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Pharmacokinetics of Bedrocan®, a cannabis oil extract, in fasting and fed dogs: An explorative study

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ABSTRACT

The aim of this study was to explore the pharmacokinetics of the two main active compounds (THC and CBD) contained in the cannabis oil extract Bedrocan® in fasting and fed dogs. Bedrocan® (20% delta-9-tetrahydrocannabinol [THC] and 0.5% cannabidiol [CBD]) was administered at 1.5 and 0.037 mg/kg THC and CBD, respectively in fasted and fed dogs according to a 2 × 2 cross over study design. The quantification of the two active ingredients was performed by LC/MS. No detectable concentrations of CDB were found at any collection time. THC was quantifiable from 0.5 to 10 h, although there was large inter-subject variability. Fed dogs showed a longer absorption phase (Tmax 5 vs 1.25 h) and lower maximal blood concentration (7.1 vs 24 ng/mL) compared with the fasted group. A larger AUC was found in the fasted group; the relative oral bioavailability in fed animals was 48.22%.

In several EU countries and some states of the USA, medical marijuana is an option for humans seeking relief from various ailments (Abrams, 2018). As cannabis is now sold as an oil extract, it has also become a conceivable option for the treatment of dogs. There are a number of cannabis oil extracts on the market, each characterized by a specific ratio in the amount of the two main active ingredients: delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is a psychoactive agent that is used in humans in a number of acute pain disorders (Farrell, 1998; Campbell et al., 2018). Although it has been hypothesized to be a useful alternative to narcotics in terms of good efficacy and reduced addiction potential (Boehnke et al., 2016), THC is still considered a recreational/addictive compound and is subject to special regulations in some countries (Abuhasira et al., 2018). In contrast, CBD is a non-psychotropic component of cannabis, sold as a herbal supplement with potential use in a variety of clinical presentations, including chronic and inflammatory pain, seizures, and anxiety (Burstein, 2015; dos Santos et al., 2015; Zuardi et al., 2017).

There is anecdotal evidence of medicinal cannabis benefiting dogs with a range of clinical signs and diseases including seizures, nausea and other gastrointestinal signs, stress and anxiety, arthritis and pain associated with cancer (Nolen, 2013), but minimal information on its pharmacology and pharmacokinetics in dogs is available. An essential prerequisite to understand the pharmacological action of cannabis oil extract, presumably related to the blood level of the active ingredients, is the evaluation of its pharmacokinetics in the target species. This study aimed to explore the pharmacokinetics of the two main active compounds (THC and CBD) in cannabis oil extract after oral administration of Bedrocan® in fasting and fed dogs.

The animal experiment was approved by the local welfare ethics committee and carried out in accordance with the European law (2010/63/UE). Six healthy, intact female, adult (5–7 years) Labrador dogs were used. The dogs were determined to be clinically healthy based on physical examination and serum chemistry and haematological analyses. Animals were evaluated daily for 7 days from the study completion for visible adverse effects by specialized personnel. Dogs were randomly assigned to one of two treatment groups (Research Randomizer software), using an open, single-dose, two-treatment, two-phase, cross-over design. During the first phase, group I was administered with Bedrocan® (olive oil containing 20% THC and 0.5% CBD) at 1.5 mg/kg THC and 0.0375 mg/kg CBD after fasting over-night, while

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group II was fed prior to and after administration of the same Bedrocan® dose. Bedocan® is a galenical formulation that can be prepared/sold only in authorized pharmacies. Canned dog food was provided as half the total amount 15 min before dosing, with the remainder provided immediately after Bedrocan® administration. On each study day, dogs were housed in individual cages and strictly monitored for potential coprophagia for 24 h. A one-month wash-out period was observed between the phases, then the treatment groups were reversed and the experiment was repeated. To facilitate blood sampling, 30 min before the commencement of the study, an 18 gauge soft cannula was inserted in the right medial saphenous vein. Blood samples (1 mL) were withdrawn at 5, 15, 30, 45 min and 1, 1.5, 2, 4, 6, 8, 10, 24, 36, 48, 72 and 96 h after administration of Bedrocan® and immediately frozen and stored at −20 °C.

Samples (whole blood) were analysed for THC and CBD content by a validated LC/MS method. Briefly, THC-d3 and CBD-d3 were added to 100 µL of blood as internal standards (5 ng/mL), followed by precipitation with 200 µl of acetonitrile. After shaking and centrifugation (3000 *g*, 5 min), the supernatant was diluted 1:1 with the LC mobile phase (10 mM ammonium formate buffer and MeOH) and transferred into a LC vial. An aliquot (25 µl) of the sample was automatically injected onto the LC-HRMS system (a Q Exactive Orbitrap mass spectrometer coupled to a Dionex UltiMate 3000 with TurboFlow technology). For the quantification of THC and CBD, the ionization source in positive ion mode was used. Discharge voltage was set to 3.7 kV and the vaporizer temperature was optimized to 450 °C. The flow rates of sheath, auxiliary and sweep gas that provided best results were 40, 15 and 2 arbitrary units (AU), respectively. The capillary temperature was set to 320 °C. The mass spectrometer acquired full scan data (100–700 amu) in positive mode at resolving powers of 70,000 FWHM.

The calibration curves (for THC and CBD) demonstrated good linearity over the range of 1–100 ng/mL with the coefficient of correlation higher than 0.997. LOD and LOQ for both analytes were 0.3 and 1.0 ng/mL respectively. The inter-day and intra-day coefficients of variation at three different concentrations (1, 10 and 20 ng/mL) were all below 12.7%, meanwhile the mean recovery ranged from 92 ± 5% (THC) or 88 ± 6% (CBD), meeting the requirements of criteria of EMA guide lines for bioanalytical method validation (Anonymus, 2011).

Pharmacokinetic analyses were performed according to a non-compartmental model (WinNonlin 5.3). Pharmacokinetic data are expressed as median and range (min-max), while THC blood concentrations are expressed as mean and standard error.

Several varieties of cannabis for the oil extract preparations are present on the market (Bedrocan®, Bedrolite®, Bediol®, Bedica®, Bedrobinol®, … etc). Among these, Bedrocan® was selected for this study for several reasons: i) it is the most widely used cannabis among those offered by the Dutch ministry (in Italy) and has been used more in research than other varieties; ii) it has the highest content in THC and smaller in CBD; iii) any likely effect that can be seen the continuation of the study by pharmacodymic tests, could be addressed almost completely to THC rather than to a combination of THC and CBD. The dose of Bedrocan® chosen in this study was within the range of doses clinically used in humans (THC 30 to 200 mg/person/day). No sign of excitation/sedation or visible adverse effects were detected in the dogs following the treatment. No detectable concentrations of CDB were found at any time. This is unsurprising given its low concentration in the Bedrocan® formulation and the reported low oral bioavailability of this compound in dogs (below 20%; Samara et al., 1988).

THC levels in blood were quantified from 30 min to 10 h after Bedrocan® administration (Fig. 1). The THC concentrations were highly variable between subjects in the same treatment group, which corresponds with results previously reported in dogs (Garrett and Hunt, 1977). This variability might be due to different absorption rate or to a different metabolism among dogs. An intravenous administration of THC might clarify the issue, but this study did not plan it. In the fed group the Tmax was significantly prolonged compared to the fasted group with two dogs reporting values of 8 and 10 h. In these two animals, it was not possible to correctly evaluate the elimination phase and consequently they were not included in the calculation of the pharmacokinetic parameters. Fed dogs showed slower but longer absorption (Tmax, 5 vs 1.25 h) and lower maximal blood concentration (7.1 vs 24 ng/mL) compared with the fasted group (Table 1).

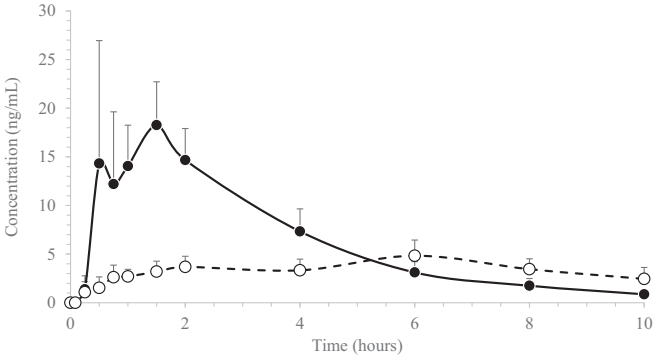

Fig. 1. THC average blood concentration vs time after oral administration of Bedrocan (1.5 mg/kg THC) in fasting (—●—) and fed (—○—) dogs.

A significant increase in the AUC was found in the fasted group. THC is a lipophilic compound and should have increased bioavailability in the fed condition. However, it was administered as an olive oil solution and the lipophilicity of the oil solution might have maximized the THC bioavailability in the fasting status, while when administered in fed status, the meal might have acted as an absorbent, slowing the absorption phase and delaying the Tmax, this pattern was found in the pharmacokinetic parameters. However, further studies are needed to confirm this speculation.

An earlier study using 14-C labelled THC reported longer detection of THC concentrations over time than those reported in this study (Garrett and Hunt, 1977) with a half-life of 10 h. This difference might be due to the fact that the use of isotopes does not allow the user to differentiate if the signal to the detector is due to the parental compound or to the likely metabolites formed in vivo. In addition a potential consideration is that in the present study Bedrocan® instead of pure THC was administered. Bedrocan® also contains other compounds (mainly therpenoids) extracted from the plant that might have affected

Table 1
Median pharmacokinetic parameters and min-max range of THC following oral administration of Bedrocan (1.5 mg/kg THC content) in fed (n = 4) and fasted (n = 6) dogs.

	Fed		Fasted	
Parameter	Median	Min-max	Median	Min-max
r^2	0.95	0.91–0.99	0.94	0.89–0.99
λz (1/h)	0.38	0.22–0.80	0.40	0.2–0.85
$T_{1/2}$ λz (h)	1.86	0.86–3.01	1.74	0.80–3.5
T_{max} (h)	5.00	0.75–8	1.25	0.5–4
C_{max} (ng/mL)	7.10	3.6–11.4	24.34	9.2–77.1
AUC_{last} (h ng/mL)	24.00	7.84–58.65	69.94	20.26–95.43
$AUC_{0-\infty}$ (h ng/mL)	29.74	8.6–61.23	74.25	22.05–100.18
MRT_{last} (h)	5.47	1.08–7.34	3.98	1.49–7.07
F%[#]	48.22	23.73–63.16		

r^2 = correlation coefficient; λz = terminal phase rate constant; $T_{1/2}$λz = terminal half-life; T_{max} = time of peak; C_{max} = peak plasma concentration; AUC_{last} = area under the plasma concentration-time curve; $AUC_{0-\infty}$ = area under the plasma concentration-time curve extrapolated to infinity; MRT = mean resident time; F% = relative bioavailability. [#]Value calculated on 4 dogs.

the pharmacokinetics of pure THC. Finally, although the analytical method used in the present study was sensitive with a LOQ of 1 ng/mL, it might not be as sensitive as the method used in the isotope study (Garrett and Hunt, 1977). Comparison between the blood concentrations found in the two studies is not possible because of the different units used (ng/ml vs A_T/D0' [A_T: total radioactivity per ml of blood; D0':total radioactive dose]). All these factors might have contributed to the discrepancy in half-life values between the two studies.

A shortcoming of the present study is that it did not detect the metabolites that are known to be produced in humans: Nor-9-carboxy-Δ9-tetrahydrocannabinol (inactive) and 11-hydroxy-Δ9-tetrahydrocannabinol (active) (Fraser and Worth, 2004). The quantification of this latter molecule in blood could be important in order to fully attribute the results of pharmacodynamic studies. Indeed, 11-hydroxy-Δ9-tetrahydrocannabinol has been shown to moderate the effects of THC itself, which may help explain the difference in subjective effects seen between occasional and regular users of cannabis (Burstein et al., 1986, 1987).

Although the high variation in blood concentrations drastically reduced the power of this study, THC appeared to have a higher bioavailability in fasted dogs. Further studies are necessary to confirm these preliminary findings.

Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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