Original article

Pharmacokinetics and pharmacodynamics (PK/PD) of irbesartan in Beagle dogs after oral administration at two dose rates

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Abstract

Irbesartan (Irb) is an angiotensin II type 1 receptor antagonist widely used in humans to treat hypertension. Age-related diseases such as hypertension are increasingly being diagnosed in dogs and there is the need for new drugs. The PK/PD of Irb was tested in Beagle dogs. Ten healthy Beagles were orally administered two dose rates (2 and 5 mg/kg), according to a cross over study design. Blood collections for PK analysis and systolic blood pressure (SBP), heart and respiratory rate, mucous membranes colour, capillary refill time and temperature evaluations were performed at scheduled intervals. The drug plasma concentration was dose dependent. The dogs administered 5 mg/kg showed a significant reduction in SBP, while in those receiving 2 mg/kg, this parameter was minimally affected. A counter clockwise hysteresis showed no direct correlation between SBP and plasma concentrations. The minimum effective concentration was theorized to be within the range 550-800 ng/mL. Although further studies are necessary, 5 mg/kg seems to be the more appropriate dose to obtain a hypotensive effect in Beagle dogs.

Key words: irbesartan, angiotensin receptor blocker, pharmacokinetics, pharmacodynamics, dogs

Introduction

Irbesartan (Irb) is an angiotensin II type 1 receptor antagonist (angiotensin receptor blocker [ARB]). In terms of mechanism of action, Irb prevents effects of angiotensin II such as vasoconstriction and aldosterone-mediated Na^+ retention, through a selective binding to angiotensin II type 1 receptors (AT1). Beside AT1 receptors, angiotensin II type 2 receptors (AT2) have also been described in several organs and tissues (Ruilope 1997, McConnaughey et al. 1999). However, Irb shows a higher affinity (more than 8500 times) for AT1 receptors versus AT2 receptors and does not show any agonistic activity on AT2 receptors (Ruilope 1997).

Activation of the renin-angiotensin system plays an important role in the development and progression of hypertension as well as heart failure by promoting vasoconstriction, retention of sodium and water, and via other mechanisms (Sweet and Rucinska 1994,

Bramlage et al. 2009). Clinical evidence suggests that these effects are mediated in part by stimulation of the AT1 receptor site by angiotensin II. Consequently, AT1 inhibitor drugs' class is considered essential in the treatment of symptomatic patients with hypertension and heart failure; indeed several studies report both experimental and clinical evidence that ARBs have beneficial effects in these diseases (Awan and Mason 1996, Croom and Plosker 2008).

Since last decade, hypertension in dogs has been increasingly diagnosed. That is mainly due to the humanized style of life of this animal species (Hoffman and Perkins 2008). Trends in pets mirror those in humans. Pets are living longer and as a result, are more susceptible to age-related diseases: cancer, arthrosis, metabolic disorders, obesity, etc. Some of these diseases result in hypertension and cardiac dysfunction (German 2006).

Unfortunately, the drug armamentarium available for the veterinarian is quite small compared to the active ingredients used in human medicine because of the relatively low economic interest. It follows that there are much fewer studies on specific target animal species. The use of human drugs in animal species can be a dangerous practice with reported cases of severe toxicity (Giorgi et al. 2012) and death (Ilkiw and Ratcliffe 1987). To the best of authors' knowledge, only 2 complete studies have been carried out in Irb treated dogs (Huang et al. 2005a,b). These studies report the pharmacokinetic-pharmacodynamic (PK/PD) of Irb and Irb plus hydrochlorothiazide in renal hypertensive dogs. Unfortunately, the dose administered to these animals was very high and would be unacceptable for long term treatment of clinical patients. As the importance of PK/PD modelling approaches is widely appreciated in drug research (Derendorf et al. 2000), the aim of the present research was to assess the PK/PD of Irb after oral administration at two dose rates to healthy Beagle dogs.

Materials and Methods

Materials

Pure powder (> 99.8% purity) of Irb hydrochloride was supplied by Sigma Aldrich (St. Louis, MI, USA). Pure powder (> 99.8% purity) of losartan, used as internal standards (IS), was purchased from LCG Promochem (Milan, Italy). HPLC grade methanol (MeOH), acetonitrile (ACN), dichloromethane (CH_2Cl_2) and diethyl ether (Et_2O) were purchased from Merck (Darmstadt, Germany). Analytical grade orthophosphoric acid and sodium hydroxide were obtained from BDH (Milan, Italy). HPLC grade water

was obtained by distilling deionised water produced by a Milli-Q Millipore Water System. All the other reagents and materials were of analytical grade and supplied from commercial sources.

Animals and experimental design

Ten male Beagle dogs, aged 4-7 years, with a bodyweight (BW) of 7-11 kg, were used. The dogs were previously determined to be clinically healthy on physical examination, serum chemistry and haematological analyses. The Institutional Animal Care and Use Committee of the Chungnam National University (CNU) approved the study protocol.

Animals were randomly assigned to two treatment groups, using an open, single-dose, two-treatment, two-period, cross-over study design. Each subject in group I received a single oral dose of 2 mg/kg of Irb (galenic capsules prepared by the CNU internal pharmacy), at 9:00 am after a 12 h overnight fast. Each subject in group II received a single oral dose of 5 mg/kg of Irb in the same conditions as those given to group I. The oral doses were selected based on both previous clinical evidence reported by the CNU Vet Hospital and translational dose calculations (Plumb 2008). An interval of 3 weeks was left between the first round of blood collection and the second administration of Irb to ensure complete metabolism and excretion. Prior to the study, the dogs were fed commercial dog food (Hill's Science Diet Pet Food) to avoid any potential influence of food impurities (i.e. preservatives) in the blood. 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.

A catheter was placed into the right cephalic vein to facilitate blood sampling. Blood samples (3 mL) were collected at 0, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10 and 24 h after administration of Irb, and placed in collection tubes containing lithium heparin. The blood samples were centrifuged at 1,000 g within 30 min of collection, and the harvested plasma was stored at -70ºC until use, within 15 days of collection.

High performance liquid chromatography (HPLC)

The concentrations of Irb in plasma were evaluated using HPLC, according to the method previously described by Shakya et al. (2007), with slight modifications. The HPLC system was an LC Jasco (Como, Italy) consisting of quaternary gradient system (PU 980 plus), with an integrated degasser and a multi

lambda fluorescence detector (FL 2020 plus). Data was processed using Borwin software (Jasco, Japan). Chromatographic separation assay was performed with a Poroshell C18 analytical column (100 x 3.0 mm inner diameter, 2.7 μm particle size, Agilent) maintained at 25ºC. The mobile phase consisted of potassium dihydrogen phosphate buffer (containing 0.06% triethylamine, pH 4.2) and ACN (60:40, v/v) at a flow rate of 0.3 mL/min. Excitation and emission wavelengths were set at 259 and 385 nm, respectively. The elution of the substances was isocratic. Calibration curve for the analyte comprised of 10 points, covering 5-2500 ng/mL. These concentrations were made from pooled stock solution of 10 μg/mL Irb in MeOH by diluting with drug free canine plasma. The limit of quantification (LOQ) was determined as analyte concentrations giving signal-to-noise ratios of 10. The precision value for the analyte was always lower or equal to 10% (CV%), while accuracy was higher than 92%. The LOQ of 10 ng/mL was obtained.

Preparation of plasma samples

Briefly, 100 μL of IS solution (20 μg/mL) were added to a 15 mL polypropylene screw cap tube (Sarsedt) containing 0.5 mL of plasma. After vortex-mixing, 150 μL of orthophosphoric acid (1 M) was added and the tube vortexed again. Then 6 mL of extraction solvent ($Et_2O:CH_2Cl_2$ 7:3 v/v) was added, the tube was vortexed (30 sec) and shaken for 5 min and then centrifuged for 10 min at 15,625 *g* (rotor radius 10 cm). Five mL of the organic layer was transferred into a clean 15 mL polypropylene screw cap conical tube, vortexed and shaken with 0.2 mL of back-extraction solvent (0.05 M sodium hydroxide pH ≥ 11) for 5 min and centrifuged for 10 min at 15,625 g (rotor radius 10 cm). The aqueous phase was frozen at -80ºC, the organic layer was discarded and the aqueous layer thawed and acidified with 100 μL of 0.2 M orthophosphoric acid. Aliquots of 20 μL were injected onto the HPLC system.

Pharmacokinetic evaluations

The pharmacokinetic calculations were carried out using WinNonLin v 5.3 (Pharsight Corp). Maximum concentration (C_{max}) of Irb in plasma and the time required to reach C_{max} (T_{max}) were predicted from the data. The area under the concentration vs. time curve ($AUC_{0-\infty}$) was calculated using the linear trapezoidal rule.

Changes in plasma concentration of Irb were evaluated using the compartmental analysis, and the relative pharmacokinetic parameters were determined using standard compartmental equations (Gabrielsson and Weiner 2002).

Pharmacodynamic evaluation

After each blood sampling, dogs were assessed for the following clinical end points: heart and respiratory rate, mucous membranes colour and capillary refill time (oral mucosa), temperature (rectal) and systolic blood pressure (SBP) by Doppler method (Parks Doppler Ultrasonic Flow Detector System model 811 [NV, USA], equipped by infant flat probe) (Wernick et al. 2012). All the measurements were performed by the same operator.

The heart and respiratory rate were evaluated over a minute. Pulse rate and quality were assessed by palpation of the proximal femoral arteries while simultaneously listening to the heartbeat at the level of IV-V intercostals space. The mucous membrane colour was classified as normal, pale, congested and blue; capillary refill time was considered normal if less than two seconds. The Doppler probe (with contact gel applied) was placed over the radial artery to measure the SBP. The cuff was placed slightly above the elbow than inflated at least 30 points above the pressure at which the Doppler sounds disappeared. Gradually the pressure's cuff was reduced until the operator clearly heard blood flow. At that time, the pressure value was read and recorded.

Statistical analysis

Data sets were evaluated using the ANOVA test. Correlations between the value groups were carried out by Pearson's test. The results were presented as means (±SEM). All analyses were conducted using GraphPad InStat (GraphPad Software). In all experiments, differences were considered significant if the associated probability level (*P*) was lower than 0.05.

Results

There were no adverse effects in the dogs either during the treatment or in the following 2 days.

Pharmacokinetics

After both administrations, Irb concentrations were detectable in plasma for up to 10 h (Fig. 1). Four

out of 10 dogs administered 2 mg/kg showed Irb plasma concentrations below the limit of quantification of the method at 10 h. Both the average plasma concentrations and the $AUC_{0-\infty}$ values were dose-dependent ($P < 0.05$). The T_{max} for both groups was detected at 4 h after the drug administration. The half-life (HL) of the disposition phase was calculated as significantly different between the two dose rate administrations (1.3 and 3.7 h for the 2 and 5 mg/kg administration, respectively). The average pharmacokinetic parameters for both the treatments are reported in Table 1.
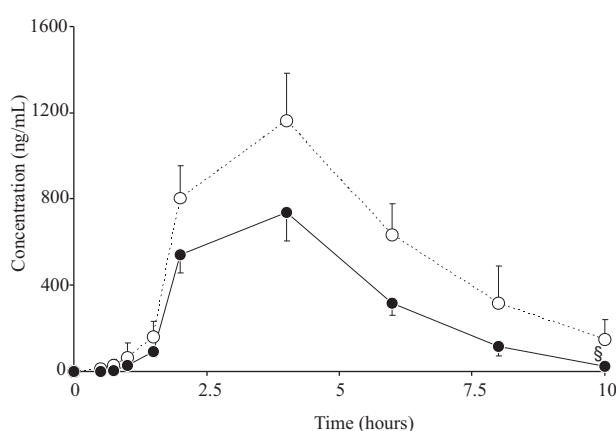
§ Value calculated in 6 out 10 dogs.
Fig 1. Mean plasma concentrations vs. time curves of Irb after single oral administrations at 2 mg/kg (–●–) and 5 mg/kg (–o–) in healthy Beagle dogs (n=10).

Table 1. The average of selected pharmacokinetic parameters after oral administration of Irb at 2 and 5 mg/kg in healthy Beagle dogs.

| Parameters	2 mg/kg	5 mg/kg
R^2	0.960 ± 0.031	0.968 ± 0.030
$AUC_{0-\infty}$ (μg h/L)	2.30 ± 0.27	5.47 ± 1.20
Cl (L/h/kg)	0.35 ± 0.19	0.46 ± 0.22
VD (L/kg)	1.89 ± 0.87	2.54 ± 1.25
HL alpha (h)	0.38 ± 0.28	0.64 ± 0.34
HL beta (h)	1.30 ± 0.23	3.70 ± 1.87
T_{max} (h)	2.83 ± 0.31	5.52 ± 2.33
C_{max} (mg/mL)	0.439 ± 0.06	0.44 ± 0.16

R^2 = correlatopn coefficient; $AUC_{0-\infty}$ = area under the plasma concentration-time curve extrapolated to infinity; Cl = clearnce; VD = volume of distribution; HL alpha = distribution half-life; HL beta = disposition half-life; T_{max} = time of peak; C_{max} = peak plasma concentration.

Pharmacodynamics

The heart rate was extremely variable among the subjects. After both the administrations, no significant variations were found in this parameter (data not shown). Temperature, respiratory rate, mucous membrane colour and capillary refill time, were evaluated to be within normal range and did not significantly vary.

The mean SBP was also quite variable among the subjects. The 2 mg/kg administration of Irb produced a significant reduction in this parameter at 4 h only, while the 5 mg/kg dose resulted in a significant reduction in this parameter over a longer period (2-10 h) (Fig. 2).
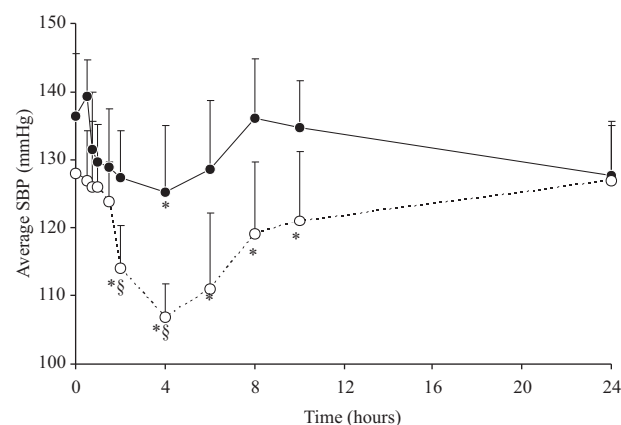
* Significantly different from T0.
§ Significantly different from the 2 mg/kg group.
Fig 2. Mean SBP variation vs. time plots after single oral administration of Irb at 2 mg/kg (–●–) and 5 mg/kg (–o–) in healthy Beagle dogs (n=10).

PK-PD integration

The PK/PD integration was only carried out on the SBP data set derived from the higher dose administration, as the lower dose had a minimal affect on this parameter.

There was no direct correlation between mean SBP and Irb plasma concentration. The effect vs. concentration plot (Fig. 3) showed a counter clockwise hysteresis loop.
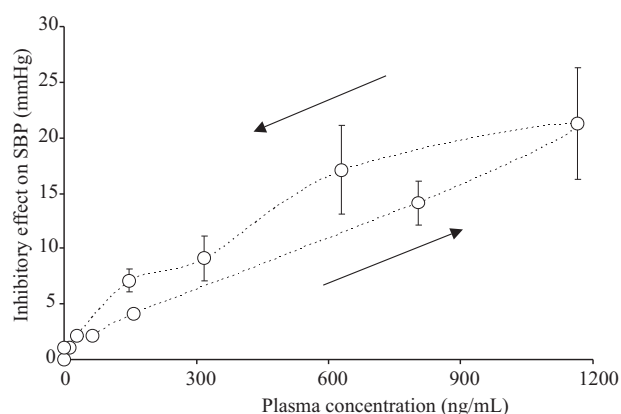

Fig 3. Relationships between the average inhibitory effect on SBP vs. plasma concentrations after single oral administration of Irb at 5 mg/kg in healthy Beagle dogs (n=10).

Discussion

Irb is a drug used in humans to prevent the effects of angiotensin II such as vasoconstriction and aldosterone-mediated sodium retention, through selective binding to angiotensin II receptors (Borghi and Cicero 2012). Irb is one of the most prescribed drugs in patients affected by cardiovascular disease because of its effectiveness and safety profile. Irb has a high bioavailability, long duration of action and small potential for pharmacological interaction due to the nature of the enzymatic pathway involved in its metabolism (Borghi and Cicero 2012).

Irb is also starting to be used in veterinary medicine (Plumb 2008). The anecdotic dose range used in canine patients is 2-5 mg/kg. This range has been translated from humans, a practice that has already been demonstrated as dangerous (Giorgi 2012, Giorgi and Owen 2012a,b, Pierini et al. 2012). Specific PK/PD studies in the target species are critical for the optimal use of drugs. To the best of authors' knowledge, only two manuscripts report the PK/PD of Irb in renal hypertensive dogs (Huang et al. 2005a,b). Both these studies used a dose of 30 mg/kg. This dose rate was far from that anecdotally used in practice so the experimental data from those manuscripts has limited application. The present research is the first description of the PK/PD of Irb in healthy dogs treated with clinically relevant doses.

The PK profiles reported in the present study are similar to the PK profiles previously reported in renal hypertensive dogs (Huang et al. 2005a,b). The T_{max} (4 h) was equivalent, as were the $AUC_{0-\infty}$ values if normalized by the mg of drug administered (36.5 [30 mg] vs 5.5 [5 mg]). In contrast, the HL beta value reported in renal hypertensive dogs was longer than the value found in the present study. This difference might be triggered by the disease or by the higher dose of Irb administered.

In contrast, the PK profiles reported in humans are quite diverse. They show a faster absorption and a shorter T_{max} (about 2h) (Marino and Vachharajani 2002). This could be the result of differences in metabolic rate between dogs and humans (Martignoni et al. 2006). When the human subjects were administered doses similar to the higher dose administered in the present study, the resulting T_{max} was about 3 times higher. These differences in absorption rate and C_{max} could be triggered by a different oral bioavailability, reported as 98% in humans, but never tested in dogs. In agreement with this speculation, earlier studies reported a drastic reduction of drug oral bioavailability in dogs when compared to humans (Giorgi et al. 2009, 2012).

Irb did not considerably affect heart rate or the other clinical parameters investigated in the present study. This might support the excellent safety profile reported in humans (Simon et al. 1998). In contrast, Irb produced a significant decrease in SBP. This is in line with earlier studies carried out in renal hypertensive dogs. As previously reported, there was no direct correlation between SBP and plasma concentration (Huang et al. 2005b).

The 2 h sampling of the 5 mg/kg group was the first time point reporting a significant reduction in SBP corresponding to a plasma concentration of about 800 ng/mL. The 2 mg/kg group at the same time (2 h), reported no significant reduction in SBP and an average plasma concentration of 550 ng/mL. From these preliminary results it may be postulated that the minimum effective concentration is within the 550-800 ng/ml plasma concentration range. In agreement with this speculation, at 4 h the lower dose group reported a significant reduction in SBP with a plasma concentration of Irb of about 700 ng/mL. This speculation cannot be extended to the descending part of the curve calculated from the higher dose group. Indeed, it is suspected that once selective binding of the drug to AT1 receptors (effective site on the wall of blood vessels) takes place, it is irreversible and irrespective of falling Irb plasma concentrations, thus extending the hypotensive effect. The counter clockwise hysteresis could be presumably triggered by the time needed for the drug distribution from central compartment to AT1 receptors (Huang et al. 2005b). Further studies are required to clarify this issue.

The data generated in this study needs to be considered with the caveat that Beagles (particularly as they are of similar age, weight and life condition) are a relatively genetically homogeneous population and that other breeds could potentially have idiosyncratic reactions to the drug as with other drugs such as ivermectin sensitivity in certain Collie dogs (Fassler et al. 1991). Cythochrome P450 polymorphisms in different dog breeds could have potential implications for Irb pharmacokinetics in clinical studies and a wider variety in the general dog population might be expected. Additionally, the fact that the present findings are generated by a small sample size with only male subjects should also be taken into consideration as, in humans, gender differences (Hallberg et al. 2004, Lam et al. 2012) in drug metabolism (Borobia et al. 2009) have been demonstrated.

Conclusion

In conclusion, this is the first report on the PK/PD of Irb when administered within the clinical dose range used in veterinary medicine. The plasma con-

centrations are dose dependent and the 5 mg/kg dose appeared to produce an effective, long-lasting hypotensive effect. Further studies are now required to examine a large sample size of clinical patients to confirm the actual effective plasma concentration range.

Conflict of interest statement

Acknowledgements

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