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Can toxicokinetics of (synthetic) cannabinoids in pigs after pulmonary administration be upscaled to humans by allometric techniques?

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ABSTRACT

Being advertised and distributed as attractive substitutes of cannabis, synthetic cannabinoids (SC) are gaining increasing relevance in forensic and clinical toxicology. As no data from controlled human studies are available, SC are sold and consumed without the knowledge of their toxicokinetic (TK) and toxicodynamic properties. Hence, animal models coupled with mathematical approaches should be used to ascertain those properties. Therefore, a controlled pig TK study allowing for extrapolation to human data was performed. For this purpose, eleven pigs received a single pulmonary dose of 200 µg/kg body weight each of Δ9-tetrahydrocannabinol (THC), 4-ethylnaphthalene-1-yl-(1-pentylindole-3-yl)methanone (JWH-210) as well as 2-(4-methoxyphenyl)-1-(1-pentyl-indole-3-yl)methanone (RCS-4) via an ultrasonic nebulizer. Blood and urine samples were repeatedly drawn over 8 h. Serum-concentration-time profiles of the parent compounds were determined using LC-MS/MS. Urine specimens were analyzed by LC-HR-MS/MS in order to elucidate the main metabolites. Maximum serum concentrations were reached 10–15 min after beginning of nebulization and amounted to 66 ± 36 ng/mL for THC, 41 ± 11 ng/mL for JWH-210, and 34 ± 8.9 ng/mL for RCS-4. The serum-concentration-time profiles of THC, JWH-210, and RCS-4 were best described by three-compartment models with first order absorption and elimination processes. Absorption from the lungs to serum was modeled by first-order processes. The determination of the bioavailability yielded 23.0%, 24.2%, and 45.7% for THC, JWH-210, and RCS-4, respectively. Furthermore, the developed THC model was upscaled to humans using allometric scaling techniques. A successful prediction of human concentration-time profiles could be done. Also the metabolic patterns were in good agreement with human data. In conclusion, these findings are the first reported regarding the TK properties of SC after pulmonary administration to pigs. The presented method of TK serves as an appropriate predictor of human TK of cannabinoids.

1. Introduction

Synthetic cannabinoids (SC) were originally synthesized in the context of structure–activity relationship studies [1–3], but have increasingly been consumed as a substitute of cannabis to elude the narcotics law. Especially during the last six years, the number of newly emerged SCs has significantly increased in Europe [4]. SC often exhibit higher affinities to the cannabinoid receptor (CB) 2 as well as CB1. This receptor is responsible for the psychoactive, but also antiemetic and analgesic effects [5], explaining the usefulness in the treatment of i.e. nausea during cancer treatment or cachexia in the context of an acquired immune deficiency syndrome. Beyond that, even higher potencies have been observed as compared to Δ^9-tetrahydrocannabinol (THC) [6–8], resulting in unpredictable psychoactive effects, ending up in intoxications with life-threatening conditions or even death [5,9]. Regarding these issues, SC have become a tremendous public health concern and are gaining increasing relevance in clinical and forensic toxicology. Unlike THC, whose toxicokinetic (TK) and toxicodynamic properties have extensively been examined [10–17], neither preclinical safety-data nor TK data from controlled studies are available for SC.

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However, explicit TK data are indispensable when interpreting analytical data from individuals who are either impaired or have overdosed following drug use (i.e. time of ingestion and plasma concentrations related to clinical observations), but in addition and especially if precise expert opinion in forensic cases is required (i.e. driving under the influence of drugs). Only few SC TK data have been obtained so far, which are based on in-vitro or few systematic animal studies, single case reports, or self-experiments with only one or two participants [18–20]. Recently, Toennes et al. [21] conducted a systematic human study in order to assess adverse effects and TK of JWH-018 after inhalation. However, this database obtained in these pioneer studies has to be supplemented for a even more comprehensive characterization of the TK properties of this drug class.

A pilot study to establish a pig model suitable for cannabinoid TK studies after intravenous (i.v.) administration of THC, 4-ethylnaphthalene-1-yl-(1-pentylindole-3-yl)methanone (JWH-210), and 2-(4-methoxyphenyl)-1-(1-pentyl-indole-3-yl)methanone (RCS-4) [22–25] and allowing for prediction of human data applying a mathematical approach and allometric scaling techniques [23] has provided first knowledge concerning that issue. In this study, a three compartment model as well as an allometric scaling exponent of 0.75 on each TK parameter described the data best [23].

Nevertheless, the most common route of SC consumption is smoking [6]. Furthermore, inhalation of vaporized cannabinoids via electronic cigarettes has also been reported [26]. Thus, an administration set-up reflecting authentic user habits should be established using the former developed pig model in order to supplement the data obtained after i.v. administration. Therefore, the aim of the present study was to elucidate the TK of THC as well as the two SC JWH-210 and RCS-4 after standardized pulmonary administration to ventilated anesthetized pigs using an ultrasonic-assisted nebulizer [27]. For this purpose, the concentration-time profiles should be determined in a first step. For evaluation of the bioavailability, the data obtained from the pulmonary administration experiments should be compared to those determined in the i.v. study. In a second step, the concentration-time profiles should be modeled and assessed whether the THC model can predict published data in humans by upscaling the THC pig model to humans using allometric techniques. In a third step, simulations of different human dosing scenarios should be performed for JWH-210 and RCS-4. At last, the main urinary metabolites should be identified by liquid-chromatography high-resolution mass spectrometry (LC-HR-MS/MS). Results should be compared with those detected after i.v. administration and finally correlated to human data.

2. Materials and methods

2.1. Chemicals and reagents

Glacial acetic acid per analysis (p.a.), isopropanol p.a., acetone Supra Solv, methanol Supra Solv, formic acid EMSURE, and di-potassium hydrogen phosphate EMSURE were obtained from Merck (Darmstadt, Germany). High pressure (HP) LC grade water was purchased from VWR-International (Darmstadt, Germany) and ethanol p.a. and HPLC grade acetonitrile from Sigma-Aldrich (Steinheim, Germany). Ammonium formate (analytical grade) was obtained from Fluka (Neu-Ulm, Germany), methanolic solution of THC (0.1 mg/mL), THC pharmaceutical grade for drug administration (Dronabinol, DAC 2008, 98.5% purity), JWH-210 (solid), and RCS-4 (solid) were purchased from THC Pharm (Frankfurt/Main, Germany). THC-d3, 11-hydroxy-THC (11-HO-THC), 11-OH-THC-d_3, and 11-nor-9-carboxy-THC (THC-COOH), and THC-COOH-d_3 ethanolic solution (0.1 mg/mL each) was obtained from LGC/Promochem (Wesel, Germany) and methanolic solutions of *N*-hydroxypentyl JWH-210, *N*-pentanoic acid JWH-210, *N*-hydroxypentyl RCS-4, *N*-pentanoic acid RCS-4 (0.1 mg/mL each), JWH-210-d_9 (1 mg/mL) and RCS-4-d_9 (5 mg/mL) were from Cayman Europe (Tallinn, Estonia). JWH-210 for drug administration was provided by the German Federal Criminal Police Office (Wiesbaden, Germany) and RCS-4 was purchased as 'research chemical' from an internet provider [28]. An in-house test of purity using a commercially available reference substance revealed a purity of 96%.

The phosphate buffer (0.1 M, pH 9) was prepared by dissolving 22.82 g di-potassium hydrogen phosphate in 1 L deionized water. For chemical and reagents used in the i.v. study see [23].

2.2. Animals

As already described in a previous study [22–25], all experiments were performed in accordance with the German legislation on protection of animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (permission number: 69/2013). Eleven domestic male pigs (Swabian Hall strain; body weight (BW) 40.5 kg – 49.8 kg) were used for the pulmonary study and twenty-four pigs (Swabian Hall strain; BW 34.4 kg – 60.8 kg) were used in the i.v study [23]. The animals had free access to tap water and daily standard chow. They were kept fasting a night before the experiment with free access to water.

2.3. Surgical procedures

Surgical procedures have already been described elsewhere [22–25]. Briefly, after premedication with an intramuscular injection of ketamine hydrochloride (30 mg/kg, Ursotamin; Serumwerk Bernburg, Bernburg, Germany), xylazine hydrochloride (2.5 mg/kg, Rompun; Bayer, Leverkusen, Germany), and atropine (1 mg, Braun, Melsungen, Germany), analgosedation was maintained by isoflurane (2–4%, Forene, AbbVie, Ludwigshafen, Germany). Animals were mechanically ventilated with a mixture of oxygen and air (1:2 vol/vol; FiO_2 of 0.30; Respirator ABV-U; F. Stephan GmbH, Gackenbach, Germany) and volume cycled with a tidal volume of 10–12 mL/kg. The left ear vein was catheterized for fluid replacement (sodium chloride 0.9% [8 mL kg^{-1} h^{-1}], Braun, Melsungen, Germany) and a triple-lumen 7F (Certofix Trio, Braun, Melsungen, Germany) central venous catheter was placed into the jugular vein for sample collection and monitoring of mean central venous pressure. A suprapubic catheter (Cystofix, Braun, Melsungen, Germany) was inserted into the bladder for urine sample collection. The animals were then allowed to stabilize for 10–15 min.

The surgical procedures performed in the i.v. study were published elsewhere [23].

2.4. Study design

At first, a stock solution of 7.5 mg/mL of THC, JWH-210, and RCS-4 each was prepared in ethanol. The appropriate volume of the solution (1080–1328 μL) was diluted with ethanol to obtain a 200 μg per kg BW dose in a total volume of 2 mL, respectively. This dose was administered within 12 min by nebulization of THC, JWH-210, and RCS-4 applying the inspiration-triggered mode (< 0.2 mL/min) of the implemented M-neb flow$^+$ ventilation ultrasonic nebulizer MN-300/7 (Nebutec, Elsenfeld, Germany) and delivering of the aerosol through the inspiratory limb and the tracheal tube into the lungs of the ventilated pigs. The administration set-up has already been described in detail in a previous study [27].

Blood samples (about 10 mL each) were drawn prior and 1, 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min after the beginning of the administration (t = 0 min: starting of nebulization). Specimens were centrifuged at 1250*g* for 15 min to obtain serum. Urine specimens were collected before administration and then hourly up to 480 min. All samples were stored at −20 °C until analysis.

The i.v. study design was published elsewhere [23].