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Original article

Pharmacokinetic profiles of 5 mg/kg ibudilast, a phosphodiesterase inhibitor, orally administered to dogs in fasted and non-fasted states. A preliminary study.

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Abstract

Ibudilast (AV-411) is a non-selective inhibitor of cyclic nucleotide phosphodiesterase (PDE). It is currently marketed for human use in Asian countries for the treatment of asthma, cerebrovascular disorders and ocular allergies. Ibudilast has also been found to have an analgesic action for neuropathic pain at doses 5-10 times higher than those used in asthma therapy. Six healthy Labrador dogs were randomly assigned to two treatment groups using an open, single-dose, two-treatment, two-phase, cross-over design (2x2 Latin-square). Dogs in group 1 (n=3) were fasted for at least 10 hours overnight before the beginning of the experiment and 4 h following dosing while dogs in group 2 (n=3) received food *ad libitum*. During the first phase, each dog in group 1 and 2 received a single dose of 5 mg/kg ibudilast administered orally. After 1-week wash-out period the groups were rotated and the experiment was repeated. The analytical method, validated for dog plasma, was shown to be linear in the range 0.10–20 µg/mL. The limit of detection (LOD) and quantification (LOQ) were 0.03 and 0.1 µg/mL, respectively. No behavioural or health alterations were observed in the animals during or after the study. Ibudilast was detectable in plasma for up to 24 h showing a wide variability between animals. Although no statistically significant differences were observed in the present study between the fed and fasted states, examination of the raw data suggests that an effect may be present. The wide degree of variation observed in area under the curve (AUC) suggests that the investigation of population pharmacokinetic modelling is warranted.

Keywords: pharmacokinetics; ibudilast; dog; non-selective cyclic nucleotide phosphodiesterase inhibitor

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Introducion

Nowadays, pets are being treated as members of the family and pet owners demand a high level of treatment. Companion animals are living longer with the appearance of more commonly manifesting age-related diseases that may involve chronic neuropathic pain, for which our science needs to be prepared (Giorgi 2012a). The challenge for drug discovery and development is to undercover innovative veterinary therapies that will provide more effective pain relief whilst being safe and having reduced side effects (Giorgi and Yun 2011, Giorgi 2012, a,b, Burgess and Williams 2014, Kim et al. 2014, De Vito et al. 2015, Łebkowska-Wieruszewska et al. 2017).

AV-411, ibudilast, is a non-selective inhibitor of cyclic nucleotide phosphodiesterase (PDE). It mainly inhibits the phosphodiesterases PDE3A, PDE4, PDE10 and PDE11 with the half maximal inhibitory concentration (IC_{50}) ranging from approximately 1–10 μ M. It can also inhibit other PDE families but to a lesser extent (Gibson et al. 2006). This active compound is currently marketed in human medications in Japan and other Asian countries. It is clinically used at the oral dose of 10-30 mg/person to treat asthma, cerebrovascular disorders (Rolan et al. 2009) and ocular allergies (Yokogaki et al. 2002). Ibudilast has been found to suppress the production of a number of inflammatory mediators such as TNF- α , interleukins (1- β and 6) and nitric oxide, it also suppresses the production of reactive oxygen species in a concentration dependent manner (Kawanokuchi et al. 2004, Mizuno et al. 2004). All these mediators are known to act on nociceptive neurons to induce, maintain and enhance the pain state (Watkins and Maier 2003). It is not surprising that ibudilast has some pain relieving action, which has been shown to occur at doses 5-10 times higher than those used in asthma therapy in several rat models of neuropathic pain (Ledeboer et al. 2007). Ibudilast has also been found to enhance acute morphine analgesia, attenuating its tolerance and withdrawal (Hutchinson et al. 2009). The mechanism of action of ibudilast in pain relief is still unclear yet, however, it has been speculated that PDE inhibition may not have a role and that the regulation of glial function is likely to be involved (Rolan et al. 2009).

It is well known that the food present in the stomach reduces its emptying process, and at the same time increases the motility of the digestive tract, increasing the passage through the intestines. Delayed arrival of the drug into the small intestine, where absorption of most drugs occurs, may delay absorption and, consequently, incomplete absorption of the administered dose (Welling 1997). On the other hand, food, especial-

ly with high rate of fat, can increase the absorption of lipophilic drugs (Dongowski et al. 2005). Hence, the aim of this study was to evaluate the pharmacokinetic profiles of 5 mg/kg ibudilast orally administered to dogs in fasted and non-fasted states.

Materials and Methods

The experiment was carried out in accordance with the European law (Directive 2010/63/EU), and the Local Ethics Committee in Lublin approved the study protocol (36/2014).

Six adult, healthy, intact Labradors, 1 male and 5 females, aged between 3 and 6 years, with body masses in the range of 34–40 kg, were enrolled in the study. The dogs were determined to be clinically healthy based on physical examination and serum chemistry and haematological analyses. The animals were evaluated daily (up to 1 week after the completion of the study) for visible adverse effects by licensed veterinarian. Two weeks after completion of the study, the dogs underwent a health-check for physical and behavioural abnormalities.

The dogs were randomly assigned to two treatment groups (6 slips of paper marked with the numbers 1 to 6 in a box), using an open, single-dose, two-treatment, two-phase, cross-over design (2x2 Latin-square). The dogs in group 1 (n=3) were fasted for at least 10 hours overnight before the beginning of the experiment and 4 h following dosing while, the dogs in group 2 (n=3) received food *ad libitum*. Water, in both groups, was provided *ad libitum*. During the study, the dogs were fed commercial dog food (Hill's Science Diet Pet Food). In order to avoid the possibility of coprophagia impacting on the study, the dogs were kept in individual boxes for 24 h and observed closely during this period.

During the first phase, each dog in group 1 and 2 received a single dose of 5 mg/kg ibudilast administered orally (KETAS[®], Kyorin Pharmaceutical Co. Ltd, Japan). The pain relieving action triggered by ibudilast occurs at 5-10 times the clinical dose (10-30 mg/person) (Ledeboer et al. 2007). Based on the average human body weight of 60-70 kg, the normalized dose considered to cause pain relief was estimated to be 5 mg/kg. The doses were prepared by weighing and partitioning the marketed drug (10 mg/capsule). To ensure that the capsules were swallowed and entered the stomach, 10 mL of water were given to dogs at the same time as the capsules were administered.

A one-week washout period was observed between the two phases, then the groups were rotated and the experiment was repeated. At the completion of the study each animal (n=6) received ibudilast in fasted

and fed status. To facilitate blood sampling, 30 min before drug administration, a 18 gauge soft cannula (Vasofix Braunule, Luer Lock; B Braun Melsungen AG, Germany) was inserted into the medial saphenous vein, and fixed in place with a cohesive flex wrap bandage (Andover Healthcare, Petflex, MA, USA). Blood samples (2 mL) were collected and transferred to tubes containing lithium heparin at 5, 15, 30, 45 min and 1, 1.5, 2, 4, 6, 8, 10, 24 and 48 h after administration of ibudilast. Samples were immediately centrifuged at 2000 g for 10 minutes and the harvested plasma was stored at -80°C until analysis was performed, within 30 days of collection.

The analytical method was based on previous studies with slight modifications (Yoon et al. 2010, Cho et al. 2013) and validated for dog plasma following the European Medicines Agency (EMA) guideline on bio-analytical method validation (EMEA 2009). Briefly, within-run and between-run accuracy and precision were assessed on quality control samples (QC samples) and determined by replicate analyses using 3 determinations of different concentration levels: LOQ, medium QC and high QC. The HPLC system (LC Jasco, Italy) consisted of a quaternary gradient system (PU 2089) and an MD 1510 multi-wavelength detector (DAD). The chromatographic separation assay was performed with a column Synergi polar-RP 150x4-6-mm (Phenomenex, Bologna, Italy) preceded by a security guard column with the same stationary phase. The system was maintained at 25°C. The mobile phase consisted of ACN:AcONH₄ (20 mM) solution, pH 6 (45:55, v/v) at a flow rate of 1 mL/min. The wavelength was set at 320 nm. Sample extraction was performed in a 15 mL polypropylene vial. A 500 µL aliquot of plasma was added to 100 µL of internal standard metoclopramide (IS) (12.5 µg/mL) and vortexed for 60 sec. NaOH 1 N 100 µL was added. Six mL of AcOEt:CH₂Cl₂ (5:1 v/v) was added, then the sample was vortexed (30 sec), shaken (100 osc/min, 10 min) and centrifuged at 3000 g for 10 minutes at 10°C. Four mL of the supernatant was collected and transferred into a separate vial. The organic phase was evaporated under a gentle stream of nitrogen at 40°C and reconstituted with 500 µL of the mobile phase. Fifty µL of this latter solution was injected onto the HPLC-DAD.

Pharmacokinetic analysis of ibudilast was performed using WinNonlin 5.3.2 software program (Certara, Princeton, NJ, USA) according to a non-compartmental model. Pharmacokinetic variables were evaluated using Student's *t* test to determine statistically significant differences between the treatment groups. Both pharmacokinetic parameters and ibudilast plasma concentrations are presented as means ± standard error (SE) (normality tested by Shapiro-Wilk test).

All analyses were conducted using GraphPad InStat (GraphPad Software). In all experiments, differences were considered significant if $p < 0.05$.

Results

No behavioural or health alterations were observed in the animals during or after the study. The HPLC method was validated using control dog plasma. Briefly, ibudilast was linear in the range 0.10–20 µg/mL. The intra-day repeatability was measured as coefficient of variation and was <6.1%, whereas accuracy, measured as closeness to the concentration added on the same replicates, was <5.9%. The limits of detection (LOD) and quantization (LOQ) were 0.03 µg/mL and 0.1 µg/mL, respectively.

The average plasma concentration vs. time curves after oral administration in fasted and fed dogs are reported in Fig. 1. Ibudilast was detectable in plasma of dogs for up to 24 h following administration with a wide variability between animals in both the fed and fasted groups. At 48 h after administration, the drug concentrations dropped below the LOQ of the method. The complete pharmacokinetic parameters obtained in the present study are reported in Table 1. No statistically significant differences between groups were noted.

Discussion

Pharmacokinetics of ibudilast in different animal species shows considerable intra- and inter-species variability (Sanftner et al. 2009). The maximal drug concentrations (C_{\max} 6.27 and 5.24 µg/mL in fed and fasted groups, respectively) found in this study, if normalized for the dose, were in agreement with those reported in an earlier study (Fanbo et al. 2000) after 10 mg/kg oral administration in dogs (C_{\max} 9.68 µg/mL). In contrast, Beagle dogs orally administered 1 mg/kg (Sanftner et al. 2009) showed a lower value (C_{\max} 0.7 ng/mL). The AUC_{24h} values (52.906 hr*µg/mL and 31,909 hr*µg/mL in fasted and fed states, respectively) observed in the present study, are comparable with those reported in dogs (Fanbo et al. 2000) but much higher than those reported in rats, rabbits, donkeys and minipigs at the dose of 1 mg/kg (range 0.0003 to 0.0869 µg*h/mL). Finally, concerning the half-life of elimination ($HL_{\lambda z}$), values from the present study did not differ significantly between the treatment groups or earlier studies in dogs (Fanbo et al. 2000). However, a considerable interspecies variability for this parameter was found when values from this study are compared with those obtained in previous studies on rats, rabbits, donkeys and minipigs (Sanftner et al. 2009). The differ-

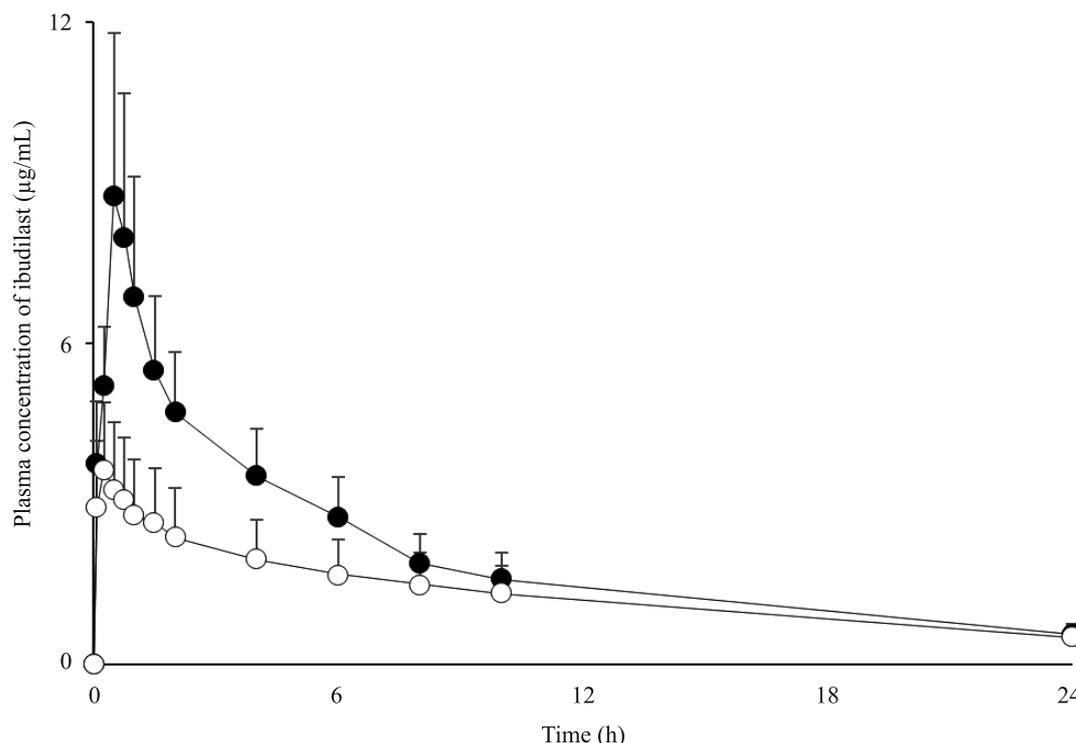


Fig. 1. Mean (\pm SE) plasma concentration time profile after oral administration of ibudilast in fasted (-o-) (n=6) and fed (-o-) (n=6) dogs.

Table 1. The mean pharmacokinetic parameters after oral administration of ibudilast at the dose of 5 mg/kg in fasted and fed dogs (n=6).

Parameters	Units	Fasted			Fed		
		Mean	SE	Range	Mean	SE	Range
$HL_{1/2}$	hr	10.37 \pm 0.44		9.9-10.79	6.80 \pm 1.00		5.02-9.58
T_{max}	hr	0.28 \pm 0.21		0.83-0.5	0.56 \pm 0.33		0.083-1.5
C_{max}	μ g/mL	6.27 \pm 1.25		5.000-7.509	5.24 \pm 1.20		1.917-7.527
AUC_{24h}	hr* μ g/mL	52.91 \pm 17.25		32.411-63.562	31.91 \pm 11.71		62.77-265.44
$AUC_{0-\infty}$	hr* μ g/mL	65.52 \pm 21.04		41.23-78.27	36.01 \pm 14.46		62.78-75.207
Vz/F	mL/kg	1258.00 \pm 545.73		928-1888	2618.26 \pm 1023.50		918-5498
Cl/F	mL/hr/kg	83.35 \pm 32.85		64-121	332.59 \pm 152.32		66-759
$AUMC_{0-\infty}$	hr*hr* μ g/mL	914.57 \pm 271.12		601.60-1077.70	412.18 \pm 197.22		44.48-959.44
$MRT_{0-\infty}$	hr	14.06 \pm 0.46		13.81-14.59	9.17 \pm 1.29		6.75-12.75

$HL_{1/2}$ half-life of the elimination phase; T_{max} time of peak concentration; C_{max} peak plasma concentration; AUC_{24h} area under the plasma concentration-time curve; $AUC_{0-\infty}$ area under the plasma concentration - time curve extrapolated to infinity; Vz/F apparent volume of distribution; Cl/F apparent clearance; $AUMC_{0-\infty}$ area under the first moment curve; $MRT_{0-\infty}$ mean resident time.

ences observed in the pharmacokinetic parameters of ibudilast in different animal species might be partially attributable to the different dosage formulation, routes of administration (Sanftner et al. 2009), drug metabolism (Martignoni et al. 2006), and breed (Labrador vs Beagle) (Toutain and Ferran 2010). Further studies are needed to clarify these differences.

Although no statistically significant differences were observed in the present study between the fed and fasted states, examination of raw data suggests that there may be an effect on the area under the curve

(AUC), but that a wide degree of variation is present. The magnitude of variation was greater than anticipated, and consequently, the power of the study to detect a difference between the groups was reduced. Another explanation for the high variability is that the number of tablets given to each dog and the large amount of drug administered, may have resulted in variation in ibudilast absorption in individual dogs (Letendre et al. 2016).

Considering this, although not statistically significant, the results of this preliminary study justify further

investigation into the potential effects of feeding on the pharmacokinetics of ibudilast in larger populations of animals. The wide degree of variation observed in AUC suggests that an investigation of population pharmacokinetic modelling is warranted.

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