Pharmacokinetics of methylphenidate following two oral formulations (immediate and sustained release) in the dog

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Abstract Methylphenidate (MPH) is an immediate-release (IR) or sustained-release (SR) drug used to treat attention-deficit hyperactivity disorder. Eight dogs were randomly assigned to two treatment groups, using an open, single-dose, two-treatment, two-period, randomized, crossover design. Each subject received a single dose of 20 mg *d,l*-MPH IR or SR tablet. After blood collections at specific times, the concentrations of *d,l*-MPH in plasma were evaluated by high-performance liquid chromatography. Following both IR and SR oral administration of *d,l*-MPH, the animals did not show any side effects, except that mild hyperkinesia was observed in a few subjects belonging to the IR treatment group. After both administrations, the concentration data for *d,l*-MPH in plasma displayed a characteristic, one-compartment drug model. The relative bioavailability of the SR formulation was $30.58 \pm 13.73\%$. Significant differences between the two administrations were found in $T_{\max}$, $C_{\max}$, AUC, and CI. Despite low drug concentrations in the blood, the SR formulation ensured uniformity of *d,l*-MPH plasma concentrations and, thus, a simpler and easier titration. In conclusion, the tested dosage appears to be too low for clinical application in canines, and an increase in dosing is suggested. Further pharmacodynamics studies are necessary to support this speculation.

Keywords Dog · Methylphenidate · Neurobehavioral disorders · Pharmaceutical formulations · Pharmacokinetic

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Abbreviations

ADHD	attention-deficit hyperactivity disorder
LOQ	limit of quantification
IR	immediate release
SR	sustained release
AUC	area under the curve

Introduction

Methylphenidate (MPH) is a psycho-stimulant drug primarily used for treating attention-deficit hyperactivity disorder (ADHD), one of the main neurobehavioral disturbances in children. Similar to other amphetamines, its mechanism of action is yet to be fully clarified. Some authors speculate that ADHD could result from disruption of dopamine receptors and that MPH may inhibit dopamine re-uptake, thereby increasing the concentrations of the neurotransmitter within synaptic gaps (Volkow et al. 1998). MPH undergoes first-pass metabolism, dramatically reducing its oral bioavailability by 19 and 23% in the rat and monkey, respectively (Faraj et al. 1974). Its rapid metabolism (de-esterification) leads to a short half-life (about 2–3 h), requiring multiple administrations in humans (Meyer et al. 2000). It is for this reason why sustained release (SR) formulations are currently used in humans to obtain prolonged effects. Unfortunately, several inter-individual variations in dose-effectiveness ratios have been reported in humans (Shader et al. 1999). Thus, the pharmacokinetics of the drug have a pivotal role in the prediction of therapeutic efficacy, therapy failure, or unexpected side effects.

Neurobehavioral alterations such as obsessive-compulsive disorders (chewing and swallowing materials, continuous licking, barking with variations in tones, aggressiveness, etc.) have recently been described in the dog. For the treatment of these pathologies, beyond the classical drugs such as benzodiazepines, tricyclic antidepressants, progestins, and barbiturates, MPH has also been considered. No pharmacokinetic data concerning the dog are available. Hence, the aim of the present study was to develop a new high-pressure liquid chromatography (HPLC) method for the quantification of MPH in dog plasma and to evaluate its pharmacokinetics after same strength IR and SR oral tablet administrations.

Materials and methods

Eight male beagle dogs, aged 3–6 years and weighing 7–19 kg, were used. Animals were assigned to two treatment groups, using an open, single-dose, two-treatment, two-period, randomized, crossover design. Each subject received a single dose of 20 mg *d,l*-MPH in the morning after fasting for 12 h overnight. Dogs were given either an IR or SR tablet. The washout period was 1 week. A catheter was placed into the right cephalic vein to facilitate blood sampling. Blood samples (5 mL) were collected at 0, 0.5, 1, 1.5, 2, 4, 6, 8, and 10 h after administration of *d,l*-MPH, placed in tubes containing lithium heparin, and centrifuged at $3,000\times g$. The harvested plasma was stored at -70°C until analyzed within 30 days of collection.

The concentrations of *d,l*-MPH in plasma were evaluated using the HPLC method, according to Zhu et al. (2007). Briefly, the HPLC system was an LC Workstation Prostar (Varian Corporation, Walnut Creek, CA, USA) with a 363 spectrofluorometric detector and 10- μL loop. Chromatographic separations were performed on a Hypersil GOLD C18 analytical column (250 mm $\times$ 4.6 mm, 5- μm , Thermo, Milan, Italy) maintained at room temperature. The mobile phase consisted of acetonitrile:water (55:45, v/v) with a flow rate

Fig. 1 Plasma concentrations of *d,l*-MPH following either IR or SR oral *d,l*-MPH administration

of 1.5 mL/min. Excitation and emission wavelengths were 330 and 460 nm, respectively. Samples were prepared by adding 0.5 mL plasma to 25 μ L 1-methyl-3-phenylpropylamine solution (0.005 mM) and 0.5 mL of 0.2 M sodium carbonate (10 mM) buffer (pH 10.5). After vortex-mixing, 1.0 mL butyl chloride/acetonitrile (4:1, v/v) was added, then the tube was shaken and centrifuged for 15 min at 4,000 $\times$ g. The organic layer was collected, and the inorganic phase was extracted once. The two organic phases were combined and evaporated to dryness with N_2 at 30°C. Twenty-five microliters sodium carbonate (10 mM) buffer (pH 9) and 50 μ L 4-(4,5-diphenyl-1H-imidazole-2-yl) benzoyl chloride (1 mM) were added to the residue, vortexed, and kept at room temperature for 30 min. Finally, the mixture was added to 25 μ L concentrated ammonia and injected onto HPLC. Pharmacokinetic analysis was performed using the MW Pharm 3.5 (Groningen, The Netherlands).

Table 1 Main pharmacokinetic parameters

Parameter		IR	SR
AUC	(h μ g/mL)	69.23 $\pm$ 19.23	21.17 $\pm$ 9.51*
Cl	(L/h)	0.27 $\pm$ 0.09	0.92 $\pm$ 0.49*
Vd	(L)	0.31 $\pm$ 0.21	0.62 $\pm$ 0.39
$T_{1/2}$ el	(h)	0.85 $\pm$ 0.49	0.65 $\pm$ 0.82
K_{01}	(L/h)	1.10 $\pm$ 0.50	6.10 $\pm$ 10.44
Ka	(L/h)	24.30 $\pm$ 13.47	20.75 $\pm$ 23.69
$T_{1/2}$ abs	(h)	0.22 $\pm$ 0.22	0.22 $\pm$ 0.30
T_{max}	(h)	0.21 $\pm$ 0.07	0.46 $\pm$ 0.29*
C_{max}	(μ g/mL)	60.58 $\pm$ 31.26	18.70 $\pm$ 7.73*
R^2		0.90 $\pm$ 0.16	0.92 $\pm$ 0.12

Results

Animals did not show side effects following either IR or SR oral *d,l*-MPH administration. However, in a few subjects, mild hyperkinesia was observed in the IR treatment group. Although the plasma concentration of *d,l*-MPH was highly variable among the subjects, the drug was detected in the plasma of all IR dogs, while in three SR dogs, it was below the limit of quantification (LOQ) of the method. The pharmacokinetics of *d,l*-MPH are best described by a mono-compartmental model. The parameters were normalized to body-weight. Both plasma concentrations reached C_{max} within 30 min and were below the LOQ after 6 h (Fig. 1). The main pharmacokinetic parameters are reported in Table 1. The relative bioavailability of the SR formulation was 30%. Significant differences ($p < 0.05$) were found for AUC, clearance, T_{max} , and C_{max} .

Discussion

In the dog, the large variations shown in plasma concentrations of MPH are in accordance with a previous study in the rat where doses between 0.5 and 5 mg/kg did not generate corresponding increases in kinetic curves (Aoyama et al. 1990). Dogs were given 1.69 mg/kg (normalized average) MPH, resulting in plasma concentrations similar to those reported for SR (Markowitz et al. 2003) and IR (Zhu et al. 2007) in humans. This latter formulation has shown a faster absorption phase (T_{max}) (0.21 vs. 2 h) and a shorter half-life (0.8 vs. 3 h) than in humans. In human medicine, the recent use of SODASTM (spheroidal oral drug absorption system) or OROS[®] (osmotic controlled-release oral delivery system) technology led to the biphasic release of MPH. Unfortunately, the monophasic SR formulation used in this study did not allow for any comparison. In humans, the effective plasma concentrations range from 1 to 10 µg/mL. After IR tablet administration, this range is largely exceeded in the dog, suggesting potential side effects especially in the time period near to the maximum plasma peak (T_{max}). The SR tablet administration showed more gradual pharmacokinetics but with large inter-subject variations. In conclusion, the SR formulation shows a more linear plasma concentration, appearing most suitable for use in the dog. Further studies that involve the influence of weight on the pharmacokinetic profile need to be performed to carefully determine the optimal dose to minimize the risk of both side effects and low efficacy.

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