Ibuprofen in healthy volunteers: safety, tolerability and pharmacokinetics with single and multiple doses

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Ibuprofen is an oral drug approved in Asia for asthma.
- Tolerability of 10-mg regimens has been described previously.
- Published pharmacokinetics (PK) are limited: single or 7-day repeat oral administration of 10 mg in healthy male Asian volunteers.

WHAT THIS STUDY ADDS

- Safety/tolerability and PK of a single 30-mg dose and a 30-mg twice daily (b.i.d.) 2-week regimen in male and female healthy volunteers.
- Higher-dose regimens are relevant for testing in new neurological indications.
- LC-MS/MS analytics for quantification of plasma and urine levels of ibuprofen parent and its primary metabolite (6,7-dihydrodiol-ibuprofen).

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AIMS

To investigate the safety, tolerability and pharmacokinetics (PK) of ibuprofen after a single-dose and a multiple-dose regimen.

METHODS

Healthy adult male ($n = 9$) and female ($n = 9$) volunteers were evaluated over a 17-day stay in a Phase 1 unit. Subjects were randomized 1 : 3 to either oral placebo or ibuprofen at 30-mg single administration followed by 14 days of 30 mg b.i.d. Complete safety analyses were performed and, for PK, plasma and urine samples were analysed for ibuprofen and its major metabolite.

RESULTS

Ibuprofen was generally well tolerated. No serious adverse events occurred. Treatment-related adverse events included hyperhidrosis, headache and nausea. Two subjects discontinued after a few days at 30 mg b.i.d. because of vomiting. Although sample sizes were too small to rule out a sex difference, PK were similar in men and women. The mean half-life for ibuprofen was 19 h and median T_{max} was 4–6 h. Mean (SD) steady-state plasma C_{max} and AUC_{0-24} were 60 (25) ng mL⁻¹ and 1004 (303) ng h mL⁻¹, respectively. Plasma levels of 6,7-dihydrodiol-ibuprofen were approximately 30% of the parent.

CONCLUSIONS

Ibuprofen is generally well tolerated in healthy adults when given as a single oral dose of 30 mg followed by 30 mg b.i.d. (60 mg day⁻¹) for 14 days. Plasma PK reached steady state within 2 days of starting the b.i.d. regimen. Exposure to ibuprofen was achieved of a magnitude comparable to that associated with efficacy in rat chronic pain models.

Introduction

Chronic neuropathic pain such as that associated with diabetic neuropathy can be a severe and disabling disorder that typically presents as spontaneous pain, hypersensitivity, burning, tingling and other unpleasant sensations. Ibutilast (3-isobutyl-2-isopropylpyrazolo-[1,5-a]pyridine) is a Japanese oral drug that has been registered and used in Asia for >15 years for the treatment of asthma and, more recently, for post-stroke dizziness. Although ibutilast is known to be a relatively nonselective phosphodiesterase (PDE) inhibitor, it has been discovered that another aspect of its pharmacology, i.e. glial cell regulating activity, may enable its use for the treatment of neuropathic pain [1, 2]. Glial cells (microglia, astrocytes and oligodendrocytes) have been shown to play a key role in neuropathic pain in animal models, at least in part via proinflammatory cytokine release [3]. For example, administration of the glial-regulating and anti-inflammatory cytokine, interleukin-10, can result in long-term, significant attenuation of neuropathic pain in standardized animal models of this condition [4]. Alternatively, systemic administration of ibutilast is well-tolerated, attenuates glial cell activation in the central nervous system (CNS), and attenuates mechanical allodynia in several rat models of neuropathic pain [2]. More recently, animal pharmacology studies have yielded support for the utility of ibutilast in opioid withdrawal, wherein glial activation may contribute to tolerance and dependence [5].

Apart from its glial-attenuating activity, ibutilast has some attractive 'drug-like' features, including low molecular weight (230 D), good CNS partitioning (2) and reportedly acceptable oral pharmacokinetics (PK) in humans [6]. Moreover, according to the Japanese package inserts for the marketed delayed release capsule forms, Ketast® and Pinatos®, ibutilast appears to have an excellent tolerability and safety profile at approved dose levels of 10 mg administered twice or thrice daily (20 or 30 mg day⁻¹).

To our knowledge, ibutilast ('AV411' by Avigen designation) has not been previously evaluated in a classic Phase 1 protocol except for studies confined to Asian men with dosing over ≤8 days and at doses no greater than 30 mg day⁻¹ [6, 7]. Based on preclinical work [2], previous human PK data [6, 7] and preliminary assessments of exposure–response relationships in patients [8], we predict that pain relief in patients is likely to require doses greater than those approved and currently used for asthma and other conditions in Asia (i.e. >30 mg day⁻¹). Hence, the purpose of this study was to investigate the safety, tolerability and PK of a 'higher-dose' regimen. Here, we present findings for ibutilast in male and female subjects (predominately White) in a Phase 1, double-blind, randomized, placebo-controlled study with single oral doses of 30 mg followed by oral doses of 30 mg b.i.d. (60 mg day⁻¹) for 14 days.

Methods

Study population and design

The study was of double-blind and placebo-controlled design. The planned enrolment was 16 healthy subjects (eight male and eight female), aged 18–70 years. Subjects were randomized to receive ibutilast or placebo in a 3 : 1 ratio in blocks of four with separate randomization schedules for men and women; hence 12 subjects were to receive ibutilast (six male and six female) and four were to receive placebo (two male and two female). Subjects who withdrew from the study could be replaced. The trial was approved by the Research Ethics Committee of Royal Adelaide Hospital (Adelaide, South Australia, Australia) and was conducted in accordance with Good Clinical Practices for Trial of Medicinal Products, as well as the Declaration of Helsinki and its amendments. All subjects gave their written informed consent before participation in the study.

Male and female subjects of any ethnic group were eligible for participation, provided they were able to provide written informed consent and met the following criteria: nonsmoking; 18–70 years old, no clinical abnormality (in the investigator's clinical judgment) in the laboratory and urine analyses; normal renal function with glomerular filtration rate >90 ml min⁻¹; liver enzymes less than twice the upper limit of normal; screening ECG with QT interval adjusted for heart rate within normal limits in the investigator's clinical judgment. Furthermore, all subjects had to agree to use barrier contraceptive methods during the course of this study (hormonal contraceptive alone was not acceptable) and female subjects of child-bearing potential had to have a negative pregnancy test on study day 1. The occurrence of the following prevented study enrolment, warranting exclusion: known hypersensitivity to Pinatos® (ibutilast or excipients); conditions that might affect drug absorption, metabolism or excretion; untreated mental illness, current drug addiction or abuse or alcoholism; blood donation in the past 90 days or poor peripheral venous access; platelets <100 000 mm⁻³ and/or a history of thrombocytopenia; confirmed diagnosis of chronic liver disease, e.g. chronic hepatitis B, hepatitis C infection, autoimmune disease, alcoholic or neoplastic liver disease; positive for human immunodeficiency virus; pregnant or nursing mother; history of clinically significant cardiovascular, pulmonary, endocrine, neurological, metabolic or psychiatric disease; receipt of an investigational drug in the past 30 days; or inability to swallow large capsules.

Subjects were resident in a clinical research unit for the study duration. A single administration of 30 mg was administered on day 1 followed by 14 days of b.i.d. dosing beginning on day 3. Standard meals were served at 07.00, 12.00 and 18.00 h and were given at least 1 h before dosing at approximately 08.00 and 20.00 h.

Drug The study used a generic delayed-release ibutilast product, Pinatos®, which was obtained from the Japanese

manufacturer, Taisho Pharmaceuticals Industries, Ltd (Koka, Japan) and produced under Japanese current Good Manufacturing Practice guidelines. In order to blind the drug product, over-encapsulation was performed using Swedish-orange capsules. The critical release analytical procedures (Japanese marketed product release criteria) were repeated on the over-encapsulated drug product. Visual placebos were produced by over-encapsulating white capsules filled with mannitol. Each capsule of drug product contained 10 mg of ibudilast and additives inherent to the Pinatos® product, some of which prolong dissolution of ibudilast in the human gastrointestinal tract.

Clinical safety assessments

Adverse events were coded using the MedDRA Version 9.0 coding dictionary. Treatment-emergent adverse events (TEAEs) were defined as adverse events that started after the first dose of study drug or worsened in intensity after the first dose of study drug.

Blood sampling for haematology and chemistry were drawn at screening, day 1, day 6, day 10 (haematology only), day 13 (haematology only), day 16, day 20 and at a follow-up visit, 90 days after the last dose. Urinalysis was performed on the same days as the blood except for days 10 and 13.

Vital signs were assessed at screening and in the morning (before taking study medication when it was a dosing day) on each day from day 1 to day 17 and again on day 20. A 12-lead ECG was obtained at screening, day 1 (predose and 4 h post dose), day 6 and day 16 (before the morning dose and 4 h after the evening dose) and day 20.

Pharmacokinetics

Blood samples for ibudilast and its primary metabolite (6,7-dihydrodiol-ibudilast) were drawn on day 1 predose and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 32 and 48 h after dosing; predose on day 5; on day 6 predose (at 0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h after the morning dose; predose on days 10 and 13; on day 16 predose (at 0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 22, 26 and 32 h after the morning dose; and a final sample drawn in the morning of day 20. On each occasion, a 2-ml volume of blood was drawn into a K₂ ethylenediamine tetraacetic acid vacutainer tube; blood was promptly processed into plasma by centrifugation in a refrigerated unit. Urine samples were collected from 0 to 8 h and from 8 to 16 h after the morning dose on day 1, day 6 and day 16. Plasma samples and urine specimens were collected for assay of ibudilast and its metabolite and stored frozen at approximately -70°C.

For each subject, the PK variables were calculated by noncompartmental methods from the plasma concentrations of ibudilast and 6,7-dihydrodiol-ibudilast. The following PK variables were calculated for day 1 (after a single dose): (i) maximum concentration (C_{max}); (ii) time of the maximum concentration (T_{max}); (iii) area under the curve

during the dosing interval ($AUC\tau$); (iv) area under the curve from the time of dosing to 24 h after dosing (AUC_{0-24}); (v) area under the curve from the time of dosing extrapolated to infinity ($AUC_{0-\infty}$); and (vii) terminal half-life ($t_{1/2}$). The following PK variables were calculated for day 6 and day 16 (after multiple doses): (i) C_{max} at steady state ($C_{max,ss}$); (ii) T_{max} at steady state ($T_{max,ss}$); (iii) minimum concentration at steady state ($C_{min,ss}$); (iv) $AUC\tau$ at steady state ($AUC\tau_{ss}$); (v) AUC_{0-24} at steady state ($AUC_{0-24,ss}$); and (vi) $t_{1/2}$ (day 16 only). The following three accumulation ratios were calculated for day 6/day 1, day 16/day 1 and day 16/day 6:

$$1 \text{ RA1} = C_{max,ss}/C_{max}$$

$$2 \text{ RA2} = AUC\tau_{ss}/AUC\tau$$

$$3 \text{ RA3} = AUC\tau_{ss}/AUC_{0-\infty}$$

Bioanalytics

Plasma and urine samples were evaluated for ibudilast using a bioanalytical method that was validated in accordance with Food and Drug Administration guidance [9]. To prepare the samples for analysis, ibudilast and the corresponding internal standard (AV1040, a structurally-related ibudilast analogue with a favourable fractionation pattern) were isolated from plasma and urine by addition of 0.2% formic acid in acetonitrile. Centrifugation was used to isolate the supernatant fraction. The supernatant fractions were analysed by high-performance liquid chromatography (LC) in conjunction with a triple quadrupole mass spectrometer that used electrospray ionization in tandem with positive ionization (MS/MS) under the following LC-MS/MS conditions:

Analytical column: Fluophase RP, 2.1 × 50 mm, 5 µm

Temperature: 35°C.

Mobile phase (A): 0.2% formic acid in water

Mobile phase (B): 0.2% formic acid in acetonitrile

Mass spectrometer: Sciex API 5000 with Turbo Spray Source, electrospray ionization positive injection volume: 10 µl

Gradient

Time (min)	Flow (µl/min)	A (%)	B (%)
0.00	450	75	25
0.50	450	75	25
2.50	450	5	95
4.00	450	5	95
4.01	450	100	0
4.50	450	100	0
4.51	450	75	25
6.50	450	75	25

The accuracy was $100 \pm 10\%$, the precision was $\leq 10\%$ (SD/mean) and the lower limit of quantification was 1.00 ng ml^{-1} .

Figure 1

Chemical structure of ibuprofen and its major oxidative metabolite, 6,7-dihydrodiol-ibuprofen

In addition to measuring ibuprofen, the LC-MS/MS assay was used to analyse plasma and urine samples for 6,7-dihydrodiol-ibuprofen (Figure 1). Because chemical synthesis of the metabolite was not possible, it was purified from rat and rabbit liver. As only a small amount of 6,7-dihydrodiol-ibuprofen was obtainable, the purified material was used to compare the LC-MS/MS signals for ibuprofen and the metabolite. The results showed that the calibration curves for the two molecules were reproducibly parallel over concentrations ranging from 1 to 1000 ng mL⁻¹. The mean ratio of the two curves was used to estimate a 'correction factor' multiplier used to deduce concentrations of 6,7-dihydrodiol-ibuprofen from ibuprofen calibration curves.

Statistical analyses

Descriptive and inferential statistics were performed using SAS release 8.02 (SAS Institute Inc., Cary, NC, USA). For continuous variables, the summary statistics of *N*, geometric mean, arithmetic mean (referred to as 'mean'), standard deviation about the arithmetic mean (SD), median, minimum and maximum were used. Where change from baseline was evaluated, baseline was defined as the last measurement before the first dose of study drug; the baseline mean for those subjects with change from baseline data at each time point was also evaluated. For categorical data, the number and percentage were used in the data summaries. No imputations were made for missing data.

PK parameters were derived by noncompartmental analysis using WinNonlin 5.0.1 (Pharsight Corp., Mountain View, CA, USA). All PK parameters were calculated on an individual subject basis. Summary statistics were calculated for each profile day [after a single dose on day 1 (30 mg) and after multiple doses on day 6 and day 16 (30 mg, b.i.d.)]. All plasma and urine concentrations determined to be <1.00 ng mL⁻¹ [below the quantifiable limit (BQL) for ibuprofen and 6,7-dihydrodiol-ibuprofen] were substituted to half the limit of quantification (0.50 ng mL⁻¹) for the calculation of the descriptive statistics of the concentrations and for use in the plasma concentration–time profiles, except at 0 h, where the concentration was taken as

zero. All BQL values in the terminal phase or between two quantifiable concentrations were excluded from the PK variable calculations except at time points before the *C*_{max}, where such data were to be taken as zero. AUCs were calculated by the logarithmic-linear (log-linear) trapezoidal method. For parameters derived through estimation of the terminal slope (*t*_{1/2} and AUC_{0–∞}), linear regression was performed of the logarithm of plasma concentration vs. time during the apparent elimination phase.

Results

Disposition of subjects and demographics

Of the 18 healthy volunteers who were randomized, four subjects (all of whom received ibuprofen) did not complete the study to day 20. Of these four subjects, two were withdrawn by the investigator because of nausea and vomiting and two withdrew for nonstudy-related reasons and were replaced by subjects assigned to the ibuprofen treatment.

In terms of demographics, the mean age of all subjects (ibuprofen and placebo groups) was 29.3 (SD 11.6) years and the median age of subjects in the ibuprofen group was the same in the placebo group, 25 years. The male : female ratio was 1 : 1, as expected by the randomization sequence. Mean height, weight, and body mass index (BMI) were similar in both groups (height 174 and 177 cm for ibuprofen and placebo groups; weight 77 and 78 kg, respectively; BMI 25 for each). Ethnic background was predominantly White (11% Asian).

Safety

TEAEs were of mild to moderate intensity, and incidence (Table 1) was similar in both the ibuprofen group [12 (85.7%) subjects] and the placebo group [three (75.0%) subjects]. Nine (64.3%) of the subjects from the ibuprofen group and one (25%) subject from the placebo group had TEAEs of moderate intensity, whereas the remainder of subjects had TEAEs considered mild. The three most frequently reported TEAEs were experienced in subjects from both groups;

Table 1

Adverse events

	Ibutilast (n = 14) n (%)		Placebo (n = 4) n (%)	
	Total	Possibly or probably related to study medication	Total	Possibly or probably related to study medication
Number of subjects experiencing a TEAE	12 (85.7)	11 (78.6)	3 (75.0)	2 (50.0)
Skin and subcutaneous tissue disorders	7 (50.0)	7 (50.0)	2 (50.0)	2 (50.0)
Hyperhidrosis*	7 (50.0)	7 (50.0)	2 (50.0)	2 (50.0)
Gastrointestinal disorders	5 (35.7)	4 (28.6)	3 (75.0)	2 (50.0)
Nausea	3 (21.4)	3 (21.4)	1 (25.0)	1 (25.0)
Vomiting	2 (14.3)	2 (14.3)	0	0
Abdominal pain	0	0	1 (25.0)	1 (25.0)
Diarrhoea	1 (7.1)	1 (7.1)	1 (25.0)	0
Stomach discomfort	0	0	1 (25.0)	0
Nervous system disorders	6 (42.9)	4 (28.6)	1 (25.0)	0
Headache	6 (42.9)	4 (28.6)	1 (25.0)	0
Disturbance in attention	1 (7.1)	1 (7.1)	0	0
Tremor	1 (7.1)	1 (7.1)	0	0
Infections and infestations	3 (21.4)	ND	0	0
Upper respiratory tract infection	2 (14.3)	ND	0	0
Blood and lymphatic system disorders	1 (7.1)	1 (7.1)	0	0
Leukopenia	1 (7.1)	1 (7.1)	0	0
General disorders and admin. site conditions	1 (7.1)	1 (7.1)	0	0
Feeling abnormal	1 (7.1)	1 (7.1)	0	0
Investigations	1 (7.1)	1 (7.1)	0	0
Gamma-glutamyl transferase increased	1 (7.1)	1 (7.1)	0	0

*Excessive sweating. Percentages are based on the number of subjects in the Safety Set. ND, not determined. TEAE, treatment-emergent adverse event.

these were as follows: hyperhidrosis [seven (50%), two (50%) subjects, respectively], headache [six (42.9%), one (25.0%) subjects, respectively] and nausea [three (21.4%), one (25.0%) subjects, respectively]. The TEAEs of nausea [three (21.4%) subjects] and vomiting [two (14.3%) subjects] that were considered to be possibly or probably related to study medication were all (with the exception of one subject with nausea in the placebo group) reported by subjects from the ibutilast group. Two women in the ibutilast group discontinued study medication because of several vomiting incidents in the first few days of b.i.d. dosing. As nausea has been associated with ibutilast (Ketas® and Pinatos® Package Inserts), this TEAE was not unexpected. There were four subjects (28.6%) from the ibutilast group with single episode headaches that were considered to be possibly or probably related to study medication and none from the placebo group.

Assessment of the haematology and serum chemistry data at the treatment level showed that the mean changes from baseline at various time points were small and inconsistent, with no marked differences between the ibutilast and placebo groups. Whereas laboratory tests showed a weak trend for increases in mean alkaline phosphatase (ALP) values in the ibutilast group, all values remained in the normal range. In one subject, leukopenia considered possibly related to study medication was reported on day 13. However, the baseline white blood cell count (WBC) was $4.35 \times 10^9 \text{ l}^{-1}$ (close to the lower limit of the reference range of 4×10^9 – $11 \times 10^9 \text{ l}^{-1}$), yet $3.88 \times 10^9 \text{ l}^{-1}$ on day 1 and

then on day 13 it was $3.69 \times 10^9 \text{ l}^{-1}$ and normal again at $4.13 \times 10^9 \text{ l}^{-1}$ on day 16. Another subject had an increase of γ -glutamyl transferase value at day 16 to 78 U l^{-1} from a baseline of 60 U l^{-1} that was considered to be clinically significant and possibly related to study medication. Another subject's urine was normal at baseline, but on day 20 the subject's urine had a WBC urinary count of $40 \mu\text{l}^{-1}$ and a red blood cell urinary count of $20 \mu\text{l}^{-1}$. This was asymptomatic, and on day 23 a repeat urinalysis was normal. Urinalysis abnormalities were recorded as TEAEs and considered unrelated to study medication.

In terms of vital sign measurements, no consistent changes from baseline were observed in blood pressure, temperature, respiratory rate or pulse. On the basis of the 12-lead ECG analysis, there were no changes relative to baseline in waveform morphology or interval duration measurements.

Pharmacokinetics

The ibutilast concentration–time plots for men and women (Figure 2) show that the PK profiles were essentially the same in the two sexes; thus, the data from this study suggest that sex may not be an important determinant of ibutilast PK. Because of the apparent lack of an important difference between the sexes, the concentration–time plots and PK parameters for men and women were combined to assess trends in the data and to compute the summary statistics (geometric means and SDs).

Figure 2

Mean plasma concentrations of ibuprofen in men and women. Plasma concentration vs. time profile of Ibuprofen following a single 30-mg oral dose from $n = 7$ men (●) and $n = 7$ women (□). Data presented are mean ($\pm$ SD)

Figure 3

Mean trough plasma concentrations of ibuprofen. Trough ibuprofen plasma concentrations from $n = 14$ subjects receiving a 30-mg single administration on day 1, followed by $n = 10$ subjects receiving 30 mg b.i.d. from day 3 to day 16. Data represents mean ($\pm$ SD)

A plot of the mean trough concentrations of ibuprofen (Figure 3) suggests that steady state was reached by day 5, 2 days after the start of the b.i.d. regimen. As indicated in Table 2, the geometric mean $C_{max,ss}$ concentration for ibuprofen was approximately 60 ng mL^{-1} and $C_{min,ss}$ was approximately $20\text{--}30 \text{ ng mL}^{-1}$. Correspondingly, at steady state, the peak (C_{max}) was about two to three times higher than the trough (C_{min}).

The geometric mean $t_{1/2}$ was approximately 19 h. The median T_{max} (4–6 h) was similar during the b.i.d. regimen (days 6 and 16) and after a single dose (day 1). As shown in Figure 4, during the b.i.d. regimen C_{max} appeared to be somewhat higher after the morning dose than after the evening dose. As further shown in Figure 4, the PK profile for ibuprofen was shifted upward on days 6 and 16 relative to day 1; relative to the single dose (day 1), the b.i.d.

regimen had a roughly twofold increase in plasma concentrations of ibuprofen. This upward shift was reflected in the exposure parameters (AUC and C_{max}). The geometric mean $AUC_{\tau,ss}$ during the bid regimen ($516.6 \text{ ng h mL}^{-1}$ for day 6 and $506.5 \text{ ng h mL}^{-1}$ for day 16) was approximately double the AUC_{τ} after a single dose ($246.3 \text{ ng h mL}^{-1}$); correspondingly, the accumulation ratio RA_2 was about 2 (Table 2). The geometric mean C_{max} was 87% higher during the b.i.d. regimen than after a single dose (accumulation ratio $RA_1 = 1.87$ for both day 6 and day 16).

The geometric mean $AUC_{\tau,ss}$ during b.i.d. dosing ($516.6 \text{ ng h mL}^{-1}$ for day 6 and $506.5 \text{ ng h mL}^{-1}$ for day 16) was about 10% smaller than the $AUC_{0-\infty}$ after a single dose, which corresponded to an RA_3 of approximately 0.9. That the RA_3 was <1.0 shows that there was no unexpected accumulation of ibuprofen from the single dose to the multiple doses, and the multiple dose plasma concentrations were as predicted under linear PK.

The results for 6,7-dihydrodiol-ibuprofen largely confirmed the results for ibuprofen, although these data were highly variable. The mean plasma 6,7-dihydrodiol-ibuprofen concentration–time profiles for men were slightly higher than those for women (data not shown), but at individual sample times the range of concentrations of the 6,7-dihydrodiol in men overlapped those of women, suggesting that sex differences (if any) were unimportant. Maximum plasma concentrations of 6,7-dihydrodiol-ibuprofen were achieved for most subjects within 10 h of administration of the first dose (day 1) and each of the doses during the multiple dose phase (days 6 and 16). The C_{max} for the metabolite on day 1 ranged from 2.48 to 53.4 ng mL^{-1} , from 4.95 to 91.6 ng mL^{-1} on day 6 and from 4.86 to 65.2 ng mL^{-1} on day 16. As shown in Table 3, these C_{max} values tended to be about 20–50% of the C_{max} for

Table 2
Plasma pharmacokinetic parameters for ibudilast

		Day 1 (n = 14)	Day 6 (n = 10)	Day 16 (n = 10)
AUCτ or AUCτss	ng h ml ⁻¹	246 (83.6)	517 (157)	507 (145)
AUC0-24 or AUC0-24,ss	ng h ml ⁻¹	373 (133)	987 (302)	1000 (303)
AUC0-∞	ng h ml ⁻¹	610 (321)	NC	NC
Cmax or Cmax,ss	ng ml ⁻¹	32.0 (9.38)	59.9 (19.6)	59.9 (25.2)
Tmax or Tmax,ss*	h	5.0 (3.0–8.0)	3.53 (2.0–16.0)	4.0 (1.0–22.0)
t1/2	H	19.2 (8.53)	NC	NC
Cmin,ss	ng ml ⁻¹	NA	27.5 (8.31)	22.1 (10.0)
RA1	[Cmax,ss/Cmax]	NA	1.87 (0.38)	1.87 (0.63)
RA2	[AUCτss/AUCτ]	NA	2.12 (0.36)	2.08 (0.31)
RA3	[AUCτ/AUC0-∞]	NA	0.90 (0.26)	0.88 (0.26)
RA1 day 16/day 6	[Cmax,ss/Cmax]	NA	NA	1.00 (0.19)
RA2 day 16/day 6	[AUCτss/AUCτ]	NA	NA	0.98 (0.09)

*For Tmax and Tmax,ss, the median (rather than the geometric mean) is reported and the values in parentheses are the ranges of observed values. Values reported are the geometric means (SD).

Figure 4
Mean plasma concentrations of ibudilast on days 1, 6 and 16. Plasma concentration vs. time profile of ibudilast in n = 10 subjects receiving 30 mg b.i.d. from day 3 to day 16. Mean plasma levels on day 6 (▲) and day 16 (○) from the b.i.d. regimen are contrasted with n = 14 subjects receiving a single 30-mg dose on day 1 (●). All data are means

the parent drug. A comparable ratio of metabolite : parent was observed with the AUCs.

Urine samples (which were collected from 0 to 8 h and from 8 to 16 h after the morning dose) had concentrations of 6,7-dihydrodiol-ibudilast either equal to those of ibudilast (day 1; Table 3) or up to approximately 40 times greater than those of ibudilast (days 6 and 16). The fraction of the dose recovered in the urine as unchanged drug averaged ≤0.01% on day 1 (0–16 h), and the fraction recovered as 6,7-dihydrodiol-ibudilast averaged ≤0.05%. Whereas ibudilast concentrations in the urine of most subjects were <3 ng ml⁻¹ at all time points, the group mean urine concen-

Table 3
Metabolite/parent ratios of 6,7-dihydrodiol-ibudilast

	Day 1 (n = 12)	Day 6 (n = 10)	Day 16 (n = 10)
Plasma AUCτ or AUCτss	0.25 (0.64)	0.22 (0.70)	0.18 (0.35)
Plasma Cmax or Cmax,ss	0.48 (0.63)	0.26 (0.69)	0.26 (0.56)
Urine 0–8 h	1.00*,† (16.2)	31.2* (23.6)	37.8* (24.8)
Urine 8–16 h	1.00*,† (22.2)	43.8* (27.4)	28.6* (23.2)

*Median value is reported. †n = 14. Values reported are the geometric means (SD).

trations of 6,7-dihydrodiol-ibudilast were 5–8 ng ml⁻¹ on day 1, 40–46 ng ml⁻¹ on day 6 and 30–52 ng ml⁻¹ on day 16.

Discussion

Ibudilast has been marketed in Japan and other Asian countries for >15 years for the treatment of bronchial asthma and, more recently, post-stroke dizziness. For the indications approved in Japan, patients typically are prescribed 10 mg orally twice daily (for asthma) or thrice daily (for cerebrovascular disorders), corresponding to respective doses of 20 and 30 mg day⁻¹. In a population of about 15 000 patients treated with the drug in Japan, a relatively low incidence (3.39%) of adverse events was noted (Ketas® package insert; Kyorin Pharmaceutical Co., Tokyo, Japan). The most common adverse events reported were increased liver enzyme levels in 0.30–0.36% of patients, anorexia (0.58%) and nausea (0.56%). Rare cases of thrombocytopenia (reduced platelet counts) were also reported.

In a double-blind, placebo-controlled study of 238 subjects with post-stroke dizziness, for which the dose was

30 mg day⁻¹ for 8 weeks, there were no significant differences in adverse drug reactions between the ibutilast and placebo groups [10]. Primary adverse effects in the ibutilast group were gastrointestinal distress (11.2%), including anorexia (4.3%), and hepatic dysfunction. In an open-label, 2-year study of ibutilast in 937 individuals examining the recurrence of stroke (dosed 30 mg day⁻¹), adverse events, primarily gastrointestinal, were reported in 2.3% of subjects, and the drug was discontinued in 1.8% of subjects [11].

Based on our preclinical work [2], previous human PK data [6, 7], and preliminary assessments of exposure–response relationships in patients [8], we predict that pain relief in patients is likely to require doses greater than those approved and currently used for asthma and other conditions in Asia (i.e. >30 mg day⁻¹). Clinical experience regarding larger doses of ibutilast is limited. However, two studies have reported using higher dose regimens in patients in multiweek trials with acceptable tolerability and no significant adverse events. Notably, in a study of 30 asthma patients, 13 were given 40 mg day⁻¹ of ibutilast for 6 months, which was well tolerated [12]. The incidence of adverse events overall was <10%, and none was serious. Gastrointestinal symptoms (nausea, abdominal discomfort) were mentioned as specific adverse events, but no further details were provided. In addition, ibutilast was given at 60 mg day⁻¹ to 11 patients with multiple sclerosis for 4 weeks [13]. The drug was generally well tolerated, with no serious adverse events and adverse events limited to gastrointestinal distress. None appeared to be treatment-limiting. There was one case of early withdrawal by an elderly patient with heart palpitations, which resolved within 1 month of drug discontinuation.

The present study was a Phase I, randomized, double-blind, placebo-controlled investigation in healthy subjects that assessed the safety, tolerability and pharmacokinetics of ibutilast when administered as a single oral dose of 30 mg followed by multiple oral doses of 30 mg b.i.d. (60 mg day⁻¹) for 14 days; 14 subjects were administered ibutilast and four were administered placebo.

The safety data indicate that ibutilast was generally well tolerated. The incidence of TEAEs was similar in both the ibutilast and placebo groups. The three most frequently reported TEAEs [hyperhidrosis (excessive sweating), headache and nausea] were experienced by subjects from both groups. The TEAEs of headache (four subjects) and nausea (three subjects, including two subjects with vomiting who withdrew from the study) were considered to be possibly or probably related to ibutilast. Nausea, which has been previously reported with ibutilast (Ketas® and Pinatos® Package Inserts) and other PDE inhibitors [14], was not unexpected. Interestingly, in the current study, headache and nausea were most frequently observed early in the multiday dosing period. Ibutilast appears to penetrate well into the CNS [LM Sanftner, JA Gibbons, MI Gross, BM Suzuki, FCA Gaeta, KW Johnson, unpublished data]; it is therefore

possible that the TEAEs of headache and nausea are centrally triggered.

Nine subjects experienced TEAEs of hyperhidrosis that were considered to be possibly or probably related to study medication; however, the incidence was similar across both ibutilast and placebo groups, and therefore environmental factors could not be ruled out. There were no clear, consistent changes from baseline or individual shifts in laboratory parameters, and no consistent changes from baseline in vital signs or ECG were observed. There were no findings of thrombocytopenia or elevated liver enzymes, both of which are reported in the Pinatos® package insert as potential adverse events. Overall, ibutilast administered at 60 mg day⁻¹ was well tolerated.

The PK data showed no apparent difference between men and women in ibutilast PK profiles. Given the small sample sizes used in this study, this is not considered definitive evidence that sex has no impact on ibutilast PK. Nevertheless, this result is in contrast to some findings in some animal species, in which profound sex differences were observed [LM Sanftner, JA Gibbons, MI Gross, BM Suzuki, FCA Gaeta, KW Johnson, unpublished data]. Another divergence from animal PK was observed in time dependency. Whereas rats may show increases in ibutilast clearance during multiple dose exposures to the drug [LM Sanftner, JA Gibbons, MI Gross, BM Suzuki, FCA Gaeta, KW Johnson, unpublished data], the current data from the Phase 1 study are consistent with time-invariant PK. For example, the median T_{max} (4–6 h) was similar after multiple dosing (days 6 and 16) and after a single dose (day 1). Furthermore, the day 16/day 6 exposure ratios (RA1, $C_{max,ss}/C_{max}$ and RA2, $AUC_{\tau,ss}/AUC_{\tau}$) had geometric mean values of approximately 1, suggesting stationariness during the multiple-dose phase.

The data for 6,7-dihydrodiol-ibutilast showed that plasma exposure to this metabolite was roughly 20–50% of the exposure to the parent drug. The urine data, which showed a higher ratio of the metabolite to the parent drug, provided evidence that biotransformation to 6,7-dihydrodiol-ibutilast followed by urinary excretion of this metabolite is an important pathway for ibutilast elimination.

The PK results of the current study mostly corroborate the findings of two studies in the Japanese literature that describe oral ibutilast in Asian male subjects. These studies used 10-mg Ketas® capsules (Kyorin Pharmaceutical Co.), a delayed-release ibutilast product, of which Pinatos® is the generic equivalent. The first of these examined single-dose ibutilast at 10 mg [7] and the second examined multiple-dose ibutilast at 10 mg given three times daily for 8 days [6]. Both studies also found that the metabolite 6,7-dihydrodiol-ibutilast circulated in plasma in concentrations that were roughly about half those of the parent molecule. In addition, approximately 50% of the circulating levels of 6,7-dihydrodiol-ibutilast were in the form of conjugated products, the nature of which was not

identified (e.g. glucuronide, glutathione, etc.). Urine appeared to be the primary route (60%) of elimination. Little or no parent drug was seen in the urine and excretion of metabolites was primarily via the 6,7-dihydrodiol metabolite, approximately 85% of which was present in the urine as conjugated products. In the multiple-dose study, steady-state peak plasma levels of ibudilast and the 6,7 dihydrodiol metabolite and its conjugates were achieved within 3 days and appeared to be sustained for the next 5 days.

Although the PK findings of the present study mostly agree with the results of the previous studies [6, 7], there were differences in the $t_{1/2}$, which was shorter in the Japanese studies than in the current study (roughly 8–12 h vs. 19 h). In addition, there were differences in the dose-normalized plasma exposure, which tended to be higher in the Japanese studies. In the present study, 30 mg of ibudilast resulted in a geometric mean C_{max} of 32.0 (SD 9.38) ng ml⁻¹ and a geometric mean $AUC_{0-\infty}$ of 609.8 (SD 321.2) ng h ml⁻¹ (Table 2). When normalized per mg of dose, these parameters correspond to 1 ng ml⁻¹ per mg (C_{max}) and 20 ng h ml⁻¹ per mg ($AUC_{0-\infty}$). In the Japanese studies, the corresponding dose-normalized parameters were nearly 3 ng ml⁻¹ per mg (C_{max}) and 40 ng h ml⁻¹ per mg ($AUC_{0-\infty}$). Thus, whereas the terminal half-life tended to be shorter in the Japanese studies, exposure per mg of ibudilast administered was about two times higher. The difference in the terminal half-life may be related to differences in the sampling schedule, which was longer in the present study (48 h vs. 24–32 h). However, the reason for the exposure difference is unknown. It is possible that the racial composition of the cohorts may partially account for the exposure difference, the current trial comprising mostly Whites and the Japanese trials comprising entirely Asians. Although one of the two Asian subjects in the study had the highest exposure at steady state, considering the dataset as a whole, however, was inconclusive, as both of the Asian subjects had exposures close to the group mean.

In conclusion, the results of this study have indicated that ibudilast (AV411) was generally well tolerated in healthy adults when given as a single oral dose of 30 mg followed by 30 mg b.i.d. (60 mg day⁻¹) for 14 days. Plasma PK reached steady state within 2 days of starting the twice-daily dosing regimen, and yielded exposures comparable to efficacy in animal models of neuropathic pain.

Competing interests

L.H., E.C., D.J., M.I.G., J.B.D., L.M.S. and K.W.J. are employed by, and possess stock options for, Avigen Inc., the developer of ibudilast (AV411). P.R. was Principal Investigator in the clinical trial and was not compensated as a consultant. J.A.G. was a paid consultant for Avigen Inc. The authors gratefully acknowledge the medicinal chemistry and development contributions of Dr Federico Gaeta, the support of Dr Ken

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