Pharmacokinetic studies on the (synthetic) cannabinoids JWH-210, RCS-4, and Δ9-tetrahydrocannabinol in pigs

Dissertation
zur Erlangung des Grades
des Doktors der Naturwissenschaften
der Naturwissenschaftlich-Technischen Fakultät III -
Chemie, Pharmazie, Bio- und Werkstoffwissenschaften
der Universität des Saarlandes

von
Nadine Schäfer
Saarbrücken
2016

Tag des Kolloquiums:	10.06.2016
Dekan:	Univ.-Prof. Dr.-Ing. Dirk Bähre
Berichterstatter:	Univ.-Prof. Dr. rer. nat. Dr. h. c. Hans H. Maurer Univ.-Prof. Dr. rer. nat. Rolf W. Hartmann
Vorsitz:	Univ.-Prof. Dr. rer. nat. Marc Schneider
Akad. Mitarbeiter:	Dr. rer. nat. Martin Frotscher

Die folgende Arbeit entstand unter der Anleitung von Herrn Professor Dr. Dr. h.c. Hans H. Maurer und Herrn Professor Dr. Peter H. Schmidt in der Abteilung Experimentelle und Klinische Toxikologie der Fachrichtung 2.4 Experimentelle und Klinische Pharmakologie und Toxikologie der Universität des Saarlandes in Homburg/Saar und im Institut für Rechtsmedizin der Universität des Saarlandes in Homburg/Saar von Februar 2011 bis Oktober 2015.

Mein besonderer Dank gilt:

Herrn Professor Dr. Dr. h.c. Hans H. Maurer und Herrn Professor Dr. Peter H. Schmidt für das interessante Dissertationsthema, die Möglichkeit des selbständigen, wissenschaftlichen Arbeitens, die Teilnahme an nationalen und internationalen Fachkongressen, für die Unterstützung und die ständige Diskussionsbereitschaft (auch bis in den späten Abend hinein),

Herrn Professor Rolf W. Hartmann für die Übernahme des Koreferats,

Herrn Professor Dr. Michael D. Menger und Herrn Professor Dr. Matthias W. Laschke sowie dem gesamten Team des Instituts für Klinisch-Experimentelle Chirurgie in Homburg für die Bereitstellung von experimentellem und personellem Know-how, ohne das die Studie nicht durchführbar gewesen wäre,

Herrn Juniorprofessor Dr. Thorsten Lehr und Herrn Jan-Georg Wojtyniak für die Durchführung der pharmakokinetischen Modellierungen,

Herrn Dr. Andreas H. Ewald, Herrn PD Dr. Markus R. Meyer, Frau Dr. Jessica Welter-Lüdeke und Herrn Andreas G. Helfer für die fachliche Expertise und Diskussionsbereitschaft,

meinen medizinischen Kolleginnen und Kollegen, insbesondere Herrn Dr. Mattias Kettner und Frau Dr. Julia Schlote für die tatkräftige Unterstützung bei der Durchführung der Tierexperimente, und Frau Ann-Katrin Kröll für das Korrekturlesen der Dissertation,

Herrn Dietmar “Karl“ Bregel für seine Geduld, seine ständige Diskussionsbereitschaft, seine fachliche Expertise, seine immerwährende Unterstützung bis weit über das normale Maß hinaus und für die gemeinsame, unvergessliche Bürozeit,

Herrn Benjamin Peters für seine grenzenlose Unterstützung, Hilfsbereitschaft und Geduld, ohne die es manchmal schwer gewesen wäre, durchzuhalten, und für seine fachliche Expertise,

Herrn Stefan “Tim“ Bregel für den technischen Support und dem gesamten Team der Rechtsmedizin Homburg für die herzliche Aufnahme und ständige Hilfsbereitschaft,

Dr. Kristina Y. von Känel für das Daumendrücken bei meinem ersten internationalen Vortrag und für ihr offenes Ohr, Jasmin Senzig und Nadja Walle für die Unterstützung und Hilfsbereitschaft,

meiner Freundin Irina Altendorfer für die bereits im Studium entstandene Freundschaft, die bis heute, trotz größerer Entfernung besteht,

meiner besten Freundin Jenny Martin, die mich immer unterstützt hat, auch (oder vor allem wenn) es mir schlecht ging,

meinen Schwiegereltern und meiner Schwägerin Desirée für die langjährige Unterstützung,

meinen Eltern und Großeltern dafür, dass sie mich fortwährend unterstützt haben und mir ermöglicht haben, meinen Weg zu gehen,

meiner Schwester Nicole, die mich immer aufgebaut hat, wenn es mir schlecht ging und an mich geglaubt hat,

meinem Mann Christoph für seine grenzenlose Geduld und Unterstützung, dafür, dass ich meinen Frust abbauen durfte und dass er immer an meiner Seite stand und an mich geglaubt hat.

Für Oma und Opa!

„Wer glaubt, etwas zu sein, hat aufgehört, etwas zu werden.“

Sokrates (468-399 v. Chr.)

TABLE OF CONTENTS

1 GENERAL PART

1.1 Introduction

1.1.1 Cannabinoid (CB) Receptor Agonists

1.1.1.1 Δ9-Tetrahydrocannabinol (THC)

The cannabis plant *(Cannabis sativa*) has been discovered millennia ago and ancient cultures have already benefited from its therapeutic properties [1]. Until today, great importance is attributed to *Cannabis sativa* as medicinal plant. Due to its analgesic, antiemetic, and appetite stimulating effects, it is often used in the treatment of e.g. multiple sclerosis, cancer, or neurodegenerative diseases such as Huntington disease [2,3]. Besides its medicinal usage, psychoactive effects of cannabis, such as euphoria and alteration of conscious perception have already been utilized by our ancestors [1,4]. Nowadays, cannabis is one of the commonly consumed drugs of abuse worldwide [5] and has become an issue in forensic toxicology, especially in driving under the influence of drugs (DUID) cases [6-8]. In addition, this drug is associated with a high tolerance, dependence, and withdrawal potential [9-11].
The cannabis plant contains more than 60 cannabinoids, with THC (Fig. 1) being the principal psychoactive ingredient [12]. It has been isolated in the 1960s [13], resulting in an increasing interest of researchers. This research also included the elucidation of THC metabolism [14] and the main metabolites were found to be the psychoactive 11-hydroxy-THC (11-HO-THC; Fig. 1) and the pharmacologically inactive 11-nor-9-carboxy-THC (THC-COOH; Fig. 1).

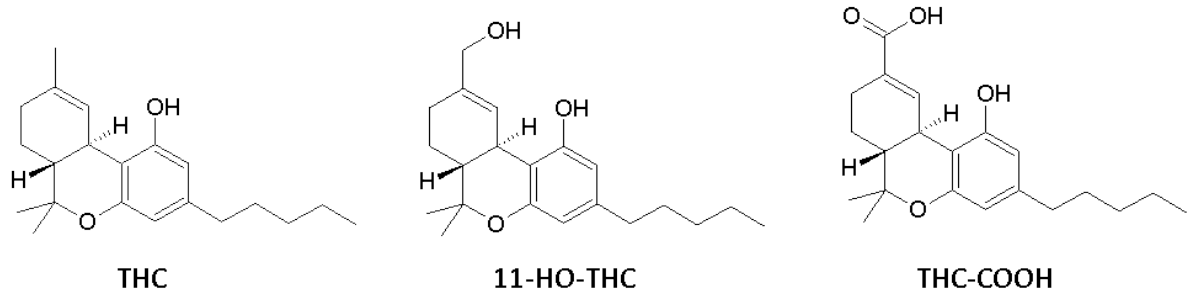
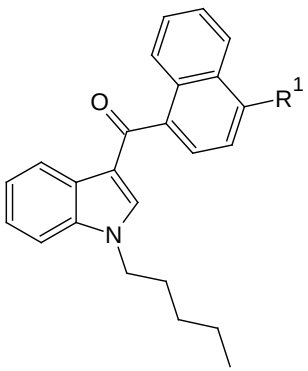


Fig. 1 Structure of THC and its main metabolites.

Only few years ago, two G-protein-coupled receptors, CB_1 and CB_2 [15-17], were identified to be responsible for the pharmacological actions of cannabinoids e.g. by inhibition of the adenylyl cyclase activity with a consecutive decrease in cyclic adenosine monophosphate (cAMP) accumulation, resulting in the inhibition of cAMP-dependent kinases [18,19]. The central nervous system (CNS) is thought to be the predominant localization of CB_1 receptors, with highest densities in the basal ganglia, substantia nigra, globus pallidus, cerebellum, and hippocampus, giving the ultimate explanation for the psychoactive effects [19]. On the contrary, CB_2 receptors are mainly detected in peripheral cells of the immune system, but also (with very low densities) in microglial cells of the CNS [19]. THC was identified to bind with similar affinity to both receptors and act as a partial agonist [20-22]. The identification and cloning of the CB receptors led to the discovery of the endocannabinoid system [23,24] and to a boom in the cannabinoid researching field. Many so-called nonclassical cannabinoids, structurally distinct from THC, were synthesized, promising to be alternative compounds with higher therapeutic potential and higher affinity to the CB_1 receptor, but with less adverse effects [25].

1.1.1.2 Synthetic cannabinoids (SCs)

A few series of these non-classic cannabinoids e.g. napthoylindole derivatives were synthesized by J.W. Huffman for structure-activity relationship studies [26-28]. According to the initials of their inventor, these compounds were called JWH compounds. Two examples of napthoylindole derivatives are naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) and 4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-210; structures depicted in Fig. 2).



$R^1 = H$ JWH-018

$R^1 = C_2H_5$ JWH-210

Fig. 2 Examples of naphthoylindole derivatives.

In comparison to THC, SCs often displayed higher affinity to CB_1 receptors [26-28]. Unlike its natural pendant, these chemically derived CBs often appeared to be full agonists on CB receptors, characterized by considerably high efficiencies [29-31]. Accordingly, rising consequences, such as undesirable psychoactive effects, led to a diminished experimental ambition in pharmacological research [25].

However, taking advantage of these properties, SCs have become attractive legal alternatives to cannabis as recreational drugs a few years ago. In 2008, JWH-018 was identified as one of the first active ingredients in a so-called herbal incense product called 'Spice' [30,31]. Before this identification, it was the common belief that the contained herbs such as *Leonotis leonuru* ('Lion's ear/tail', 'Wild dagga' [30]) were responsible for the pharmacological effects that were observed after consumption of these herbal products. Instead, it was found that the SCs were supplementary added (mainly sprayed) to the herbal contents [30,32].

Until then, various SCs have increasingly been synthesized in clandestine laboratories and distributed via the internet in herbal incenses [30,33]. Not only naphthoylindoles, but also other groups of SCs, such as the benzoylindole 2-(4-methoxyphenyl)-1-(1-pentylindol-3-yl)methanone (RCS-4; Fig. 3), were uncovered in herbal incenses [34].

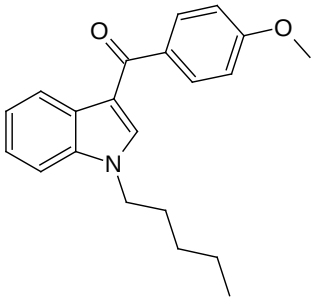


Fig. 3 Structure of the benzoylindole RCS-4.

However, the actual type and concentration of the contained SCs were notably fluctuating, even from one batch to another of the same product. After having successfully banned certain types of derivatives, the next generation of SCs followed by distributing them under the same brand in order to circumvent law [35]. Figure 4 shows the number of distribution of the newly identified new psychoactive substances (NPS), notified by the European (EU) Early Warning System (EWS). It is apparent that the number of SCs significantly increased over the last six years.

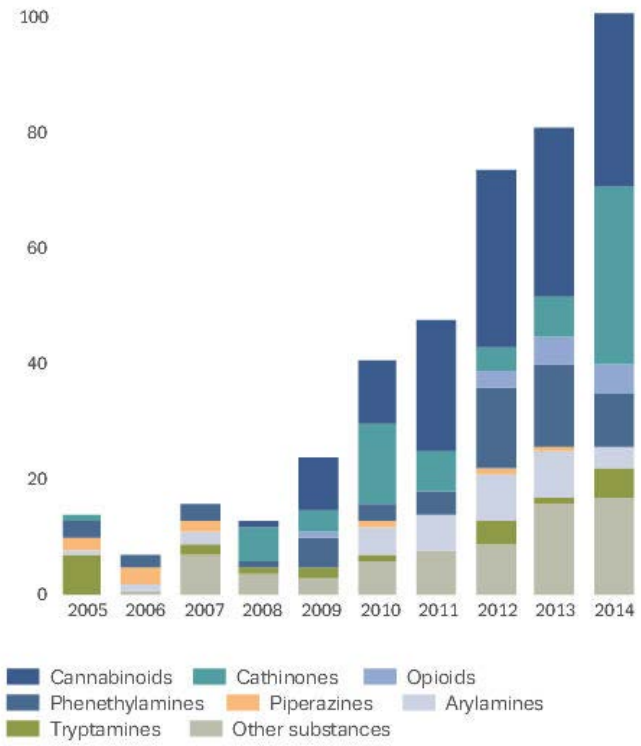


Fig. 4 Number and categories of newly identified NPS notified by the EU EWS from 2005-2014 (data and graph of the European Monitoring Centre [36]).

Officially, the herbal incenses were marked with the advice ‘not for human consumption’. Obviously, they were not used to condition the air of a room, but rather misused (mainly smoked) as recreational drugs. The prevalence of SC use differs between EU countries and the United States (US). In Germany, lifetime levels of the use of herbal mixtures among students (15 to 18 years) were 7% in 2009, 9% in 2010, 7% in 2011 and 7% in 2012 [33]. In France, a global survey for adults (18 to 64 years) revealed SC experimentation rates of 1.7% in 2014 [33]. Regarding the US, a survey about NPS use by adolescents for the years 2012-2014 resulted in 3.5% SC use in the past 12 months by 8th graders, 7.2% of 10th graders, and 8.3% of 12th graders [37]. Besides the legal status of new emerging SCs, along with their cannabis-related effects, consumption is additionally triggered by the facts that these substances are affordable, easily accessible via the internet, and not covered by routine toxicological analysis [38]. In fact, SC consumption became even more forensically relevant in terms of DUID cases, because of a potential impairment of the driver [39,40].

Withal, it has to be considered that these compounds are sold and consumed without safety pharmacological tests. Due to their high potency and efficacy together with the highly dynamic market and an uncontrolled production of the herbal incenses, users are always exposed to a risk of strong psychoactive and unpredictable toxic effects. Numerous cases of intoxications have been reported with life-threatening conditions accompanied e.g. by tachycardia, somnolence, anxiety, nausea, vomiting, seizures, hyperglycemia, hypokalemia, agitation, hallucination, and acute psychosis [41-44]. A case series of mono-intoxications with JWH-210 provided more knowledge of adverse effects and clinical manifestations of SCs [45]. Alarming symptoms such as seizures, agitation, and severe vomiting, all atypical symptoms for cannabis, can be explained by the fact that SCs are full CB_1 receptor agonists [46]. In addition, even deaths involving SCs have been reported [43,47-50]. Similar to THC, SCs can cause tolerance, dependence, and withdrawal [38].

Taking all these findings together, SCs have become a tremendous public health concern and are gaining an increasing importance in clinical and forensic toxicology. When interpreting analytical data of impaired or poisoned persons in concern of e.g. the time of intake or concentration at a particular time, explicit pharmacokinetic (PK) data are indispensable, especially when it comes to profound expert opinion in e.g. DUID cases. Even though systematic controlled administration studies are needed, the lack of pre-clinical safety studies does not allow for conducting human studies. Thus, comparable standards by use of animal models have to be established. One important step in elucidating PK data should be the assessment of serum-concentration-time profiles in comparison to those obtained from the already well-studied THC (see chapter 2.3).

1.1.2 Detection Methods in Biological Matrices

Understanding the PK of a drug requires the determination of the concentration in biological matrices, especially in blood/serum. Regarding the quantification of THC and its main metabolites HO-THC and THC-COOH, many studies using blood and urine as standardized biological specimens have been published. As summarized in different reviews, in the past, gas chromatography-mass spectrometry was the gold standard in detecting and quantifying cannabinoids after solid-phase extraction (SPE) or liquid-liquid extraction (LLE) [51-53]. In recent years, liquid-chromatography-

tandem mass spectrometry (LC-MS/MS) has increasingly been applied [51,54,55]. As a result, more sensitive procedures with short runtimes and reduced sample preparation could be developed. For molecule ionization, electrospray (ESI) or atmospheric pressure chemical ionization (APCI) were used, however, APCI appeared to be more suitable for cannabinoid detection as compared to ESI [51]. For more details see Chapter 2.1.

Regarding SCs determination in biological matrices, several reviews dealing with mainly LC-MS/MS based methods after e.g. SPE or LLE have been published recently [56-59]. Despite that, none of these methods allowed for simultaneous determination of THC, SCs and their metabolites needed to elucidate PK properties in blood. A new LC-MS/MS assay using one ionization technique should therefore be developed and validated according to national and international guidelines ([60,61]; see Chapter 2.1).

1.1.3 Pharmacokinetics

1.1.3.1 Metabolic Patterns

When conducting a study on PK, the elucidation of the metabolic patterns of the drug under investigation should be included. In order to obtain more insight into analytical data interpretation in cases of suspected intoxication or in abstinence monitoring programs, doping analysis, and workplace drug testing, the knowledge of the analytical target for urinalysis, which is basically preferred in these cases, is necessary.

In general, endogenous and exogenous lipophilic compounds, such as drugs, are metabolized (mainly by the liver) to more hydrophilic and often pharmacologically inactive compounds that can be excreted, mainly via urine. The metabolic reactions proceed in two different phases. In so-called functionalizing phase I reactions, compounds are modified by e.g. hydroxylation, oxidation, epoxidation, or dealkylation. These reactions are often catalyzed by cytochrome P450 monooxigenases (CYP; [59,62]). Following the phase I reactions, the modified compounds are further conjugated e.g. with glucuronic or sulfuric acid in phase II reactions by conjugating enzymes, such as uridine 5'-diphospho-glucuronosyltransferases or sulfotransferases.

In the matter of THC, nearly 100 metabolites have been identified. Main phase I reactions are hydroxylation (principally at C-11 to 11-HO-THC) and further oxidation to the carboxylic acid (THC-COOH). These phase I metabolites, as well as THC itself, are extensively glucuronidated [63].

Regarding SCs, recently published reviews summarized the results of *in vitro* and *in vivo* biotransformation studies [59], and provide a comprehensive overview of developments in urinalysis of SC metabolites [64]. SCs are very lipophilic compounds and primarily excreted into urine via their metabolites. The main metabolic phase I steps include aromatic and aliphatic hydroxylation, followed by further oxidation of aliphatic hydroxy metabolites to ketones or carboxylic acids, *N*-dealkylation, oxidative defluorination (of those SCs containing a fluorine atom), and *O*-demethylation (of those containing a methoxy group; [59,64]). The main metabolic phase II reactions were observed to be glucuronidation or sulfation, while the major metabolites seem to be extensively glucuronidated [59].

Two approaches are commonly used for metabolite elucidation. At first, the *in vitro* assay with liver microsomes or hepatocytes [65,64]. In comparison to data derived from authentic cases, this method offers the advantage of unequivocally assessing the metabolites of the known parent compound. One disadvantage is that the formed metabolites are not necessarily excreted into urine and thus, urinary metabolite patterns may differ. Therefore, a confirmation in authentic urine specimens is needed. As already mentioned above, no pre-clinical data are available as far as SCs are concerned, and except for self-experiments (allowed in some countries), controlled human studies are not possible. For this purpose, authentic human urine from clinical or forensic cases can be used [64]. However, this approach is limited by the fact that type and dosage of the consumed drug/s, as well as time and frequency of consumption are mainly unknown. Hence, experimental studies by using animal models (*in vivo*) under controlled conditions remain as a second common approach [64]. One advantage of animal models as compared to the *in vitro* assay is that they reflect systemic processes following drug intake similar to human pathways. Furthermore, excretion patterns covering a certain period of time can be pointed out. Yet, species differences regarding metabolism rate and types of formed metabolites, attributable to differences in enzyme patterns, remain possible [66]. The most common animals used for such experiments were rats [67-70]. However, pigs as large mammals, are not only advantageous when it comes to the quantity of

specimen sampling, but also convenient for scientific research on metabolism [71-73]. This is underlined by the fact that they are closely related to humans in terms of CYP enzyme patterns [74], anatomical structures as well as physiological properties regarding e.g. cardiovascular, urogenital, nutrition, and digestive systems [75,76]. In the comprehensive PK study conducted in this work, it should therefore be examined if the pig reflects human metabolic THC and SC patterns (see Chapter 2.2).

1.1.3.2 Distribution in blood and tissues

When investigating PK properties of a substance, the route of administration should be deliberated. The distribution of a substance strongly depends on the route of administration and hence, on the bioavailability. After pulmonary administration, which is the primary route for THC and SCs (by smoking; [59,63]), the bioavailability is less than 100%, depending on different issues such as frequency, depth of puffs, and amount of pyrolytic destruction. Oral ingestion (sometimes chosen for THC and SC administration [59,63]) also results in less than 100% bioavailability, because the whole amount of the substance usually does not pass bio membranes and first-pass metabolism can reduce the absorbed amount. Anyhow, passing bio membranes and reaching the circulation is a basic principal in developing pharmacological activity. In addition, the concentration of the parent compound can be reduced during the first liver passage due to biotransformation. Focussing on THC and SCs, pulmonary or oral administrations reflect authentic user habits. Nevertheless, using these routes of administration, the exact amount of substance reaching the circulation remains speculative. In contrast, an intravenous (i.v.) administration bears the advantage of a 100% bioavailability. Conclusively, the whole amount of unchanged drug reaches the systemic circulation and thus providing an exact quantification to establish a PK model.

A PK study is often associated with a so-called compartmental analysis. In detail, this complex mathematical model yields additional help in predicting and describing the concentration-time profile of a substance by means of parameters, such as area under the blood-concentration-time curve (AUC), elimination half-lifes ($t_{½}$), volumes of distribution (Vd), or clearances (Cl). The AUC is defined as the integral of the concentration-time curve, $t_{½}$ of a substance is that time, the drug concentration needs to halve, Vd is the apparent volume in which a drug is distributed, and Cl is the

volume of blood cleared of the substance per time unit. The parameters are related to each other, as described by different equations [77]. In the context of compartmental analysis, it has to be distinguished between two models. In a one-compartmental model, the body acts as a single compartment, into which the substance is distributed. On the other hand, describing the PK of a substance by a multi-compartment model, does not only consider the distribution into a central compartment (blood and rapidly equilibrating tissues), but also the distribution into one or few peripheral compartments (slowly equilibrating tissues), as well as a redistribution between them [77]. A typical concentration-time profile of a substance after i.v. administration described by a three-compartment model is schematically presented in Fig. 5. It consists of a distribution, elimination and tissue release phase (α, β, γ phase)

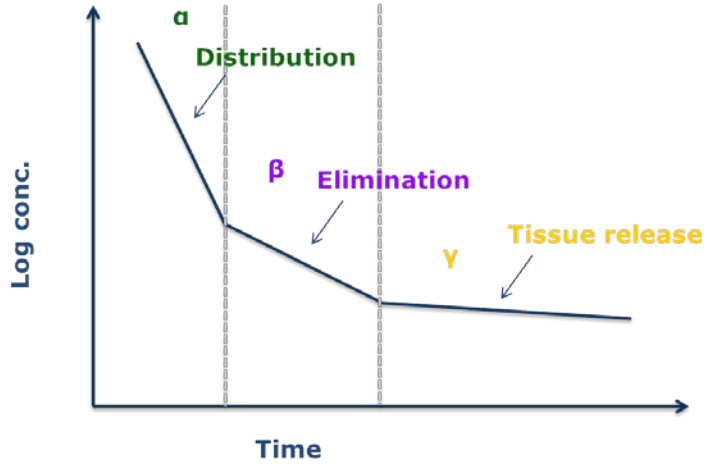


Fig. 5 Concentration-time profile of a substance described by a three-compartment model.

As far as THC is concerned, the PK has extensively been described in animals and humans using different routes of administration [63,78]. Seeing that, THC concentrations rapidly declined after i.v. administration and at the end of smoking, respectively. As opposed to the inhalation of THC, i.v. administration resulted in higher concentrations due to issues already discussed above. The significant decrease of THC concentrations was typically attributable to a (mainly hepatic) metabolism and a rapid distribution into highly perfused tissues (e.g. brain, lung, liver). After oral administration, concentrations were significantly lower because of degradation of THC in the stomach and an extensive first-pass metabolism [63,78]. THC has been identified to fit multi-compartment models [63]. For detailed

information see Chapter 2.4. By reason of its high lipophilic nature, THC was stored in adipose tissue and could be redistributed into the bloodstream, resulting in long terminal $t_{½}$ [63,78]. Especially after chronic consumption, longer windows of detection owing an accumulation and retarded release from adipose tissue, have to be expected. The storage in adipose tissue displays an important issue that has to be considered when interpreting analytical data of forensic toxicological cases. In this context, the usually unknown consumption habits of chronic users have to be additionally taken into consideration.

This phenomenon has also been discussed for SCs, because they are also very lipophilic compounds [79]. Though, as already mentioned, detailed knowledge on SC PK properties is lacking. In a review by Castaneto et al. [59], recent investigations on blood and tissue distribution in humans and animals have been summarized. Unlike the few systematic animal studies that have been conducted, the majority of the human studies were self-experiments with only one or two participants. Apart from that, SC tissue distribution has been described in several fatal cases [50,80-83]. Even so, as already mentioned, data from authentic cases always bear the disadvantage of certain imponderables. SCs seem to behave similar to THC after administration, resulting e.g. in a rapid decrease of concentrations and accumulation in brain and adipose tissue. For more details see Chapter 2.4. Nevertheless, data are limited and should be completed by systematic controlled animal studies, as human studies are not approved for ethical reasons. As an alternate to expensive mammals, such as dogs and monkeys, pigs are increasingly used in preclinical toxicological testing of pharmaceuticals [76] and they are a common model in pharmacological studies, especially to assess PK properties of substances [73,84,85]. In addition, the pig was found to be suitable to elucidate the THC PK [71,86]. SC PK properties including blood and tissue distribution in comparison to THC should therefore be assessed using the pig (see Chapter 2.4).

1.2 Aims of the dissertation

Synthetic cannabinoids are becoming an increasing public health concern. Pharmacokinetic data are indispensable for a reliable interpretation of analytical data from clinical and forensic toxicological cases to provide substantial expert opinion in e.g. poisoning or Driving-Under-the-Influence-of-Drugs cases. However, pre-clinical pharmacologically studies are not available and controlled human studies are not allowed. Thus, systematic controlled animal models have to be established. Therefore, the aims of this dissertation are given below:

- Development of an analytical LC-MS/MS method for the simultaneous determination of JWH-210, RCS-4, THC, and their main metabolites in pig and human serum, whole blood, and urine
- Elucidation of metabolic patterns of the studied drugs in pig urine using LC-high resolution (HR)-MS/MS and comparison with published human data
- Distribution of the studied SCs and THC including a representative choice of specimens, such as relevant organs, tissues, and important body fluids e.g. bile, in addition to blood and urine, after i.v. administration to pigs
- Determination of concentration-time profiles of the investigated SCs after i.v. administration to pigs in comparison to that of THC
- Modeling of the concentration-time profiles and assessment whether this model can predict published THC data in humans

2 PUBLICATION OF THE RESULTS

The results of the dissertation studies were published in the following papers:

2.1 Simultaneous LC-MS/MS determination of JWH-210, RCS-4, delta9-tetrahydrocannabinol, and their main metabolites in pig and human serum, whole blood, and urine for comparing pharmacokinetic data [87] (DOI: 10.1007/s00216-015-8605-6); http://link.springer.com/article/10.1007%2Fs00216-015-8605-6

2.2 Metabolic patterns of JWH-210, RCS-4, and THC in pig urine elucidated using LC-HR-MS/MS – Do they reflect patterns in humans? [88] (DOI: 10.1002/dta.1995; hyperlink not yet provided)

2.3 Pharmacokinetics of (synthetic) cannabinoids in pigs and their relevance for clinical and forensic toxicology [89]

(DOI: 10.1016/j.toxlet.2016.04.021);
http://www.sciencedirect.com/science/article/pii/S0378427416300790

2.4 Distribution of JWH-210, RCS-4, and ∆9-Tetrahydrocannabinol after intravenous administration to pigs [90]

(accepted by mail of 04/26/2016; DOI and hyperlink not yet provided)

3 DISCUSSION AND CONCLUSIONS

The presented studies provide data on the PK of the two model SCs JWH-210 and RCS-4, as well as of THC obtained after controlled i.v. administration to pigs. Their major phase I and phase II metabolites and their metabolic patterns were elucidated in urine specimens. Regarding JWH-210, the major metabolic pathways were hydroxylation at the ethyl side chain or *N*-pentyl side chain and combinations of them followed by glucuronidation. The analysis of the *N*-hydroxypentyl glucuronide (detectable for 3-4 h) might be helpful to prove JWH-210 consumption. Concerning RCS-4, the main metabolic steps were hydroxylation at the methoxyphenyl moiety or *N*-pentyl side chain followed by glucuronidation as well as *O*-demethylation followed by glucuronidation or sulfation. In urinalysis, targets for RCS-4 intake might be the glucuronides of the *N*-hydroxypentyl, hydroxy-methoxyphenyl (detectable for at least 6 h), and the *O*-demethyl-hydroxy metabolites (detectable for 4 h). Regarding THC, THC glucuronidation, 11-hydroxylation, followed by carboxylation and glucuronidation was observed. SC parent compounds could not be detected in urine in contrast to THC. Apart from some minor differences, the results of this pig study were consistent with the results obtained from human hepatocytes and/or human cases. This suggests that pigs are a suitable model for the elucidation of (human) SC metabolism.

In a next step, an LC-MS/MS method was developed and fully validated to determine simultaneously THC and the SCs in blood and urine and to assess the serum-concentration-time profiles of these substances. Subsequently, a population PK three-compartment model was successfully developed describing the serum-concentration-time profiles. Based on that pig PK model, human THC exposure was successfully predicted, proposing that pigs together with PK modeling technique may be a useful tool for prediction of human PK of SCs. Since no preclinical safety studies have been performed before marketing and thus no PK data are available, this may help in interpretation of authentic clinical and toxicological cases. Following this pilot study, an administration by smoking, reflecting better the habits of users, the application of an extended sampling protocol, and the usage of a higher number of animals might further improve the predictive performance of the model.

In a last step, a representative collection of specimens, including relevant organs, tissues, and important body fluids, such as bile, was conducted to investigate major distribution patterns of the examined drugs. Regarding the parent compounds, the distribution patterns were similar with two exceptions. RCS-4 and THC persisted in lung tissues, while JWH-210 was stored in the kidneys. Insofar, toxicological analysis of these organs is recommended in forensic postmortem toxicology. Storage of the three drugs in adipose and muscle tissue could be observed, implying these matrices to be appropriate for SC and THC postmortem detection, particularly if other specimens are no more available. Brain tissue could also serve as alternative specimen. As far as the metabolites are concerned, concentrations were basically highest in tissues involved in metabolism and/or elimination. *N*-Hydroxypentyl RCS-4 (HO-RCS-4) was the most prevalent metabolite found in almost every tissue or body fluid. Kidney tissue was suitable for detection of THC-COOH, HO-RCS-4, and RCS-4 pentanoic acid. Though, bile fluid was the most suitable specimen for SC and THC metabolite detection.

In conclusion, the presented results indicate that this pig model served as valid model for elucidating PK properties of SCs. Moreover, further systematic PK studies of other, new emerging SCs should be performed applying this particular model. Furthermore, important knowledge on the PK of this hazardous drugs class might be offered using pigs and the PK modeling approach. This is expected to improve the interpretation of analytical data obtained from misuse and poisoning cases and finally to provide scientifically based expert opinion in e.g. DUID cases.

4 REFERENCES

1. Zuardi AW (2006) History of cannabis as a medicine: a review. Rev Bras Psiquiatr 28:153-157.

2. Koppel BS, Brust JCM, Fife T, Bronstein J, Youssof S, Gronseth G, Gloss D (2014) Systematic review: Efficacy and safety of medical marijuana in selected neurologic disorders: Report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology 82 (17):1556-1563.

3. Sarfaraz S, Adhami VM, Syed DN, Afaq F, Mukhtar H (2008) Cannabinoids for Cancer Treatment: Progress and Promise. Canc Res 68 (2):339-342.

4. Li H-L (1978) Hallucinogenic plants in Chinese herbals. J Psychedelic Drugs 10 (1):17-26.

5. European Monitoring Centre for Drugs and Drug Addiction (2016) Cannabis drug profile http://www.emcdda.europa.eu/publications/drug-profiles/cannabis.

6. Beirness DJ, Davis CG (2006) Driving under the influence of Cannabis. CCSA=CCLAT, Ottawa (Kanada)

7. Wolff K, Brimblecombe R, Forfar J, Forrest A, Gilvarry E, Johnston A, Morgan J, Osselton M, Read L, Taylor D (2013) Driving under the influence of drugs. Report from the Expert Panel on Drug Driving (March 2013).

8. Fergusson DM, Horwood LJ, Boden JM (2008) Is driving under the influence of cannabis becoming a greater risk to driver safety than drink driving? Findings from a longitudinal study. Accident Anal Prev 40 (4):1345-1350.

9. Budney AJ, Hughes JR (2006) The cannabis withdrawal syndrome. Curr Opin Psychiatry 19 (3):233-238.

10. Kaymakcalan S (1981) The addictive potential of cannabis. Bull Narc 33 (2):21-31.

11. McRae AL, Budney AJ, Brady KT (2003) Treatment of marijuana dependence: a review of the literature. J Subst Abuse Treat 24 (4):369-376.

12. ElSohly MA, Slade D (2005) Chemical constituents of marijuana: The complex mixture of natural cannabinoids. Life Sci 78 (5):539-548.

13. Mechoulam R, Gaoni Y (1965) A Total Synthesis of dl-Δ1-Tetrahydrocannabinol, the Active Constituent of Hashish1. J Am Chem Soc 87 (14):3273-3275.

14. Wall ME (1971) The in vitro and in vivo metabolism of tetrahydrocannabinol (THC). Ann N Y Acad Sci 191 (1):23-39.

15. Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34 (5):605-613.

16. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346 (6284):561-564.

17. Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365 (6441):61-65.

18. Turu G, Hunyady L (2010) Signal transduction of the CB1 cannabinoid receptor. J Mol Endocrinol 44 (2):75-85.

19. Mechoulam R, Parker LA (2013) The endocannabinoid system and the brain. Annu Rev Psychol 64:21-47.

20. Burkey TH, Quock RM, Consroe P, Roeske WR, Yamamura HI (1997) Δ9-Tetrahydrocannabinol is a partial agonist of cannabinoid receptors in mouse brain. Eur J Pharmacol 323 (2–3):R3-R4.

21. Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq M-C (1998) Complex pharmacology of natural cannabinoids: Evidence for partial agonist activity of Δ9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. Life Sci 63 (1):PL1-PL6.

22. Govaerts SJ, Hermans E, Lambert DM (2004) Comparison of cannabinoid ligands affinities and efficacies in murine tissues and in transfected cells expressing human recombinant cannabinoid receptors. Eur J Pharm Sci 23 (3):233-243.

23. De Petrocellis L, Di Marzo V (2009) An introduction to the endocannabinoid system: from the early to the latest concepts. Best Pract Res Clin Endocrinol Metab 23 (1):1-15.

24. Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258 (5090):19461949Elbatsh.

25. Seely KA, Prather PL, James LP, Moran JH (2011) Marijuana-based Drugs: Innovative Therapeutics or Designer Drugs of Abuse? Mol Interv 11 (1):36-51.

26. Huffman JW, Dai D, Martin BR, Compton DR (1994) Design, synthesis and pharmacology of cannabimimetic indoles. Bioorg Med Chem Lett 4 (4):563-566.

27. Huffman JW (2009) Cannabimimetic indoles, pyrroles, and indenes: structure–activity relationships and receptor interactions. In: The cannabinoid receptors. Humana Press NY, 49-94

28. Wiley JL, Smith VJ, Chen J, Martin BR, Huffman JW (2012) Synthesis and Pharmacology of 1-Alkyl-3-(1-naphthoyl)indoles: Steric and Electronic Effects of 4- and 8-Halogenated Naphthoyl Substituents. Bioorg Med Chem 20 (6):2067-2081.

29. Tai S, Fantegrossi WE (2014) Synthetic Cannabinoids: Pharmacology, Behavioral Effects, and Abuse Potential. Curr Addic Rep 1 (2):129-136.

30. European Monitoring Centre for Drugs and Drug Addiction (2009) Understanding the 'Spice' Phenomenon http://www.emcdda.europa.eu/publications/thematic-papers/understanding-spice-phenomenon.

31. Auwärter V, Dresen S, Weinmann W, Müller M, Pütz M, Ferreirós N (2009) 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? J Mass Spectrom 44 (5):832-837.

32. European Monitoring Centre for Drugs and Drug Addiction (2016) Synthetic cannabinoids and 'Spice' drug profile http://www.emcdda.europa.eu/publications/drug-profiles/synthetic-cannabinoids.

33. European Monitoring Centre for Drugs and Drug Addiction (2015) Perspectives on drugs: Synthetic cannabinoids in Europe http://www.emcdda.europa.eu/topics/pods/synthetic-cannabinoids.

34. Denooz R, Vanheugen J-C, Frederich M, de Tullio P, Charlier C (2013) Identification and Structural Elucidation of Four Cannabimimetic Compounds (RCS-4, AM-2201, JWH-203 and JWH-210) in Seized Products. J Anal Toxicol 37 (2):56-63.

35. Dresen S, Ferreirós N, Pütz M, Westphal F, Zimmermann R, Auwärter V (2010) Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. J Mass Spectrom 45 (10):1186-1194.

36. European Monitoring Centre for Drugs and Drug Addiction (2015) European drug report: http://www.emcdda.europa.eu/edr2015.

37. Patrick ME, O'Malley PM, Kloska DD, Schulenberg JE, Johnston LD, Miech RA, Bachman JG (2015) Novel psychoactive substance use by US adolescents: Characteristics associated with use of synthetic cannabinoids and synthetic cathinones. Drug Alcohol Rev doi: 10.1111/dar.12372.

38. Spaderna M, Addy PH, D'Souza DC (2013) Spicing things up: Synthetic cannabinoids. Psychopharmacology 228 (4):525-540.

39. Musshoff F, Madea B, Kernbach-Wighton G, Bicker W, Kneisel S, Hutter M, Auwärter V (2013) Driving under the influence of synthetic cannabinoids ("Spice"): a case series. Int J Legal Med 128 (1):59-64.

40. Karinen R, Tuv SS, Øiestad EL, Vindenes V (2015) Concentrations of APINACA, 5F-APINACA, UR-144 and its degradant product in blood samples from six impaired drivers compared to previous reported concentrations of other synthetic cannabinoids. Forensic Sci Int 246:98-103.

41. Harris CR, Brown A (2013) Synthetic cannabinoid intoxication: a case series and review. J Emerg Med 44 (2):360-366.

42. Hermanns-Clausen M, Kneisel S, Hutter M, Szabo B, Auwarter V (2013) Acute intoxication by synthetic cannabinoids - Four case reports. Drug Test Anal 5:790-794.

43. Kronstrand R, Roman M, Andersson M, Eklund A (2013) Toxicological findings of synthetic cannabinoids in recreational users. J Anal Toxicol 37:534-541.

44. Schneir AB, Baumbacher T (2012) Convulsions associated with the use of a synthetic cannabinoid product. J Med Toxicol 8 (1):62-64.

45. Hermanns-Clausen M, Kithinji J, Spehl M, Angerer V, Franz F, Eyer F, Auwärter V (2016) Adverse effects after the use of JWH-210 – a case series from the EU Spice II plus project. Drug Test Anal doi: 10.1002/dta.1936.

46. Auwärter V, Kneisel S, Hutter M, Thierauf A (2012) Synthetische Cannabinoide. Rechtsmedizin 22 (4):259-271.

47. Behonick G, Shanks KG, Firchau DJ, Mathur G, Lynch CF, Nashelsky M, DJ J, C. M (2014) Four postmortem case reports with quantitative detection of the synthetic cannabinoid, 5F-PB-22. J Anal Toxicol 38 (8):559-562.

48. Patton AL, Chimalakonda KC, Moran CL, McCain KR, Radominska-Pandya A, James LP, Kokes C, Moran JH (2013) K2 Toxicity: Fatal case of psychiatric complications following AM2201 exposure. J Forensic Sci 58 (6):1676-1680.

49. Shanks KG, Dahn T, Terrell AR (2012) Detection of JWH-018 and JWH-073 by UPLC-MS-MS in postmortem whole blood casework. J Anal Toxicol 36 (3):145-152.

50. Schaefer N, Peters B, Bregel D, Kneisel S, Auwärter V, Schmidt PH, Ewald AH (2013) A fatal case involving several synthetic cannabinoids. Toxichem Krimtech 80 (Spec Iss):248-251.

51. Foltz RL (2007) Mass Spectrometric Methods for Determination of Cannabinoids in Physiological Specimens. In: ElSohly MA (ed) Marijuana and the Cannabinoids. Humana Press, Totowa, NJ, 179-204

52. Cody JT, Foltz RL (1995) GC/MS analysis of body fluids for drugs of abuse. CRC Press, Boca Raton, FL

53. Staub C (1999) Chromatographic procedures for determination of cannabinoids in biological samples, with special attention to blood and alternative matrices like hair, saliva, sweat and meconium. J Chromatogr B Biomed Sci Appl 733 (1–2):119-126.

54. Kraemer T, Paul LD (2007) Bioanalytical procedures for determination of drugs of abuse in blood. Anal Bioanal Chem 388 (7):1415-1435.

55. Ferreirós N (2013) Recent advances in LC–MS/MS analysis of Δ9-tetrahydrocannabinol and its metabolites in biological matrices. Bioanalysis 5 (21):2713-2731.

56. Namera A, Kawamura M, Nakamoto A, Saito T, Nagao M (2015) Comprehensive review of the detection methods for synthetic cannabinoids and cathinones. Forensic Toxicol 33 (2):175-194.

57. Meyer MR, Peters FT (2012) Analytical Toxicology of Emerging Drugs of Abuse—An Update. Ther Drug Monitor 34 (6):615-621.

58. Znaleziona J, Ginterová P, Petr J, Ondra P, Válka I, Ševčík J, Chrastina J, Maier V (2015) Determination and identification of synthetic cannabinoids and their metabolites in different matrices by modern analytical techniques – a review. Anal Chim Acta 874:11-25.

59. Castaneto MS, Wohlfarth A, Desrosiers NA, Hartman RL, Gorelick DA, Huestis MA (2015) Synthetic cannabinoids pharmacokinetics and detection methods in biological matrices. Drug Metab Rev 47 (2):124-174.

60. Peters FT, Paul LD, Musshoff F, Aebi B, Auwaerter V, Kraemer T, Skopp G (2009) Anhang B zur Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen Anforderungen an die Validierung von Analysenmethoden. Toxichem Krimtech 76 (3):185-208 (https://www.gtfch.org/cms/files/GTFCh_Richtlinie_Anhang%120B_Validierung Version%201.pdf).

61. Peters FT, Drummer OH, Musshoff F (2007) Validation of new methods. Forensic Sci Int 165 (2–3):216-224.

62. Stout SM, Cimino NM (2014) Exogenous cannabinoids as substrates, inhibitors, and inducers of human drug metabolizing enzymes: a systematic review. Drug Metab Rev 46 (1):86-95.

63. Grotenhermen F (2003) Pharmacokinetics and Pharmacodynamics of Cannabinoids. Clin Pharmacokinet 42 (3):327-360.

64. Peters FT (2014) Recent developments in urinalysis of metabolites of new psychoactive substances using LC–MS. Bioanalysis 6 (15):2083-2107.

65. Peters FT, Meyer MR (2011) In vitro approaches to studying the metabolism of new psychoactive compounds. Drug Test Anal 3 (7-8):483-495.

66. Pasanen M (2004) Species differences in CYP enzymes. Monografías de la Real Academia Nacional de Farmacia.

67. Grigoryev A, Savchuk S, Melnik A, Moskaleva Nj, Dzhurko J, Ershov M, Nosyrev A, Vedenin A, Izotov B, Zabirova I, Rozhanets V (2011) Chromatography–mass spectrometry studies on the metabolism of synthetic cannabinoids JWH-018 and JWH-073, psychoactive components of smoking mixtures. J Chromatogr B 879 (15–16):1126-1136.

68. Grigoryev A, Melnik A, Savchuk S, Simonov A, Rozhanets V (2011) Gas and liquid chromatography–mass spectrometry studies on the metabolism of the synthetic phenylacetylindole cannabimimetic JWH-250, the psychoactive component of smoking mixtures. J Chromatogr B 879 (25):2519-2526.

69. Hsin-Hung Chen M, Dip A, Ahmed M, Tan ML, Walterscheid JP, Sun H, Teng B-B, Mozayani A (2015) Detection and Characterization of the Effect of AB-FUBINACA and Its Metabolites in a Rat Model. J Cell Biochem doi: 10.1002/jcb.25421.

70. Jang M, Yang W, Shin I, Choi H, Chang H, Kim E (2014) Determination of AM-2201 metabolites in urine and comparison with JWH-018 abuse. Int J Legal Med 128 (2):285-294.

71. Brunet B, Doucet C, Venisse N, Hauet T, Hebrard W, Papet Y, Mauco G, Mura P (2006) Validation of large white pig as an animal model for the study of cannabinoids metabolism: application to the study of THC distribution in tissues. Forensic Sci Int 161 (2-3):169-174.

72. Lundahl A, Tevell Åberg A, Bondesson U, Lennernäs H, Hedeland M (2014) High-resolution mass spectrometric investigation of the phase I and II metabolites of finasteride in pig plasma, urine and bile. Xenobiotica 44 (6):498-510.

73. Sjögren E, Bredberg U, Lennernäs H (2012) The pharmacokinetics and hepatic disposition of repaglinide in pigs: mechanistic modeling of metabolism and transport. Mol Pharm 9 (4):823-841.

74. Anzenbacher P, Soucek P, Anzenbacherova E, Gut I, Hruby K, Svoboda Z, Kvetina J (1998) Presence and activity of cytochrome P450 isoforms in minipig liver microsomes. Comparison with human samples. Drug Metab Dispos 26:56-59.

75. Bode G, Clausing P, Cervais F, Loegsted J, Luft J, Nogues V, J. S, Project SGotR (2010) The utility of the minipig as an animal model in regulatory toxicology. J Pharmacol Toxicol Methods 62:196-220.

76. Swindle MM, Makin A, Herron AJ, Clubb Jr FJ, Frazier KS (2012) Swine as Models in Biomedical Research and Toxicology Testing. Vet Pathol 49:344-356.

77. Gabrielsson J, Weiner D (2001) Pharmacokinetic and pharmacodynamic data analysis: concepts and applications, vol 2. CRC Press, Sweden

78. Huestis MA (2007) Human cannabinoid pharmacokinetics. Chem Biodivers 4 (8):1770-1804.

79. Kneisel S, Teske J, Auwärter V (2014) Analysis of synthetic cannabinoids in abstinence control: long drug detection windows in serum and implications for practionners. Drug Test Anal 6 (1-2):135-136.

80. Saito T, Namera A, Miura N, Ohta S, Miyazaki S, Osawa M, Inokuchi S (2013) A fatal case of MAM-2201 poisoning. Forensic Toxicol 31 (2):333-337.

81. Hasegawa K, Wurita A, Minakata K, Gonmori K, Nozawa H, Yamagishi I, Watanabe K, Suzuki O (2015) Postmortem distribution of MAB-CHMINACA in body fluids and solid tissues of a human cadaver. Forensic Toxicol 33 (2):380-387.

82. Hasegawa K, Wurita A, Minakata K, Gonmori K, Nozawa H, Yamagishi I, Watanabe K, Suzuki O (2015) Postmortem distribution of AB-CHMINACA, 5-fluoro-AMB, and diphenidine in body fluids and solid tissues in a fatal poisoning case: usefulness of adipose tissue for detection of the drugs in unchanged forms. Forensic Toxicol 33 (1):45-53.

83. Hasegawa K, Wurita A, Minakata K, Gonmori K, Yamagishi I, Nozawa H, Watanabe K, Suzuki O (2015) Identification and quantitation of 5-fluoro-ADB, one of the most dangerous synthetic cannabinoids, in the stomach contents and solid tissues of a human cadaver and in some herbal products. Forensic Toxicol 33 (1):112-121.

84. Shimshoni JA, Britzi M, Sobol E, Willenz U, Nutt D, Edery N (2015) 3-Methyl-methcathinone: Pharmacokinetic profile evaluation in pigs in relation to pharmacodynamics. J Psychopharmacol 29:734-743.

85. Mogi M, Toda A, Iwasaki K, Kusumoto S, Takehara H, Shimizu M, Murayama N, Utoh M, Yamazaki H (2012) Simultaneous pharmacokinetics assessment of caffeine, warfarin, omeprazole, metoprolol, and midazolam intravenously or orally administered to Microminipigs. J Toxicol Sci 37 (6):1157-1164.

86. Brunet B, Hauet T, Hebrard W, Papet Y, Mauco G, Mura P (2010) Postmortem redistribution of THC in the pig. Int J Legal Med 124 (6):543-549.

87. Schaefer N, Kettner M, Laschke MW, Schlote J, Peters B, Bregel D, Menger MD, Maurer HH, Ewald AH, Schmidt PH (2015) Simultaneous LC-MS/MS determination of JWH-210, RCS-4, delta9-tetrahydrocannabinol, and their main metabolites in pig and human serum, whole blood, and urine for comparing pharmacokinetic data. Anal Bioanal Chem 407:3775-3786.

88. Schaefer N, Helfer AG, Kettner M, Laschke MW, Schlote J, Ewald AH, Meyer MR, Menger MD, Maurer HH, Schmidt PH (2016) Metabolic patterns of JWH-210, RCS-4, and THC in pig urine elucidated using LC-HR-MS/MS – Do they reflect patterns in humans? Drug Test Anal DOI: 10.1002/dta.1995

89. Schaefer N, Wojtyniak J-G, Kettner M, Schlote J, Laschke MW, Ewald AH, Lehr T, Menger MD, Maurer HH, Schmidt PH (2016) Pharmacokinetics of (synthetic) cannabinoids in pigs and their relevance for clinical and forensic toxicology. Toxicol Lett in press doi: 10.1016/j.toxlet.2016.04.021.

90. Schaefer N, Kettner M, Laschke MW, Schlote J, Ewald AH, Menger MD, Maurer HH, Schmidt PH (2016) Distribution of JWH-210, RCS-4, and ∆9-Tetrahydrocannabinol after intravenous administration to pigs. Curr Neuropharmacol accepted by mail of 04/26/2016

5 ABBREVIATIONS

11-HO-THC	11-hydroxy-THC
APCI	atmospheric pressure chemical ionization
AUC	area under the concentration-time curve
cAMP	cyclic adenosine monophosphate
CB	cannabinoid
Cl	clearance
CNS	central nervous system
CYP	cytochrome P450
DUID	driving under the influence of drugs
ESI	electrospray ionization
EU	European
EWS	Early Warning System
i.v.	intravenous
JWH-018	naphthalen-1-yl-(1-pentylindol-3-yl)methanone
JWH-210	4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone
LC-MS/MS	liquid-chromatography tandem mass spectrometry
LLE	liquid-liquid extraction
NPS	new psychoactive substances
PK	pharmacokinetic
RCS-4	2-(4-methoxyphenyl)-1-(1-pentylindol-3-yl)methanone
SCs	synthetic cannabinoids
SPE	solid-phase extraction
$t_{½}$	elimination half-life
THC	Δ9-tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-THC
US	United States
Vd	volume of distribution

6 SUMMARY

Synthetic cannabinoids (SCs) are gaining increasing importance in clinical and forensic toxicology. Neither preclinical safety data nor data obtained from controlled human studies are available. Thus, alternative models for assessment of pharmacokinetic (PK) data have to be established for improved interpretation of authentic clinical and forensic cases. In the presented studies, the PK of the two SCs JWH-210 and RCS-4 has been elucidated after intravenous administration to pigs. In addition, the PK of tetrahydrocannabinol (THC) has been assessed to examine the comparability of porcine and human PK. The major urinary metabolic SC pathways were aromatic and aliphatic hydroxylation, as well as *O*-demethylation (RCS-4) followed by glucuronidation or sulfation (RCS-4). Regarding THC, THC glucuronidation, hydroxylation and carboxylation followed by glucuronidation were the known major metabolic steps. With few exceptions, the results of this pig study were in good agreement with those obtained from human hepatocytes and/or human cases. A population PK modeling approach revealed that a three-compartment model described best the concentration-time profiles. In addition, the pig PK model has proven to be suitable for prediction of human THC exposure. The investigation of distribution patterns of the drugs revealed that particularly the analysis of lungs, kidneys, brain, adipose and muscle tissue, and bile is recommended in postmortem toxicology.

7 ZUSAMMENFASSUNG

Synthetische Cannabinoide (SC) gewinnen zunehmende Bedeutung in der klinischen und forensischen Toxikologie. Weder präklinische Sicherheitsdaten, noch Daten aus kontrollierten Humanstudien sind verfügbar. Die Etablierung alternativer Modelle zur Auswertung von Pharmakokinetik (PK)-Daten ist notwendig, um eine verbesserte Interpretation authentischer Daten zu gewährleisten. In den vorgestellten Studien wurde die PK der beiden SC JWH-210 und RCS-4 nach intravenöser Gabe in Schweinen aufgeklärt und ebenfalls Tetrahydrocannabinol (THC) bestimmt, um die Vergleichbarkeit der PK in Schwein und Mensch zu überprüfen. Die Hauptmetabolismuswege der SC im Urin waren aromatische und aliphatische Hydroxylierung sowie *O*-Demethylierung (RCS-4), gefolgt von Glucuronidierung oder Sulfatierung (RCS-4). Hauptmetabolismusschritte von THC waren, wie bekannt, THC-Glucuronidierung, Hydroxylierung und Carboxylierung, gefolgt von Glucuronidierung. Mit wenigen Ausnahmen stimmen die Ergebnisse gut mit denen aus Studien mit humanen Hepatozyten oder aus Fallberichten überein. Ein populationskinetischer Modellierungsansatz ergab, dass ein Drei-Kompartiment-Modell die Konzentrations-Zeit Profile am besten beschreibt. Außerdem scheint das Schweinemodell geeignet zur Vorhersage humaner THC Exposition zu sein. Die Ermittlung der Verteilungsmuster der Drogen zeigte, dass insbesondere die Analyse von Lungen, Nieren, Gehirn, Fett- und Muskelgewebe und Galle in der Postmortem-Toxikologie empfehlenswert ist.