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RESEARCH PAPER

Intrapulmonary and intravenous administrations of dihydroergotamine mesylate have similar cardiovascular effects in the conscious dog

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Background and purpose: The effects of intrapulmonary artery (i.p.a.) administration of dihydroergotamine mesylate (DHE) were evaluated.
Experimental approach: Conscious beagle dogs ($n=4$) were given DHE via the i.p.a. or i.v. route as two 0.014 mg kg^{-1} doses and a 0.14 mg kg^{-1} dose given 60 min apart. A recovery period of $\geqslant$45 h occurred before crossover to the alternative route. Physiological parameters were monitored by telemetry or direct measurement, and venous blood samples were collected for pharmacokinetic assessments.
Key results: No meaningful differences between i.v. and i.p.a. treatments were observed for heart rate, systemic pressures and vascular pressures. Aortic resistance increased 8, 27 and 70%, respectively, following three doses of i.v. DHE compared with 11, 37 and 57%, respectively, with i.p.a. DHE. Carotid artery resistance increased 22, 40 and 87%, respectively, following three doses of i.v. DHE, compared with 17, 45 and 67%, respectively, following i.p.a. DHE. Increases in coronary artery resistance were of similar magnitude following i.v. and i.p.a. DHE administration. Increases in left ventricular systolic and diastolic pressures were seen following all doses of i.v. and i.p.a. DHE. Changes following DHE 0.014 mg kg^{-1} were minimal and not clinically significant. With DHE 0.14 mg kg^{-1} by either route, emesis was the most common adverse event.
Conclusions and implications: DHE has comparable effects delivered via simulated deep inhalation (i.p.a.) or i.v. administration. The risk of cardiovascular complications is unlikely to be greater following inhalation of DHE.
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Keywords: aerosol; bioavailability; cardiovascular side effects; dihydroergotamine; pharmacokinetic/pharmacodynamic model; pulmonary delivery; toxicity

Abbreviations: AUC, area under the concentration-time curve; C_{max}, peak plasma concentration; DHE, dihydroergotamine mesylate; i.p.a., intrapulmonary artery; LV, left ventricular; PA, pulmonary artery

Introduction

Therapy for acute migraine includes non-steroidal anti-inflammatory drugs, migraine-specific therapies such as ergots and triptans, and adjuvant therapies for associated symptoms (for example, nausea) such as metoclopramide (Bendtsen and Jensen, 2006; Ramadan and Buchanan, 2006). The effectiveness of acute migraine therapy varies among drugs and drug classes, from one situation to the next, and even between one attack and the next within the same migraineur (Goadsby *et al*., 2002). When appropriately used, acute migraine therapy generally results in pain relief for 60–70% of acute migraine attacks (Ferrari *et al*., 2001).

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Most migraine patients prefer oral over intranasal or parenteral routes of administration (Dowson *et al*., 2005). However, parenteral drugs are often utilized for severe or refractory migraine. Indeed, US physicians prefer injectable dihydroergotamine mesylate (DHE) in such situations (Lipton and Bigal, 2005). DHE has been available for over 60 years and is now available for administration by intranasal, s.c., i.m. and i.v. routes. (Silberstein, 2000; Saper and Silberstein, 2006). The inconsistent pharmacokinetic (PK) profile and hence response to intranasal DHE have meant that this route is not favoured among patients and prescribers, and the i.v. or i.m. routes are painful and inconvenient for use by patients (Saper *et al*., 2006). A novel inhaled DHE formulation is under development for patients with migraine, which aims to provide convenient administration with rapid and complete relief, particularly when

their pain is severe. Inhaled administration of DHE has been shown to provide a similar systemic exposure to DHE as demonstrated by the area under the concentration time curve (AUC) for a predicted equivalent systemic dose, to administration of DHE by the i.v. route (Shrewsbury *et al*., 2008). In our experimental system, delivery of DHE by injection into the intrapulmonary artery (i.p.a.) has been used to mimic the delivery of DHE by deep inhalation.

Triptans and ergots, including DHE, carry risks of cardiovascular (CV) complications in susceptible individuals, perhaps related to their agonist activity at the 5-HT_{1B} receptor (Dodick *et al*., 2004). Labelling for both classes of drugs includes warnings about the risk of coronary artery spasm and even heart attacks (Imitrex Package Insert; Imitrex Label, 2006). Theoretically, these risks may be compounded by i.p.a. administration, as minimal dilution of drug concentration occurs before reaching the heart, thus potentially resulting in higher concentrations of DHE in the left heart and coronary vasculature than when administered via the peripheral i.v. route.

An inhaled formulation of DHE is under clinical development for the treatment of acute migraine. To explore the effects of DHE on the coronary arteries and other CV parameters, this study was conducted to assess the CV safety and systemic PK of DHE delivered directly into the pulmonary artery of conscious beagle dogs.

Methods

Animals

This study was approved by an Institutional Animal Care and Use Committee. Four 7- to 11-month-old naïve male beagle dogs, weighing 9.4–11.6 kg, were purchased from Covance Research Products Inc., Denver, PA, USA. Animals were quarantined in accordance with standard operating procedures for handling research animals, and routine pathology screening, physical examinations and clinical observations were performed to verify the health of the dogs. Animals were housed individually in stainless steel cages at 64–84 °F temperature and 30–70% humidity with a 12 h:12 h light–dark cycle. Food and water were supplied *ad libitum* except during restraint periods for experimentation.

Preparation procedures

Animals were acclimatized progressively over 6 days to accept up to 4 h of sling restraint and then trained for an additional 6 days of restraint prior to treatment. Animals were prepared prior to starting the study with physiological monitors to measure systemic arterial pressures, pulmonary arterial (PA) pressures, heart rate, left ventricular (LV) pressures, coronary blood flow, carotid artery flow and aortic blood flow. Each animal was surgically implanted with a radio telemetry unit (D70-PCTP; Data Sciences, St Paul, MN, USA), while the sensing leads (pressure and ECG) were placed by performing a left lateral thoracotomy incision and securing the telemetry unit on the external left thorax beneath the latissimus dorsi muscle. A LV pressure catheter was inserted into the left ventricle and sutured in place. A systemic pressure catheter was placed in the descending aorta and secured in place. The positive and negative ECG leads were placed (one on the cardiac apex and one submuscularly on the external right cranial thorax) so that the ECG signal derived from the implanted leads emulated a standard Lead II ECG). An ultrasonic flow probe (3 mm; Transonics, London, UK) was placed on the left circumflex coronary artery. A second flow probe (10 mm) was placed on the descending aorta to measure systemic flow. A third flow probe (6 mm) was placed on the left carotid artery to evaluate cerebral arterial flow changes. All flow probe leads were tunnelled to exit percutaneously at the dorsal midline. The external portions of the flow probe leads were protected by jackets and collars placed on the dogs. Additionally, a Schwann–Ganz catheter was placed percutaneously under brief anaesthesia (i.v. propofol) prior to each animal's i.p.a. dosing to provide a dosing route to the PA and to measure PA pressures. Direct i.p.a. administration was selected to investigate the effects of pulmonary delivery on the heart and major arteries instead of inhaled delivery to avoid variability in dose delivery, which can arise from variable respiratory patterns and physiological and behavioural responses to inhalation delivery systems. In addition, animals cannot be trained to take deep inhalations while at rest—the instructions that would be given in clinical use. Furthermore, use of conscious animals avoided the potential confounding effect of anaesthesia on CV parameters. Once implanted, the Schwann–Ganz catheter was maintained percutaneously and protected by the dog's jacket for the duration of the i.p.a. dosing period. Schwann–Ganz catheter was removed on completion of that period and was not re-inserted for i.v. dosing. Thus, PA pressures were not monitored during i.v. administration.

Drugs and dosage protocol

DHE was supplied from commercial sources (DHE 45; Novartis Pharmaceuticals, East Hanover, NJ, USA). The test formulation was prepared by diluting the product with phosphate-buffered saline (Nexell Therapeutics Inc, Irvine, CA, USA) and sonicating it until the formulation was visually uniform. The preparation was then filter sterilized (Millex 0.22 μm Durapore filters) into sterile amber glass bottles.

Each animal served as its own control in a randomized, crossover design, receiving three doses of DHE, 60 min apart, on each dosing day. After obtaining baseline data, dogs were given 15 mL saline (vehicle) and monitored for 15 min. Subsequently, each dog received an initial dose of DHE 0.014 mg kg^{-1} via either i.p.a. or a cephalic vein (i.v.). Following a 60-min CV monitoring period, a second DHE 0.014 mg kg^{-1} dose was administered via the same route, followed after a further 60 min of monitoring, by a third and higher dose of 0.14 mg kg^{-1} (that is, 10 times the putative clinical dose). Each animal had a recovery period of at least 45 h before crossover dosing to the other route of administration. The 0.014 mg kg^{-1} dose was chosen to allow extrapolation to humans where the approved maximum single dose of i.v. DHE is 1.0 mg to a 70 kg adult (DHE 45; Novartis Pharmaceuticals) and would provide approximately 0.014 mg kg^{-1} of DHE on a body weight basis.

Table 1 Monitored haemodynamic parameters

Parameters measured
Systemic arterial BP (systolic, diastolic, mean)
Pulmonary arterial pressure (Schwann–Ganz catheter)
Heart rate
Left ventricular pressure (systolic and diastolic)
Coronary blood flow ($Flow_{cor}$)
Carotid artery (left) blood flow
Aortic blood flow ($Flow_{aorta}$)
Parameters calculated
Systemic vascular resistance ($MSAP/Flow_{aorta}$)
Pulmonary vascular resistance ($MPAP/Flow_{aorta}$)
Coronary vascular resistance ($MSAP/Flow_{cor}$)

Abbreviations: MPAP, mean pulmonary arterial pressure; MSAP, mean systemic arterial pressure.

Data collection

Haemodynamic (Table 1) and electrocardiographic data were collected continuously from 15 min prior to dosing (that is, baseline) to 60 min after the third dose was administered. All signals were collected by Notocord Hem Ver. 4.1 software. Signals were continuously sampled with analogue-to-digital signal conversion through an A/D board at a rate of 250 Hz. Only ECG signals had filters of a respiratory baseline remover and a 50 Hz notch filter. Values were derived from the raw waveform data into an MS Excel spreadsheet using embedded Notocord macros. The derived data were summarized as a 10 min mean for baseline, 5 min means through the 15 min vehicle monitoring, 1 min means for the first 30 min post-dose of DHE and 5 min means for the second 30 min after each dose. MS Excel was used to generate means, and SAS software was utilized to perform all subsequent estimations and analyses.

Parameters recorded included systemic arterial BP (mean, systolic and diastolic), PA pressure, heart rate, LV pressure (systolic and diastolic), left circumflex coronary artery blood flow, left carotid artery blood flow and aortic blood flow. Resistance values were calculated based on each arterial flow and the systemic mean BP.

Blood samples for PK modelling were collected from the cephalic vein pre-dose and at 5, 15, 30 and 60 min after each dose of DHE. Samples were obtained from the contralateral cephalic vein during the i.v. dosing period. All samples were placed in heparinized tubes, centrifuged and the plasma drawn off and frozen at $-70\,^{\circ}C$ until analysis (Charles River Laboratories, Montreal, Quebec, Canada). Clinical observations were recorded for any abnormalities seen following dosing while the animals were in their restraint slings for CV monitoring.

Statistical analysis

Two primary analysis strategies were employed. A repeated measures analysis was utilized for the full data set to evaluate treatment effects (i.v. vs i.p.a.) at each dose, on a time point by time point basis. The second strategy used a contrast statement between doses, on a time point by time point basis, to evaluate dose effects (0.014 mg as the first dose vs 0.014 mg as the second dose vs 0.14 mg as the third dose). The specific model was a crossover analysis of covariance, including factors for baseline, treatment, period, sequence and subject within sequence and was used to estimate treatment and dose effects, as well as model assumptions including period and carryover effects. As the results indicated no period or carryover effects, treatment and dose effects were then assessed based on point estimates of least-square means, change from baseline and 90% confidence intervals. All *P*-values presented were two-tailed and were considered significant if less than 0.10 due to the small sample sizes utilized in the study.

Results

Heart rate, arterial pressure and electrocardiographic changes

No differences between i.v. and i.p.a. treatments were observed for any of these parameters (heart rate, arterial pressure and electrocardiographic changes). The initial dose of DHE $0.014\,mg\,kg^{-1}$ i.v. or i.p.a. resulted in minimal elevations in heart rate and systemic pressures (systolic, diastolic and mean BP) of $\leqslant 12\%$ for approximately 10 min post-dose (Figure 1). Repeat dosing of DHE $0.014\,mg\,kg^{-1}$ i.v. or i.p.a. resulted in 10–16% increases in systemic pressures that were sustained over the 60-min monitoring period. This second dose had a minimal lowering effect on the heart rate, possibly as a baro-reflex to the increased pressure. The third dose of DHE $0.14\,mg\,kg^{-1}$ i.v. or i.p.a. resulted in an initial 25% increase in heart rate that lasted approximately 5 min. At this high dose, emesis or attempted emesis was observed in all dogs. Marked increases in systemic pressures of 20–45% with a peak effect at approximately 10 min post-dose were seen following the resolution of emesis. During the balance of this post-dose period, heart rates were approximately 20% lower, which also may have been a baro-reflex response. All pressures returned to pre-dose values by 60 min post-dose. No significant changes in QRS, PR or QT intervals were observed (Figure 2). For comparison between doses, no consistent significant dose effect (that is, first dose vs high dose) was observed for heart rate; however, a significant ($P<0.1$) dose effect was present for systolic and diastolic BPs with DHE $0.14\,mg\,kg^{-1}$. Changes in BP following this third dose of DHE, regardless of the route of administration, were significantly larger than changes following either of the first two doses of DHE (Table 2 and Figure 1).

PA pressures

As previously indicated, PA pressures were recorded only during i.p.a. administration. All three doses of DHE resulted in short-lived 30–50% increases in systolic and diastolic pulmonary pressures that lasted 10–15 min. The magnitude and time course of changes were similar for each of the three doses and similar to the patterns seen for systemic pressures.

LV pressures

No significant differences between i.v. and i.p.a. treatments were observed for any of these parameters (LV pressures). Overall, increases of $<10\%$ at the $0.014\,mg\,kg^{-1}$ dose and up to 25% at the $0.14\,mg\,kg^{-1}$ dose in both LV systolic and

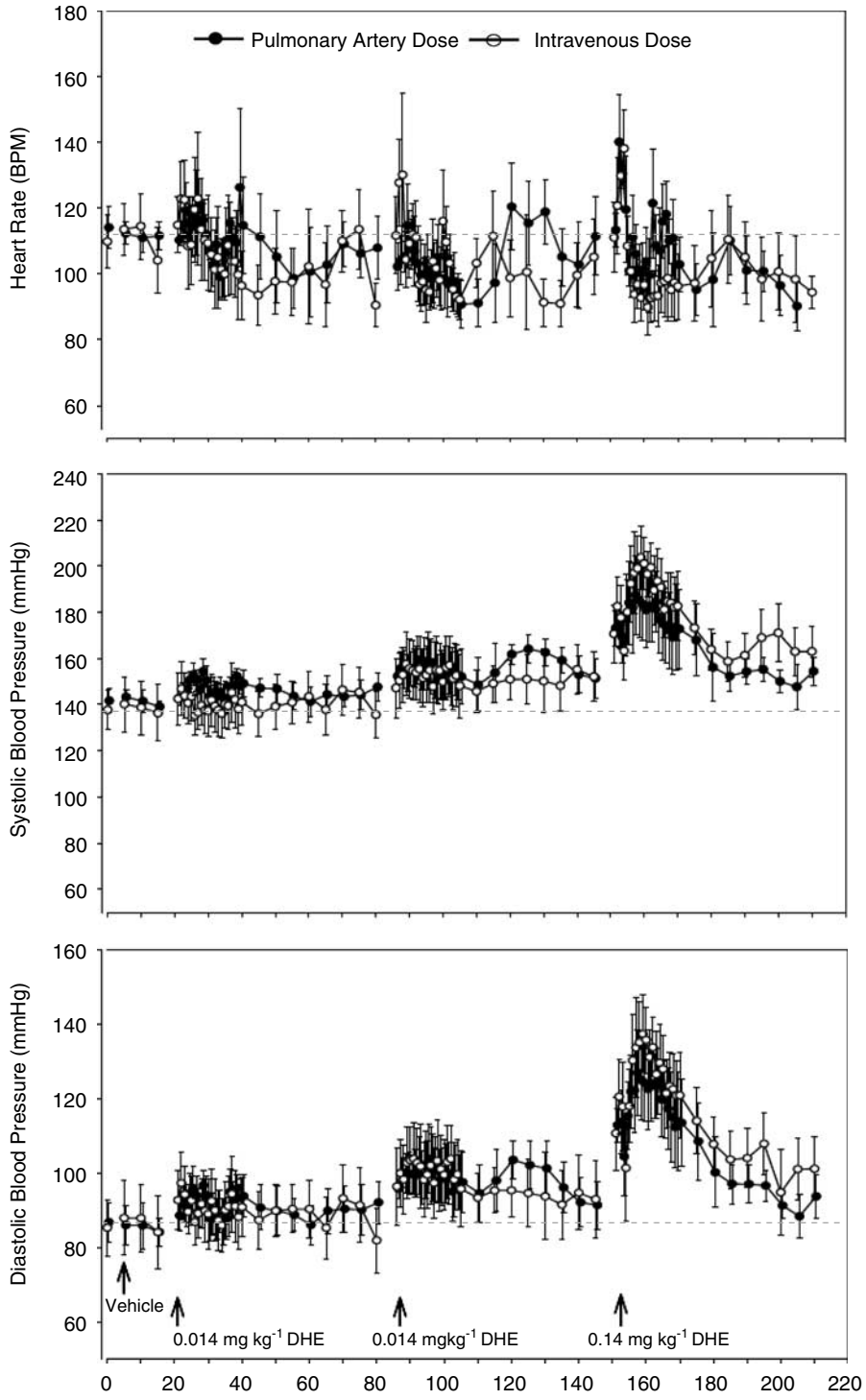

Figure 1 Effect of dihydroergotamine mesylate (DHE) on absolute heart rate, systolic and diastolic BP (mean ± s.e.) following sequences of three i.v. or three i.p.a. injections in beagle dogs. Horizontal line approximates baseline value of parameters for both routes of administration.

diastolic BPs were seen following all doses of i.v. and i.p.a. DHE in a similar pattern to changes in systemic pressures (Figure 3). Peak effects occurred at approximately 10 min post-dose, and values declined to near pre-dose values within approximately 30 min after dosing. The increase in LV diastolic BP was consistent with ergots having a vasoconstrictive effect on the venous system. For the assessment of dose effect, these results suggest that the changes of LV pressures at most time points following the third dose of DHE were significantly ($P<0.1$) larger than the comparable time points following either of the two previous doses of DHE.

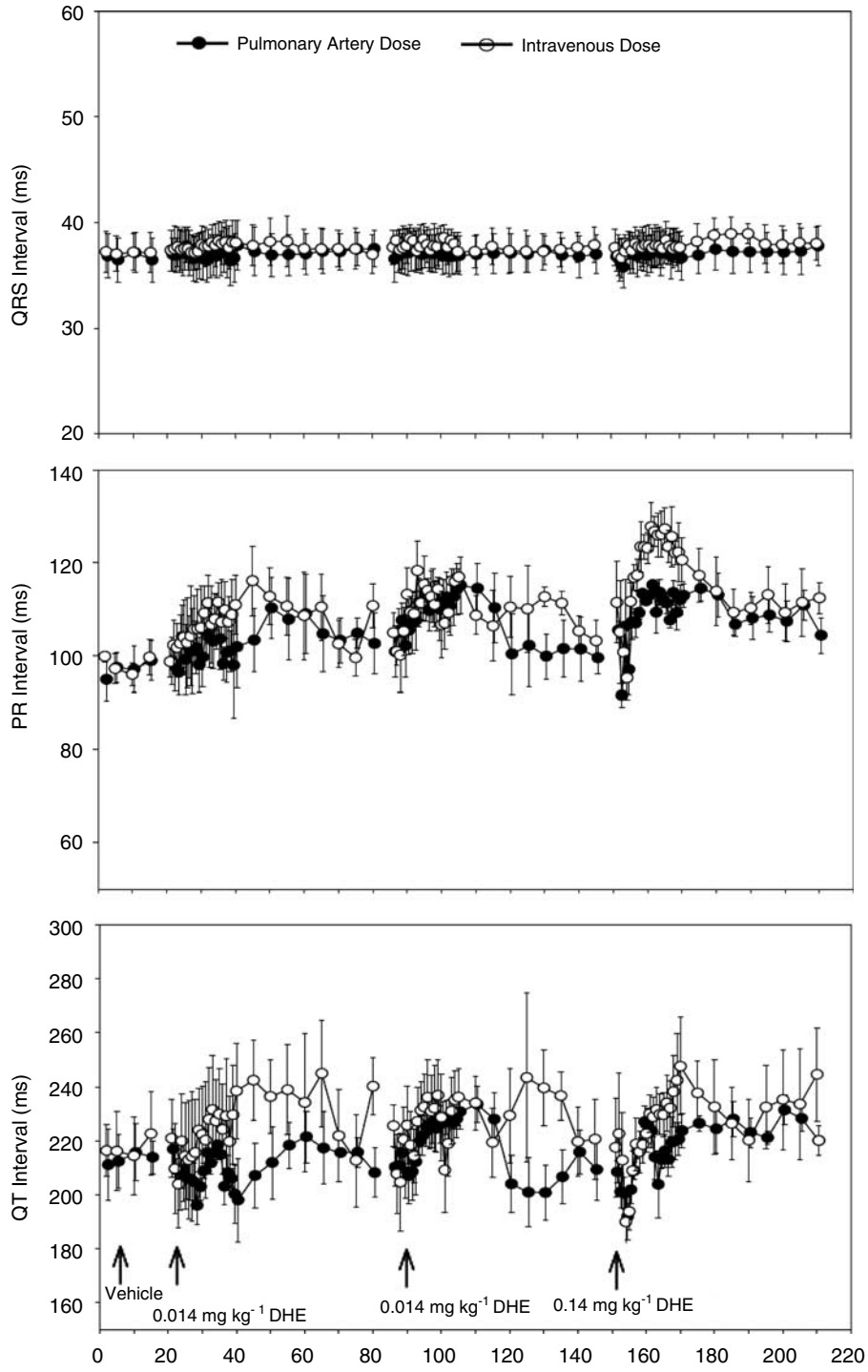

Figure 2 Effect of dihydroergotamine mesylate (DHE) on absolute QRS, PR and QT intervals of the ECG (mean ± s.e.) following sequences of three i.v. or three i.p.a. injections in beagle dogs.

Vascular resistance and flows

No consistent differences between i.v. and i.p.a. treatments were noted for any of these calculated resistances (vascular resistance and flows). The initial dose of DHE 0.014 mg kg^{-1} i.v. resulted in an increase in aortic resistance of 8% and carotid and coronary arterial resistance increases of 22 and 25% through the 60-min monitoring period (Figure 4). The second dose of DHE 0.014 mg kg^{-1} i.v. resulted in an increase in aortic resistance of 27%, and carotid and coronary arterial resistances were increased by 40 and 72% across the 60-min monitoring period. The third dose of DHE 0.14 mg kg^{-1} i.v. resulted in a marked increase in vascular resistances with a mean of 70, 87 and 112% for the aorta, carotid and coronary arteries, respectively. Values then returned to pre-dose levels.

When administered i.p.a., the initial dose of DHE 0.014 mg kg^{-1} resulted in an increase in aortic resistance of

Table 2 Summary of treatment and dose effects (i.p.a. dosing)

Variables	*Time points with significant treatment effect*[a]	*Time points with significant dose effect*[b]
Heart rate		
Dose 1	None	Reference
Dose 2	None	10, 20*
Dose 3	None	None
SBP		
Dose 1	None	Reference
Dose 2	None	10, 60
Dose 3	None	5*, 10*, 15*, 20*, 30*, 60*
DBP		
Dose 1	None	Reference
Dose 2	None	10*, 15*
Dose 3	None	5*, 10*, 15*, 20*, 30*, 60
LVSBP		
Dose 1	None	Reference
Dose 2	None	10, 15, 60
Dose 3	60	5*, 10*, 15*, 20*, 30*, 60*
LVDBP		
Dose 1	5, 20*	Reference
Dose 2	None	10
Dose 3	None	5, 10*, 15*, 20*
Carotid resistance		
Dose 1	60*	Reference
Dose 2	None	None
Dose 3	None	5*, 10*, 20*
Aorta resistance		
Dose 1	None	Reference
Dose 2	30*	10*, 15, 20*, 30*
Dose 3	60	5*, 10*, 15*, 20*, 30*, 60*
Coronary resistance[c]		
Dose 1	5*, 10	Reference
Dose 2	10, 15*, 30*, 60	5*, 10*, 20*
Dose 3	20*	5*, 10*, 15*, 20*, 30, 60

Abbreviations: DBP, diastolic BP; LVDBP, left ventricular diastolic BP; LVSBP, left ventricular systolic BP; SBP, systolic BP.
[a]Repeated measures crossover implemented on a dose by dose basis, and time point by time point basis.
[b]Dose effect is evaluated at a time point by time point basis, from the repeated measures crossover model utilizing contrast between doses. Additional analyses were performed utilizing time = 0 for dose 1 and the preceding 60 min response for doses 2 and 3. Results were consistent with those presented.
[c]Results are confounded by missing records observed from animal 103.
The presented time points are those with *P*-values <0.10; **P*-value <0.05. Only results on selected time points are evaluated (5, 10, 15, 20, 30 and 60 min post-dose).

11% and carotid and coronary arterial resistance increases of 17 and 33%, respectively, through the 60-min monitoring period (Figure 4). The second dose of DHE 0.014 mg kg^{-1} i.p.a. resulted in an increase of aortic resistance by 37%, and carotid and coronary arterial resistances were increased by 45 and 69%, respectively, across the 60-min monitoring period. The third dose of DHE 0.14 mg kg^{-1} i.p.a. resulted in mean increases in vascular resistances of 57% for the aorta, 67% for the carotid and 79% for the coronary artery. Values then returned to pre-dose levels. Regardless of the route of administration, significant ($P<0.1$) dose effects were observed at 10, 15, 20 and 30 min between the first and second doses of DHE 0.014 mg kg^{-1} for aortic resistance, and at most time points between the first (0.014 mg kg^{-1}) and third doses (0.14 mg kg^{-1}) of DHE.

The first 0.014 mg kg^{-1} dose of DHE i.v. or i.p.a. produced an initial rapid increase of 10–37% then a progressive decline of 8–20% from baseline in carotid, aortic and coronary blood flows at 60 min (Figure 5) that coincided with increases in carotid, aortic and coronary flow resistance. The second dose of 0.014 mg kg^{-1} DHE produced an initial increase in flows of 7–26% followed by a decline of 6–27% from baseline at 60 min. The third dose of 0.14 mg kg^{-1} DHE produced an initial increase of 13–40% in carotid and coronary blood flow and minimal change in aortic blood flow, but flows were 12–21% below baseline values at 60 min.

PK parameters

Time to peak plasma concentration (T_{max}), peak plasma concentration (C_{max}) and AUC over the subsequent 60 min (AUC_{0-60}) were similar between i.v. and i.p.a. administration for each of the three doses administered (Table 3 and Figure 6).

Clinical observations

Emesis was observed in all dogs 2–4 min after i.v. administration of DHE 0.14 mg kg^{-1}, and in three of the four dogs 2–5 min after i.p.a. administration of the same high dose; the fourth dog experienced retching but no vomiting. Emesis was not observed following any administration of DHE 0.014 mg kg^{-1}. No other abnormal clinical observations were noted. All dogs were in good health at the end of the study and were returned to the experimental pool of test animals.

Discussion

This study was designed to evaluate the preclinical safety and CV effects of a projected clinical dose of DHE, delivered via the pulmonary artery to simulate an inhalation bolus. The results of this study indicate that equivalent doses of i.v. and i.p.a. DHE produce comparable PK and pharmacodynamic effects, with no increased risk for adverse CV responses via i.p.a. delivery. In fact i.p.a. delivery generally resulted in numerically smaller amplitude changes than i.v. dosing, although these differences were not statistically significant.

Although a second full 1.0 mg dose in humans would be contraindicated for acute migraine within any 24-h period (and certainly at 1 h), this experiment sought to explore the comparative CV effects of excessive systemic levels of DHE, and thus dosing of DHE 0.014 mg kg^{-1} by either route of administration was repeated at approximately 60 min from the first dose and this resulted in greater systemic exposures as indicated by C_{max} and AUC_{0-60} values that were approximately twofold that of the first dose. Similarly, the DHE 0.14 mg kg^{-1} third dose delivered 60 min later resulted in C_{max} and AUC_{0-60} values that were 15–20 times greater than seen after the first dose. At all dose levels, the PK

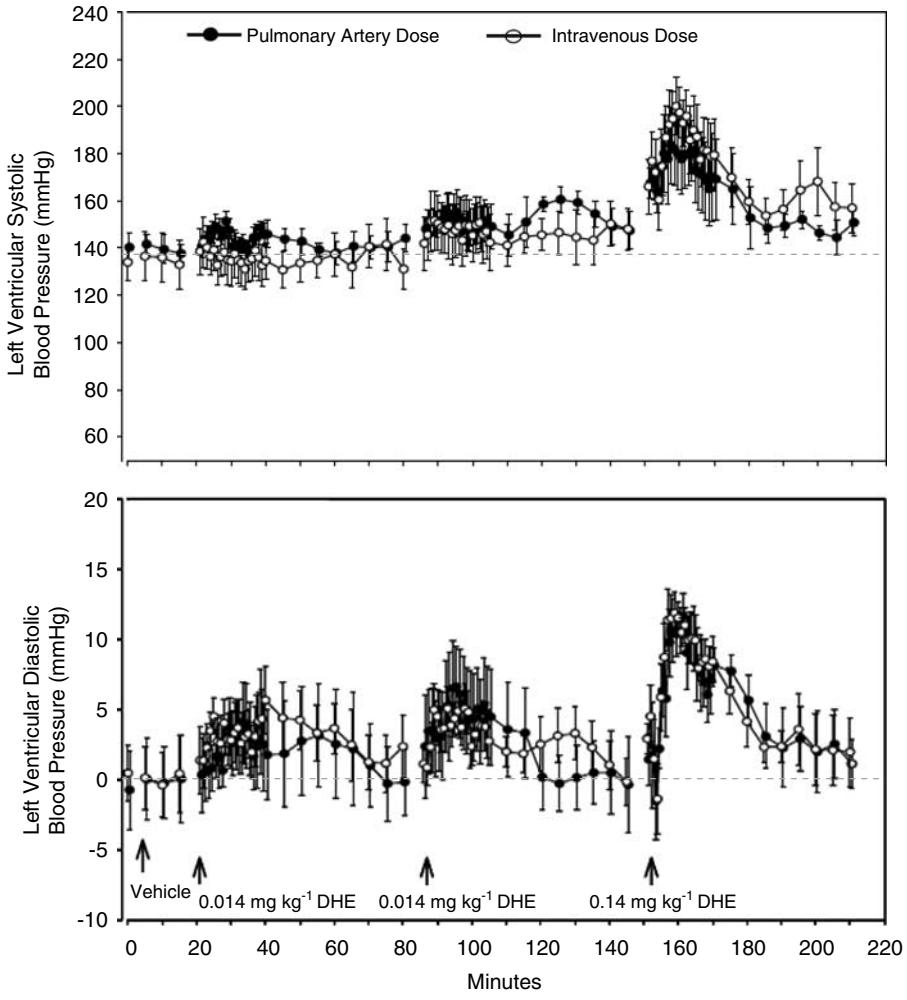

Figure 3 Effect of dihydroergotamine mesylate (DHE) on absolute left ventricular systolic and diastolic BPs (mean ± s.e.) following sequences of three i.v. or three i.p.a. injections in beagle dogs. Horizontal line approximates baseline value of parameters for both routes of administration.

profiles of i.v. and i.p.a. administrations were similar, and repeated doses of DHE to an equivalent cumulative dose of approximately 12-fold the approved clinical i.v. dose did not produce any significant clinically observable adverse effects on the dogs.

Few, mild, transient effects on CV parameters were observed following the first or second administration of DHE 0.014 mg kg^{-1}, and the responses were similar for i.p.a. and i.v. Initial administration of DHE 0.014 mg kg^{-1} by either route produced minimal changes in heart rate and haemodynamic parameters, systemic BPs, aortic resistance and carotid resistance. Repeat dosing of DHE 0.014 mg kg^{-1} produced approximately twice the magnitude of change in these parameters compared with the first DHE dose, which correlated with changes in systemic exposure. However, because of statistical confounding factors, it could not be determined whether this was an effect of drug accumulation (although both C_{max} and AUC_{0-60} were almost double after the second dose of 0.014 mg DHE than after the first), sensitization by the animals to the effects of DHE or some other unknown effects unrelated to treatment. The *ex vivo* contractile response to DHE persisted in human coronary arteries, even after repeated washings (MaassenVanDenBrink *et al*., 1998).

Following administration of DHE 0.14 mg kg^{-1} (10 times the clinical dose), marked increases in vascular and systemic pressures were observed with minimal increases in heart rate. These changes persisted for approximately 5 min and were temporally associated with emesis that occurred at this dose level that was 10 times the usual human dose of i.v. DHE on a milligram per kilogram basis. Following the initial changes, and after emesis ceased, there were marked increases in systemic and ventricular pressures and increases in calculated carotid and aortic resistance with minimal changes in heart rate, which were accompanied by relatively modest changes in vascular flow rates. Vascular pressure increases with DHE are consistent with its known vasoconstrictive effects, primarily on venous capacitance vessels (increases in LV end diastolic pressure) (Aellig, 1984). This vasoconstrictive property is likely to be the result of DHE agonism at the 5-HT_{1B} and 5-HT_{2A} receptors, although interactions at receptors of different classes (for example, α_1, α_2 and β-adrenoceptors; dopamine D_1 and D_2 receptors) where DHE effectively binds may have also contributed to the

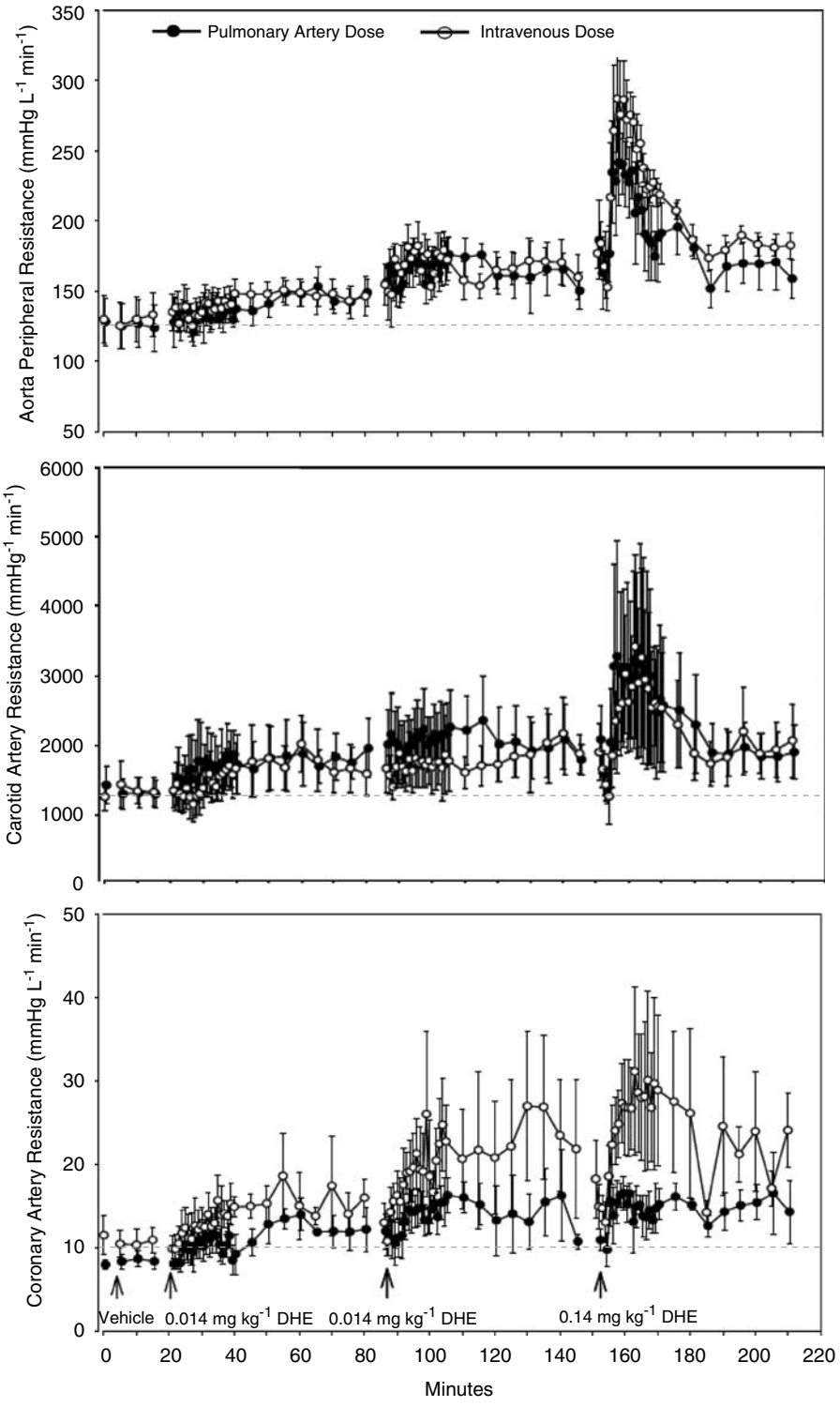

Figure 4 Effect of dihydroergotamine mesylate (DHE) on calculated resistance (mean ± s.e.) of the aorta, left carotid artery or left circumflex coronary artery following sequences of three i.v. or three i.p.a. injections in beagle dogs. Horizontal line approximates baseline value of parameters for both routes of administration.

observed vasoconstriction (Saper and Silberstein, 2006). Elevations in LV diastolic pressure could result in increased cardiac output which, in conjunction with only minimal increased arterial constriction, probably contributed to the observed increases in pulmonary and systemic pressures.

Coronary arterial blood flow was increased for up to 10 min following each dose level and demonstrated similar magnitudes of increase at all dose levels. It is unclear what was the mechanism of action underlying this increase in coronary arterial flow, and it is counter-intuitive to the

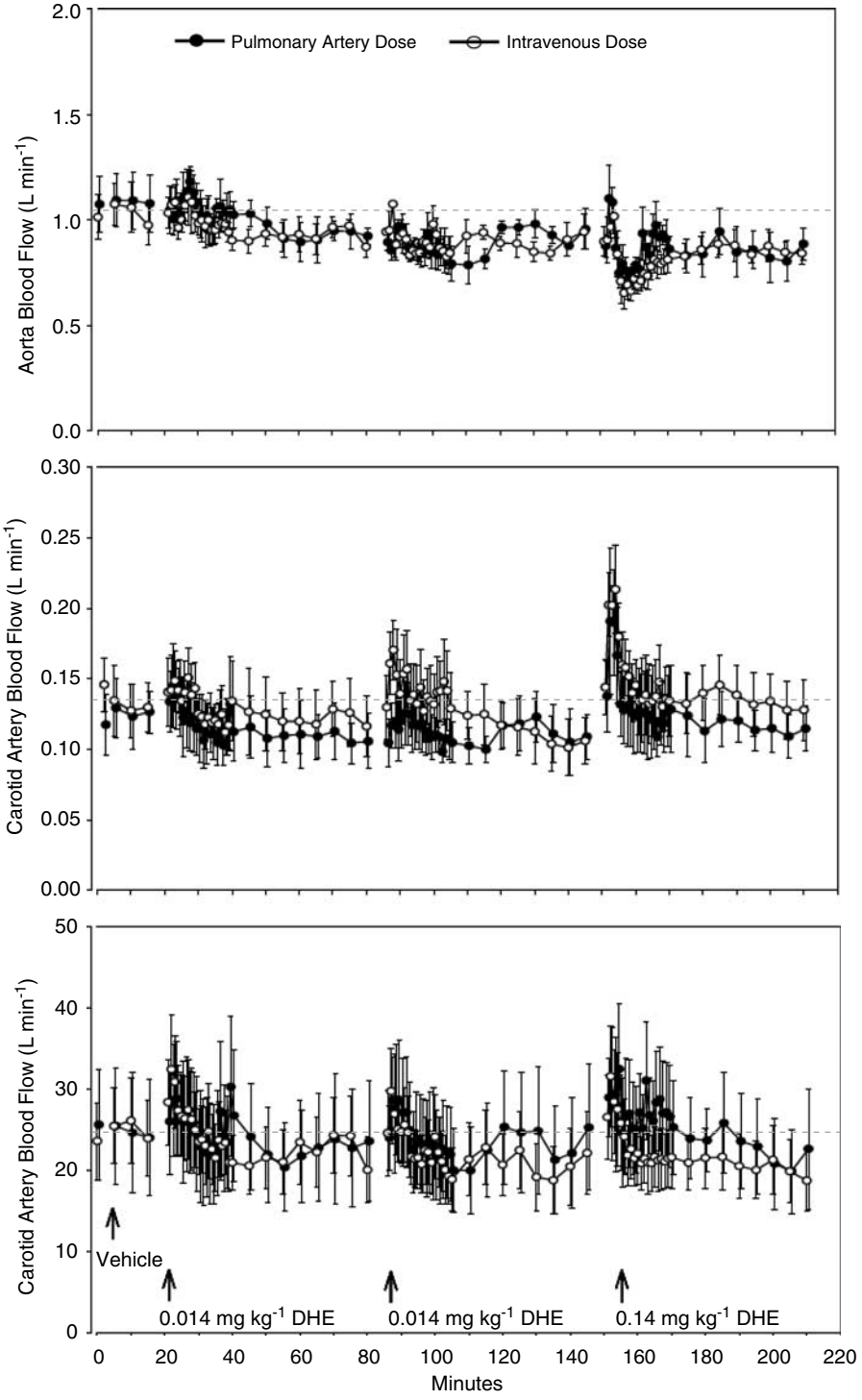

Figure 5 Effect of dihydroergotamine mesylate (DHE) on aortic, left carotid artery and left circumflex coronary artery blood flow (mean ± s.e.) following sequences of three i.v. or three i.p.a. injections in beagle dogs. Horizontal line approximates baseline value of parameters for both routes of administration.

known vasoconstrictor effect of DHE, suggesting that, at least within the coronary circulation, effects from other non-vasoconstrictive receptors may be more important than previously thought. However, further work is needed to reproduce and explain this phenomenon but, if reproduced, it would be reassuring. After approximately 10 min, coronary arterial flow returned to levels that were either similar to baseline or even slightly lower. Similarly, coronary arterial resistance was markedly increased at all dose levels following i.v. administration, with the largest changes seen after the

Table 3 Pharmacokinetic parameters following three successive doses of DHE via i.p.a. or i.v. administration in conscious dogs

Dose	*0.014 mg kg^{-1}*			*0.014 mg kg^{-1}*			*0.14 mg kg^{-1}*		
Start time	*0 min*			*60 min*			*120 min*		
	T_{max} *(min)*	C_{max} *(ng mL^{-1})*	AUC_{0-60} *(ng min mL^{-1})*	T_{max} *(min)*	C_{max} *(ng mL^{-1})*	AUC_{0-60} *(ng min mL^{-1})*	T_{max} *(min)*	C_{max} *(ng mL^{-1})*	AUC_{0-60} *(ng min mL^{-1})*
i.v. dose									
Mean	5	1.72	45.70	5	2.34	72.66	5	30.46	795.77
s.e.		0.22	5.20		0.16	5.69		3.28	85.78
Intrapulmonary artery dose									
Mean	5	1.52	44.78	5	2.70	79.66	5	30.17	825.03
s.e.		0.19	4.26		0.36	9.19		3.79	43.13

Abbreviation: DHE, dihydroergotamine mesylate.

DHE 0.14 mg kg^{-1} dose, compared with the response after i.p.a. dosing. Only slight increases in resistance were noted with i.p.a. DHE. The increase in calculated resistance was due to the greater increase in mean systemic pressure (as resistance = mean pressure/flow) compared with changes in actual blood flow. Heart rates were unchanged or slightly reduced, suggesting that myocardial oxygen demand would be unaffected.

The pressure increases are consistent with the known effects of DHE, which causes constriction primarily of the capacitance veins (that is, in increases in LV diastolic pressure) (Saper and Silberstein, 2006). Increased LV diastolic pressure can result in increased cardiac output which, in conjunction with only minimal increased arterial constriction, probably contributed to the observed increases in pulmonary and systemic pressures. Changes in pressures, flows and calculated resistances of carotid, aortic and coronary vessels were similar between i.p.a. and i.v. administration for both of the two doses of DHE 0.014 mg kg^{-1}. The changes seen following these two clinical doses of DHE 0.014 mg kg^{-1} were minimal and not considered to be significant. As expected, a greater effect was seen following DHE 0.14 mg kg^{-1}, 10-fold higher than the currently approved i.v. clinical dose. The only difference noted between the administration routes was an increase in aortic and coronary resistance following i.v. delivery of this high dose of DHE. This increased resistance response was noted immediately after dose administration and was marked by a short duration with return to pre-dose values within 20–40 min. These results are reassuring as they indicate that 10 times the approved clinical dose of i.v. DHE (1.0 mg) may not pose a safety risk after i.p.a. administration (which is 1.0 mg) (that is, after inhalation) as might have been expected.

The more marked, albeit transient, CV effects seen after the higher dose of DHE can be at least partly explained by the emesis experienced. All dogs had an acute, brief period of retching and vomiting 3–5 min after DHE 0.14 mg kg^{-1}, corresponding to the sudden changes seen in the haemodynamic and ECG data. Independent of drug effect, retching and vomiting may result in rapid and pronounced effects on CV parameters (Uchino *et al*., 2002).

This animal model was designed to simulate delivery of an inhaled bolus in humans by giving a bolus i.p.a. injection of DHE to conscious beagle dogs. This mode of delivery of DHE was used to simulate inhaled delivery because animals cannot be trained to take a forced, deep inhalation and thus i.p.a. overcomes this obstacle as well as reducing the potential large variability in drug delivery and pulmonary absorption associated with inhaled administration. This route resulted in a bolus administration of the drug to the pulmonary venous system with minimal dilution and rapid exposure of the left heart, coronary and carotid arteries, as would be expected following inhalation. In addition, the model ensured that an entire dose reached the pulmonary artery and hence the entire lung. Therefore, this model allows the investigation of simulated, rapid bolus delivery of drugs via the lung in conscious animals—previously a difficult area of research because of the practical difficulties of drug delivery to the deep lung in an animal model. This model can be used in planning inhaled drug development programmes as opposed to traditional approaches. In these, animals performing tidal breathing from a common aerosol plenum are commonly used for assessing toxicology after chronic dosing by inhalation. Such studies do not simulate bolus aerosol delivery in humans, which comprises voluntary, deep inhalations to breath-actuate pressurized metered-dose inhalers or dry powder inhalers. This model may be particularly useful for investigating the CV effects at peak exposure where the rate, and amplitude, of increasing exposure may be important.

Despite the small sample size of this study, it was reassuring to note that i.p.a. administration of DHE did not amplify the pharmacodynamic effects of equivalent doses of DHE when administered i.v. These findings in conjunction with 60 years of clinical experience with i.v. dosing suggest that inhaled DHE can be administered safely to humans (Shrewsbury *et al*., 2006; Armer *et al*., 2007).

In conclusion, this novel experimental bolus delivery of DHE via the PA to conscious dogs simulates the clinical scenario of a voluntary deep inhalation in human subjects. Examination of CV parameters after dosing (even at an excessive single dose that was 10-fold higher on a milligram per kilogram basis than the maximum single approved i.v. dose of 1.0 mg in humans) did not amplify changes in aortic, coronary, carotid or systemic vasculature that were seen following peripheral i.v. bolus dosing. Therefore, bolus

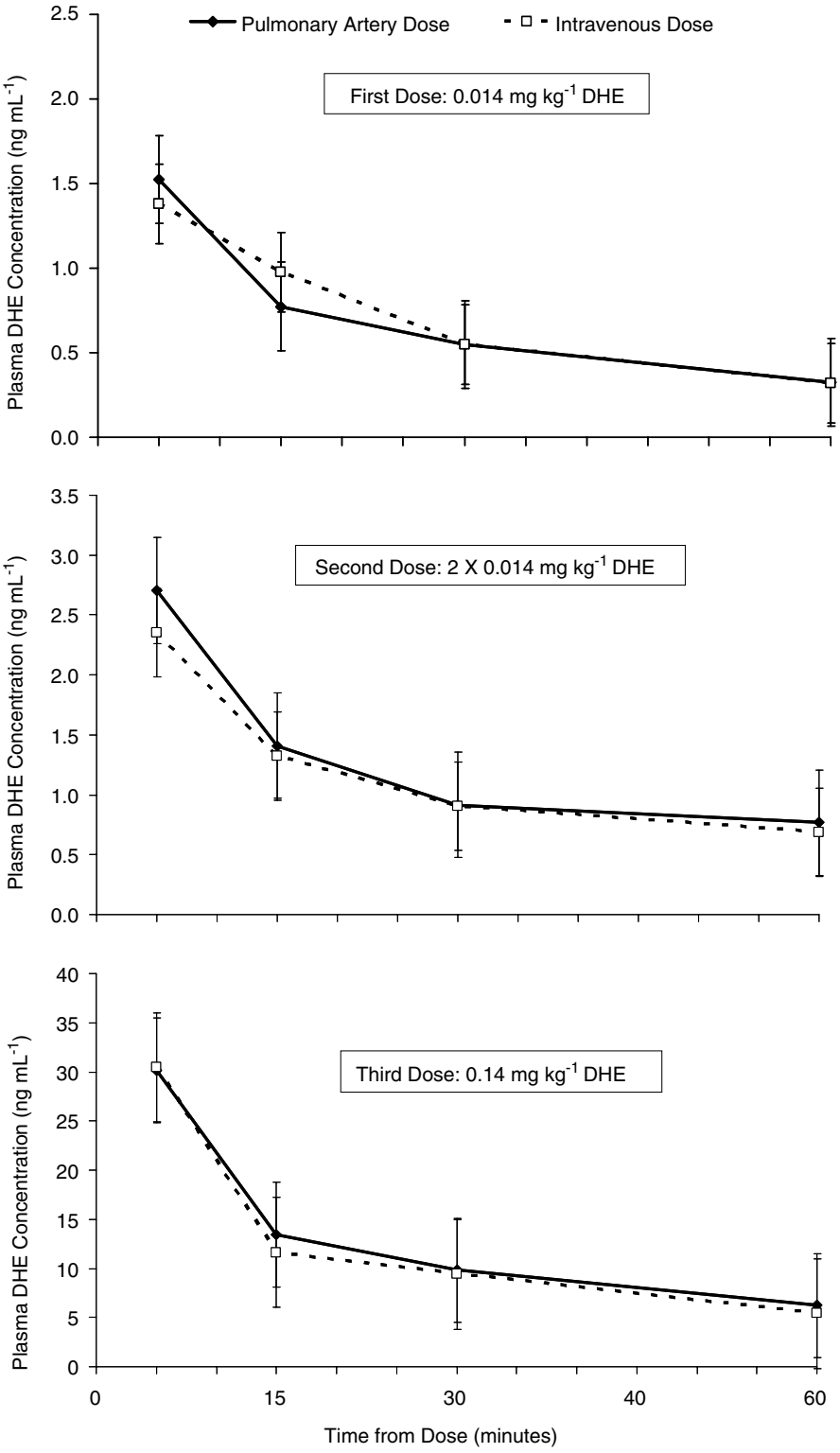

Figure 6 Mean plasma dihydroergotamine mesylate (DHE) concentrations following i.v. or i.p.a. administration of DHE 0.014 mg kg^{-1} (top) second dose (of 0.014 mg kg^{-1}) (middle) and third dose (of 0.14 mg kg^{-1}) (bottom) in beagle dogs.

inhaled dosing of DHE in humans might be expected to result in similar or fewer CV changes at a dose likely to give similar systemic exposure to known efficacious dose of i.v. DHE 1.0 mg in humans. These results extend and expand on the findings of other studies with DHE in animal models, isolated vascular tissue and humans (MaassenVanDenBrink *et al.*, 1998; Saper and Silberstein, 2006). Additional research is being undertaken using echocardiography to confirm the cardiac safety of intranasal DHE in humans. Large-scale clinical studies of inhaled DHE via a novel breath-synchronized

plume-control inhaler are now underway to explore and confirm the efficacy and safety of this method of delivery in human subjects.

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Conflict of interest

Stephen B Shrewsbury is an employee of MAP Pharmaceuticals Inc., Mountain View, CA, USA as was Jerry K Okikawa when this study was performed by Michael Stonerook at the Battelle Memorial Institute, Columbus, OH, USA, under a contract from MAP Pharmaceuticals.

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