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Pharmacokinetics and in-situ absorption studies of a new anti-allergic compound 73/602 in rats

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Abstract

Compound 73/602 (AA) is a structural analogue of vasicinone, an alkaloid present in the leaves and roots of *Adhatoda vasica* (Acanthaceae). It possesses potent antiallergic activity in mice, rats and guinea pigs. The pK_a of AA was determined to be 2.87 ± 0.19 by UV spectrophotometry. The absorption kinetics of this compound were studied in-situ using a rat gut technique at pH 2.6 and 7.4. The rate of absorption at pH 2.6 (0.0288 ± 0.004 min^{-1}) was slightly less than at pH 7.4 (0.035 ± 0.0008 min^{-1}). This characteristic behavior was attributed to the low pK_a of AA, a weekly basic compound, where nearly 35% of the compound remained in the unionized form at pH 2.6. Also, the return of compound into the mucosal lumen from the blood capillaries over a period of 2 h after administering a 2 mg dose in tail vein was less than 0.3%. Hence it was concluded that entero-enteric circulation of AA did not contribute significantly to the in-situ absorption rates. Pharmacokinetic parameters of AA were determined in male rats after administering a single 10 mg/kg intravenous dose (i.v.) and 50 mg/kg oral bolus dose. Following i.v. administration the initial decline in serum concentration was rapid with half-life of 20.2 min. After a single oral dose the concentration-time data of AA in rats was best described by a one-compartment model with equal first order absorption and apparent elimination rate constants. The half-life of the decline in serum concentration of AA following oral administration was 50.6 min, indicating absorption rate limiting disposition at the high dose given. Comparison of AUC of oral and i.v. data indicates that only about 60% of the oral dose reach the systemic circulation.

Keywords: Compound 73/602; Anti-allergic; Pharmacokinetics; Absorption

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1. Introduction

Compound 73/602, [7-methoxydeoxyvasicinone (or) 2,3-dihydro-7-methoxypyrrolo {2, 1-b} quinazolin-9(1H)-one], (AA) Fig. 1 is a structural analogue of vacicinone, an alkaloid present in the leaves and roots of *Adhatoda vasica* (Acanthaceae) (Southon et al., 1989). In the indigenous system of medicine vasicinone has been widely used as an expectorant and mild bronchial antispasmodic. This biological activity is attributed to the presence of alkaloids vasicine and vasicinone (Lahiri and Pradhan, 1964; Gupta et al., 1971). AA was synthesized (Devi et al., 1976) as one of the congeners of vasicinone to study the structure activity relationship, and was found to possess potent antiallergic activity in mice, rats and guinea pigs (Arya, 1982). Its antiallergic activity has been studied by antagonism of passive cutaneous anaphylactic reaction in mice and rats. Inhibition of histamine release, induced by passive peritoneal anaphylaxis, was tested in rats and guinea pigs. The results indicate that AA effectively inhibits reaginin mediated cutaneous anaphylaxis by 77–92% at oral doses ranging from 0.5 to 1 mg kg^{-1} (Lahiri and Pradhan, 1964). Submaximal histamine release induced by antigen was also significantly reduced (62–100%) as compared to control values and these results were comparable to those obtained with sodium chromoglycate (Brogden et al., 1974).

AA is a new antiallergic compound under development. The present study explores the pharmacokinetics and absorption characteristics of this candidate drug in rats.

2. Materials and methods

2.1. Chemicals and reagents

Compound 73/602 was synthesized and purified in this institute. Water (triple distilled) was obtained by distillation from all quartz distillation unit. All other chemicals were of either AR or HPLC grade and were used without further purification. Normal rat serum was separated from the blood collected before sacrificing normal healthy Sprague–Dawley rats of the institute's Laboratory Animal Division.

2.2. Stock and standard solutions of AA

Stock solutions of AA (100 μg ml^{-1}) was prepared by dissolving 10 mg in 100 ml of acetonitrile. It was diluted to prepare two working standards of 10 μg ml^{-1} and 1 μg ml^{-1}. The analytical standards of AA containing 10, 25, 50, 100, 250, 500, 1000, 2000 ng ml^{-1} in mobile phase were prepared by appropriately diluting the stock and working dilutions in 10 ml volumetric flasks.

For determining pK_a by UV spectrophotometry a stock solution of AA (10 mg ml^{-1}) was prepared in ethanol by dissolving 50 mg in 5 ml. This stock solution (50 μl) was pipetted in 11 volumetric flasks of 10-ml capacity and final volume was made up with appropriate pH buffers/hydrochloric acid solutions. Blanks were prepared by pipetting 50 μl of absolute alcohol into 11 volumetric flasks of 10-ml capacity and final volume was made up with the appropriate buffers/hydrochloric acid solutions. For in situ absorption studies, 2 μg ml^{-1} of AA solutions were prepared in appropriate buffers by diluting a 10 mg ml^{-1} ethanolic stock solution.

2.3. pK_a determination by UV spectrophotometry

The buffer/hydrochloric acid solutions of pH 0.7, 1.0, 3.0, 4.0, 5.0 and 12.0 fortified with AA were scanned over a wavelength of 200–400 nm and their spectra were recorded. The analytical wavelength was found to be 285 nm because a peak over a inflection was observed at this wavelength (Albert and Serjeant, 1962). The optical density of the compound in buffer/hydrochloric acid solution of pH 1.1, 1.6, 1.9, 2.3, 2.5, 2.8, 3.1, 3.4 and 3.7 were recorded at this wavelength. The

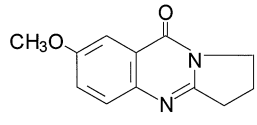

Fig. 1. Chemical structure of compound 73/602 (AA).

pK_a of the drug was calculated using the following formulae.

$$pK_a = pH + \log(d - d_M)/(d_I - d)$$

Where: d_I, the optical density of the ion at the analytical wavelength; d_M, the optical density of the neutral molecule at the analytical wavelength; d, the optical density of the mixture of ion and molecule.

2.4. Drug formulation

A solution formulation of AA (10 mg/ml) was prepared by dissolving 100 mg in 2 ml ethanol. It was then diluted with 5 ml polyethylene glycol (PEG 600) and finally the volume was made up to 10 ml with normal saline.

2.5. Animal experiments

2.5.1. Animals

Young, healthy, male, Sprague–Dawley rats weighing 200 ± 20 g were obtained from the Laboratory Animal Division of this institute. Rats were housed in groups of three in plastic cages with free access to standard pelleted food (Goldmohar laboratory animal feed, Lipton India, Chandigarh) and water. The rats were acclimatized for not less than 3 days prior to experimentation.

2.5.2. Oral administration and sampling

After an overnight fast (14–16 h) a 50 mg kg^{-1} dose of AA forumulation was administered orally. For collection of serum sample at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12 h post dose, groups of three rats were sacrificed at each time point under light ether anaesthesia. Bleeding was done from the posterior vena-cava and the separated serum was stored at -30°C until analysis.

2.5.3. Intravenous administration and sampling

Under light ether anaesthesia the jugular vein of each rat was cannulated with a silicon cannula (Silastic Medical-grade tubing, 0.025″ i.d. × 0.047″. o.d., Dow Corning Corporation Medical Products, Midland, MI) previously flushed with 20 units ml^{-1} heparin-normal saline solution. A 10 mg kg^{-1} dose of AA was injected through the tail vein. Eight serial blood samples were collected through jugular vein cannula at 0, 5, 15, 30, 60, 120, 180, 240 min and the last terminal, 360 min post dose sample was collected from posterior vena-cava just before sacrificing the animal. The blood was allowed to clot and serum was separated by centrifuging the tubes at $1000 \times g$ for 10 min at 20°C. The samples were stored at -30°C until analysis.

2.5.4. In-situ studies using rat intestinal lumen

For absorption studies the rats were fasted 16–20 h prior to experiment, however drinking water was readily accessible. The rats were anesthetized by giving an 1 mg g^{-1} i.p. injection of 25% w/v ethyl carbamate (urethane) solution 1 h prior to surgery. The in situ surgical step was similar to that described earlier (Doluisio et al., 1969). Briefly, the small intestine of an anesthetized rat was exposed by a midline abdominal incision and two L-shaped cannulae were inserted into the intestine, one isoperistaltically at the proximal end of duodenum and the other antipersistaltically at the distal end of ileum to isolate a 30 cm segment of gut. The lumen of the isolated segment was washed with 30 ml of perfusion solution containing sodium chloride 1.45×10^{-1} M, potassium chloride 4.56×10^{-3} M, calcium chloride 1.25×10^{-3} M and sodium dihydrogen phosphate 5×10^{-3} M (Diamond et al., 1970) to remove intestinal contents. The perfusion solution was then expelled and 10 ml of 2 g ml^{-1} solution of AA in appropriate buffer was filled into the system. Samples of intestinal solution (200 μl) were withdrawn from alternate syringes at periodic interval and stored at -30°C for subsequent analysis. This sampling procedure helped to mix the interstinal fluid, thereby ensuring uniform drug concentration throughout the intestinal segment (Doluisio et al., 1969). A heating lamp was positioned to assist in maintaining the preparation at around body temperature while manipulating the absorption fluids.

2.5.4.1. In-situ absorption at pH 7.4. In the absorption experiment the lumen of the isolated gut segment was filled with 10 ml of Sorensen buffer

(pH = 7.4) spiked with 2 g ml^{-1} of AA. Seven luminal samples (200 μl each) were collected at 0, 5, 10, 15, 20, 25 and 30 min interval and stored at − 30°C until analysis.

2.5.4.2. Effect of pH on in-situ absorption. The lumen was filled with the 2 μg ml^{-1} solution of AA in citrate-phosphate buffer (pH = 2.6) and the luminal samples (0.1 ml) were collected at 5 min interval for the first 30 min. Then the luminal fluid was raised into one of the syringe barrels and 20 μl of 15 M sodium hydroxide was added to the gut fluid. The fluid (now at a pH of 7.5–8.0) was then immediately returned to the gut lumen (Swintosky and Pogonowska-wala, 1982). No precipitation of AA in buffer solution was observed — by the abrupt increasing in the pH — upon addition of sodium hydroxide solution. Sampling was again carried out at 5 min intervals for the next 30 min. All the samples were stored at − 30°C until analysis.

2.5.4.3. Secretion into the lumen. The lumen was filled with drug free perfusion solution. A single 200 μl dose of the 10 mg ml^{-1} drug formulation was administered by tail vein injection to the rat. Luminal samples (200 μl each) were collected up to 2 h at 0, 5, 15, 30, 60, 90 and 120 min from the time of injection and stored at − 30°C until analysis.

2.6. HPLC analysis

2.6.1. Instrumentation

Samples of 73/602 were analysed on Kontron HPLC 600 system (Kontron, Zurich, Switzerland) fitted with a Rheodyne 7125 injector and 100 μl loop. AA was eluted on RP-C18 100 × 4.6 mm i.d., 5 m cartridge analytical column preceded by RP-C_{18} 30 × 4.6 mm i.d., 5 μm Guard column (Pierce Chemical Corporation, Rockford, USA) by a mobile phase prepared by mixing 200 ml of acetonitrile and 800 ml of potassium dihydrogen phosphate buffer (50 mM, pH 7). It was pumped at 1 ml min^{-1} after degassing by sonication for 15–20 min before use on each day. Quantitation was done on Model RF-530 variable wavelength fluorescence detector (Shimadzu Corporation, Japan) operated at 275 nm excitation and 363 nm emission wavelength connected to a C-R5A Chromatopac integrator-plotter (Shimadzu Corporation, Japan)

2.6.2. Analysis of serum

Serum samples were analysed by an HPLC assay method (Singh et al., 1994). Briefly 100–500 μl of serum was basified by the addition of 25 μl of 1M potassium hydroxide and volume of rat serum was made up to 500 μl with blank rat serum wherever necessary before processing. It was then extracted with 3 ml of diethylether. The terminal 1 ml serum samples were basified with 50 μl of 1M potassium hydroxide and extracted with 6 ml of ether. The ether extract was evaporated to dryness at 35 ± 20°C and the residue was reconstituted in 200 μl of mobile phase prior to injecting on to HPLC.

2.6.3. Analysis of luminal samples

The luminal samples were centrifuged at 40°C at 1000 × *g* for 15 min and the supernatant was vortex mixed with acetonitrile in ratio of 1:4 and directly injected onto HPLC. In experiments where the effect of pH on in-situ absorption was studied, the supernatant and acetonitrile were mixed in ratio of 1:1 and centrifuged again at 1000 × *g* for 10 min. An aliquot (50 μl) was then evaporated to dryness at 35 ± 2°C. Finally the residue was reconstituted in 100 μl of mobile phase and injected onto HPLC for analysis.

2.6.4. Calibration graph

Peak areas/heights of standard solutions of AA were used to plot the calibration curve of the compound. The standard curve thus obtained was used for calculating the concentration of the AA present in unknown solutions (luminal solutions and samples of pK_a determination by aqueous solubility method). Calibration curve using spiked serum standards were also plotted after processing the serum samples by linear regression of the weighted data, $w_i\,(y_i) = 1/y_i$ and were used to read the unknown concentrations of AA in serum.

2.6.5. Data analysis

The absorption rate constants of the compound at pH 2.6, 7.4 and 7.5 were determined from the

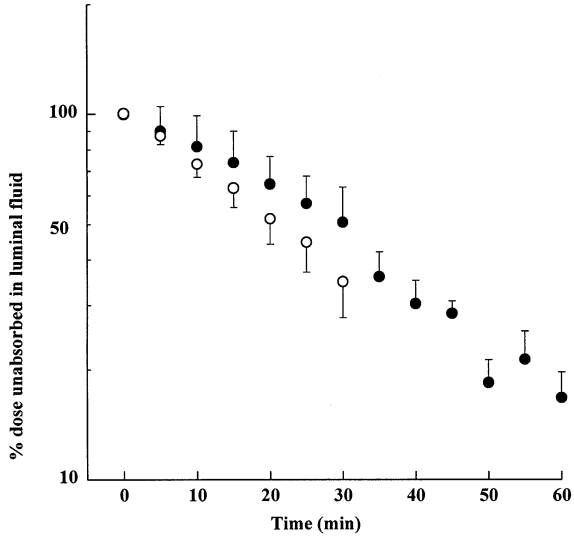

Fig. 2. Time course of AA absorption at pH 7 (○) and at pH 2.6 changed to 7.4 after 30 min (●) from the rat small intestine by in situ experiments. Results are expressed as the mean ± SD of five and four animals, respectively.

slope of the semi-logarithmic plots of mean concentration time profile at each pH by linear regression. The fitting of the curve was done using Microsoft Excel software (version 5). The absorption rate constant (K_a) was calculated as K_a = slope × 2.303.

The mean serum concentration-time data of rats receiving intravenous (i.v.) ($n = 5$) and oral dose ($n = 3$) was fitted by one and two compartment models with PCNONLIN, a nonlinear regression software (PCNONLIN, 1992). Pharmacokinetic parameters were determined by standard model dependent and model independent methods (Gibaldi and Perrier, 1975). Area under the serum concentration-time curve (AUC) was calculated using the trapezoidal rule, extrapolated to infinity by dividing the last measured concentration by terminal elimination rate constant. The fraction of oral dose absorbed (F) was calculated from ratio of dose normalized AUCs from oral and i.v. studies.

3. Results and discussion

3.1. pK_a value

A UV spectrometic method was applied for determining the pK_a of the AA. The pK_a of AA was found to be 2.87.

3.2. In-situ absorption of AA at pH 7.4

Fig. 2 shows the time course of the in situ absorption of AA from rat small intestine. It was rapidly absorbed from the intestine and > 66% of the compound was absorbed at pH 7.4 within the first half an hour of the commencement of the experiment. It appears to follow apparent first-order kinetics and the absorption rate constant of AA at this pH was calculated to be 0.035 ± 0.008 min^{-1} from the slope of the logarithmic plots of concentration time profile by linear regression.

3.3. Effect of pH on in-situ absorption

The effect of pH on the absorption rate of AA was studied in four rats by abruptly changing the pH of the luminal drug solution at the end of 30 min period. The mean% dose of AA remaining in the luminal fluid is shown in Fig. 2. Eighty three percent of the compound was absorbed by the end of an hour of which 50% was absorbed within initial thirty min at pH 2.6 and another 33% (or 66% of the remaining dose) in the next half an hour at pH 7.5. The apparent absorption rate constants were 0.0228 ± 0.004 min^{-1} at pH 2.6 and 0.03 ± 0.012 min^{-1} at pH 7.5. The rapid absorption seen in situ compared to in vivo could be due to saturation of the transport mechanism or precipitation of AA at high (50 mg kg^{-1}) oral dose. Poor solubility (Seth et al., 1990) and log P value (1.442) confirm the these findings.

The unexpectedly small difference in absorption rate found in situ when the pH was changed (giving a higher% of drug in unionized form) may be as a result of the reduction of the concentration gradient upon absorption rates (passive transport process) after first 30 min of the experiment. Using the Henderson Hasselbach equation it was calculated that at pH 2.6 nearly 35% of the compound remains

in the unionized form and is available for absorption. Likewise at pH 7.5 more than 99% of the compound is unionized. Although there is little difference in the absorption rates of AA in the two mediums, the total amount of drug absorbed from the two segments of the absorbing system (namely stomach and intestine) will differ due to difference in the surface area available for absorption, difference in the fraction of the compound ionized, and difference in the transit period in each of these segments. Hence it can be concluded that the absorption of AA will begin in the stomach but the major portion of the dose will be absorbed from the small intestine.

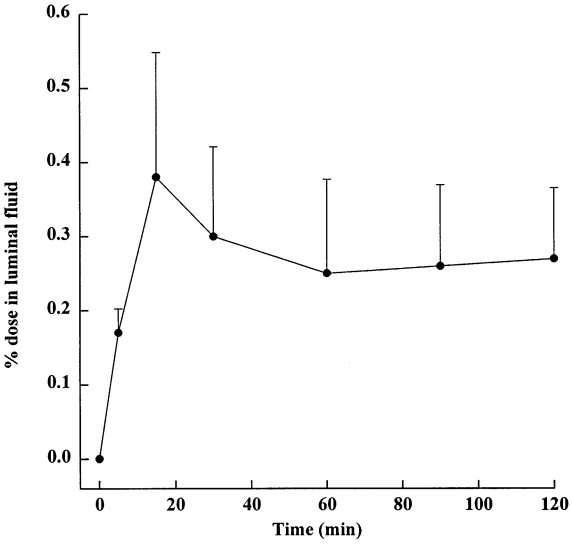

Fig. 3. Secretion of AA into the lumen of 10 cm segment of rat small intestine after administering 2 mg i.v. dose. Results are expressed as the mean ± SD of four animals.

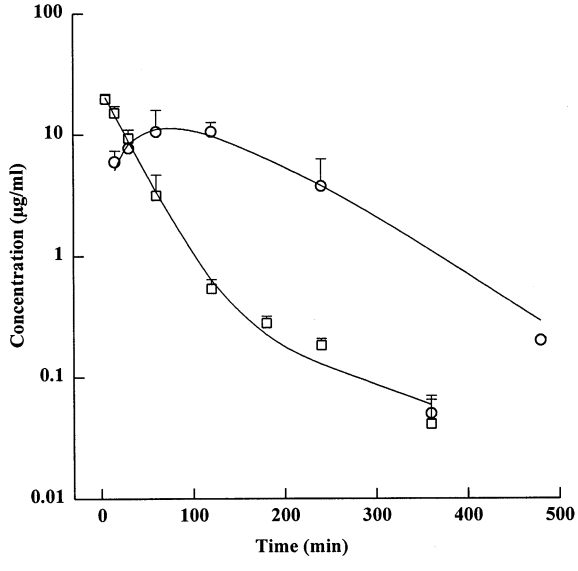

Fig. 4. Mean concentration-time profile of AA in rats after administering 10 mg kg^{-1} i.v. (○) and 50 mg kg^{-1} (●) oral dose.

3.4. Secretion of AA from blood to lumen

The secretion of compound into the lumen was studied in four rats. The mean% dose of AA secreted into the lumen of 30 cm segment of small intestine up to 2 h post i.v. dose is plotted in Fig. 3. Less than 0.3% of the total i.v. dose was secreted in the luminal fluid in 2 h. Hence enteroenteric circulation of AA does not contribute significantly to the in-situ absorption data in rats.

3.5. Pharmacokinetics of AA in male rats

Plots of mean serum concentration-time profiles of AA following administration of single 10 mg kg^{-1} i.v. and 50 mg kg^{-1} oral bolus dose in male rats are given in Fig. 4. The mean concentration time data after oral dose was best fitted by a one compartment model with equal first order input (absorption) and first order output (elimination) rate constants, i.e. ($K_a = K_e = K$) and can be mathematically expressed as:

$$C = K\ (D/V)\ t \exp(-Kt)$$

Where C is the serum concentration of AA at time t; D, Dose; and V is the volume of distribution. In this special case where $K_a = K_e$, absorption continues through the elimination phase and the terminal slope of the log C versus t plot is not linear and can not be used to determine the rate constants. Fitting of this data with general Bateman equation for one compartment body model is indeterminate and can not be used to determine pharmacokinetic parameters. Equality of K_a and K_e was also confirmed by the Bailer's criteria (Bailer, 1980) as:

$$C_{max} \cdot T_{max} = \mathrm{AUC}/e$$

Table 1
Pharmacokinetic parameters of AA after administering 10 mg kg^{-1} i.v. and 50 mg kg^{-1} oral dose

Parameter		Oral dose	i.v. Dose
k_{10}	Min^{-1}	0.0316	0.0312
k_{12}	Min^{-1}		0.0025
k_{21}	Min^{-1}		0.0671
K	Min^{-1}	0.0137	
α	Min^{-1}		0.0344
β	Min^{-1}		0.0061
C_1	ml min^{-1}	2.59	2.59
Vd	ml	190	82.9
Vd_{ss}	ml		114.3
K-HL	Min	50.6	
k_{10}HL	Min		22.2
α-HL	Min		20.2
β-HL	Min		113.7
AUC	μg min ml^{-1}	2248	772.8
MRT	Min	121	44.2
C_{max}	μg ml^{-1}	11.27	
T_{max}	Min	73	

The AUC, C_{max} and T_{max} were determined by PCNONLIN as secondary parameters and are given in Table 1.

The mean concentration-time data of i.v. study was fitted by PCNONLIN using Gauss–Newton's algorithm with Levenberg modification. The calculated parameters according to two compart-ment model with relative weighing function $w_i(y_i) = 1/y_i$ are given in Table 1. Two compartment fit was better than one compartment model on the basis of visual inspection and the smaller value of sum of weighted squared residuals in the former model. The distribution of drug to the peripheral compartment was rapid with 20 min distribution phase half-life (α-HL). The poor aqueous solubility and reasonably high octanol water partition coefficient value are in confirmation of the high volume of distribution in the i.v. pharmacokinetic data.

Non compartmental analysis of data gave mean residence time (MRT)s of AA following i.v. dose of 50 min compared to 121 min after single 50 mg kg^{-1} oral dose in rats.

4. Conclusions

The present study reports the absorption kinetics of AA by an in-situ method. The compound is a weak base with mean $pK_a = 2.87$, hence this compound will exist in unionized form throughout the GIT. In-situ absorption rates of AA were 0.0228 min^{-1} and 0.03 min^{-1} at 2.6 and 7.5 pH, respectively. Thus it can be reasonably argued that upon oral administration of the dose, absorption will begin in the stomach itself though the major fraction will be absorbed from the lower part of GIT due to relatively large surface area of the intestine, presence of greater fraction of the compound in unionized form, and longer transit period down the intestine compared to stomach. From single dose oral and i.v. pharmacokinetic studies in rats, it can be concluded that the disposition kinetics of AA in rats can be best described by a two compartment open model with first order absorption, distribution and elimination rate constants and about 58% of the oral dose reaches to systemic circulation.

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