CLINICAL PHARMACOKINETICS

Clin Drug Invest 2001; 21 (2): 129-136
1173-2563/01/0002-0129/$22.00/0

Multiple-Dose Pharmacokinetics of Atrasentan, an Endothelin-A Receptor Antagonist

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Abstract

Objective: To determine the single- and multiple-dose pharmacokinetics of atrasentan, a highly selective endothelin-A receptor antagonist that is currently being investigated for the treatment of prostate cancer and cardiovascular disorders.

Design: Phase I, randomised, placebo-controlled, double-blind, single- and multiple-dose study of orally administered atrasentan.

Methods: Single daily oral doses of 1, 5, 10, 15, 20, 25, 30 or 40mg of atrasentan or placebo were administered to healthy male volunteers (n = 72; six active and three placebo per drug administration group) on study day 1 and days 3 to 9. Atrasentan plasma concentration-time profiles for day 1 and day 9 were used to assess atrasentan pharmacokinetics.

Results: Except for the 1mg group, atrasentan plasma concentrations increased rapidly after single and multiple administration, declining thereafter biexponentially with a study-wide harmonic mean (pseudo-SD) half-life of 21 (12) hours and mean (SD) apparent total body clearance (CL/F) of 28 (9.8) L/h. For the 1mg group, there was no apparent distribution phase and the absorption was slower. Drug administration in the 40mg group was discontinued prematurely because of adverse events. Except for lower-than-predicted maximum plasma concentration (C_{max}) values for the 1mg group, drug exposure (C_{max}, trough concentration and area under the concentration-time curve) increased linearly with dose, and CL/F values were similar across groups, after single- and multiple-dose administration. Steady state was reached within 4 days of drug administration.

Conclusion: The pharmacokinetics of atrasentan are dose- and time-independent after single- and multiple-dose administration over the range of 1 to 30 mg/day.

Endothelins are a family of three paracrine/autocrine peptides (ET-1, ET-2 and ET-3) produced in a variety of tissues where they apparently act as modulators of vasomotor tone, cell proliferation and protein synthesis.[1-3] Two types of endothelin receptors, ET_A and ET_B, have been identified.[4] ET_A receptors have a high affinity for ET-1 and ET-2, whereas ET_B receptors have equal affinity for all of the ET-related peptides.[4] ET_A receptors are primarily responsible for ET-1-mediated vasoconstriction and cell proliferation.[3,4] ET_B receptors can contribute to the vasomotor tone and are thought to provide a mechanism for the clearance of endothelins as well as feedback regulation of endothelin synthesis and secretion.[4]

Atrasentan (ABT-627) is an endothelin antagonist with approximately 1800-fold selectivity for the ET_A-receptor. In a dose-dependent manner, atrasentan inhibits the increase in arterial blood pressure induced by ET-1. In addition, in several animal models, atrasentan and its racemate, A-127722, have yielded promising results that indicate that antagonism of the effects of ET-1 may be beneficial in the pathophysiological states of congestive heart failure,[5] pulmonary hypertension,[6] essential hypertension, restenosis,[7] renal failure[8] and cancer.[9] Accordingly, atrasentan is being developed to evaluate its therapeutic utility in several of these disorders. The objective of this study was to evaluate the pharmacokinetics and safety of multiple doses of atrasentan, administered once daily. Results of this study were used to guide the selection of doses for phase II studies. This is the first report of the multiple-dose pharmacokinetics of atrasentan in humans.

Participants and Methods

Participants

A total of 72 healthy adult nonsmoker male volunteers were enrolled in the study. The participants' average (SD) age and bodyweight were 31 (8.2) years and 82.4 (10.6) kg, respectively. The ethics committee at Innovex, Inc. approved the study protocol. Written informed consent was obtained from each participant after the purpose and nature of the study had been explained. Participants were screened for eligibility based upon the results of medical history, physical examination, laboratory profile, chest x-ray and electrocardiogram.

Study Design

This was a Phase I, randomised, placebo-controlled, double-blind, single and multiple oral dose study of atrasentan. Seventy-two healthy males were equally divided into eight drug administration groups. A randomisation schedule was computer-generated for each drug administration group prior to the start of the study. Within each drug administration group, the participants were assigned, in a 2:1 ratio, to receive either atrasentran or matching placebo according to the randomisation schedule. The drug administration schedules were designed such that successively higher doses were administered after safety had been determined for the previous group. Single daily oral doses of 1, 5, 10, 15, 20, 25, 30 or 40mg of atrasentan or placebo were administered to participants on study day 1 and days 3 through 9. No dose was administered on day 2. Atrasentan was formulated by Abbott Laboratories as a 1 mg/ml oral solution in a vehicle of 50% glycerin, 14% alcohol and water. The vehicle was utilised as placebo.

Participants from each dose group were confined to the research unit from day –1 to day 13. Normal research unit meals were served during confinement and participants were not permitted to engage in any strenuous activity or exercise during the study. Drug administration was accomplished after an 8-hour fast and the first post-dose meal was consumed approximately 2 hours after drug administration. Participants were asked to abstain from caffeine starting 3 days prior to confinement to the research unit on day –1 until they were released from the unit on study day 13. Blood samples were collected after the first dose on day 1 over a 48-hour interval and after the last dose on day 9 over a 96-hour interval for determination of atrasentan plasma concentrations.

Analytical Methodology

Atrasentan plasma concentrations were determined using a validated HPLC method with fluorescence detection.[10,11] The assay was specific and sensitive with a lower quantifiable limit for atrasentan of 0.21 μg/L from 1ml of plasma. The assay was unbiased with coefficients of variation (CVs) below 10%.

Pharmacokinetic Analyses

Noncompartmental Analysis

Atrasentan pharmacokinetic parameters for the first and last doses were determined using standard noncompartmental methods.[12] For the first and last dose interval, the following parameters were determined: maximum observed concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma concentration versus time curve (AUC) calculated by the trapezoidal rule, apparent total body clearance (CL/F) derived as dose/AUC, terminal phase elimination rate constant (β) and elimination half-life ($t_{½β}$) calculated as ln(2)/β. Due to the short collection period, β was not calculated after the first dose administration except for the 40mg group, where no steady-state data were available. For calculation of single-dose AUC to infinity ($AUC_∞$), the extrapolation from the last measurable concentration was calculated as the quotient of the last measurable concentration on day 2 and β determined after administration of the last dose. Additionally, pre-dose plasma concentrations (C_{min}) were determined on days 7, 8 and 9 (pre-dose samples) and day 10 (24-hour sample of day 9).

Parametric Modelling

A two-compartment open model with first-order absorption into and first-order elimination from the central compartment, and first-order intercompartmental transfer, was used to characterise atrasentan plasma concentration-time profiles. The pharmacokinetic model was fitted to the mean plasma concentration-time data from the eight drug administration groups using ADAPT II.[13]

Unweighted and mixed/proportional weighting schemes were investigated, with the latter proving more appropriate for the 10^3 range in observed concentrations. The data variance was assumed to be similar to analytical error, with a coefficient of variation (CV) of 10% at or below 1 μg/L and 5% at 250 μg/L.

Statistical Analyses

Dose proportionality and linear kinetics were assessed by analyses of covariance (ANCOVAs) for day 1 (first dose) and day 9 (last dose) pharmacokinetic parameters. The pharmacokinetic parameters analysed included t_{max}, β and dose-normalised values of C_{max}, C_{min} and AUC. The model included classification by dose level and had baseline bodyweight and age as co-variates. Because of the unusual concentration versus time profiles in the 1mg dose group, similar analyses were performed after excluding data from the 1mg dose group.

To assess attainment of steady state after multiple doses of atrasentan, an analysis of variance (ANOVA) was performed on 0-hour pre-dose concentrations (C_{min}) on days 7 and 9. An ANOVA was also performed on the change in dose-normalised AUC from the first dose ($AUC_∞/D$) to the last dose (AUC_{24h}/D). PROC GLM of SAS version 6.12 was used for the ANCOVA and ANOVA models, and type III sums of squares were used for all tests of hypotheses. A p-value equal to or less than 0.05 was considered statistically significant.

Results

Mean atrasentan plasma concentration-time profiles obtained after single- and multiple-dose administration are shown in figure 1. Except for the 1mg group, atrasentan plasma concentrations increased rapidly after single- and multiple-dose administration, reaching maximum plasma concentrations within 1 hour, declining thereafter biexponentially. The absorption after the administration of the 1mg dose was slower and the distribution phase was not apparent. Mean (SD) atrasentan pharmacokinetic parameters are presented in table I. Drug administration in the 40mg

group was discontinued prematurely because of the adverse event of headache.

Since there was no statistically significant effect due to age, this covariate was dropped from the statistical model. Bodyweight was not a significant covariate ($p > 0.05$) for all pharmacokinetic parameters tested except for dose-normalised AUC_∞ ($p = 0.016$). This may be due to randomness since it was significant for single-dose administration but not at steady state.

Maximum Plasma Concentration

The mean C_{max} values obtained after single and multiple doses of 1 to 40mg increased linearly with dose ($r^2 > 0.87$; fig. 2, top panel). The mean dose-normalised C_{max} values (C_{max}/D) after single-dose administration were similar for all groups except for the noticeably lower value for the 1mg group. The same was true for C_{max}/D on day 9. Indeed the dose effect was significant, both for days 1 and 9 ($p = 0.016$ and 0.029, respectively). However, when data from the 1mg group were excluded, the dose effect was not statistically significant ($p > 0.2$).

Time to Maximum Plasma Concentration

Except for the 1mg group, maximum plasma concentrations were attained within 1 hour after administration in all participants. For the 1mg group, after an initial increase in the concentrations within the first few hours after drug administration, secondary peaks were observed at later times.

Table I. Atrasentan pharmacokinetic parameters

Dose (mg)		Single dose					Multiple dose					
		C_{max} (μg/L)	t_{max} (h)	AUC_{24h} (μg/L • h)	AUC_∞ (μg/L • h)	CL/F (L/h)	C_{max} (μg/L)	t_{max} (h)	C_{min} (μg/L)[a]	AUC_{24h} (μg/L • h)	$t_{½β}$ (h)[b]	CL/F (L/h)
1	Mean	1.5	4.9	17	52	24	2.6	6.4	1.7	40	37	37
	SD	1.3	9.5	13	24	14	1.9	5.3	1.9	28	12	24
5	Mean	26	0.3	120	220	25	25	0.3	4.1	210	25	27
	SD	14	0.1	33	69	8.4	4.7	0.1	2.5	81	6.3	10
10	Mean	49	0.4	190	370	28	46	0.5	7.8	330	25	33
	SD	24	0.3	26	52	3.7	17	0.3	0.93	86	4.0	13
15	Mean	97	0.3	330	530	30	90	0.3	8.7	490	13	32
	SD	57	0.1	110	170	7.9	26	0.1	1.8	100	12	5.7
20	Mean	130	0.3	470	740	28	140	0.5	11	720	20	29
	SD	42	0.1	98	200	6.9	50	0.3	3.0	190	4.7	6.8
25	Mean	130	0.5	600	960	27	190	0.5	18	1100	21	25
	SD	37	0.1	150	220	6.3	97	0.1	4.8	440	4.0	7.3
30	Mean	200	0.5	750	1200	25	220	0.5	19	1100	22	29
	SD	61	0.0	78	180	3.5	92	0.1	2.9	240	6.2	6.1
40	Mean	170	0.6	1000	1600	26					20[c]	
	SD	46	0.2	170	430	6.3					3.9	

a Day 9, pre-dose concentration.

b Reported as harmonic mean and pseudo-SD.

c Calculated from the single-dose data.

AUC_∞ = area under the plasma concentration-time curve from zero to infinity; **AUC_{24h}** = area under the plasma concentration-time curve to 24 hours; **C_{max}** = maximum plasma concentration; **C_{min}** = trough (pre-dose) plasma concentration; **CL/F** = apparent total body clearance; **t_{max}** = time to C_{max}; **$t_{½β}$** = elimination half-life.

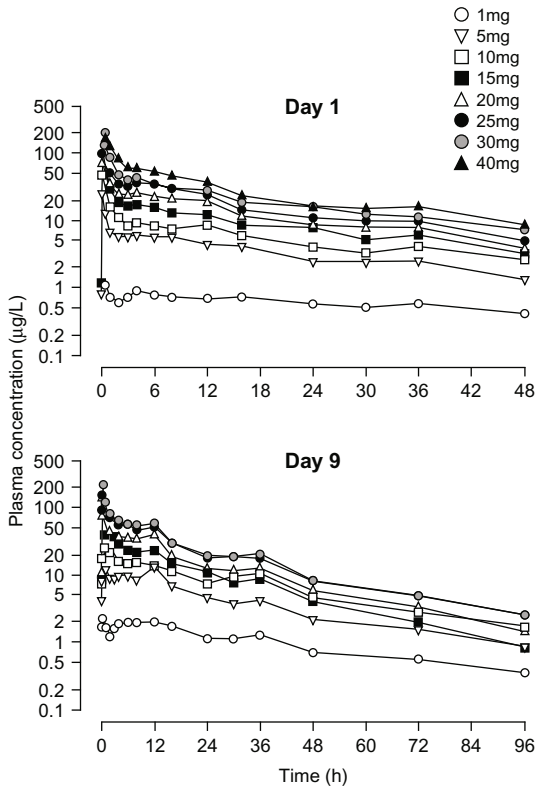

Fig. 1. Mean atrasentan plasma concentration-time profiles obtained after single- and multiple-dose administration of atrasentan.

Area Under the Concentration-Time Curve and Apparent Clearance

The mean AUC values increased dose-proportionally ($r^2 > 0.96$; fig. 2, bottom panel). The dose effect was not significant ($p > 0.149$) for dose-normalised AUC values after single- and multiple-dose administration, indicating that atrasentan pharmacokinetics are dose-independent in the dose range of 1 to 30mg.

The average AUC_∞ values obtained after single-dose administration were comparable to the AUC_{24h} values obtained after multiple-dose administration in all groups (fig. 2, lower panel). Furthermore, the difference between day 9 AUC_{24h} and first-dose AUC_∞, excluding the 1mg dose group, was not statistically significant ($p = 0.212$). These results indicate that the pharmacokinetics of atrasentan are time-independent in the dosage range of 1 to 30 mg/day.

CL/F was similar after single- and multiple-dose administration, averaging 27 (SD 12) and 30 (SD 12) L/h for days 1 and 9, respectively, suggesting that the CL/F of atrasentan is dose- and time-

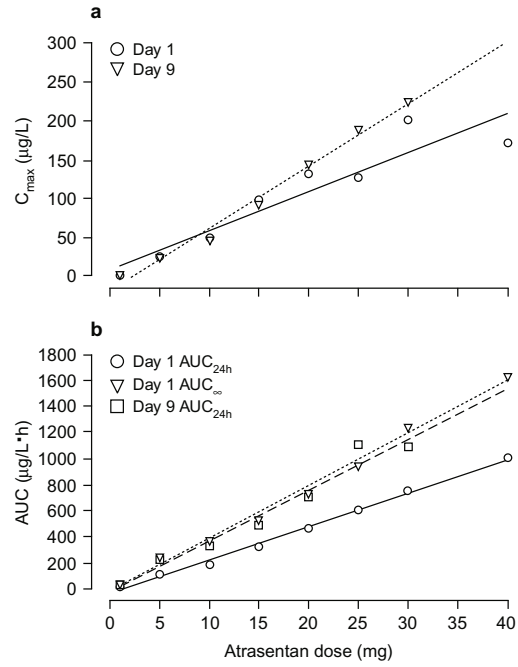

Fig. 2. Mean maximum concentration (C_{max}) [**a**] and area under the concentration-time curve (AUC) [**b**] values obtained after single (day 1) and multiple once-daily (day 9) administration of atrasentan. The lines represent the linear regression of the C_{max} or AUC values versus atrasentan dose.
Day 1 C_{max} (continuous line), $y = 9.26 + 4.97x$ ($r^2 = 0.877$); day 9 C_{max} (dotted line), $y = -17.2 + 7.89x$ ($r^2 = 0.987$). Day 1 AUC_{24h} (continuous line), $y = -32.4 + 25.6x$ ($r^2 = 0.996$); day 1 AUC_∞ (dotted line), $y = -23.9 + 40.6x$ ($r^2 = 0.994$); day 9 AUC_{24h} (dashed line), $y = -20.8 + 38.9x$ ($r^2 = 0.966$).
AUC_∞ = area under the plasma concentration-time curve from zero to infinity; **AUC_{24h}** = area under the plasma concentration-time curve to 24 hours.

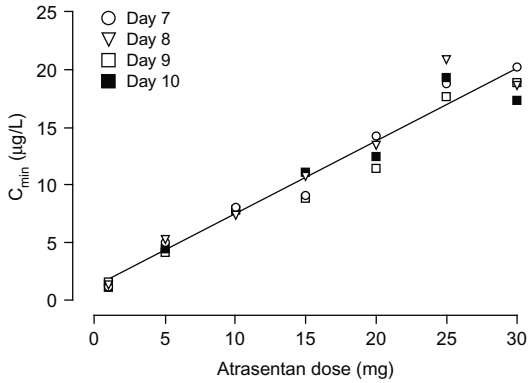

Fig. 3. Mean trough (pre-dose) concentration (C_{min}) versus atrasentan dose. Days 7, 8, 9 and 10 represent 4, 5, 6 and 7 consecutive daily doses, respectively. The continuous line represents the linear regression of the data characterised by the equation $y = 1.179 + 0.618x$ ($r^2 = 0.952$).

independent. There was no indication of any deviation from linear pharmacokinetic behaviour after 7 days of daily drug administration. The study-wide mean CL/F was 28 (SD 9.8) L/h.

Trough Concentration

The mean C_{min} values increased linearly with dose ($r^2 > 0.95$; fig. 3). The mean dose-normalised C_{min} values after multiple-dose administration were similar for all groups. The dose effect was not statistically significant ($p > 0.14$) for day 9 dose-normalised C_{min}.

The average C_{min} values were similar for days 7, 8, 9 and 10 (fig. 3), suggesting that steady state was predictably reached within 4 days of daily drug administration. Indeed, the ANOVA for dose-normalised C_{min} revealed that the difference between day 7 and day 9 was not statistically significant ($p = 0.865$).

Half-Life

In contrast to the other groups, $t_{½\beta}$ for the 40mg group was calculated from the single-dose data; therefore, the 40mg group was not included in the statistical analysis for β. Except for the 1mg group, the harmonic mean half-life obtained on day 9 ranged from 13 to 25 hours. The half-life for the 1mg group was higher, with a harmonic mean of 37 hours. The analysis for β found the test statistic for dose to be not significant ($p = 0.064$). The study-wide harmonic mean (pseudo-SD) half-life was 21 (12) hours.

Parametric Modelling

A two-compartment open model optimally characterised atrasentan plasma concentration-time data. Observed atrasentan plasma concentrations versus those predicted by the two-compartment model are shown in figure 4. The model-estimated pharmacokinetic parameters, i.e. absorption rate constant (k_a), volume of the central compartment (V_c/F), volume of the peripheral compartment (V_p/F), clearance (CL/F) and distribution clearance (CL_d/F), are presented in table II.

Initially, a single k_a was estimated for all groups. However, the plasma concentration-time profiles and noncompartmental analyses suggested that the rate of absorption was substantially slower for the 1mg group compared with the higher dose groups. Subsequently, separate absorption rate constants

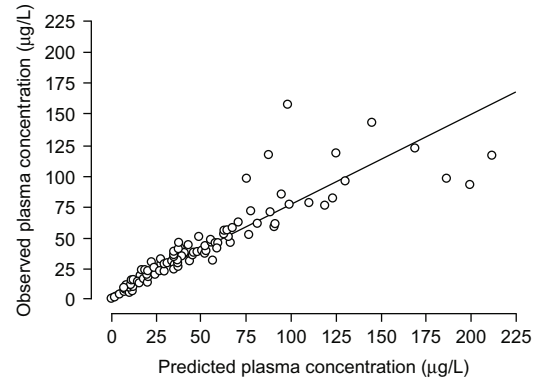

Fig. 4. Observed atrasentan plasma concentrations versus those predicted by the two-compartment model. The continuous line represents the linear regression of the data characterised by the equation $y = 3.776 + 0.716x$ ($r^2 = 0.866$).

Table II. Atrasentan pharmacokinetic parameters estimated from parametric model

Parameter and unit	Estimate	CV (%)
k_a (h^{-1})	0.16[a]	6
	1.5[b]	3
V_c/F (L)	11	74
V_p/F (L)	690	1
CL/F (L/h)	29	1
CL_d/F (L/h)	270	3
r^2	0.866	

a 1mg group.

b 5 to 40mg groups.

CL/F = apparent total body clearance; **CL_d** = apparent distribution clearance; **CV** = coefficient of variation; **k_a** = absorption rate constant; **V_c/F** = apparent volume of the central compartment; **V_p/F** = apparent volume of the peripheral compartment.

were estimated for the 1mg group and all other groups. Use of two different k_a values resulted in substantial improvement of the model fits and significant reduction of the objective function (weighted sum of squared residuals) as well as lower Akaike Information Criteria and Schwarz Criteria. The model-estimated k_a values were $0.16h^{-1}$ for the 1mg group and $1.5h^{-1}$ for all other groups.

Atrasentan pharmacokinetic parameters estimated in this study are in excellent agreement with previously reported values.[11] The precisions of the parameter estimates from ADAPT were ≤6% for all parameters except V_c. V_c was poorly estimated, with a CV of 74%. The large total apparent volume of distribution ($V_c/F + V_p/F$), approximately 700L, suggests extensive tissue distribution of atrasentan.

Discussion

Endothelins are a family of paracrine/autocrine peptide factors that contribute to cell proliferation and hormone production through a G-protein-coupled pathway.[3] Dysfunction of endothelin regulation has been implicated in several cancers, including prostate, ovarian and breast.[3,9,14,15] Atrasentan, a highly selective antagonist of ET_A receptors, is postulated to inhibit tumour growth. Atrasentan is currently under development at Abbott Laboratories for treatment of prostate and other cancers. Atrasentan has been evaluated principally in patients with hormone-refractory prostate cancer.[15] The purpose of this study was to evaluate the multiple-dose pharmacokinetics of atrasentan.

Atrasentan plasma concentrations increased approximately linearly with dose. Except for the 1mg group, atrasentan plasma concentrations increased rapidly after single- and multiple-drug administration, declining thereafter biexponentially with a study-wide harmonic mean (pseudo-SD) half-life of 21 (12) hours and mean (SD) CL/F of 28 (9.8) L/h. With the 1mg group, there was no apparent distribution phase and the absorption was slower. Except for lower than predicted C_{max} values for the 1mg group, the C_{max}, C_{min} and AUC values increased linearly with dose, and CL/F values were similar across the groups, after single- and multiple-dose administration. Therefore, it can be concluded that atrasentan pharmacokinetics are dose-independent.

There are several sources of evidence for time-independent pharmacokinetics. First, CL/F was similar after single- and multiple-dose administration. Secondly, the pre-dose plasma concentrations (C_{min}) on days 7, 8, 9 and 10 were similar (fig. 3) and the dose-normalised C_{min} values for days 7 and 9 were not statistically significantly different. Thirdly, the first-dose AUC_∞ and last-dose (day 9) AUC_{24h} values were similar (fig. 2) and not statistically significantly different (excluding the 1mg group).

A two-compartment model with linear absorption, distribution and elimination optimally characterised the plasma concentration-time data from all groups. There was no dose dependency in the apparent clearance; however, some dose dependency in the absorption was observed, mainly at the 1mg group. The large volume of distribution suggests that on average 90% of the drug in the body is in the tissue compartment. A previous study of single doses of atrasentan has shown that

atrasentan pharmacokinetics are linear at doses ≤23.25mg.[11] Nonlinearity in tissue distribution was observed when a higher dose of 139.5mg was administered. The half-maximal tissue-binding coefficient was estimated at 300 μg/L, which is above concentrations attained in the present study.

The binding affinity (K_i) of atrasentan for ET_A receptors has been determined to be 0.034 nmol/L (17.4 ng/L) in the absence of protein from *in vitro* receptor binding studies, and this binding is attenuated approximately 30-fold by plasma proteins (data on file, Abbott Laboratories). The mean (SD) steady-state C_{max} for the 1mg group was 2.6 (1.9) μg/L, approximately 5-fold higher than the K_i (after accounting for presence of plasma proteins) for ET_A receptors. Therefore, complete antagonism of ET_A receptors by atrasentan might be expected at doses of 1mg or higher.

Conclusion

Atrasentan pharmacokinetics were dose- and time-independent after single- and multiple-dose administration over the range of 1 to 30 mg/day. The study-wide harmonic mean (pseudo-SD) half-life was 21 (12) hours and mean (SD) CL/F was 28 (9.8) L/h. Steady state was achieved within 4 days of once-daily drug administration. Atrasentan plasma concentration-time data were optimally characterised by a two-compartment model with linear absorption, distribution and elimination. Oral doses of atrasentan ranging from 1 to 30mg in man achieve plasma concentrations that have demonstrated biological activity in preclinical models. This dose range will be sufficient to test the therapeutic responses of a number of disease states to selective and potent ET_A receptor antagonism.

Acknowledgements

Abbott Laboratories, Abbott Park, Illinois, USA, funded this study. E. Samara is currently at Chiron Corporation, San Francisco, CA, USA.

References

1. Levin ER. Endothelins. N Engl J Med 1995; 333 (6): 356-63
2. Raffa RB, Schupsky JJ, Martinez RP, et al. Endothelin-1-induced nociception. Life Sci 1991; 49 (11): L61-5
3. Bagnato A, Catt KJ. Endothelins as autocrine regulators of tumor cell growth. Trends Endocrinol Metab 1998; 9 (9): 378-83
4. Opgenorth TJ. Endothelin receptor antagonism. Adv Pharmacol (New York) 1995; 33 1-65
5. Borgeson DD, Grantham JA, Williamson EE, et al. Chronic oral endothelin type A receptor antagonism in experimental heart failure. Hypertension 1998; 31 (3): 766-70
6. Chen SJ, Chen YF, Opgenorth TJ, et al. The orally active non-peptide endothelin A-receptor antagonist A-127722 prevents and reverses hypoxia-induced pulmonary hypertension and pulmonary vascular remodeling in Sprague-Dawley rats. J Cardiovasc Pharmacol 1997; 29 (6): 713-25
7. McKenna CJ, Burke SE, Opgenorth TJ, et al. Selective ET(A) receptor antagonism reduces neointimal hyperplasia in a porcine coronary stent model. Circulation 1998; 97 (25): 2551-6
8. Opgenorth TJ, Adler AL, Calzadilla SV, et al. Pharmacological characterization of A-127722: an orally active and highly potent ETA-selective receptor antagonist. J Pharmacol Exp Ther 1996; 276 (2): 473-81
9. Rosano L, Salani D, Castro VD, et al. Inhibition of tumor growth and angiogenesis by ABT-627, an endothelin receptor A antagonist, in ovarian carcinoma xenografts [abstract]. Proc Am Assoc Cancer Res 2000; 41: 327
10. Bryan PD, Sapochak LB, Tames MM, et al. Determination of atrasentan by high performance liquid chromatography with fluorescence detection in human plasma. Submitted for publication
11. Samara E, Dutta S, Cao G, et al. Single dose pharmacokinetics of atrasentan, an endothelin-A receptor antagonist. J Clin Pharmacol. In press
12. Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker. 1982
13. D'Argenio DZ, Schumitzky A. A program package for simulation and parameter estimation in pharmacokinetic systems. Comput Programs Biomed 1979; 9 (2): 115-34
14. Ryan CW, Janus TJ, Vogelzang NJ, et al. A phase I study of men and women with refractory malignancies given daily dosing of an endothelin receptor antagonist [abstract]. Proc Am Soc Clin Oncol 2000; 19: 201a
15. Carducci MA, Vogelzang NJ, Daliani D, et al. A placebo controlled phase II dose-ranging evaluation of an endothelin-A receptor antagonist for men with hormone refractory prostate cancer and disease-related pain [abstract]. Proc Am Soc Clin Oncol 2000; 19: 334a

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