non-post-OP cases. In contrast, among post-OP cases, the total number of diagnoses and co-medication was higher than among the age-, sex- and ward-matched controls.

In the non-post-OP control group, metamizole was solely taken orally whereas in the corresponding case group, 20% received intravenous metamizole (Table 2). As also shown in Table 2 the mean daily metamizole dose did not differ significantly among non-post-OP cases and controls and was within the recommended range. The median treatment duration in the post-OP case group was 6 days and approximately two thirds of the cases took metamizole for 1-7 days. The median treatment duration in the non-post-OP case group was 13 days with one third taking metamizole for 1-7 days. The non-post-OP controls took metamizole per definition for at least 28 days, one third for even longer than one year.

Table 1 Patient characteristics

	Post-OP Cases (n=32)	Post-OP Controls (n=64)	p-value	Non-post OP Cases (n=25)	Non-post OP Controls (n=75)	p-value
Age [years; median (range)]						
At ADR diagnosis (cases) or at last intake (controls)	58 (16-82)	58 (16-82)	0.87[a]	51 (20-84)	79 (41-98)	<0.001[a]
Female [number (%)]	17 (53)	34 (53)	1[b]	19 (76)	57 (76)	1[b]
Body mass index [kg/m^2; median (range)][d]	25 (20-45), n = 18	26 (18-56), n = 56	0.69[a]	21 (18-47), n = 11	28 (16-40), n = 51	0.18[a]
Co-morbidities						
Total number of diagnoses [median, (range)]	5 (1-9)	2 (0-10)	<0.001[a]	4 (1-9)	6 (2-11)	0.0060[a]
Number of chronic diseases [median, (range)]	2 (0-5)	2 (0-10)	0.52[a]	3 (0-6)	4 (1-9)	0.0014[a]
Number of patients with known allergy [number (%)]	16 (50)	12 (19)	0.0015[b]	8 (32)	18 (24)	0.43[b]
Previous leucopenic episodes [number (%)][e]	3 (9)	0 (0)	0.03[c]	1 (4)	0 (0)	0.25[c]
Underlying Hepatitis C infection [number (%)][f]	3 (9)	1 (2)	0.1[c]	2 (8)	0 (0)	0.061[c]
Co-medication						
Total number of co-medications [median, (range)]	9 (1-18)	5 (1-18)	0.0024[a]	6 (1-18)	9 (0-19)	0.047[a]
Patients concomitantly receiving non-chemotherapeutic potentially myelotoxic co-medication[g] [number (%)]	10 (31)	22 (34)	0.76[b]	7 (28)	33 (44)	0.16[b]
Patients concomitantly receiving cytostatic agents [number (%)][h]	3 (9)	3 (5)	0.40[c]	6 (24)	3 (4)	0.007[c]

[a]Mann-Whitney U Test, [b]Chi-Square test, [c]Fisher exact test [d]In cases where the current body weight was missing, the last known value (last observation carried forward method) was used to calculate the BMI [e]pancytopenia n=3, bicytopenia (anemia and neutropenia) n=1 [f]serology performed during admission in five non-post-OP cases and in seven post-OP-cases and in four post-OP controls [g]Drugs with evidence 1 level for causing acute agranulocytosis (table 2) according Andersohn, et al 2007 [h]cytostatic agents included immunosuppressive-dose methotrexate, azathioprine and cancer chemotherapeutic drugs (see Supporting Table II for detailed information)

Table 2 Characteristics of metamizole therapy

	Cases post-OP (n=32)	**Controls post-OP (n=64)**	**Cases non-post-OP (n=25)**	**Controls non-post-OP (n=75)**
Formulation				
Oral, number of patients (%)	12 (38)	64 (100)	14 (56)	75 (100)
Intravenous, number of patients (%)	12 (38)	10 (16)[b]	5 (20)	0 (0)
Unknown, number of patients (%)	8 (25)	0 (0)	6 (24)	0 (0)
Mean (SD) daily dose (g)	2.19 (1.22) n= 27	na	1.99 (1.27) n=17	1.76 (0.82)[a]
Median (range) cumulative dose (g)	13.50 (0.5-94) n=25	na	21.6 (1-82.5) n=14	316 (24-3109)
Median (range) treatment duration (days)	6 (1-59) n= 29	na	13 (1-365) n= 16	187 (32-2192)
Number of patients treated for:				
1 to 7 days (%)	18 (62)	na	5 (31)	n/a
8 to 30 days (%)	8 (28)	na	8 (50)	n/a
31 to 60 days (%)	3 (10)	na	1 (6)	11 (15)
61 to 120 days (%)	0 (0)	na	1 (6)	18 (24)
121 to 180 days (%)	0 (0)	na	0 (0)	6 (8)
180 to 365 days (%)	0 (0)	na	1 (6)	14 (19)
> 365 days (%)	0 (0)	na	0 (0)	26 (35)

[a]Mann-Whitney U Test compared to cases non-post-OP p = 0.88, [b] i.v. administration followed by per oral administration
n/a not applicable, na not available

Characteristics of the leucopenia

Figure 1 shows the leucocyte and neutrophil values for the case and control subgroups; all cases experienced a distinct fall in leucocyte and neutrophil counts. Leucocyte counts measured whilst under treatment with metamizole were available for 72% of post-OP controls. Pre- and during- metamizole exposure leucocytes were available for 53% and 52% of post-OP and non-post-OP controls, respectively. Among non-post-OP controls, 69% had a during-treatment leucocyte count result, all of which were above the lower normal range. The values confirmed the clinical observation that metamizole was well tolerated (Figure 1). Regarding hemoglobin and platelet counts, no trend was seen (Supporting Figure 1).

Table 3 shows the ADR characteristics among the cases. No cases fulfilled the criteria for a “definite” causality assessment according the Naranjo scale. The three cases that had a recurrence of the ADR on rechallenge would, however, have been classified as “certain” if the WHO–UMC Probability Scale had been applied [12].

The ADR appeared during the first week of treatment in 40% and during the first two months in 93% of all cases. Nineteen patients (33%) were known to have been treated with granulocyte colony stimulating factor (G-CSF).

Four cases in the present study had a fatal outcome. In two of them, metamizole was assessed as probably being causal and in the other two as possibly causal because concomitant methotrexate and azathioprine, respectively, were also suspected. All fatal cases were females (22, 71, 75, and 76 years old). One patient in the case group was recently reexposed to metamizole and had a fatal outcome [13].

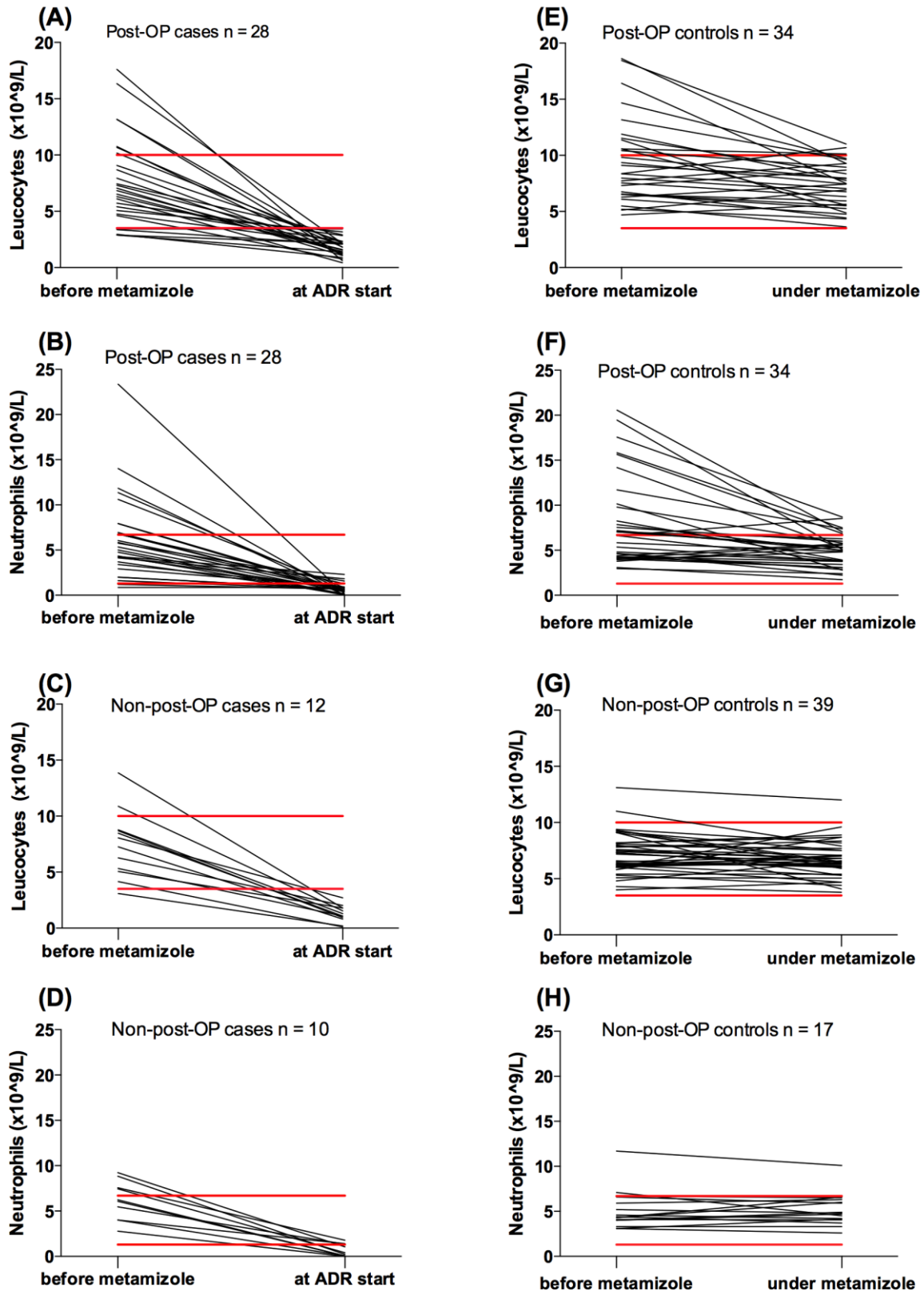

Figure 1 Leucocyte and neutrophil counts before starting metamizole and at time of diagnosis of ADR (case groups A-D) or at last follow-up whilst taking metamizole (control groups E-H). Red lines indicate upper and lower reference values (10 - 3.5 x 10^9/L for leucocytes and 6.7 - 1.3 x 10^9/L for neutrophils, respectively). ADR adverse drug reaction; n number

Table 3 Characteristic of the white blood cell disorders (all cases)

	All cases (n=57)
Naranjo Causality assessment [number (%)]	
Definite	0 (0)
Probable	20 (35)
Possible	37 (65)
Rechallenge information [number (%)]	
Known positive rechallenge	3 (5)
Known negative rechallenge	2 (4)
Rechallenge with unknown outcome	1 (2)
No known rechallenge	51 (89)
Timing of ADR	**n=49**
During therapy [number (%)]	31 (63)
1-7 days after last application [number (%)]	14 (29)
8-14 days after last application [number (%)]	2 (4)
> 14 days after last application [number (%)]	2 (4)
Latency time[a] [days; median (range)]	11 (1-368)
ADR duration [days; median (range)]	
With G-CSF (n= 16)	5.5 (1-20)
Without G-CSF (n= 21)	5 (1-21)
Unknown whether G-CSF given or not (n= 12)	5 (1-51)
Time between ADR onset and nadir [days; median (range)]	1 (0-36)
Time between nadir and recovery [days; median (range)]	4 (1-19)
With G-CSF (n= 16)	3.5 (1-19)
Without G-CSF (n= 21)	4 (1-13)
Unknown whether G-CSF given or not (n= 12)	4 (1-16)
Time between stopping metamizole and recovery in cases where ADR occurred during metamizole therapy [days; median (range)]	
With G-CSF (n= 9)	5 (1-29)
Without G-CSF (n= 15)	4 (1-19)
Unknown whether G-CSF given or not (n= 2)	6.5 (6-7)
Degree of Leucopenia (n=56)	
Leucocytes 2-3.5 x 10^9/L [number (%)]	18 (32)
Leucocytes 1-2 x 10^9/L [number (%)]	22 (39)
Leucocytes <1 x 10^9/L [number (%)]	16 (29)
Degree of Neutropenia (n=55)	
Neutrophils 1-1.3 x 10^9/L [number (%)]	10 (18)
Neutrophils 0.5-1 x 10^9/L [number (%)]	19 (35)
Neutrophils <0.5 x 10^9/L [number (%)]	26 (47)
Outcome [number (%)]	
Recovered	53 (93)
Died	4 (7)

[a]time between starting metamizole and the onset of the ADR

ADR adverse drug reaction, G-CSF granulocyte colony stimulating factor

Co-morbidities and their association with the ADR

Figure 2 shows the course of the GFR at the start of metamizole therapy and at the start of the ADR in the case groups. The GFR did not change significantly between these two time points, whether in post-OP cases nor in the non-post-OP cases. Likewise, the GFR did not change in the control groups and under metamizole therapy. Three patients in the case group (1 post-OP and 2 non-post-OP cases) and one patient in the non-post-OP control group had preexisting severe renal impairment (GFR < 30 mL/min/1.73m^2) at the start of the metamizole therapy. However, the GFR increased or remained clinically stable during the metamizole therapy in all cases (from 16 to 61.2 mL/min/1.73m^2, 24.4. to 34.0 mL/min/1.73m^2, from 21.5 to 28.3 mL/min/1.73m^2 and from 27.3 to 22.0 mL/min/1.73m^2 respectively).

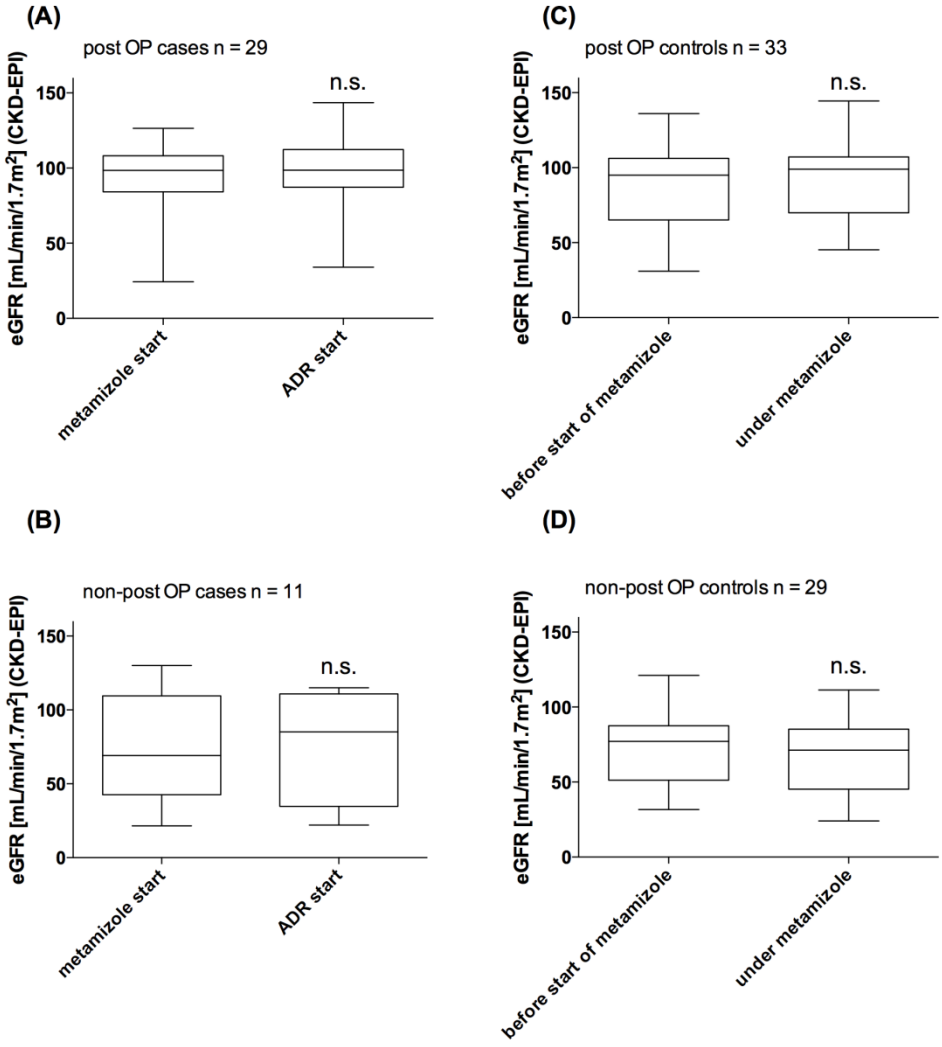

Figure 2 Estimated glomerular filtration rate (eGFR) at the start of metamizole therapy and at ADR start (case groups, left side), eGFR before the start of metamizole therapy and under metamizole therapy (control groups, right side). ADR adverse drug reaction; n number; n.s. not significant

Patients with a history of allergy (Table 1) were also analyzed in more detail (Supporting Table 1). The number of known medication and non-medication allergies was significantly higher in the post-OP case group compared to the corresponding control group (p= 0.007 and 0.014, respectively). Among non-post-OP cases and controls, there was a non-statistically significant trend towards a higher prevalence of allergies among cases compared to controls. Specifically, the number of patients with beta-lactam allergies was significantly higher (p= 0.02).

Patients with a history of previous leucopenias (Table 1) were analyzed in more detail. One patient (a post-OP case) had a preexisting bicytopenia (recurrent episodes of anemia and neutropenia) of unknown origin. The neutropenia re-emerged under metamizole and resolved again after stopping metamizole. In three cases (2 post-OP and 1 non-post-OP), a preexisting recurrent pancytopenia of uncertain origin had previously been diagnosed. In all of these cases, the leucocyte or neutrophil counts fell from a value within the normal range to a value below the lower limit of normal under metamizole. Three other cases had a preexisting hematological condition (one patient had hemophilia B, one patient had an isolated factor VII deficiency and another chronic myelocytic leukemia).

Five patients in the case group (3 post-OP and 2 non-post-OP cases) and one control patient were hepatitis C positive. Analyzing data from all cases and all controls together, there were significantly more patients with underlying hepatitis C infection among cases than controls (p= 0.008). One patient with an underlying hepatitis C infection was co-infected with hepatitis B and another with hepatitis B and HIV. No other viral infectious diseases were known to be present in either the case or the control groups.

Co-medication and their association with the ADR

The total number of patients concomitantly treated with potentially myelotoxic co-medication was similar in all groups (p= 0.76 in post-OP cases and controls and p= 0.16 in non-post-OP cases and controls, respectively) (Table 1). Potentially myelotoxic and cytostatic co-medication are listed in detail in the supporting Table 2. In 54% of all cases, co-medication other than metamizole could potentially have also caused or contributed to the ADR, however these were taken into consideration when determining the Naranjo score. The number of patients treated with concomitant cytostatic agents did not differ significantly in the post-OP case and control group (Table 1). However, in the non-post-OP groups, a greater proportion of cases than controls received concomitant cytostatic agents (p= 0.007).

Discussion

This retrospective study of metamizole-associated leucopenia identified a number of different possible risk factors, which might allow targeted and safer metamizole use. This is of particular interest in view of the increasing use of metamizole in central European countries such as Switzerland and Germany.

Previous population-based studies of drug-induced agranulocytosis found a high incidence among older patients [14, 15]. Firm conclusions, however, can only be drawn if the exposure data of specific age groups is known. Considering the current study, the significantly higher age of the non-post-OP controls does not support the assumption that age is a real risk factor for metamizole-induced leucopenia.

The opposing findings regarding the total number of diagnoses and concomitant medication among post-op and non-post-op cases and controls likely reflects the inability to age-match in the non-post-OP groups. As the findings are in opposition, we do not believe the total number of diagnoses or total number of co-medication are risk factors for the development of metamizole-associated leucopenia.

Whether intravenous administration is a risk factor - which the data from the post-OP patients might suggest - or not, cannot be fully assessed in this study because the non-post-OP controls were never candidates for intravenous therapy.

The fact that the daily doses in the non-post-OP case and control groups were similar is evidence against a typical dose-dependent toxic effect; rather it favors toxicity associated with the presence of immunological or metabolic susceptibility factors in affected patients. The median latency time of 11 days between the start of the metamizole therapy and the onset of the ADR (Table 3) lies within the 7-14 day latency time found in a previous study analyzing pharmacovigilance data [3]. Similarly, the ADR appeared within the previously described time-frame of the pharmacovigilance data study of 1417 reported cases of metamizole-induced white blood cell disorders [3] and are in agreement with other previous studies [8, 16]. These findings would support routine blood count monitoring during the first weeks of metamizole therapy. The duration of the ADR was approximately 5 days, independent of the use of G-CSF. This finding has to be interpreted in the light of the fact that the more severe cases tended to preferentially receive G-CSF, despite the paucity of data supporting the benefit of G-CSF in this setting.

No changes in GFR were seen under metamizole and there was no difference in GFR between the case and control groups (see Figure 2). Renal impairment was therefore not found to be a risk factor for the development of metamizole-induced leucopenia in the present study.

If the mechanism of toxicity has an immunological component, another possible risk factor could be a susceptibility to allergies, which our findings support. A significantly higher prevalence of allergies among non-post-OP cases compared to controls was not seen as it was in the post-OP setting, merely a trend. This may reflect different data recording practices in hospital compared to general practice. The post-OP groups – all of which were inpatients in the same hospital – however, underwent the same documentation process, so their data is more comparable. New concepts of immunological mechanisms like the p-i-concept where drugs or metabolites interact directly with T-cells may help to understand mechanisms of toxicity, which cannot easily be categorized into classical known mechanisms [17]. A genetic predisposition – which could not be examined in this study - may also be relevant.

Of special interest were the cases with preexisting hematological conditions, especially previous leucopenia. In two of the four patients with previous leucopenia, the likeliest cause was liver disease due to hepatitis C infection. In the other two patients, no reason for the peripheral leucopenia could be found in the bone marrow study. Whether underlying

hematological conditions make patients more vulnerable to the hematotoxic effects of metamizole cannot be conclusively assessed here. However, these data suggest that preexisting hematological conditions are a possible risk factor.

Our observation regarding an increased prevalence of hepatitis C infection in patients who developed a metamizole-associated white blood cell disorder compared to controls is in keeping with the increased risk of ADR in the setting of viral infection seen with other drugs [18]. An increased risk for developing ADRs has been shown in the setting of HIV, hepatitis C, EBV, HSV, HHV and CMV infection [19-22]. Asymptomatic carriers of hepatitis B with normal liver function tests were found to have impaired clearance of metamizole via oxidative pathways compared to healthy controls, leading to increased exposure to the 4-methylaminoantipyrine metabolite (carriers and controls all had slow acetylator phenotype) [23]. In Western Europe, the prevalence of hepatitis C infection is estimated as 2.4% [24]. Overall our case group showed a prevalence of 7% and the control group a prevalence of 1%. The exact mechanisms whereby viral infections cause an increased risk of ADR are not fully known, but could be caused by any combination of a reduction in immune tolerance, increased antigenicity or altered drug metabolism [18].

Regarding the co-administration of other bone-marrow toxic drugs, it is not always possible to separate out the impact the different drugs have on the ADR. This led to 54% of the cases having other co-medications which may or may not have contributed to the ADR. However, the administration of non-chemotherapeutic, potentially myelotoxic co-medication was not different between both types of cases and controls. Concomitant use of cytostatic agents was more common among cases than their respective controls. Among non-post-OP patients this difference was statistically significant, indicating that the concomitant use of cytostatic agents might be a risk factor for the development of a white blood cell disorder under metamizole therapy. In the pharmacovigilance data study, co-administration of methotrexate was identified as a risk factor for a fatal outcome [3]. In this current study, two fatalities involving concomitant immunosuppressive-dose methotrexate and azathioprine, respectively, occurred.

Limitations

Retrospective studies are associated with a number of limitations. The problem of missing data is one important aspect and may have led to the inclusion of control patients who might have gone on to develop a white blood cell disorder under metamizole, especially in the post-OP control group. Furthermore, the limited number of study subjects due to the rarity of the ADR as well as the limited number of patients who take metamizole on a long-term basis reduces study power. It is also possible that some cases were missed due to varying documentation policies. The inclusion of possible rather than only confirmed cases according to the Naranjo scoring system may have led to the inclusion of some cases where metamizole was not the likeliest cause of the white blood cell disorder. Lastly, the true prevalence of hepatitis C was not known as hepatitis C testing is not routinely performed in either the in-patient or general practice setting.

Conclusion

A history of allergy, previous leucopenic episodes, infection with hepatitis C and concomitant use of cytostatic agents (including at immunosuppressive doses) might be risk factors for metamizole-induced leucopenia and require further study. We suggest avoiding metamizole in such patients unless the benefits clearly outweigh the risks, in which case close blood-count monitoring should be performed. In general, metamizole should always be prescribed on a case by case basis balancing the risk for myelotoxicity against the ADR profiles of alternative analgesic agents.

Acknowledgements

The authors would like to cordially thank all collaborating general practitioners (including Doctors Beat Biedermann, Mathis Grehn, Edy Riesen, Peter Sigg, Radu Vornicel-Schwenck, and colleagues). A special thanks also to the Institute of Primary Health Care, University of Basel, Switzerland for the important collaboration. Thanks also to the Senglet-Foundation, the foundation for the promotion of young pharmaceutical researchers in Basel, for their financial support (LSB).

Author contribution

Performed research: LSB, HH, SH, AZ, AH, ABT. Analyzed data: LSB, HH, MH, SK, ARB, ABT. Wrote manuscript: LSB, SK, ABT. All authors contributed to the review and final approval of the manuscript.

Competing interests

The authors have no competing interests.

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3.3.1. *Supplementary material: Metamizole-induced Leucopenia: a Case-Control Study*

Supporting Table 1 Allergy history

		Post-OP cases (n=32)	Post-OP controls (n=64)	p-value	Non-post-OP cases (n=25)	Non-post-OP controls (n=75)	p-value
Patients with known medication allergies [number (%)]		13 (41)	10 (16)	0.007 [a]	6 (24)	15 (20)	0.67[a]
Median number of trigger drugs in patients with medication allergies (range)		3 (1-6)	1 (1-3)		1 (1-2)	1 (1-4)	
Trigger drugs [number (%)]:							
Beta-lactam	Total	9 (28)	6 (9)	0.02[a]	3 (12)	8 (11)	1[b]
	With skin reaction	5	2		2	4	
	With anaphylactic reaction (dyspnea)	1	-		1	-	
	With unknown reaction	3	4		-	4	
Other antibiotics		4	1		-	3	
NSAID		4	4		1	5	
Opioids		1	-		1	2	
Others		5	3		2	6	
Patients with known non-medication allergy [number (%)]		7 (22)	3 (5)	0.014[b]	4 (16)	3 (4)	0.063[b]
Allergic rhinitis		4	-		1	1	
Other allergies		5	3		3	1	
Not specified allergy		-	-		-	1	

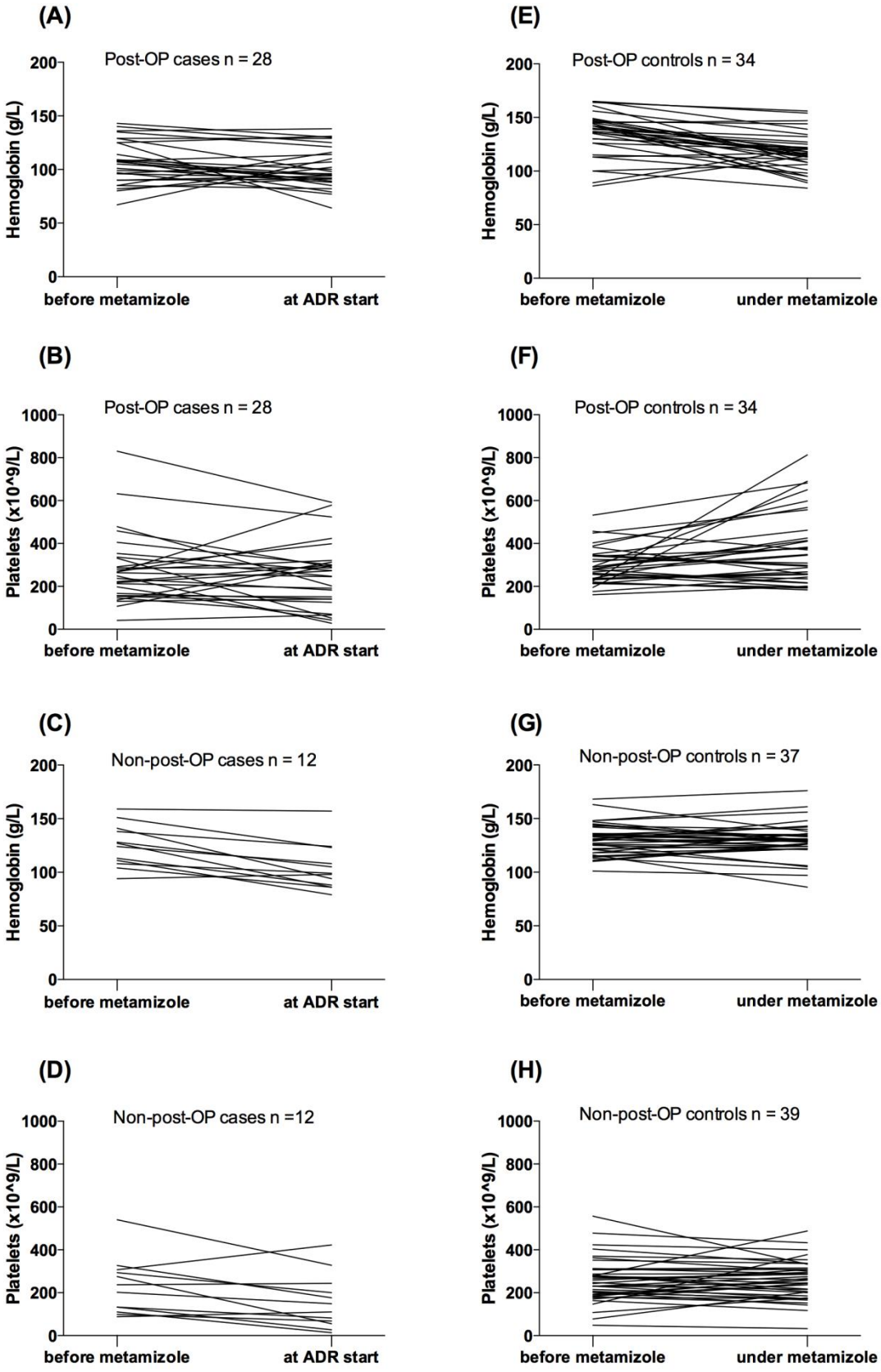
[a]Chi-Square test, [b]Fisher exact test

NSAID non-steroidal antiinflammatory drug

Supporting Table 2 Co-medication known to be associated with agranulocytosis

	Post-OP cases (n=32)	Post-OP controls (n=64)	Non-post-OP cases (n=25)	Non-post-OP controls (n=75)
Total number of co-medication	286	368	185	688
Patients concomitantly receiving cytostatic agents [number (%)]				
Methrotrexate (low-dose)	2 (6)	3 (5)	3 (12)	2 (3)
Azathioprine	-	-	2 (8)	-
Gemcitabine	-	-	-	1 (1)
Sorafenib	-	-	1 (4)	-
Ipilimumab	1 (3)	-	-	-
Cisplatin/5-Fluorouracil	-	-	1 (4)	1 (1)
Patients concomitantly receiving non-chemotherapeutic potentially myelotoxic co-medication[a] [number (%)]				
Clopidogrel	1 (3)	2 (3)	-	3 (4)
Diclofenac	-	-	-	3 (4)
Fluoxetine	1 (3)	-	-	3 (4)
Ibuprofen	5 (16)	13 (20)	4 (16)	3 (4)
Ramipril	2 (6)	4 (6)	2 (8)	15 (20)
Spironolactone	1 (3)	1 (2)	-	7 (9)
Co-prescribed drugs for which an association with the ADR could not be ruled out [number (%)]				
Beta-lactam antibiotics				
Amoxicillin/Clavulanic acid	6 (19)	3 (5)	2 (8)	1 (1)
Tazobactam/Piperacillin	10 (31)	-	2 (8)	-
Flucloxacillin	2 (6)	-	1 (4)	-
Cefepim	1 (3)	1 (2)	-	-
not specified	2 (6)	-	1 (4)	-
Other antibiotics				
Ciprofloxacin	4 (13)	3 (5)	1 (4)	1 (1)
Moxifloxacin	1 (3)	-	-	-
Vancomycin	7 (22)	-	-	-
Tobramycin	1 (3)	-	-	-
Proton-pump inhibitors				
(Es-)Omeprazole	-	9 (14)	2 (8)	11 (15)
Pantoprazole	2 (6)	28 (44)	1 (4)	39 (25)
Other drugs				
Quetiapine	1 (3)	2 (3)	1 (4)	6 (8)
Acetylsalicylic acid[b]	-	9 (14)	1 (4)	22 (29)
Mesalazine	-	-	1 (4)	-
Rifampicin	2 (6)	-	-	-
Interferon	-	-	1 (4)	-
Gabapentin	-	1 (2)	1 (4)	3 (4)
Mirtazapine	-	2 (3)	1 (4)	4 (5)
Haloperidol	1 (3)	-	-	-
Caspofungin	1 (3)	-	-	-
Pregabalin	-	2 (3)	1 (4)	7 (9)
Phenytoin	1 (3)	1 (2)	-	-

[a]Drugs with evidence 1 level for causing acute agranulocytosis (table 2) according Andersohn, et al 2007
[b]Non-post-OP Case: analgesic dose; Non-post-OP controls: 20 cardiac doses, 1 analgesic dose, 1 unknown dose



Supporting Figure 1. Hemoglobin and platelet counts before starting metamizole and at time of diagnosis of ADR (case group A-D) or at last follow-up whilst taking metamizole (control group E-H). ADR adverse drug reaction; n number

So far, the focus of our projects lay on the risks of metamizole. But for a proper evaluation of the safety profile, also the benefits of a drug have to be considered. An important question for clinicians is the question of renal tolerability of a substance. Especially for metamizole as a possible alternative for NSAIDs that are contraindicated in patients with renal insufficiency. The following clinical study should provide data for a more evidence-based use of metamizole in such situations and may deliver information about the renal pharmacology of this drug.

3.4. Effect of metamizole (dipyrone) on renal function in salt-depleted healthy subjects

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Unpublished

List of nonstandard abbreviations

4-AA	4-aminoantipyrine
4-AAA	4-N-acetylaminoantipyrine
4-FAA	4-N-formylaminoantipyrine
4-MAA	4-N-methylaminoantipyrine
AUC	area under the concentration–time curve
$AUC_{0\text{-}5h}$	partial area under the concentration-time curve from 0 to 5 hours
$AUC_{0\text{-}24h}$	partial area under the concentration-time curve from 0 to 24 hours
AUEC	area under effect curve
C_{max}	peak plasma concentration
COX	cyclooxygenase
CTCAE	common terminology criteria for adverse events
GFR	glomerular filtration rate
NSAID(s)	nonsteroidal anti-inflammatory drug(s)
Tmax	time to reach peak plasma concentration
λ_z	terminal elimination rate constant

Abstract

Introduction: The antipyretic and analgesic metamizole (dipyrone) is supposed to have better renal tolerability than NSAIDs, but there is little data available supporting this assumption.

Aims: The primary aim was to investigate the effect of metamizole on inulin clearance and urinary excretion of sodium and of the prostacyclin metabolite 6-keto-PGF1α in healthy, salt-depleted, volunteers. A secondary aim was to obtain pharmacokinetic data of the four major metamizole metabolites and to correlate these data with pharmacodynamic effects.

Methods: Subjects received a diet containing 50 mmol sodium/day one week before and during treatment with metamizole (1000 mg TID, n=8) or naproxen (500 mg BID, n=7) for 7 days. Investigations were performed after single dose (day 1) and after repetitive dosing (day 7).

Results: After single and repetitive dosing, neither metamizole nor naproxen had a significant effect on inulin clearance or sodium excretion. After repetitive dosing, there was a trend for decreased sodium excretion after naproxen but not after metamizole. Both metamizole and naproxen inhibited renal 6-keto-PGF1α excretion starting 2 hours after ingestion and lasting the entire dosing period during repetitive dosing. The two active metamizole metabolites (4-methylaminoantipyrine, 4-MAA and 4-aminoantipyrine, 4-AA) had half-lives of 3.7 (4-MAA) and 5.8 h (4-AA), respectively and they accumulated approximately 1.5 and 3 times after 7 days of repetitive dosing.

Discussion: Metamizole inhibits renal excretion of 6-keto-PGF1α similar to naproxen, suggesting that metamizole inhibits renal prostaglandin synthesis. Nevertheless, in healthy, sodium-depleted subjects, metamizole had no significant effect on inulin clearance or renal sodium excretion, whereas there was a trend to a decreased sodium excretion after repetitive naproxen dosing.

Introduction

The antipyretic analgesic metamizole (dipyrone) is supposed to have a better renal tolerability than nonsteroidal anti-inflammatory drugs (NSAIDs), but there is little data available to support this assumption [1, 2]. The mechanism of action of metamizole is currently not entirely established. Inhibition of cyclooxygenase (COX) enzymes by metamizole or metamizole metabolites has been demonstrated, but the reported IC50 for COX-1 inhibition shows high variability ranging from 2.6 µmol/L to >400 µmol/L (in comparison IC50 for COX-1 for ibuprofen 12-42 µmol/L, and for naproxen 0.3-24 µmol/L) [3-6]. Similarly, the reported IC50 for COX-2 inhibition by metamizole show high variability ranging from 4.7 µmol/L to >400 µmol/L [4, 5, 7]. Other mechanisms of action have been proposed, including stimulation of endogenous cannabinoid receptors [7, 8], involvement of glutamatergic mechanisms, inhibition of neurokinin-1 mediated responses, inhibition of the protein kinase C-dependent pathway [9], and involvement of the descending serotonergic and noradrenergic systems [10]. Regarding safety, the most often mentioned problem of metamizole is agranulocytosis (neutrophil count <500/µL), representing a severe and potentially life-threatening adverse drug reaction [11]. Additionally, metamizole can cause infusion reactions with severe hypotension, especially after rapid infusion of high doses [12].

On the other hand, metamizole shows several advantages compared to NSAIDs. Gastrointestinal toxicity is the most common adverse reaction of NSAIDs, ranging from dyspepsia to life-threatening gastrointestinal ulcerations [13, 14]. Metamizole, however, showed much less gastrointestinal toxicity, which is an argument against clinically relevant inhibition of COX-1, at least in the gastrointestinal tract [15-17]. Moreover, the cardiovascular effects associated with NSAIDs have recently gained increasing attention [14, 18, 19]. The use of NSAIDs, especially the use of selective cyclooxygenase-2 (COX-2) inhibitors, is associated with an increased risk for myocardial infarction and death, particularly in patients with known cardiovascular disease [18]. So far, no cardiovascular risk has been reported for metamizole, supporting the absence of a clinically relevant COX-2 inhibition [20].

Metamizole is a prodrug (see Figure 1). It reaches the systemic circulation after non-enzymatic cleavage to 4-N-methylaminoantipyrine (4-MAA), which can be N-demethylated

by cytochrome P450 enzymes to 4-aminoantipyrine (4-AA). 4-AA can be N-formylated to 4-N-formylaminoantipyrine or N-acetylated to 4-N-acetylaminoantipyrine (4-AAA).

The pathomechanism of impaired renal perfusion associated with NSAIDs is well established. Vasodilatory prostaglandins such as prostacyclin are crucial to maintain renal perfusion in conditions with low intravascular pressure; inhibition of renal prostaglandin synthesis by NSAIDs can therefore impair renal perfusion. However, it is not entirely clear whether metamizole is a valuable alternative to NSAIDs in such situations because little is known about the effect of metamizole on renal function. Regarding renal complications in patients treated with metamizole, mainly case reports with acute kidney injury due to impaired renal perfusion or acute interstitial nephritis have been described in the literature [21-23]. The mechanisms proposed include impairment of prostaglandin synthesis by COX-inhibition similar to NSAIDs for impaired renal perfusion and allergic reactions for interstitial nephritis [21]. In 1995, Farker and colleagues investigated the influence of metamizole and diclofenac on glomerular filtration and renal perfusion in healthy male subjects by measuring the creatinine and inulin clearances and the clearance of para-aminohippurate (PAH), respectively [1]. In their study, neither metamizole, nor diclofenac at therapeutic doses over 3 days had an effect on GFR or renal plasma flow. Since diclofenac lacked an effect on these parameters also in other studies [24-28], it appears to be difficult to demonstrate the consequences of COX inhibition on renal function in healthy volunteers. Results from studies with naproxen were not consistent, but more studies showed a decrease in the GFR if measured using inulin clearance rather than estimated using creatinine clearance [29-31].

Taking into account the wide-spread use of metamizole in certain countries and the lack of conclusive data regarding its influence on renal function, the primary aim of the current study was to examine the effect of metamizole on inulin clearance, urinary excretion of sodium and potassium and urinary excretion of the stable prostacyclin-metabolite 6-keto-prostaglandin F1α (6-keto-PGF1α) in comparison with the non-specific COX-inhibitor naproxen in healthy, salt-depleted, male volunteers. A secondary aim was to obtain pharmacokinetic data for the four major metamizole metabolites and to correlate these data with pharmacodynamic effects.

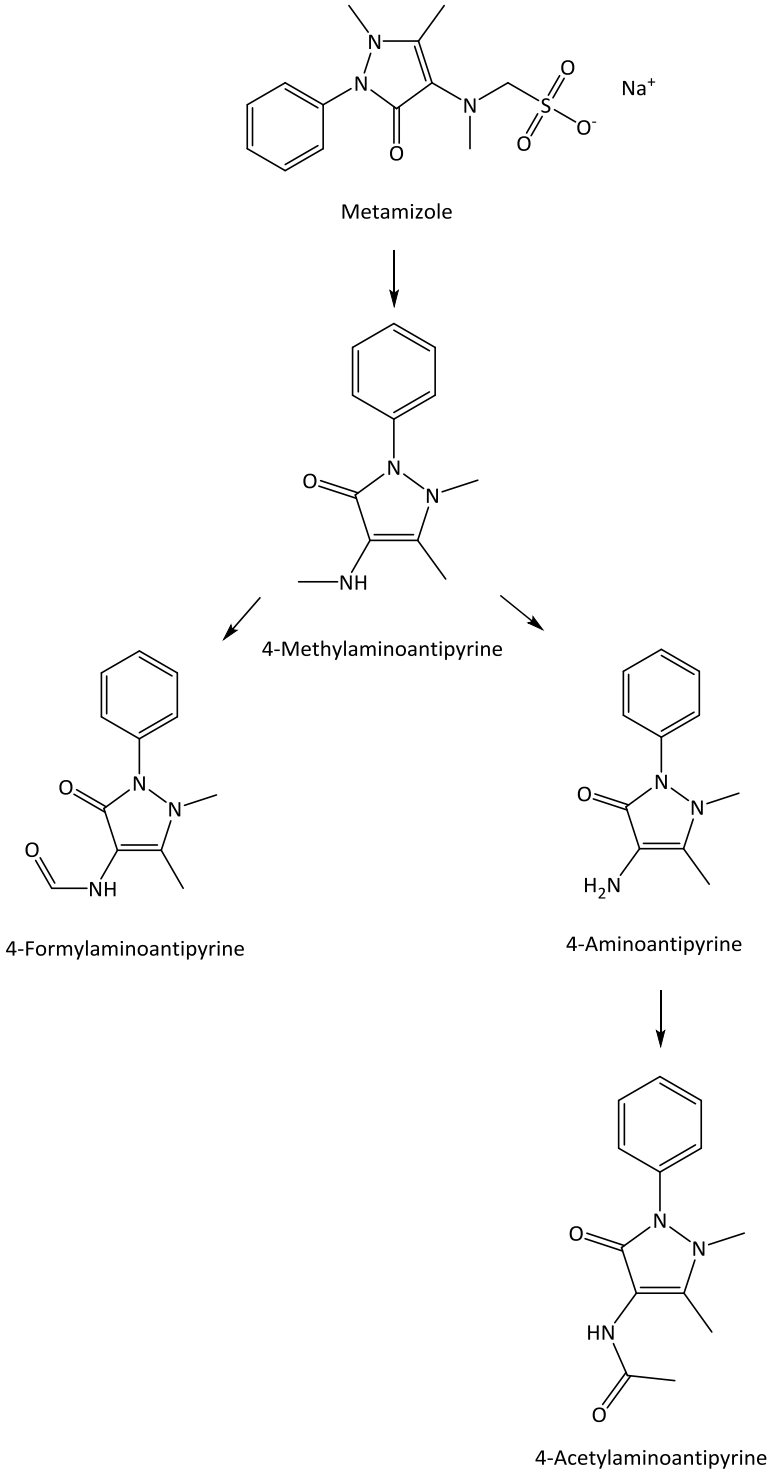

Figure 1 Metabolism of metamizole into the four primary metabolites

Methods

Materials

Metamizole was administered as Novalgin® 500mg tablets (Sanofi-Aventis Suisse SA, Vernier, Switzerland) and naproxen as Naproxen-Mepha® Lactabs 500mg (Mepha Pharma AG, Basel, Switzerland). Inulin (Inutest® 25% ampules) was from Fresenius Medical Care AG (Oberdorf, Switzerland). D-Glucose/D-Fructose Test-Combination was from R-Biopharm AG (Darmstadt, Germany), glucose-oxidase from *Aspergillus niger* and invertase from baker's yeast (*S. cerevisiae*) were from Sigma-Aldrich (Buchs, Switzerland). Citric acid, sodium citrate, and hydrogen peroxide 30% were also purchased from Sigma-Aldrich (Buchs, Switzerland).

Gradient grade water, methanol, isopropanol and formic acid of analysis grade (98-100%) for liquid chromatography were obtained from Merck (Darmstadt, Germany). Chloroform was from Carl Roth (Karlsruhe, Germany) and O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) was from Sigma-Aldrich (Buchs, Switzerland). All reference compounds and internal standards for naproxen and metamizole quantification were products of Toronto research chemicals (Toronto, Canada). 6-keto PGF1α, 6-keto PGF1α-d4, and 2,3-dinor-6-keto-PGF1alpha were obtained from Cayman chemicals (Ann Arbor, Michigan, USA). Stock solutions of naproxen (10 mg/mL), 6-Keto PGF1α-d4 (1 mg/mL) and the metamizole metabolites 4-methylaminoantipyrine (4-MAA; 10 mg/mL), 4-aminoantipyrine (4-AA; 5 mg/mL), 4-formylaminoantipyrine (4-FAA; 5 mg/mL), and 4-acetylaminoantipyrine (4-AAA; 5 mg/mL) were prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, USA). The internal standards 2,3-Dinor 6-Keto PGF1α-d9, 4-MAA-d3, 4-AA-d3, and 4-AAA-d3, and naproxen-d3 were dissolved in DMSO to a final concentration of 1 mg/mL.

Study protocol

This single-center, randomized, open-label, parallel-group study in healthy, male, salt-depleted subjects was approved by the local ethics committee (Ethikkommission beider Basel) and the Swiss Agency for Therapeutic Products (Swissmedic) and was registered at ClinicalTrails.gov (NCT01995006). Before inclusion into the study, we obtained written

informed consent and performed a physical examination including routine laboratory tests of each subject. After inclusion, subjects were randomized into two treatment groups receiving either 1000 mg metamizole three times a day (n=8 subjects) or 500 mg naproxen twice a day for 7 days (day 1 to 7) (n=7 subjects). One week before the first study day (day -7), the subjects started a low sodium diet containing approximately 50 mmol sodium per day [32, 33]. The diet was maintained during the whole study period (until the last blood sampling on day 8). Lunch and dinner with reduced sodium content were provided at the University Hospital during the whole study period. For breakfast and snacks, we delivered written dietary instructions. Urinary sodium excretion was measured in 24-hour urine on days -4, 1, and 7.

Determination of inulin clearance

On day 1, after an overnight fast, a venous catheter was placed on each forearm. After the collection of baseline plasma samples, we started an inulin infusion at 125 mg/min for 20 minutes followed by 28 mg/min for 400 minutes according to the instructions of the manufacturer. After an equilibration period of one hour, we collected a baseline urine sample for the inulin determination (t_{-1} to t_0), before subjects ingested a single dose of either 1000 mg metamizole or 500 mg naproxen (t_0). We continued to collect hourly urine and plasma samples for the determination of the respective inulin concentrations for the next five hours (t_5). The inulin clearance was calculated according the following formula:

$$Clearance_{inulin} = \frac{inulin_{urine} \times volume_{urine} \times 1.73}{inulin_{plasma} \times time \times bs} \qquad bs = \sqrt{\frac{size\ (cm) * weight(kg)}{3600}}$$

where $inulin_{urine}$ is the urinary concentration of inulin, $volume_{urine}$ the urinary volume, $inulin_{plasma}$ the inulin plasma concentration, time the collecting period, and bs the body surface. To ensure a minimal urinary flow of 2-3 mL/min (according to the manufacturer's recommendation), we instructed the volunteers to drink 700 mL of water within the first 30 min after the start of the inulin infusion, followed by additional 300 mL of water per hour until the end of the inulin infusion (t_5).

Plasma and urinary inulin concentrations were measured using an enzymatic method without deproteinization as described by Kuehnle and colleagues [34]. The assay was performed with half of the suggested volume of plasma or urine. Standard curves were linear in plasma (mean r^2 0.9994, n=17) and in urine (mean r^2 0.9991, n=12). For different concentration calibrators, the coefficients of variation for repetitive determinations were 6.2-9.6% for plasma and 9.6-11.2% for urine.

Determination of urinary sodium and potassium excretion and urinary excretion of 6-keto-PGF1-alpha

We measured sodium and potassium excretion in aliquots of hourly urine collections. Urinary potassium concentrations were analyzed using ion-specific electrodes at the central laboratory of the University Hospital Basel. Urinary sodium concentrations were analyzed at the environmental health and safety laboratory, Department of Environment and Energy Basel, using an inductively coupled plasma mass spectrometry (ICP-MS) method with a detection limit of 0.05mg/L. Concentrations of 6-keto-PGF1-alpha in urine were determined using an LC-MS/MS method at the laboratory of the division of clinical pharmacology and toxicology, University Hospital Basel.

LC-MS/MS instrumentation

Naproxen and metamizole analyses were performed on a Shimadzu HPLC system (Kyoto, Japan) coupled to an API 4000 tandem mass spectrometer (AB Sciex, Massachusetts, USA). 6-keto-PGF1-alpha was analysed on a UPLC system from Shimadzu connected to an AB Sciex API 5500 tandem mass spectrometer. The HPLC system consisted of two LC-20AD pumps, two LC-20AD XR pumps, a CTO-20AC column oven, a DGU-20A5 degassing unit, a CBM-20A controller, and a CTC HTS PAL autosampler (Zwingen, Switzerland). The UPLC system had the same configuration except that four 30-AD pumps and a SIL-30AC autosampler were used.

Metamizole LC-MS/MS settings

4-MAA, 4-AA, 4-FAA, and 4-AAA were separated on a pentafluorophenyl phase column (Luna 3 µ PFP 50x2.0 mm, Phenomenex, CA, USA) using water (A) and methanol (B) as mobile phases. Both mobile phases were supplemented with 0.1% (v/v) formic acid. The introduced samples (2 µL) were pre-column diluted via a t-piece with water 0.1% formic acid transported by pump C. The flow rate of pump C was thereby reduced from 0.3 to 0 ml/min within the first 0.5 minutes of each run. Pumps A and B initially were set at 0.1 ml/min and increased to 0.5 ml/min within the first 0.5 min. In the last 0.5 min of the run the flow rate was switched back to the initial conditions. The following mobile phase gradient program was used for separating the analytes: 0-0.25 min; 2% mobile B, 0.25-3.5 min; 2-55% mobile B, 3.51-4.5; 95% mobile B, 4.51-5.0 min; 2% mobile B. The HPLC stream was connected between minute 1 and 3 to the mass spectrometer otherwise to the liquid waste. The retention time of 4-MAA was 1.62 min, of 4-AA 1.74 min, of 4-FAA 2.43 min, and of 4-AAA 2.5 min. Chromatographic separation was carried out at 43° C.

All analytes were detected in the positive mode using electrospray ionization. The applied mass transitions and compound specific parameters are illustrated in the Supplemental Table 1. Mass spectrometer was running at an Ion source gas 1 of 50 l/min (N2), an Ion source gas 2 of 60 l/min (N2), a curtain gas of 10 l/min, a collision gas of 6 l/min, an ion spray voltage of 5500 V, and a source temperature of 600°C. Analyst software 1.6.1 (AB Sciex, MA, USA) was used to operate the LC-MS/MS system.

Naproxen LC-MS/MS settings

Naproxen was eluted on a reversed phase core-shell C18 column (Kinetex 2.6 µm, 50x2.1 mm; Phenomenex, CA, USA). Similar mobile phases were used as for the metamizole analysis. The flow rate was set to 0.5 ml/min at 45°C column oven temperature. The HPLC gradient program was as follows: 0-0.25 min; 40% mobile B, 0.25-1 min; 40-95% mobile B, 1-2 min; 95% mobile B, 2.01-2.25 min; 40% mobile B. Naproxen eluted after 1.3 minutes runtime.

Naproxen and naproxen-d3 were detected in the negative ionization mode. Mass transition and compound specific settings of the two analytes are illustrated in the Supplemental Table 1. General settings of the mass spectrometer were similar to the metamizole analysis except that the ion spray voltage was set to -4500 V.

Metamizole and naproxen quantification in plasma samples

Calibration lines were prepared in drug free blank human plasma obtained from the local blood donation center (University Hospital Basel, Switzerland). Thereby, analyte stock solutions were serially diluted with DMSO and each dilution was mixed with blank plasma at a ratio of 1:100. Calibration ranges, which included 10-11 calibrators, were established from 25-50'000 ng/mL for naproxen, from 50-50'000 ng/mL for 4-MAA, from 25-5000 ng/mL for 4-FAA, and from 5-5000 ng/mL for 4-AA and 4-AAA. A correlation coefficient of ≥0.99 (R^2) was at least required. Quality control samples were prepared at low, medium, and high concentration levels. A mean accuracy of 85-115% (80-120% at the lower limit of quantification (LLOQ)) and a precision of 15% (LLOQ: 20%) was accepted in our study.

Plasma aliquots of 50 µL were precipitated with 500 µL methanol containing the internal standards (4-AA-d3: 10 ng/mL, 4-AAA-d3: 50 ng/mL, 4-MAA-d3: 25 ng/mL, naproxen-d3: 500ng/mL). Samples were mixed with a VX-2500 multi-tube vortexer (VWR, Dietikon, Switzerland) for about 1 min and centrifuged at 4000 rpm during 30 min (Eppendorf 5810R, Hamburg, Germany). 2 µL supernatant were injected into the LC-MS/MS system.

6-keto PGF1α LC-MS/MS settings

Derivatization of the ketone group of 6-keto PGF1α using (O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride resulted in the formation of two isomers. These isomers were separated on a Kinetex C18 column (2.6 µm, 50 x 2.1 mm, Phenomenex, CA, USA). Mobile phase A was a solution 5mM ammonium bicarbonate (Sigma-Aldrich, MO, USA) in water. Isopropanol was used for mobile B. The introduced samples (50 µL) were pre-column diluted via a t-piece during the first 0.5 min of each run with mobile phase A (flow rate gradient of pump C: 0.35 to 0 mL/min). Flow rates of pumps A and B were increased

from 0.35 to 0.7 mL/min in the first 0.5 min of each run. In the last 0.5 min of the run the flow rate was switched back to the initial conditions. The following mobile phase B gradient program was used: 0-11 min; 2-23%, 11-12 min; 23-95%, 12-13 min; 95%, 13-13.5 min; 2%. HPLC and mass spectrometer were connected during minute 8 and 11.5 min of each run to monitor 2,3-dinor-6-keto-PGF1α which eluted after 9.4 min and the two PFBHA-6-keto PGF1α isomers, which had a retention time of 10.0 and 10.4 min, respectively. Column oven temperature was set at 60°C.

All analytes were detected in the negative mode using atmospheric pressure chemical ionization. The applied mass transitions and compound specific parameters are illustrated in the Supplemental Table 1. The mass spectrometer was running at an Ion source gas 1 of 45 l/min (N2), an Ion source gas 2 of 60 l/min (N2), a curtain gas of 30 l/min, the collision gas was set on medium flow, an nebulizer current voltage of -4.5 V, and a source temperature of 400°C. Analyst software 1.6.1 (AB Sciex, MA, USA) was used to operate the LC-MS/MS system.

6-keto-PGF1α quantification in urine samples

Calibration lines were prepared by spiking blank human urine with 6-keto PGF1α-d4 (in DMSO) at a ratio of 1:1000. Ten calibrators from 10 to 2500 pg/mL were used to establish a calibration line. Linearity was sufficient if the correlation coefficient was ≥0.99 (R^2). Quality control samples were prepared at low (75 pg/mL), medium (250 pg/mL), and high (750 pg/mL) concentration levels. A mean accuracy of 85-115% and a precision of 15% was accepted in our study.

We applied a modified liquid-liquid extraction technique as described by Sterz et al. [35]. Aliquots of 2 mL urine were used for analysis. Before extraction, 20 µl of acetic acid was added to each sample, followed by 8 mL of the IS (2,3-dinor-6-keto-PGF1α) in a methanol-chloroform (2:1 v/v) mixture. The samples were mixed during half an hour and incubated for another half an hour at room temperature. After adding chloroform and water (2.5 mL each), the samples were mixed during 10 minutes and afterwards centrifuged for 10 minutes at 2000 rpm. The chloroform phase was evaporated under a gentle stream of nitrogen at 40°C with a Turbo Vap LV (Caliper Life Science, MA, USA). The residue was dissolved in a 200

µl isopropanol-water-PFBHA solution (4:5:1, v/v) and incubated for 30 minutes at 60 °C for derivatization (Blatnik et al, 2010). 50 µL was injected into the LC-MS/MS system. Quantified concentrations of 6-keto-PGF1α were normalized to the creatinine concentrations of the urine samples to compensate for differences in urine dilutions.

Pharmacokinetic Analysis

Plasma samples for the pharmacokinetic evaluation were drawn before and at 1, 2, 3, 4, 5, 8, 12, and 24 hours after drug administration. Starting on day 2, the full doses were given until day 7. On day 7, the PK measurements were repeated at the same time-points as on day 1.

Plasma concentration data were analyzed using non-compartmental methods. Peak plasma concentrations (C_{max}) and time to reach C_{max} (T_{max}) were directly obtained from observed concentration-time data. The terminal elimination rate constant (λ_z) was determined by log-linear regression using at least three data points in the elimination phase. The area under the concentration-time curve (AUC) from zero to infinity after dosing ($AUC_{0\text{-}inf\_obs}$) was estimated using the linear trapezoidal method and was extrapolated to infinity based on the last observed or predicted concentration. Additionally, partial area under concentration-time curve from 0 to 5 hours ($AUC_{0\text{-}5h}$) was calculated for comparison of the two treatment days. The terminal elimination half-life was calculated using λ_z. Calculations were done using the PK Solver add-in (version 2.0) for Microsoft Excel [36].

Statistical analysis

Statistical analyses were performed using Microsoft Office Excel (Version 2010, Redmond, Washington, USA) and GraphPad PRISM (Version 6, La Jolla, California, USA). For variables with normally distributed numerical values, the arithmetic mean and standard deviation were calculated. For variables without normally distributed values, median and range were determined. Calculated inulin clearance and urinary sodium excretion values were parameterized by calculating an area under effect curve (AUEC) from time 0 to 5 hours. AUECs between the metamizole and the naproxen group were statistically compared using a Mann-Whitney-U-test whereas AUECs within a treatment group but of the different study

days were tested using a Wilcoxon-test. The course within a treatment group on one day was evaluated using a Friedman-test. Differences were considered as significant when $p \leq 0.05$.

Results

Fifteen subjects completed the study (mean age 24.3 years [range 18 to 29 years], mean BMI 23.2 kg/m^2 [range 18.6 to 27.8 kg/m^2]). Eight subjects received metamizole, 7 subjects received naproxen. Two subjects had to be excluded because of adverse reactions caused by inulin before the administration of the study drug. One of the excluded subjects developed an allergic reaction with bronchospasm (CTCAE grade 3) and the other subject suffered from vomiting after the start of the inulin infusion (CTCAE grade 1). The drugs were well tolerated, with only mild and no unexpected adverse events.

Reduced dietary sodium intake was monitored by determination of urinary sodium excretion in 24 hour urine collections during the pre-study period (day -4) and at the start (day 2) and the end (day 8) of the treatment period. Median urinary sodium excretion was in the target range of less than 60-80 mmol/day on day -3 and day 2, but exceeded the target range at the end of the dosing period (Table 1).

Table 1 Urinary sodium excretion in 24 hour urine samples

Sodium excretion [mmol/day] median (range)	**Metamizole n=8**	**Naproxen n=7**
pre-study period (day-3)	52 (29-64)	43 (29-67)
start of treatment (day 2)	53 (26-82)	52 (34-132)
end of treatment (day 8)	85 (31-127)	104 (85-121)

The concentration-time profiles of the four main metamizole metabolites after a single oral dose are shown in Figure 2 and the corresponding pharmacokinetic parameters are listed in Table 2. On day 7, subjects continued study drug intake and had two additional metamizole doses 5h and 12h or one additional naproxen dose 12h after the morning dose. Therefore, no 24 hour steady-state PK profile could be obtained after the last study drug dose. The first metabolite formed, 4-MAA, reached the highest plasma concentration of all metabolites, both after single and multiple doses. After single dose, the C_{max} values for 4-AA, 4-AAA and 4-FAA were 10 to 20 times lower than for 4-MAA. After multiple dose, the differences in the C_{max} values were less pronounced because the half-lives of 4-AA, 4-AAA and 4-FAA are longer than for 4-MAA.

The concentration-time profiles of naproxen are shown in Supplemental Figure 1 and the corresponding pharmacokinetic data are listed in Supplemental Table 2. After application of a single dose, the half-life of naproxen was in the range of 18 hours, corresponding to published values [37]. After repetitive dosing, a slight increase in C_{max} and $AUC_{0\text{-}12h}$ compared to single dose was observed.

Table 2 Pharmacokinetic parameters after a single oral dose of 1000 mg metamizole (n=8) on day 1 and after multiple doses (1000 mg TID) on day 7

	4-MAA	**4-AA**	**4-AAA**	**4-FAA**
Day 1				
$T_{1/2}$ [h]	3.7 (2.8-4.4)	5.8 (4.0-12.4)	---	16.0 (8.9-27)
T_{max} [h]	1(1-2)	5 (3-5)	12 (8-24)	8 (5-12)
C_{max} [mg/L]	14.9 (11.9-18.5)	1.2 (0.6-2.5)	1.6 (0.8-2.5)	1.9 (1.4-2.3)
$AUC_{0\text{-}24h}$ [mg/L x h]	105 (67-128)	15 (7-31)	30 (15-47)	34 (27-40)
$AUC_{0\text{-}5h}$ [mg/L x h]	58 (48-70)	4.3 (2.3-8.8)	2.9 (1.0-5.2)	5.9 (4.2-7.6)
Day 7				
C_{max} [mg/L]	21.2 (15.7-24.8)	3.0 (1.1-8.9)	8.3 (2.0-11.3)	3.5 (2.0-4.8)
C_{max} ratio MD/SD	1.35	2.22	4.85	1.77
$AUC_{0\text{-}5h}$ [mg/L x h]	82.6 (55.1-106)	13.9 (4.8-42.4)	39.1 (9.2-53.9)	16.3 (8.7-22.0)
$AUC_{0\text{-}5h}$ ratio MD/SD	1.42	3.24	13.35	2.77

Values are listed as median and ranges. $AUC_{0\text{-}24h}$ partial area under concentration-time curve from 0 to 24 hours, $AUC_{0\text{-}5h}$ partial area under concentration-time curve from 0 to 5 hours (adapted to dosing interval, before second daily dose), C_{max} maximum plasma concentration, MD multiple dose, SD single dose, $T_{1/2}$ half-life, TID three times per day, T_{max} time to maximum plasma concentration

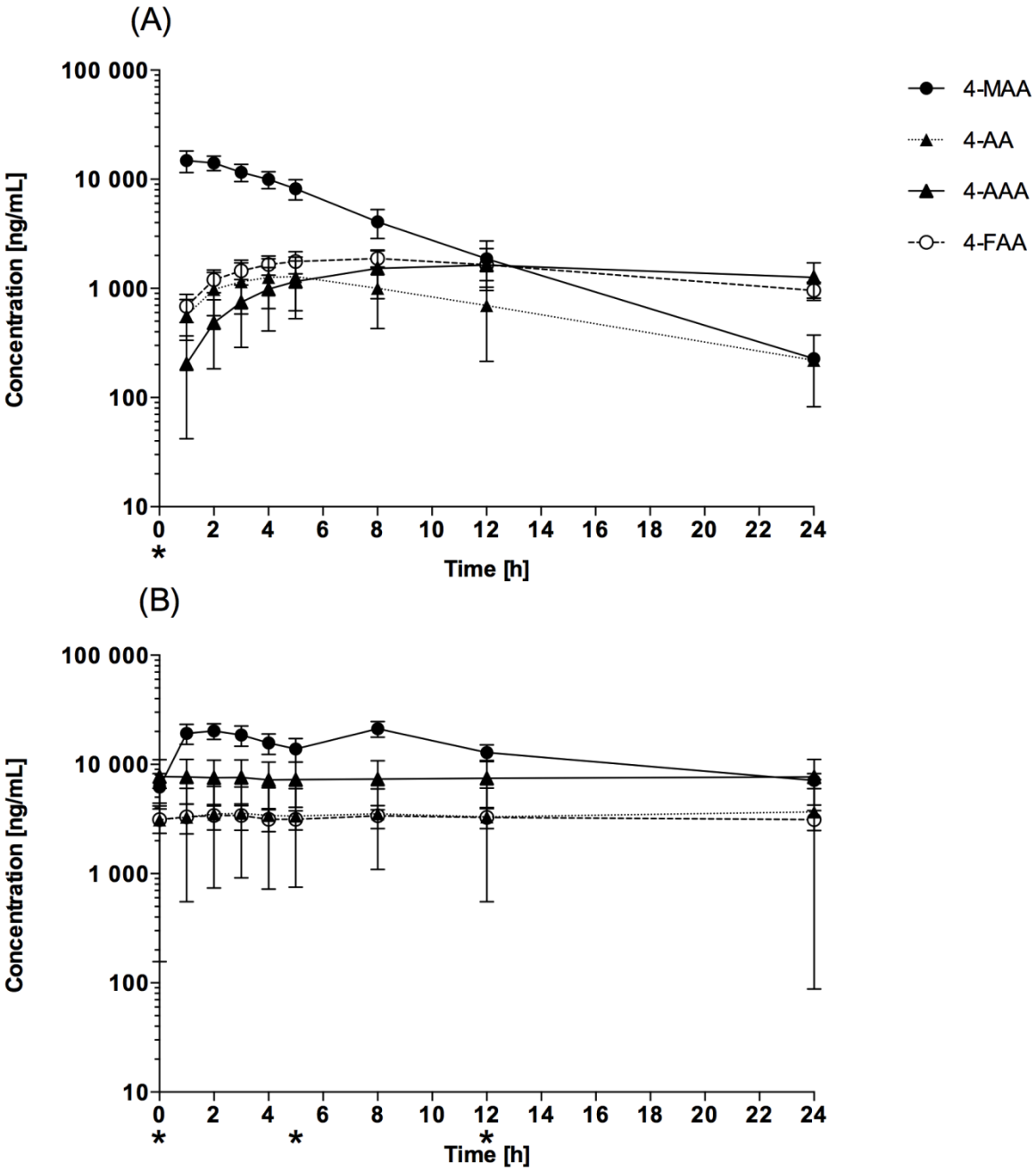

Figure 2 Mean plasma concentration of the four main metabolites of metamizole vs. time in healthy volunteers (n= 8) following a single oral dose of 1000 mg (A) and after seven days of continuous intake of 1000 mg three times per day (B). *oral drug intake

As shown in Figure 3A and B, the GFR (as reflected by the inulin clearance) increased relative to baseline in both the naproxen and the metamizole group after single and repetitive dosing. A statistical comparison between the metamizole and naproxen group did not reach statistical significance. Likewise, no significance was observed within a medication

group when day 1 and day 7 were compared. The mean GFR values at baseline were 74.5 mL/min and 82 mL/min in the metamizole and naproxen group, respectively.

The urinary fractional sodium excretion showed a trend to higher values after single dose treatment with metamizole and with naproxen, without reaching statistical significance between metamizole and naproxen or between the two study days (Fig. 3C). After 7 days of treatment with naproxen, a trend to decreased renal sodium excretion was observed, without reaching statistical significance (Fig. 3D AUEC naproxen versus metamizole, p=0.07; AUEC naproxen day 1 versus day 7, p=0.81). The highest reduction of the fractional sodium excretion was observed 3 hours after naproxen dosing on day 7, (Fig. 3D, p=0.006 compared to baseline).

As shown in Figure 3E and 3F, the effect of metamizole and naproxen on renal excretion of potassium was minimal and did not reach statistical significance between metamizole and naproxen. Detailed data of the urinary volumes, urinary sodium excretion, and urinary creatinine concentrations for the different one hour collection intervals are listed in Supplemental Tables 3 to 5.

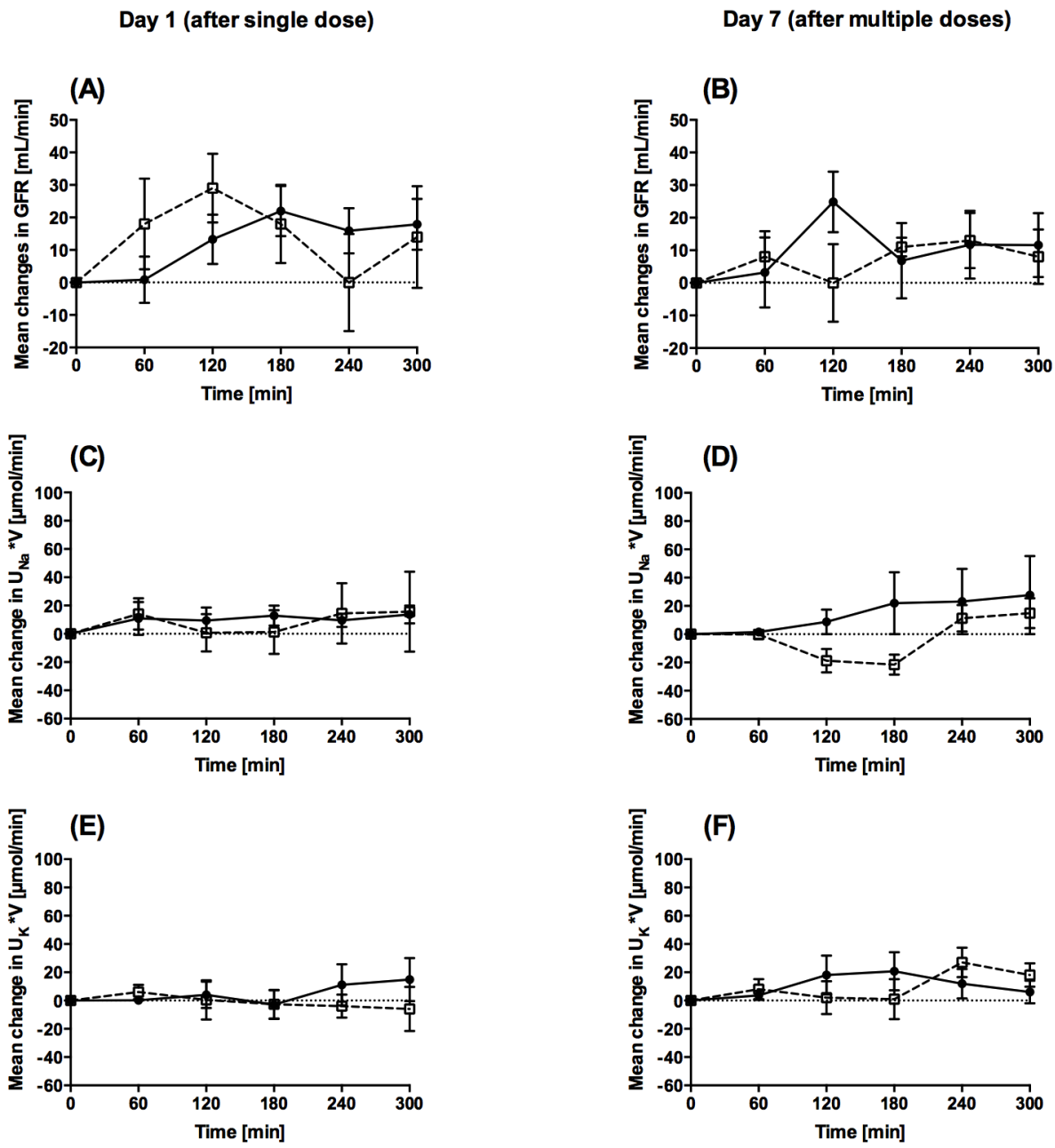

Figure 3 Mean changes in glomerular filtration rate (GFR) (A +B), fractional urinary sodium excretion (UNa) (C +D), and fractional urinary potassium excretion (UK) (E + F) measured after a single oral dose of 1000 mg metamizole (solid line) or 500 mg naproxen (dashed line) on day 1 (left panel, A, C, E) and in a steady state situation on day 7 (right panel, B, D, F). Data are presented as mean values ± SEM

The fractional excretion of urinary 6-keto-PGF1α decreased under naproxen as well as under metamizole. Figures 4A and 4B summarize the data obtained after a single dose (day 1) and at steady state (day 7). The baseline urinary 6-keto-PGF1α concentrations on day 1 at time 0 were quantifiable for all subjects for both treatments. After a single dose of metamizole, the 6-keto-PGF1α excretion started to decrease at 2 hours and remained low up the 5 hours. A similar picture was seen after a single dose of naproxen. At steady state conditions (day 7), the value before ingestion of the morning dose (time 0h) was lower than the corresponding value at day 1, indicating a long-lasting effect of both treatments on renal excretion of 6-keto-PGF1α. Since urinary excretion of 6-keto-PGF1α was already low at the beginning (time 0h) of day 7, ingestion of 500 mg naproxen or 1000 mg metamizole had almost no observable effect on quantifiable 6-keto-PGF1α over the observation period of 5 hours. In many cases concentrations of 6-keto-PGF1α dropped to levels below the limit of quantification (LLOQ, 8 pg/mL) after ingestion of metamizole or naproxen. Since values below LLOQ were set as LLOQ, the averages shown in Figure 4A and 4B overestimate the real values. Individual 6-keto-PGF1α excretion profiles combined with urine creatinine concentrations are shown in Supplemental Figure 2A and 2B. For the majority of the subjects, the urine creatinine concentrations in the 2- to 5-hour fractions were comparable to the baseline fraction, indicating that the observed decrease of 6-keto-PGF1α concentrations after metamizole or naproxen was not caused by urine dilution.

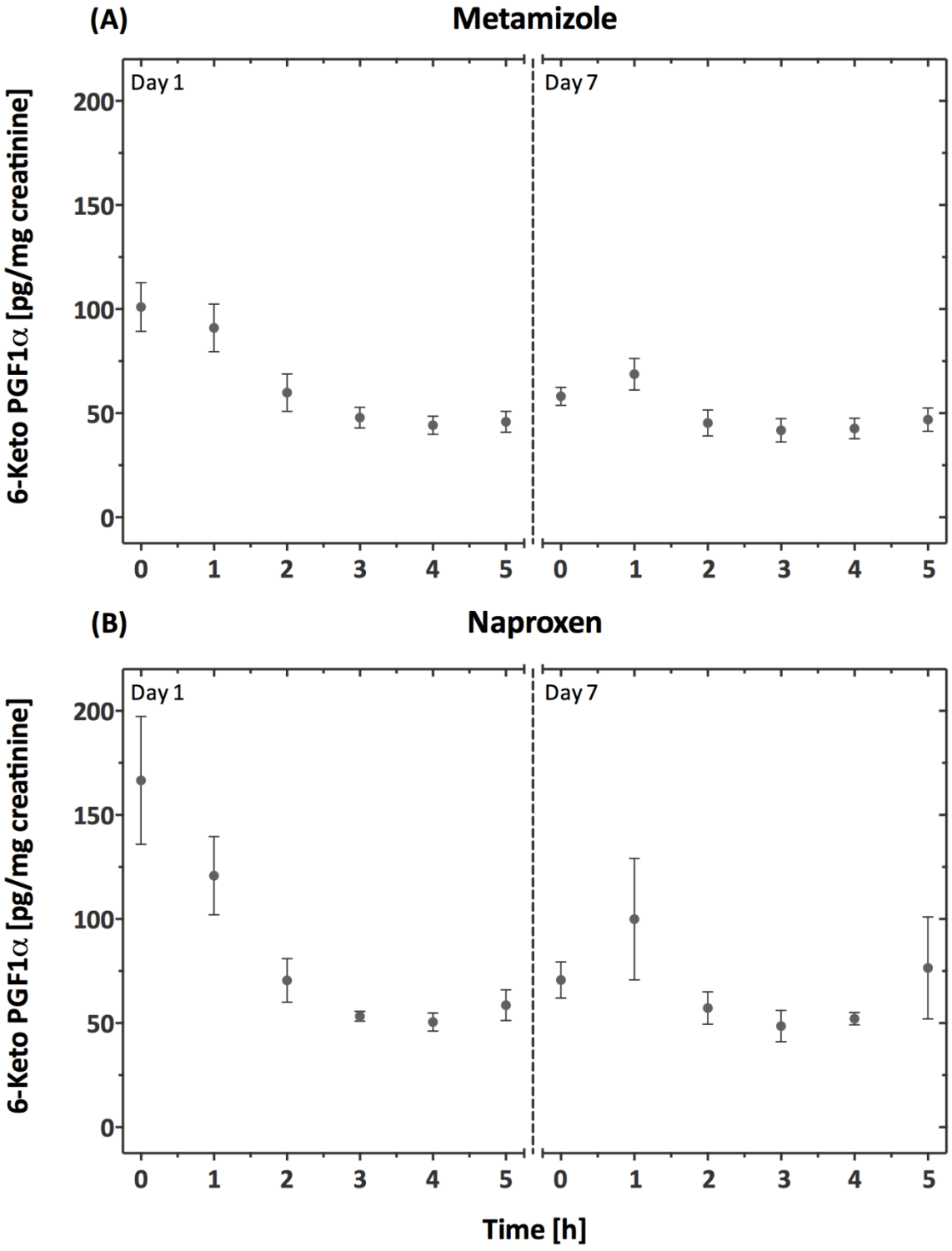

Figure 4 Mean urinary 6-keto-PGF1α excretion measured after a single oral dose of 1000 mg metamizole (A) or 500 mg naproxen (B) on day 1 and in a steady state situation on day 7

Discussion

In this study we investigated the pharmacokinetics of 4 metamizole metabolites and the effect of single and repetitive doses of metamizole on renal function compared to naproxen in salt depleted healthy human volunteers. After a single dose, both metamizole and naproxen increased inulin clearance and renal sodium excretion and decreased renal 6-keto-PGF1α excretion. After 7 days of continuous treatment, both metamizole and naproxen increased inulin clearance and decreased renal excretion of 6-keto-PGF1α. Metamizole did not affect renal excretion of sodium, whereas naproxen was associated with a decrease. None of the treatments had a relevant effect on potassium excretion after single or repeated dosing.

Pharmacokinetics

The observed pharmacokinetic data of metamizole are in line with previously published values. The main metabolite 4-MAA showed a median half-life of 3.7 hours, which is comparable to published values [38, 39]. The observed half-life for the other active main metabolite 4-AA was between reported values for slow and rapid N-acetylators (rapid acetylators 3.8 hours, slow acetylators 5.5 hours [38]). The values for T_{max} observed in the current study for 4-MAA and 4-AA were between 1 and 2 hours and between 3 and 5 hours, respectively, as reported previously for 4-MAA (1.2-1.9 hours) and for 4-AA (3.2-6.7 hours) [39]. The C_{max} of 4-MAA observed in the current study was in the higher range (14.9 mg/L in current study compared to 9.7-17.3 mg/L) and the $AUC_{0\text{-}inf}$ was higher than previously published values (107 mg/L x h in the current study compared to 64.5-95.1 mg/L x h [39]). The ratios of C_{max} and $AUC_{0\text{-}5}$ of repetitive to single doses were clearly larger than 1, indicating accumulation of metabolites. Accumulation could be expected taking into account the half-lives (3.7, 5.8 and 16.0 h for 4-MAA, 4-AA, 4-FAA, respectively) in relation to the dosing interval of 8 hours. Considering 4-MAA and 4-AA, which are both regarded as pharmacologically active metabolites [39], 4-MAA reached approximately 10 times and 7 times higher values for C_{max} and AUC compared to 4-AA after single and repetitive dosing, respectively. It can therefore be assumed that the analgesic activity of metamizole is primarily associated with 4-MAA after both single and repetitive dosing. Regarding toxicity,

whose mechanisms are currently not well established, the other metabolites may also contribute.

Previously published pharmacokinetic data have been analyzed using HPLC methods, whereas our measurements were performed using a more specific LC-MS/MS method. Since the data agree well with each other, HPLC methods appear to be specific enough for this analysis. There are no newer studies dealing with pharmacokinetic properties of the metabolites of metamizole. Studies have been published, however, trying to find genetic and non-genetic factors influencing the pharmacokinetic profile of metamizole metabolites, which may influence both the pharmacological action of metamizole and also its toxicity. [40, 41].

Effect on renal function

The low absolute values for inulin clearance suggest a methodological bias in our assessment. Compared to the estimated baseline GFR using the CKD-EPI equation, the average inulin clearance was approximately 20 mL/min lower. A reason for the low baseline values could be an incomplete distribution of inulin after the 1 hour equilibration period with changing plasma concentrations and renal excretions. Since the formula used to calculate inulin clearances presumes stable plasma levels over the entire observation interval, the calculated clearances may be biased. Another reason for this observation could be an incomplete voiding of the bladder by the subjects. On the other hand, it has previously been reported that measured creatinine clearances can exceed corresponding inulin clearances, possibly due to additional tubular creatinine secretion. In one study, the ratio of measured creatinine clearance to inulin clearance varied between 1.14 and 2.27, which is similar to our findings [42].

Surprisingly, the inulin clearance numerically increased with time in both treatment groups after drug intake, suggesting an increase rather than the expected decrease in GFR. We interpreted the increase in inulin clearance as a consequence of the water load applied in order to maintain a sufficient urinary flow. Interestingly, although the extent of the water load was the same on both study days (day 1 and day 7), the increase in inulin clearance was less pronounced after repetitive compared to single dosing, in particular for naproxen. In

comparison, in a previous clinical study with a comparable study design, there was also no decrease in GFR after single dose naproxen, but a fall in the GFR in the steady state situation [33]. Since naproxen, which was used as a positive control, was not associated with a measurable effect on inulin clearance in the current study, it is difficult to evaluate the effect of metamizole on GFR. Under the conditions of our experiment, metamizole did not affect inulin clearance both after a single and during repetitive dosing.

The results for urinary sodium excretion are similar to those obtained for inulin clearance. After a single dose, both metamizole and naproxen increased sodium excretion, explained best by the water load applied to the study subjects. After repetitive dosing at day 7, however, the sodium excretion was not affected by metamizole but decreased in the subjects treated with naproxen (Figure 3C and 3C). In a previous study, the sodium clearance decreased already after a single dose of naproxen [33]. A possible reason for this difference could be the more pronounced salt depletion in the previous compared to the current study, as suggested by the lower sodium excretion at baseline before drug application (26.1 to 36.7 mmol/day vs. 43 to 52 mmol/day in the current study) despite aiming at an identical sodium ingestion of 50 mmol/day.

Regarding the urinary 6-keto-PGF1α excretion, we observed the expected inhibition in the naproxen group (Figure 4). The inhibition started at 2 h after ingestion, which corresponds to the T_{max} of naproxen. Since the values were below the detection limit in most subjects at the morning of day 7 before drug ingestion, 6-keto-PGF1α excretion was still impaired in most subjects after the doing interval of 12 h for naproxen. Interestingly, metamizole also inhibited renal excretion of 6-keto-PGF1α (Figure 4). After a single dose, maximal inhibition was reached at 2 h, correlating with T_{max} for 4-MAA, but not for the other metabolites. At day 7, before ingesting metamizole morning dose, 6-keto-PGF1α was detectable in 4 of subjects, but the average appeared to be slightly lower than at baseline. Regarding T_{max} and half-life of the active metamizole metabolites, 4-MAA and 4-AA, these results suggest that the initial decrease in renal 6-keto-PGF1α excretion has mainly been caused by 4-MAA, but that 8 h after dosing 4-AA may also have contributed to the effect.

A decrease in renal prostaglandin excretion after metamizole intake has already been shown in a study with healthy volunteers [43] and in a study in patients with liver cirrhosis [2]. Clinical experience suggests a better renal tolerability of metamizole compared to

NSAIDs. The pathomechanism of decreased glomerular filtration associated with NSAIDs is known to be based on the inhibition of renal prostaglandin synthesis. The vasodilatory prostacyclin is important to maintain sufficient renal perfusion in conditions with low intravascular pressure. Since our subjects may not have reached a sufficient extent of sodium depletion leading to a low intravascular pressure situation (see limitation section), renal prostaglandin synthesis may not have been critical to maintain renal perfusion in our subjects. This could be an explanation why we did not observe a significant effect on inulin clearance and sodium excretion in the naproxen group. Another possible explanation for the better renal tolerability of metamizole despite the inhibition of vasodilatory renal prostaglandin synthesis could be a direct vasodilatory effect of metamizole, counteracting reduced renal availability of vasodilatory prostacyclin. Such a vasodilatory effect of metamizole has been described before in animal models for vasospasm [44, 45]

In 14 patients with liver cirrhosis (50% with ascites, Child-Pugh score 7.0 ± 1.5), metamizole did not reduce renal function when used for short time periods (up to 72h) [2]. Since patients with liver cirrhosis are at high risk for renal complications after prostaglandin synthesis inhibition [46, 47], these results support the assumption of a better renal tolerability of metamizole compared to NSAIDs in patients with low intravascular filling pressures. The current study, which shows a similar inhibition of renal 6-keto-PGF1α excretion for metamizole and for naproxen suggests that renal excretion of 6-keto-PGF1α may not be the ideal marker to differentiate between the renal adverse effects of NSAIDs and metamizole. In this situation, measurement of the renal plasma flow would probably allow a better differentiation of the net effect of metamizole on renal vascular tone compared to NSAIDs.

Limitations

The most important limitation of the current study is the lack of effect of naproxen which was used as positive control on inulin clearance. Based on a previous report [33], the expected effect of naproxen on GFR was a reduction of approximately 20% after seven treatment days. The compliance with the low-sodium diet is most probably a crucial factor to render healthy subjects vulnerable for the renal effects of NSAIDs. Measurement of

sodium excretion in 24 hour urine collections showed that the subjects were compliant at the beginning but not at the end of the repeated dosing period (Table 1). This could be the reason for the lacking effect of naproxen on inulin clearance and sodium excretion during repetitive dosing at day 7. A second weakness of study is the small number of subjects included, which was chosen based on practical considerations. A third weakness is the lack of a placebo group which would have helped to demonstrate the effect of the water load on inulin clearance and renal sodium excretion.

Conclusions

Metamizole inhibits renal excretion of the prostacyclin metabolite 6-keto-PGF1α similar to naproxen, suggesting that metamizole inhibits renal prostaglandin synthesis. Despite inhibition of renal 6-keto-PGF1α excretion, metamizole had no significant effect on inulin clearance or renal sodium excretion in healthy, moderately sodium-depleted subjects, whereas naproxen decreased sodium excretion. Renal tolerability of metamizole in patients at risk might be explained by a direct vasodilatory effect of metamizole counteracting reduced renal availability of vasodilatory prostacyclin. Further studies in more susceptible individuals, looking also at renal plasma flow have to be conducted to evaluate differences in the effects on renal function between metamizole and NSAIDs that would explain the better renal tolerability of metamizole observed in the clinical setting.

Acknowledgements

The authors would like to thank Claudia Bläsi, Luisa Baselgia, and Andrea Stoller for their valuable assistance during the clinical work.

Financial support

LSB was supported by a grant of the Senglet-Foundation. SK and MH were supported by grant of the Swiss National Science Foundation (31003A_156270 and 31003A_160206/1, respectively).

Conflict of Interest

The authors report no competing interests regarding this study.

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3.4.1. *Supplementary material: Effect of metamizole (dipyrone) on renal function in salt-depleted healthy subjects*

Supplemental Table 1 Mass transitions and compound specific settings

ID	Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	DP (V)	CE (V)	CXP (V)	EP (V)
4-AA	204.2	56.2	30	71	39	8	10
4-AA-d3	207.2	59.1	30	46	39	10	10
4-AAA	246.2	83.1	30	46	43	14	10
4-AAA-d3	249.2	84.1	30	61	43	6	10
4-FAA	232.2	204.1	30	46	19	12	10
4-MAA	218.2	56.1	30	31	16	10	10
4-MAA-d3	221.2	56.1	30	46	37	8	10
Naproxen	229.1	168.8	50	-40	-36	-15	-10
Naproxen-d3	232.1	170.8	50	-45	-40	-9	-10
6-Keto PGF1α	564.1	306.1	100	-155	-36	-21	-10
6-Keto PGF1α-d4	568.1	310.1	100	-155	-36	-21	-10
2,3-Dinor 6-Keto PGF1α-d9	545.2	259.0	100	-210	-28	-17	-10

Supplemental Table 2 Pharmacokinetic parameters after a single oral dose of 500 mg naproxen (n=7) on day 1 and after multiple doses (500 mg BID) on day 7

	Naproxen
Day 1	
T1/2 [h]	18.4 (12.8-21.8)
Tmax [h]	2(2-5)
Cmax [µg/mL]	63.2 (51.7-76.0)
$AUC_{0\text{-}inf\_obs}$ [µg/mL *h]	1293 (889-1528)
$AUC_{0\text{-}12h}$ [µg/mL *h]	497 (398-560)
Day 7	
Tmax [h]	2 (1-3)
Cmax [µg/mL]	85.7 (67-101)
Cmax ratio MD/SD	1.37
$AUC_{0\text{-}12h}$ [µg/mL *h]	754 (572-817)
$AUC_{0\text{-}12h}$ ratio MD/SD	1.52

Values are listed as median and ranges. $AUC_{0\text{-}inf\_obs}$ area under the concentration-time curve from zero to infinity extrapolated based on the last observed or predicted concentration, $AUC_{0\text{-}12h}$ partial area under concentration-time curve from 0 to 12 hours, C_{max} maximum plasma concentration, MD multiple dose, SD single dose, $T_{1/2}$ half-life, BID twice daily, T_{max} time to maximum plasma concentration

Supplemental Table 3 Median sodium excretion (hourly intervals) of the two study days

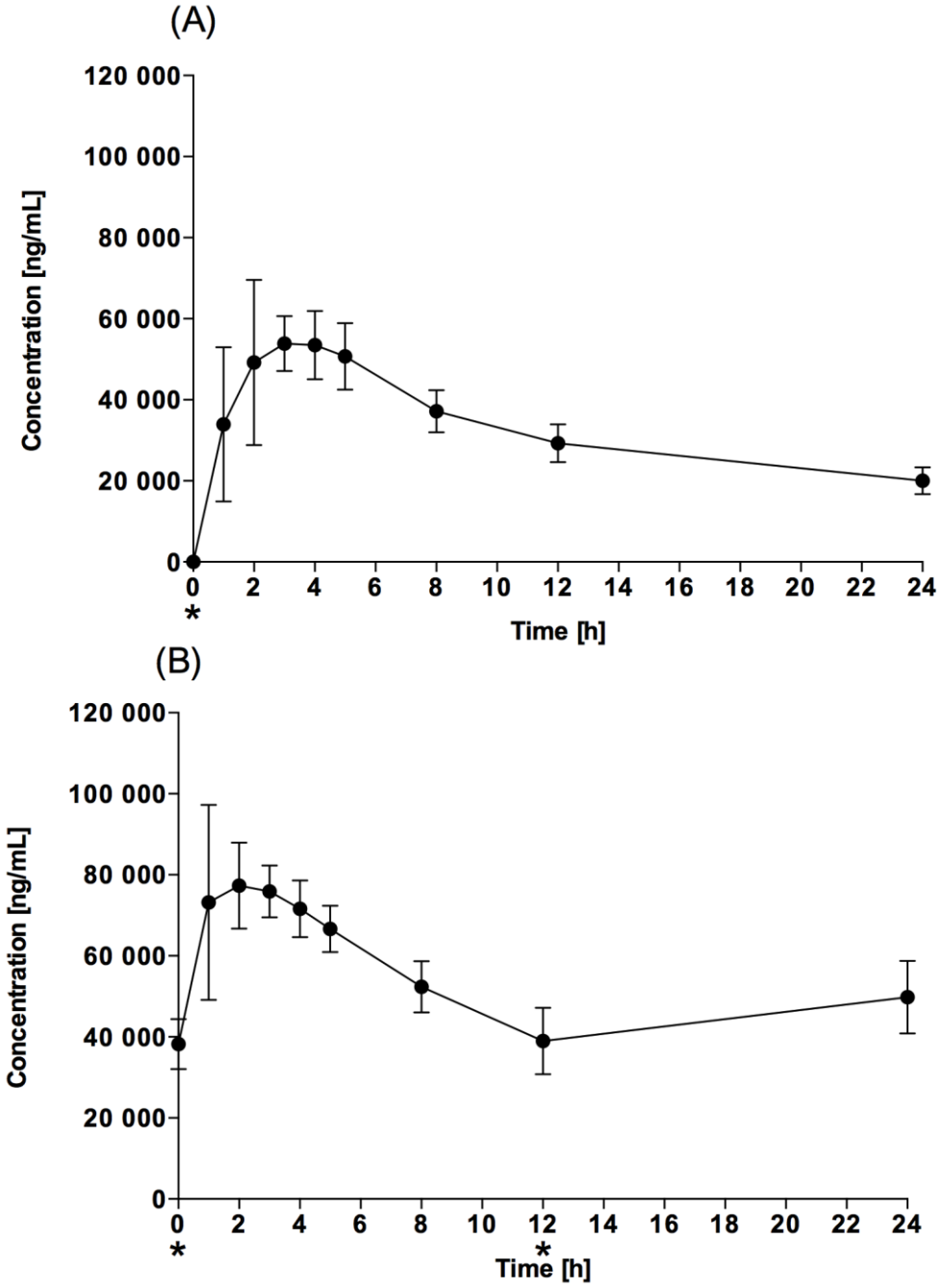
Sodium excretion [mmol/L], median (range)		Metamizole n=8	Naproxen n=7
Day 1	Baseline (t-1 to t0)	3 (2-8)	5 (2-35)
	1h (t0-t1)	3 (2-8)	5 (2-19)
	2h (t1-t2)	3 (1-8)	3 (1-17)
	3h (t2-t3)	4 (1-8)	3 (1-16)
	4h (t3-t4)	4 (1-10)	3 (0-18)
	5h (t4-t5)	5 (2-10)	5 (1-18)
Day 7	Baseline (t-1 to t0)	5 (4-31)	10 (4-13)
	1h (t0-t1)	4 (5-13)	9 (4-15)
	2h (t1-t2)	5 (2-18)	8 (3-12)
	3h (t2-t3)	9 (5-25)	10 (5-19)
	4h (t3-t4)	8 (5-63)	13 (7-21)
	5h (t4-t5)	9 (6-18)	15 (6-25)

Supplemental Table 4 Median urinary volume (hourly intervals) of the two study days

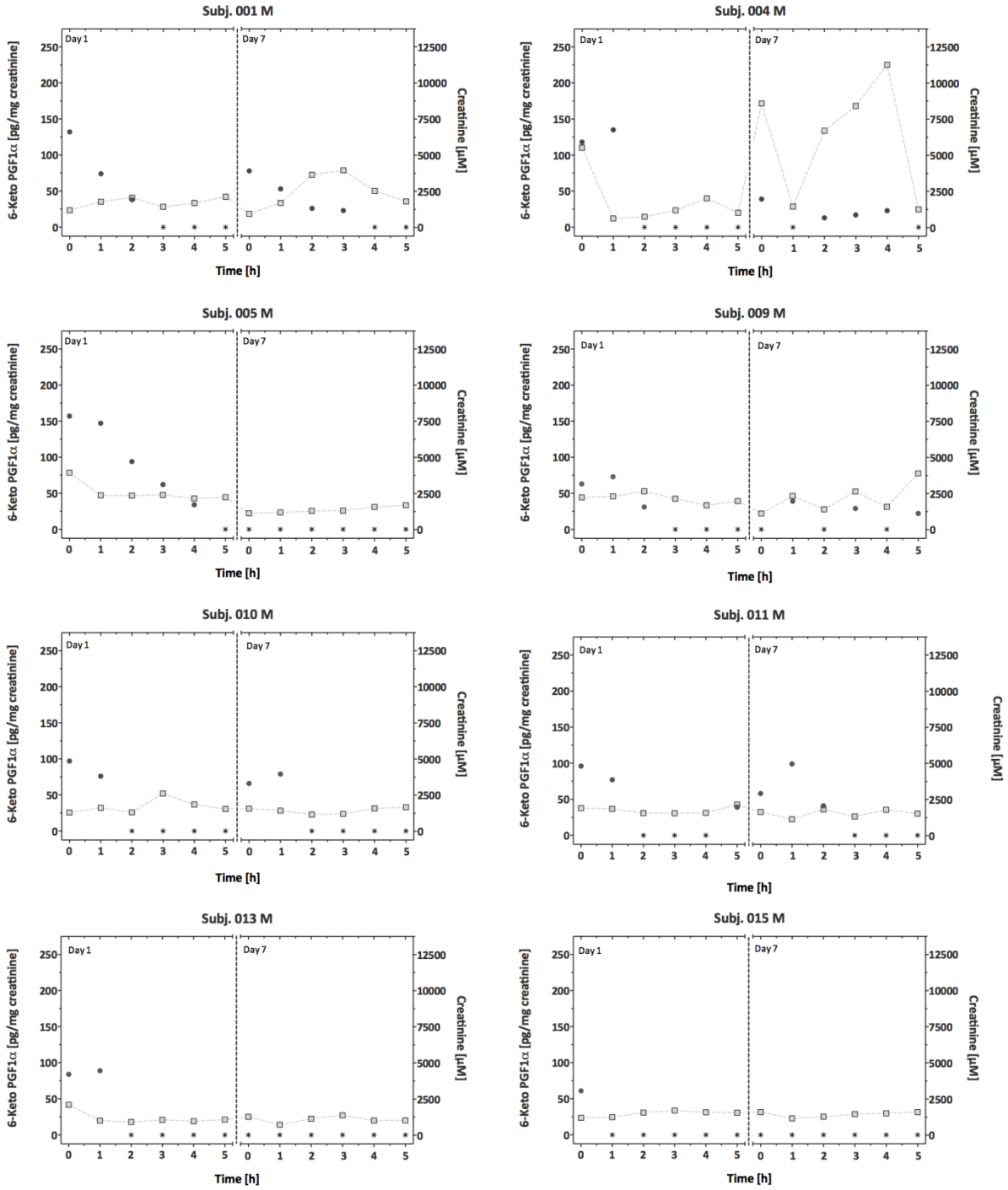
Urinary volume [mL/h], median (range)		Metamizole n=8	Naproxen n=7
Day 1	Baseline (t-1 to t0)	310 (75-520)	590 (40-700)
	1h (t0-t1)	435 (260-720)	520 (270-660)
	2h (t1-t2)	420 (210-550)	410 (210-480)
	3h (t2-t3)	320 (240-720)	350 (275-490)
	4h (t3-t4)	390 (210-620)	325 (240-440)
	5h (t4-t5)	380 (140-560)	320 (120-560)
Day 7	Baseline (t-1 to t0)	400 (60-610)	530 (370-680)
	1h (t0-t1)	440 (220-630)	550 (370-720)
	2h (t1-t2)	425 (80-760)	480 (260-720)
	3h (t2-t3)	350 (55-640)	360 (260-440)
	4h (t3-t4)	365 (42-560)	410 (315-460)
	5h (t4-t5)	380 (210-600)	365 (240-580)

Supplemental Table 5 Median urinary creatinine concentration (hourly intervals) of the two study days

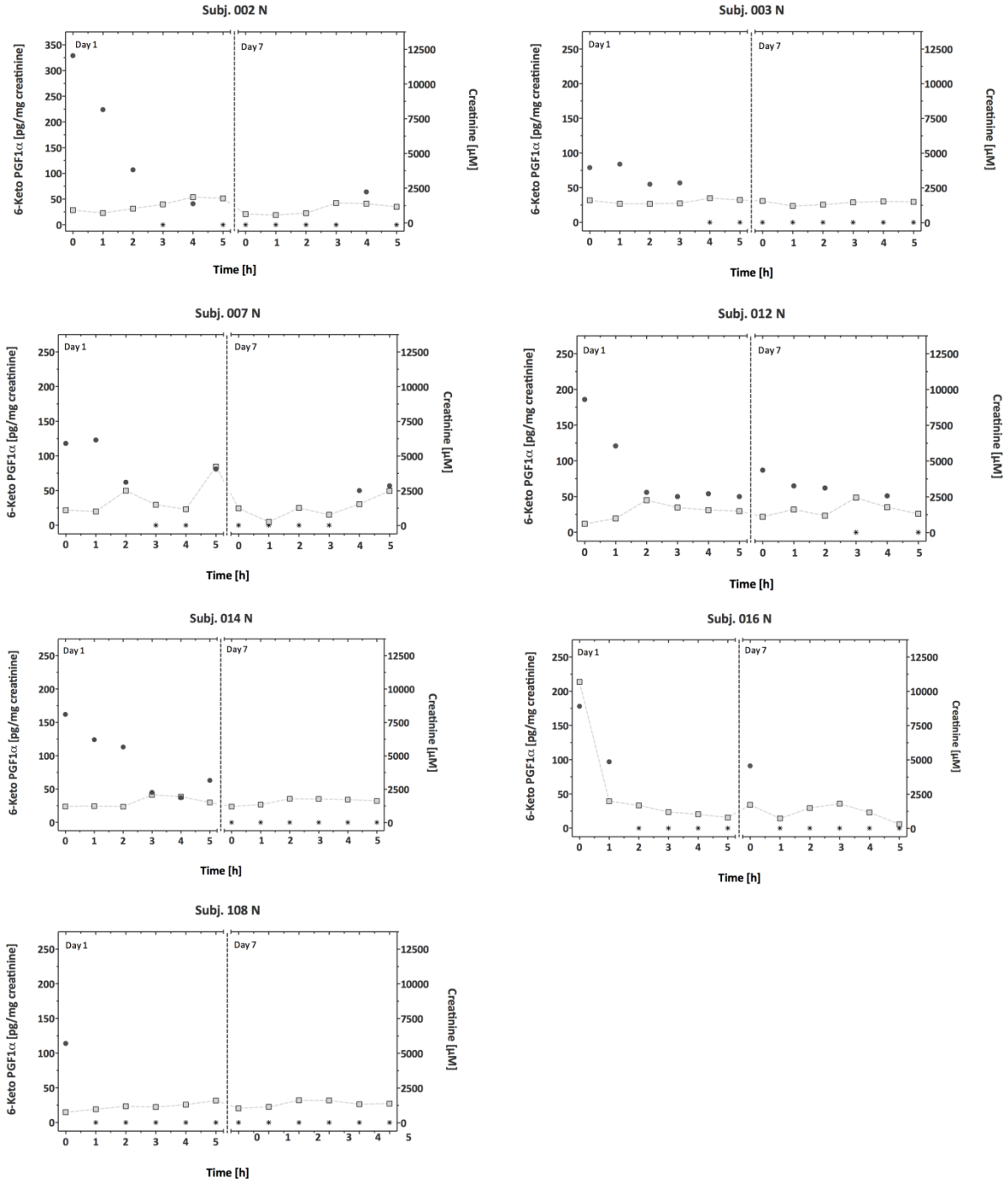
Urinary creatinine concentration [µmol/l], median (range)		**Metamizole n=8**	**Naproxen n=7**
Day 1	Baseline (t-1 to t0)	2004 (1188-5525)	1109 (617-10691)
	1h (t0-t1)	1704 (631-2381)	1019 (714-2000)
	2h (t1-t2)	1563 (743-2664)	1358 (1029-2516)
	3h (t2-t3)	1633 (1070-2624)	1397 (1140-2083)
	4h (t3-t4)	1697 (970-2159)	1575 (1045-1947)
	5h (t4-t5)	1770 (1023-2243)	1604 (798-4255)
Day 7	Baseline (t-1 to t0)	1426 (942-8591)	1216 (634-1724)
	1h (t0-t1)	1309 (719-2335)	1146 (269-1622)
	2h (t1-t2)	1352 (1149-6699)	1296 (703-1793)
	3h (t2-t3)	1415 (1214-8413)	1606 (781-2444)
	4h (t3-t4)	1589 (1017-11260)	1532 (1179-1770)
	5h (t4-t5)	1634 (1025-3900)	1384 (317-2495)



Supplemental Figure 1 Mean plasma concentration of naproxen vs. time in healthy volunteers (n= 7) following a single oral dose of 500 mg (A) and after seven days of continuous intake of 500 mg twice a day (B). *oral drug intake



Supplemental Figure 2A Single profiles of urinary 6-keto-PGF1α excretion (closed circles) corrected to the urinary creatinine concentration (open squares) in healthy salt-depleted volunteers (n= 8) following a single oral dose of 1000 mg metamizole (Day 1) and after seven days of continuous intake of 1000 mg three times a day (Day 7). * symbol for concentration below the quantification limit



Supplemental Figure 2B Single profiles of urinary 6-keto-PGF1α excretion (closed circles) corrected to the urinary creatinine concentration (open squares) in healthy salt-depleted volunteers (n= 7) following a single oral dose of 500 mg (Day 1) naproxen and after seven days of continuous intake of 500 mg twice a day (Day 7). * symbol for concentration below the quantification limit

4. Discussion – Conclusion – Outlook

4.1. Discussion

4.1.1. *Pharmacovigilance data analysis and case-control study: What is new? Clinical relevance of our findings*

In both the data from the pharmacovigilance analysis and the results of the case-control study, the large number of patients developed neutropenia or agranulocytosis within 1-7 days after starting metamizole was striking (52% of the international dataset in the pharmacovigilance study and between one to two thirds in the post-OP and non-post-OP cases). In addition, the data showed that most reactions occurred within the first two months of therapy (>90%). This is of great importance regarding patient monitoring after starting metamizole. Especially during the first weeks, the physicians and patients should be vigilant for symptoms of myelotoxicity. On the other hand, patients, who have tolerated metamizole well over a long period (>2 months), have a low (although not negligible) risk of reacting with hematotoxicity in the future.

The provided sale figures of all Swiss metamizole preparations confirmed that the use of metamizole has increased over the last years in Switzerland. The corresponding increase in reporting rate of fatalities due to metamizole-induced white blood cell disorders demonstrates the severity of this adverse reaction, despite broad-spectrum antibiotics and G-CSF as therapeutic options. Additionally, the sale figures allowed us to estimate a minimal incidence rate (0.46-1.63 per million person-days) which was in the range of the IAAA study [4]. Assuming underreporting, the real incidence must be higher. Our data, however, are nearer to the data from studies that have found rare incidences. Most probably, the truth lies somewhere in-between and is likely to be population-specific.

The analysis of the seven reported fatal cases which occurred between 1991 and 2012 in Switzerland confirmed the previously described poor prognostic factors namely age >65 years, septicemia and/or shock, and neutrophil count below 0.1G/L [54]. Additionally, we identified female sex and co-treatment with methotrexate as potential risk factors for a fatal outcome. Of course, as soon as patients have a myelotoxic co-medication, the effect of the two treatments on the adverse event cannot be kept apart. In our case-control study, the

non-post-operative case group had significantly more concomitant treatments with cytostatic agents compared to the non-post-operative controls. On the other hand, we did not see this in the post-operative comparison group. We know that metamizole is prescribed as an analgesic for oncological patients. Co-treatment with cytostatic agents is therefore not uncommon. But since an additive effect cannot be ruled out, especially concomitant methotrexate (four of seven fatal cases between 1991 and 2012), also at an immunosuppressive dose, should be used with caution.

The female predominance for fatalities can partially be explained by the fact that more women suffer from autoimmune disease and therefore are treated with methotrexate. But in general, more cases of female patients with metamizole-induced white blood cell disorders are reported, both in the pharmacovigilance analysis as well as in the characterized cases managed at the University Hospital Basel. Final conclusions about female sex as a risk factor can only be drawn with sex-specific exposure data. Since we used a sex-matched design in the case-control study, this question cannot be answered with this study. However, The larger number of affected women might be explained by the higher exposure of females to analgesics [99].

The same problem arises regarding age as risk factor. The age-matched design of the post-operative comparison groups did not allow evaluation of age as a risk factor. The non-post-operative comparison group was not age-matched. The age of the control group was significantly higher. Since these control patients were selected as long-term analgesic users, the fact that they were older can be seen as a selection bias. The fact that more cases involving older people are reported in many studies (also in our pharmacovigilance study), might be explained by a higher exposure of old patients to analgesics (analogous to the gender-question). The characterization of all the collected cases, however, clearly showed that patients from every age group can be affected.

The fact that the adverse events mostly occur under a therapeutic dose and the mean daily doses for the non-post-operative comparison group did not differ significantly, is a hint against dose-dependent toxicity. Furthermore, overdoses are not usually accompanied by a fall of neutrophil granulocytes [90]. Our data do confirm this observation.

The toxicity seems to be associated with the presence of immunological and/or metabolic susceptibility factors in affected patients. The analysis of our case-control study showed a remarkably large number of patients with a positive allergy history among the post-operative cases. However, there was no statistically significant difference in the non-post-operative comparison group. Since the source of the post-operative comparison groups was identical (electronic medical reports from the hospital) but different for the non-post-operative comparison groups (electronic medical reports from the hospital and medical reports of general practitioners), the post-operative comparison was probably more homogenous and therefore more accurate. Nevertheless, it is possible that patients with a predisposition to allergic reactions have a susceptibility factor that makes them vulnerable to developing leucopenia under metamizole.

Another potential risk factor that we identified in our case-control study was an underlying hepatitis C infection. The higher prevalence of an underlying hepatitis C infection in the case group fits to the previously made observation, that patients with viral infections including HIV, hepatitis C, EBV, HSV, HHV and CMV show an increased risk of developing adverse drug reactions [100-103]. The mechanisms behind this observation are not entirely clear, but could be caused by a combination of impaired immune tolerance, increased antigenicity and/or altered drug metabolism [93]. As an example for the altered drug metabolism, it is known that asymptomatic carriers of hepatitis B with normal liver function tests were found to have impaired clearance of metamizole via oxidative pathways compared to healthy controls, leading to increased exposure of the 4-methylaminoantipyrine metabolite (carriers and controls all had slow acetylator phenotype) [104]. Finally it must also be considered, that patients with hepatitis C could develop myelotoxicity due to the hepatitis C infection itself [105-107].

In the case-control study, four of the in total 57 cases had previous leucopenic episodes in their medical history, whereas no patients in the control group were known to have a history of leucopenia. In two cases, the likeliest cause for the blood cell disorder was liver cirrhosis due to hepatitis C infection (for example due to hypersplenism-induced cytopenias [107]), thus another potential identified risk factor. In the other cases, the disorder was of unknown origin. The absolute number of cases is small and metamizole as a cause of the leucopenia can be questioned as these patients may have developed the disorder without

metamizole. However, there remains a possibility that these patients are more vulnerable. Therefore, metamizole should be used with caution in patients who have had pervious leucopenic episodes.

4.1.2. *Pharmacovigilance data analysis and case-control study: Methodological problems and limitations*

In both the pharmacovigilance data analysis and the retrospective case-control study, the problems of missing data and heterogeneity of documentation policies must be considered. The international pharmacovigilance dataset enabled the characterization of a large case collective. But without a control group, some questions cannot be answered (for example age or sex as risk factors). Therefore, we decided to perform a case-control study. But the question of how to identify the correct controls was a challenge. The first approach was to focus on a minimal continuous treatment duration of at least 28 days to ensure that control patients truly did not develop leucopenia. Since we did not find such long-term metamizole users in the hospital setting, we sought them in the outpatient settings. As a consequence, the control group largely consisted of older patients with chronic pain disorders. Since this patient collective differed strongly from the case group (mostly post-operative patients), we decided to split our cases in post-operative and non-post-operative cases and to collect an additional control group for the post-operative cases. These control patients were age-, sex- and ward matched to cases for the analysis. The disadvantage of this design is, however, the loss of ability to evaluate the effect of the matched factors. To further improve the study quality, a prospective design would be beneficial. But since the incidence of the adverse effect is low, the study period would have to be extremely long.

The potential risk factors identified in the case-control study are based on a relatively small number of subjects. Additionally, our inclusion criteria were less restrictive than in other studies. As an example, Huber et al only included cases with a neutrophil count of less than 0.5 x 10^9/L, normal counts in other blood cell types, and excluded patients with concomitant cytostatic treatments [14]. Our approach included patients with a fall in leucocyte count below 3.5 x 10^9/L and did not exclude patients with additional abnormalities in other blood cell counts. Additionally, we also included patients with concomitant

cytostatics, if the blood counts were normal before starting metamizole. The causality of metamizole as the cause for the adverse drug reaction was assessed using the Naranjo Score [108]. The relatively open inclusion criteria make the results more disputable since the effects of several influences cannot be kept completely apart. But it allowed the assessment of potential additive effects and allowed also to study milder courses of the adverse drug reaction.

4.1.3. *Clinical study about renal safety of metamizole: What is new? Clinical relevance of our findings*

Regarding the supposed better renal tolerability of metamizole compared to conventional NSAIDs, data from our clinical study did not deliver finally answers to support this assumption. Nevertheless, the study delivered interesting results. Especially the fact, that metamizole seems to inhibit synthesis of renal prostacyclin to a similar degree as naproxen, was unexpected. However, two other studies have also observed this inhibition in healthy subjects and in patients with liver cirrhosis [109, 110]. Since patients with liver cirrhosis are at increased risk for renal complications after prostaglandin synthesis inhibition [111, 112], these results support the assumption of a better renal tolerability of metamizole in patients at risk compared to NSAIDs. The current study, which shows a comparable inhibition of renal 6-keto-PGF1α excretion for metamizole and for naproxen suggests that renal excretion of 6-keto-PGF1α may not be the ideal marker to differentiate between the renal adverse effects of NSAIDs and metamizole. Renal tolerability of metamizole in patients at risk might be explained by a direct vasodilatory effect of metamizole counteracting reduced renal availability of vasodilatory prostacyclin. Such a vasodilatory effect of metamizole has been described before in animal models for vasospasm [113, 114]. Further studies in more susceptible individuals, looking also at renal plasma flow have to be conducted to evaluate differences in the effects on renal function between metamizole and NSAIDs that would explain the better renal tolerability of metamizole observed in the clinical setting.

4.1.4. *Clinical Study about renal safety of metamizole: Methodological problems and limitations*

The most important limitation of the current study is the lack of effect of naproxen (our positive control) on inulin clearance. There are several possible reasons for this observation, but the level of the salt-depletion seems to play a key role. Due to the only modest reduction in sodium intake during the course of the study, renal prostaglandin synthesis may not have been critical to maintain renal perfusion in our subjects. Although all subjects reached the target values (<60-80 mmol urinary sodium per day) before the first study day, urinary sodium excretion increased during the course of the study, indicating failing compliance with the dietary restriction. As a consequence, for another study, we would change several diet settings to render study participants more susceptible to COX inhibition. First of all, the compliance should be checked more often. Further, the duration of the diet could be shortened. The run-in time could be reduced to three or four days (instead of one week) with a single dose of a loop diuretic to boost initial sodium depletion. The allowed daily sodium intake should also be reduced to about 10 mmol per day. Preferably, the subjects should be in-house to ensure controlled intake also of breakfast and in-between meals.

Another important point we would change is the equilibration time of the inulin infusion. The duration should be increased to two hours before start of the baseline collection (instead of one as suggested in the manufacturer's recommendation) to ensure that the inulin reaches stable concentrations in the plasma. This is necessary for the proper use of the traditional formula used to estimate inulin clearance. To avoid urinary samples and therefore incomplete bladder voiding, a single-shot inulin method could be used as well, based on a computer-based two-compartment model. This would also solve the questions concerning the appropriate equilibration time of metamizole. And, finally, the hourly water load to ensure hourly urine samples should be reduced, as it caused highly diluted urine samples, complicating quantification of sodium concentrations. This would also reduce the "water load effect", which increased GFR on the study days and masked potential drug effects on GFR. The high water load also caused many urinary 6-keto-PGF1α concentrations below the lower limit of quantification (LLOQ). In the literature, different methods of handling values below the LLOQ are described [115, 116], but every method has its limitations. In a first approach, we decided to set all values which were below the LLOQ to LLOQ, thereby overestimating the true concentrations. Since we did not observe a decrease

for the urinary sodium excretion, the sodium concentrations have to be determined using a more sensitive method to be able to see whether the effects of metamizole on sodium excretion really are different compared to Naproxen. Regarding the urinary 6-keto-PGF1α excretion, we were able to see the inhibition under naproxen since the collected baseline samples were quantifiable and the following samples under treatment fell under the LLOQ, an effect which was not caused by increased urine dilution. Therefore, we were able to show the inhibition, but we were not able to exactly quantify the extent of the inhibition.

In general, the question remains whether differences in the effect on renal function between metamizole and a conventional NSAID can really be demonstrated in healthy salt-depleted volunteers with a low sodium diet as the only stressor. [117] This also leads to the question whether it would be more promising to expose more susceptible individuals such as e.g. patients with liver cirrhosis [109], which on the other hand may raise ethical concerns.

4.2. Conclusions

From the results of our studies, we can conclude that:

- Metamizole-induced agranulocytosis remains a severe adverse drug reaction despite therapeutic options.
- Female sex, triple blood cell line dyscrasia, older age, and concomitant treatment with methotrexate were identified as risk factors for a fatal outcome.
- The minimal incidence rate of metamizole-induced agranulocytosis seems to be between 0.46-1.63 per million person-days.
- A large proportion of cases (around 50%) occurred during the first 7 days of metamizole treatment.
- A history of allergy, previous leucopenic episodes, infection with hepatitis C and concomitant use of cytostatic agents (including at immunosuppressive doses) might be risk factors for metamizole-induced leucopenia and require further study.
- Metamizole inhibits renal excretion of the prostacyclin metabolite 6-keto-PGF1α similar to naproxen, suggesting that metamizole inhibits renal prostaglandin synthesis.
- Despite inhibition of renal 6-keto-PGF1α excretion, metamizole had no significant effect on inulin clearance or renal sodium excretion in healthy, sodium-depleted subjects, whereas naproxen showed a trend to decreased sodium excretion.
- Further studies in more susceptible individuals, looking also at renal plasma flow have to be conducted to evaluate differences in the effects on renal function between metamizole and NSAIDs that would explain the better renal tolerability of metamizole observed in the clinical setting.

In general, metamizole should always be prescribed on a case by case basis balancing the risk for myelotoxicity against the ADR profiles of alternative analgesic agents.

4.3. Outlook

The characterization of cases and the search for risk factors is limited by the nature of the feasible study designs. Of course, our identified possible risk factors need further confirmation in a larger case collective. Since the identification of patients at risk of developing this adverse reaction prior to exposure is crucial, a known genetic predisposition would be a fundamental step in the safe use of this compound. This would allow to prevent exposure of at-risk patients, therefore avoiding morbidity and mortality, and saving expenses for the treatment without losing the drug due to withdrawal from the market. For clozapine-induced agranulocytosis, several reports indicate that specific HLA variants may be associated with this adverse event [118-120]. For metamizole-induced agranulocytosis, data from current genomic technologies are lacking. Therefore, a genetic association study to identify involved pathways and potential predictive markers (such as HLA-types) in an appropriate number of cases and controls should be the next step.

Concurrently, the uncovering of the mechanism behind the toxicity including the resolution of the question whether a metabolic toxicity on granulocyte progenitors and/or an immune-mediated toxicity cause this adverse reaction would facilitate the understanding of this adverse drug reaction. The knowledge of the mechanism of toxicity may help to understand risk factors and perhaps also offers therapeutic options and/or prevention possibilities and may be of importance regarding the development of new, safer, compounds.

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Curriculum vitae

Personal Data

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Education

10/2012 – today	PhD thesis „Clinical safety of metamizole“
	Division of Clinical Pharmacology and Toxicilogy, University Hospital Basel
	Departement of Pharmaceutical Science, University Basel
	Under supervision of Prof. Dr. phil., Dr. med. Stephan Krähenbühl
09/2012	Swiss Federal Diploma Degree in Pharmacy
09/2010 – 09/2012	Master of Science in Pharmacy, University Basel
	Master thesis subject „GLP-1 release in NAFLD/NASH patients“
	Gastroenterology, Department of Biomedicine, Basel
	Under supervision of Prof. Dr. med. Christoph Beglinger and
	Prof. Dr. phil., Dr. med. Stephan Krähenbühl
09/2007 – 08/2010	Bachelor studies in pharmaceutical sciences, University Basel

2003 – 2007 Matura, Kurzzeitgymnasium Musegg Luzern, focus Biology and Chemistry, complementary focus Psychology and Education

Academic Teaching Experience

10/2012 – today Supervision of master's theses:

- 2013: "A Retrospective and Descriptive Analysis of Spontaneous Individual Case Safety Reports Concerning Metamizole. Analysis of Haematological, Nephrological, and Gastrointestinal Adverse Drug Reactions from the WHO and Swissmedic". Candidate: Alexandra Tramonti, MSc in pharmacy.
- 2014: "Metamizol induzierte Agranulozytose. Eine Fallserie". Candidate: Hala Hassna, MSc in pharmacy.
- 2015: "Dosisanpassung bei Patienten mit Leberinsuffizienz." Candidate: Raife Ibrahimova, MSc in pharmacy.

Practical training:

- Therapeutic drug and organ function monitoring; master students in pharmacy

Working Experience

10/2012 – today Creating and editing of recommendations for dose adjustments in patients with hepatic insufficiency for the Clinicial Decsision Support System published by the HCI Solutions AG, Bern, Switzerland.

07/2013 – 12/2014 Deputy as pharmacist (individual days): Weiherschloss-Apotheke, Bottmingen, Switzerland.

09/2011 – 06/2012 Assistant pharmacist within the context of the education for the Masters Degree and Federal Diploma Degree in Pharmacy. Sonnen-Apotheke, Basel, Switzerland.

Advanced Trainings and Lectures

05/2015 Spitalpharmazie / Klinische Pharmazie, Bruderholzseminar "Rund um die Leber", Department Clinical Sciences, Advanced Studies University Basel

06/2014 Study Manager Research Lunch, "Erfassung, Beurteilung und Melden von AEs und SAEs", Department Anesthesia, Clinical Trial Unit University Hospital Basel

03/2013	Basis course Good Clinical Practice for Investigator/Study Manager Clinical Trial Unit University Hospital Basel
03/2013	Advanced course Good Clinical Practice for Sponsor/Investigator Clinical Trial Unit University Hospital Basel
2013	Scientific Writing, "A Lecture Course for Students of Pharmaceutical and Biomedical Science", University of Basel
06/2013	CTU Thementag: Das neue Humanforschungsgesetz Clinical Trial Unit University Hospital Basel
2013	Seminar on Drug Discovery and Development, University of Basel
2012-2015	Research Seminar Clinical Pharmacology and Toxicology, University of Basel
2012-2014	Montagsfortbildung der medizinischen Poliklinik und der klinischen Pharmakologie, University Hospital Basel

Attendance and Presentations at Congresses

2015	SGIM (Swiss Society of General Internal Medicine), annual meeting, Basel, Switzerland Poster and Poster-Presentation: „Metamizole-induced White Blood Cell Disorders: a Case-Control Study"
2013-2015	Annual Research Meeting, Department of Pharmaceutical Science, University of Basel, Switzerland Poster 2014: "Hematologic Safety of Metamizole – Analysis of Pharmacovigilance Data"

Publications

Blaser LS, Tramonti A, Egger P, Haschke M, Krähenbühl S, Rätz Bravo AE. Hematological safety of metamizole: retrospective analysis of WHO and Swiss spontaneous safety reports. Eur J Clin Pharmacol. 2015 Feb;71(2):209-17.

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Blaser LS, Hassna H, Hofmann S, Holbro A, Haschke M, Rätz Bravo AE, Zeller A, Krähenbühl S, Taegtmeyer AB. Leucopenia associated with Metamizole: a Case-Control Study. Submitted.

Blaser LS, Duthaler U, Bouitbir J, Taegtmeyer AB, Liakoni E, Krähenbühl S, Haschke M. Effect of metamizole (dipyrone) on renal function in salt-depleted healthy subjects. Unpublished.

Blaser LS, Ibrahimova R, Krähenbühl S, Rätz Bravo AE. Dosisanpassung bei Patienten mit Lebererkrankung. Unpublished, manuscript in progress.