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Research Paper

In-situ absorption, protein binding and pharmacokinetic studies of S002-853, a novel antidiabetic and antidyslipidaemic flavone derivative in rats

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Abstract

Objectives The aim of the study was to investigate the in-situ absorption kinetics, plasma protein binding and pharmacokinetic characteristics of a novel synthetic flavone derivative, S002-853, which shows pronounced antidiabetic and antidyslipidaemic activity.

Methods Quantification of S002-853 in plasma was performed by the LC-MS/MS method and in-situ sample analysis was carried out by the HPLC-UV method.

Key findings The absorption rate constant was 0.274/h in a mild alkaline environment, which S002-853 experiences in the intestine following oral dose administration. Plasma protein binding was found to be $26.37 \pm 2.58\%$ at a concentration of 1 μg/ml. The pharmacokinetic parameters were determined in male rats after administration of a single 40 mg/kg oral dose and 10 mg/kg intravenous dose. The peak plasma concentration (C_{max}) was found to be 60.93 ng/ml at 8 h after oral administration. Irregular concentration–time profiles with secondary peaks were observed after oral dose administration. The elimination half-life of the compound was 19.56 h and 16.30 h after oral and intravenous doses, respectively. Comparison of the AUC after oral and intravenous dosing of S002-853 indicates that only about 29.48% (bioavailability) of the oral dose reaches the systemic circulation.

Conclusions In-situ study of S002-853 shows slow absorption from the gastrointestinal tract. S002-853 also shows low plasma protein binding. The pharmacokinetic parameters after oral and intravenous dose reveal low oral bioavailability and high mean residence time.

Keywords in-situ absorption; pharmacokinetics; protein binding; rat plasma; S002-853

Introduction

Type-2 diabetes is one of the most common chronic diseases and is associated with co-morbidities such as obesity, hypertension, hyperlipidaemia and cardiovascular disease, which taken together comprise the 'metabolic syndrome'. A worldwide survey reported that diabetes mellitus (type-2) affects 10% of the population.[1] The synthetic hypoglycaemic agents used in clinical practice have serious side effects.[2] Therefore, the search for more effective and safer antidiabetic agents has become an area of active research.

S002-853 is a novel *R*(−)/*S*-(+)-2-(3, 5-bis (benzyloxy-phenyl)-7-(3-tert-butylamino-2-hydroxypropoxy)-chromen-4-one synthesised by the Central Drug Research Institute (CDRI; Lucknow, India), which has enormous potential as an antidiabetic along with its significant antidyslipidaemic activity (Patent pub WO/2008/018089) (Figure 1). A flavonoid as a basic skeleton was selected by CDRI to optimise phamacophoric properties to achieve the desired activity and property profile. Flavonoids are among the most ubiquitous groups of polyphenolic compounds in foods of plant origin. As integral constituents of the diet, they may exert a wide range of beneficial effects on human health. Flavonoids produce such biological effects through their free radical scavenging antioxidant actions and metal ion chelating ability.[3] Flavonoid-rich extract of *Eugenia jambolana* has long been known for its antidiabetic activity in traditional medicines.[4,5] The metabolism and pharmacokinetics of flavonoids has been an area of active research in the last decade. The profiles indicate considerable differences among the different types of dietary flavonoids so that the most

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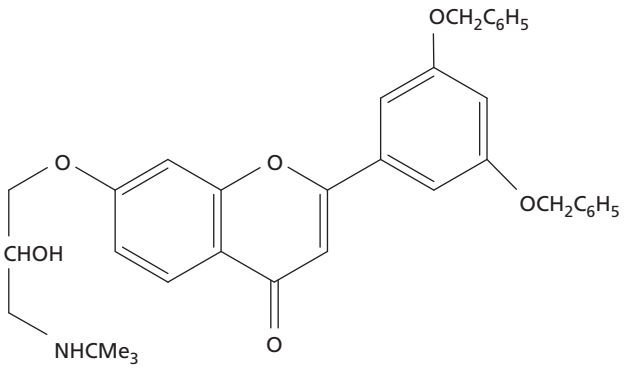

Figure 1 Chemical structure of S002-853

abundant flavonoids in the diet do not necessarily produce the highest concentration of flavonoids or their metabolites *in vivo*.[6]

It was essential to characterise S002-853 in terms of its absorption profile in the gastrointestinal tract, plasma protein binding, pharmacokinetic profile and oral bioavailability. Characterisation by pharmacokinetic studies in model animals is required for toxicological and preclinical studies and also for extrapolation of the pharmacokinetics and pharmacodynamics to humans.[7] This study reports the new findings of in-situ absorption kinetics, plasma protein binding and pharmacokinetic parameters after intravenous and oral dose administration of synthesised flavone derivative S002-853 in rats. Study samples were analysed using validated HPLC-UV and LC-MS/MS methods for the compound S002-853.[8]

Materials and Methods

Chemicals and reagents

Pure reference standard CDRI candidate drug S002-853 (Figure 1) and centchroman used as an internal standard (IS) in LC-MS/MS method were synthesised and purified (>99%) in the Medicinal Chemistry Division, CDRI (Lucknow, India). HPLC-grade methanol and acetonitrile were purchased from Sisco Research Laboratories (Mumbai, India). Analytical grade ammonium acetate and glacial acetic acid were purchased from E. Merck Ltd (Mumbai, India). Charcoal granular 4–14 mesh and dextran (average MW 71 200) were procured from Sigma (St Louis, USA). Dulbecco's phosphate buffered saline (DPBS) (Ca and Mg ion free) was procured from HiMedia Laboratories (Mumbai, India). Analytical-grade potassium dihydrogen orthophosphate (KH_2PO_4) and dimethylformamide (DMF) were supplied by S.D. Fine chemicals (Biosar, India). Polyethylene glycol-400 (PEG) was purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Methylcellulose (MC) was purchased from Aldrich Chemical Company (St Louis, USA). Water of 18.2 MΩ cm was obtained from a Milli-Q Ultrapure Water Purification Systems (Bangalore, India). Drug-free rat blood for plasma was collected from healthy male Sprague–Dawley rats provided by the division of laboratory animals of CDRI (Lucknow, India).

Stock and standard solutions of S002-853 for analytical methods

For the LC-MS/MS method, mother stock solutions of 1 mg/ml concentration for S002-853 and IS were prepared by dissolving 10 mg of the compound, made up to a final volume of 10 ml, in methanol. Working stock solutions were obtained by step-wise dilution of the mother stock solution in mobile phase. Analytical standards and calibration standards for plasma samples of protein binding and pharmacokinetic studies were prepared over the concentration range 0.78–400 ng/ml.[8]

For the HPLC-UV method, a stock solution of 100 μg/ml was prepared by dissolving 10 mg of the compound S002-853, made up to a final volume of 100 ml, in acetonitrile. Calibration standards for in-situ absorption were prepared in the range of 15.6–2000 ng/ml by a serial dilution method.

Animals

Young, healthy male Sprague–Dawley rats, 250 ± 25 g, were obtained from Laboratory Animal Division of the Institute. The rats were housed in plastic cages under standard laboratory conditions with a regular 12-h day–night cycle. The rats were acclimatised to this environment for at least two days before the start of the experiments. The rats were fasted overnight (8–12 h) before experiments, although water was freely provided. The rats were cared for in accordance with principles of the guide for care and use of laboratory animals (Department of health education and welfare, number [NIH] 85–32). Studies were approved by the institutional Animal Ethics Committee (Ethical approval 58/08/PKM/IAEC).

In-situ study using rat intestinal lumen

Drug formulation for in-situ absorption

Ten milligrams of S002-853 was weighed and dissolved in acetonitrile and the volume made up to 10 ml to give a final concentration of 1 mg/ml; 100 μl of this stock was spiked into 19 900 μl of Sorensen buffer[9] of pH 7.4 to give a final concentration of 5 μg/ml. The contents were slowly agitated in a thermostatic incubator shaker for 12 h at 37°C. The solution was centrifuged at 3000*g* for 15 min and the supernatant collected was used for in-situ absorption study.

Experimental procedure

The rats were anaesthetised by administering urethane solution intraperitoneally[9] (1 mg/g body weight). The small intestine of anaesthetised rats was exposed by midline incision on the abdominal region. A 10-cm intestinal loop was prepared[10,11] by inserting two silicone cannulae, one isoperistaltically at the proximal end of the duodenum and the other antiperistaltically at the distal end of the ileum, and tied with silk suture. The loop was washed with 30 ml perfusion solution (composition in M: NaCl 0.145, KCl 0.0045, $CaCl_2$ 0.00125, NaH_2PO_4 0.005)[9] to clear the intestinal contents. The free ends of the cannula were dipped into the reservoir. The perfusion solution was then replaced with 10 ml of formulation prepared for in-situ absorption study. The drug solution was recirculated with the help of a peristaltic pump at a flow rate of 0.9 ml/min. Samples (200 μl) of the intestinal solution were withdrawn from the reservoir (Figure 2) at 0, 0.083, 0.16, 0.33, 0.5, 0.75, 1 and 1.5 h and were stored at −60°C for subsequent analysis. The

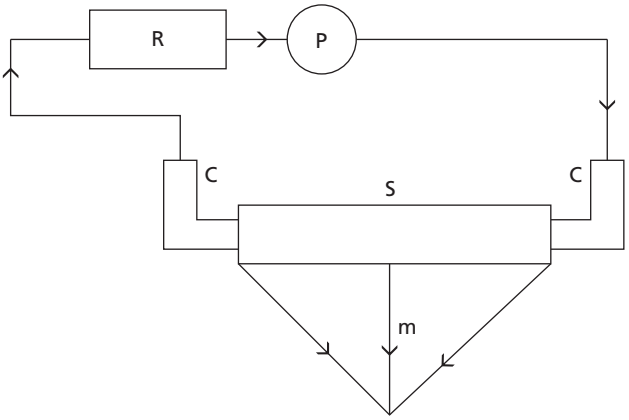

Figure 2 Schematic diagram representing the experimental setup for the recirculation perfusion technique. S, perfused intestinal segment; C, cannula; m, jejunal mesenteries; P, peristaltic pump; R, reservoir

entire experiment was performed under a closed hood in which heating lamps were used to maintain the temperature at 37 ± 1°C.[10–12]

In-vitro protein binding study

The dextran-coated charcoal suspension was prepared by adding 0.66 g of dextran-coated charcoal to 100 ml of DPBS (9.5 g/l). The mixture was stirred with a magnetic stirrer at room temperature until the charcoal became suspended. This suspension was prepared at least 18 h before use and stored at 5–10°C for not more than 30 days. The stored charcoal mixture was re-suspended before use.[11–13]

The study was carried out in triplicate at 1 μg/ml concentration of S002-853. The spiked plasma was allowed to equilibrate for 15 min before the start of the study. Charcoal suspension (6.0 ml) was transferred into a 30-ml glass tube, centrifuged at 3000*g* for 15 min at 25°C, and the supernatant DPBS was carefully decanted off. Spiked plasma (6.0 ml) was then added onto the charcoal pellet under continuous stirring at 37 ± 1°C. Serial samples (200 μl) were withdrawn at 0.083, 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6 and 8 h in 0.6-ml polypropylene micro-centrifuge tubes and centrifuged immediately at 11 000*g* for 2.5 min at 37°C. Supernatant was separated and immediately transferred into 1.5-ml micro-centrifuge tubes and stored at −60°C until analysis.

Pharmacokinetic study

Drug formulation for oral and intravenous dose administration

An oral dose formulation of S002-853 was prepared in an aqueous suspension of 0.5% methylcellulose. The strength of the oral formulation was 15 mg/ml for a dose of 40 mg/kg in rats. The intravenous formulation was prepared in DMF–PG–water (5 : 4 : 1 v/v) and finally filtered through a 0.2-μm filter before administration. The strength of the intravenous formulation was 7.5 mg/ml for a dose of 10 mg/kg.

Dose administration and sample collection

The oral dose formulation was administered with an oral feeding needle. Blood samples were withdrawn at 0.25, 0.5, 1, 1.5, 2, 4, 8, 10, 12, 18, 24, 30, 36, 48, 60, 84, 96 and 120 h post dose in clean and heparinised glass tubes. Two blood samples were drawn from each rat. An initial sample (≈ 0.6 ml) was withdrawn by cardiac puncture with a 24G needle under light ether anaesthesia and the terminal blood sample was withdrawn from the inferior vena cava.[11] Plasma was separated by centrifugation at 1500*g* for 10 min at 4°C within 1 h of sampling and stored at –60°C until analysis. The volume of plasma utilised for sample analysis was 0.2 ml.

For the intravenous dose rats were anaesthetised by light ether and the formulation was administered through the caudal vein after dilation with xylene. Blood samples were drawn at 0.083, 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36 and 48 h post intravenous dose. Blood samples were withdrawn in a similar way as described for oral administration.

Pharmacokinetic data analysis

The concentrations of S002-853 were calculated by comparing the detector response(s) with the calibration curves in plasma drawn from the same batch. Computer-aided fitting of the individual data sets provided the initial estimates of pharmacokinetic parameters. These initial estimates were used in the final curve fitting and estimation of pharmacokinetic parameters, such as AUC, V_d, CL and t½, etc., were carried out using WinNonlin software (SCI consultants). The models were tested using Gauss–Newton algorithm with one- and two-compartment models. Decision on the suitability of a particular compartment model in explaining the data was taken based on the correlation coefficient, Akaike information criteria,[14] Schwarz value[15] and the scatter of residuals. The elimination half-life (t½β) was obtained from the formula of 0.693/β. The values of total body clearance (CL_{Total}) and the apparent volume of distribution (V_d) were calculated as dose/$AUC_{0-\infty}$ and dose/$\beta AUC_{0-\infty}$, respectively. The formula of $AUMC/AUC_{0-\infty}$ was applied to determine the mean residence time (MRT) values. The mean absorption time (MAT) and absorption rate constant (K_a) were calculated as $MRT_{p.o}$–$MRT_{i.v.}$ and 1/MAT, respectively.[16] The absolute bioavailability was calculated as the ratio between the AUC value for oral and intravenous routes after dose normalisation.

Sample analysis

HPLC-UV analysis of in-situ samples

An HPLC pump system (LC-10AT *VP* with FCV-10AL *VP*, Shimadzu) with a degasser (DGU-14A, Shimadzu), auto-injector (SIL-10AD *VP*, fixed with a 100-μl loop) was used to load the samples on a Pierce Spheri-5 cyano column (100 × 4.6 mm i.d., 5 μm) with a cyano guard column (30 × 4.6 mm, 5 μm). The mobile phase consisting of acetonitrile with ammonium acetate buffer (10 mM, pH 4.0) in a ratio of 60 : 40 (v/v) was used under isocratic condition at a flow rate of 1 ml/min. S002-853 was monitored at 310 nm with a UV detector (SPD-10A UV-VIS) and chromatograms were

integrated using Compaq Presario 3200 with Class-VP integrating software.

The in-situ samples (100 μl) were diluted with a 400 μl mixture of acetonitrile and ammonium acetate buffer (pH 4.0, 10 mM) in a ratio of 80 : 20 (v/v) and vortexed for 1 min. The samples were centrifuged at 10 000*g* for 5 min; the supernatant was collected and analysed over the concentration range 15.6–2000 ng/ml.

LC-MS/MS analysis of plasma samples

Mass spectrometric detection was performed on API 4000 LC-MS/MS (Applied Biosystems, MDS Sciex, Toronto, Canada) with Analyst 1.4 software. The mass spectrometer was operated with an electrospray ion source (ESI) in positive ion mode and the analytes were quantified using multiple reaction-monitoring (MRM) mode. The optimised precursor (hydrogen adducts of analytes, $M+H^+$) to product ion transitions were monitored as m/*z* 580.3 → 524.2 and m/*z* 458.4 → 119.1 and used for quantification of S002-853 and IS, respectively.

A Series 200 HPLC system consisting of quaternary low-pressure gradient pumps, on-line degasser and auto sampler with temperature controlled sample tray (Perkin-Elmer instruments, Norwalk, USA) was used. An isocratic mode was used to deliver the mobile phase at a flow rate of 0.75 ml/min. Chromatographic separation was achieved on Pierce Spheri-5, cyano column (30 mm × 4.6 mm i.d., 5 μm). Elution was carried out using methanol–ammonium acetate buffer (pH 4.6, 10 mM) (90 : 10 v/v) as mobile phase. The chromatographic run time was 6.0 min and the injection volume was optimised to 20 μl. Plasma samples were prepared by a simple and efficient liquid–liquid extraction process using diethyl ether.

Results

In-situ absorption of S002-853

To check the adsorption of S002-853 in silastic cannulae, the formulation of 5 μg/ml in Sorensen buffer was taken in a beaker and circulated through the silastic cannulae for 90 min. The adsorption was found to be <5% with silastic cannulae.

During recirculating perfusions, the absorption of S002-853 was evaluated by measuring its disappearance from the perfusate, which followed first-order kinetics. The time dependence of the luminal concentration C can be written as:

$$\ln C_t/C_o = -K_{dis}.t \qquad (1)$$

where C_o and C_t are the luminal concentration of the model compound at time 0 and t, respectively, and k_{dis} is the first-order disappearance rate constant. The disappearance rate constant k_{dis} was calculated from plots of $\ln(C_t/C_o)$ versus time by linear regression.[17]

The $\ln C_t/C_o$ versus time plot for the disappearance of S002-853 from the intestinal lumen solutions is shown in Figure 3. It appeared to follow apparent first-order kinetics and the absorption rate constant was calculated to be 0.274/h and the absorption half-life ($t_{1/2\alpha}$) was 2.53 h.

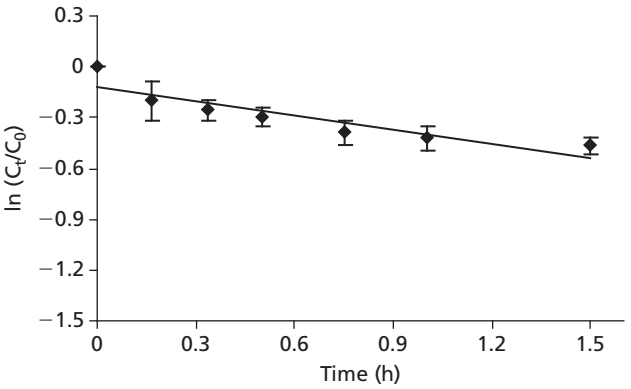

Figure 3 Mean $\ln(C_t/C_0)$–time profile of S002-853 in the in-situ absorption study. Data are means ± SD, $n = 3$

In-vitro protein binding study

The method was based on charcoal adsorption kinetics and operated under non-equilibrium conditions. It involved measuring the time-course of decline of the concentration of bound drug when the free drug was progressively removed by charcoal adsorption. The association and dissociation of drug with proteins was a dynamic phenomenon with charcoal acting as a sink for free drug removal. The percentage binding was then estimated from the decline of percentage of drug remaining in the supernatant after the addition of charcoal. Dextran-coated charcoal was used since the dextran coating effectively prevents charcoal from adsorbing plasma proteins, which would lead to erroneous results.

Percent S002-853 remaining in the supernatant rat plasma versus time data was fitted to a two-compartment model, intravenous bolus non-linear regression program on Win-Nonlin 5.1 (Figure 4). The model is described by the following biexponential equation:

$$B_t = A_1 e^{-\alpha t} + A_2 e^{-\beta t} \qquad (2)$$

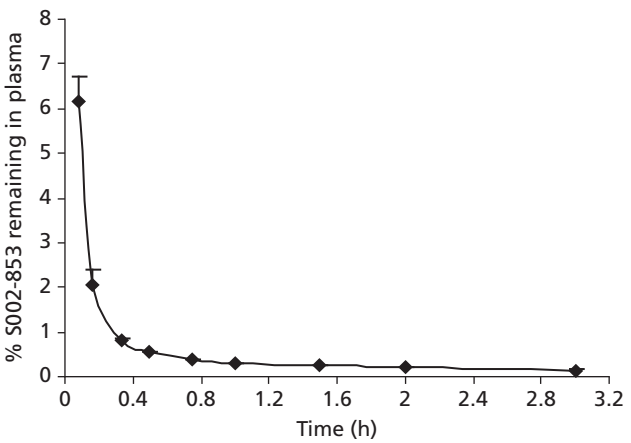

Figure 4 Percentage of S002-853 remaining in pooled rat plasma after adding charcoal suspension 1 μg/ml in three sets. Data are means ± SD, $n = 3$

where B_t is % bound at time t, A_1 and A_2 are Y intercepts and α and β are distribution and disposition rate constants for the two phases, respectively. The extent of protein binding was given by the C_{max} value (at time t = 0, i.e. Bt).

S002-853 showed low plasma protein binding, 26.37 ± 2.58%, at a concentration of 1 μg/ml. α and β were found to be 18.78 ± 2.20 and 0.89 ± 0.02/h, respectively.

Pharmacokinetic studies

The plasma concentration–time curve obtained after administration of a single 40 mg/kg oral dose to rats is presented in Figure 5a. After the oral dose, qualitative visual examination of the data indicated the presence of multiple peaks in the plasma concentration–time profile of S002-853. The maximum plasma concentration (C_{max}) obtained was up to 60.93 ng/ml at 8 h, followed by a second C_{max} of 48.3 ng/ml at 30 h and a third C_{max} of 45.3 ng/ml at 60 h. The compound was monitored in plasma from the first post-dose sampling time point (15 min), and could be followed up to 120 h post dose. The pharmacokinetic parameters were determined by fitting data for non-compartmental analysis using WinNonlin (Version 5.1). The terminal half-life for S002-853 was 19.56 h. The other pharmacokinetic parameters of S002-853 after oral administration are presented in Table 1. The MRT of S002-853 was 43.20 h, which was considerably higher than that observed in the single-dose intravenous pharmacokinetic study (22.77 h).

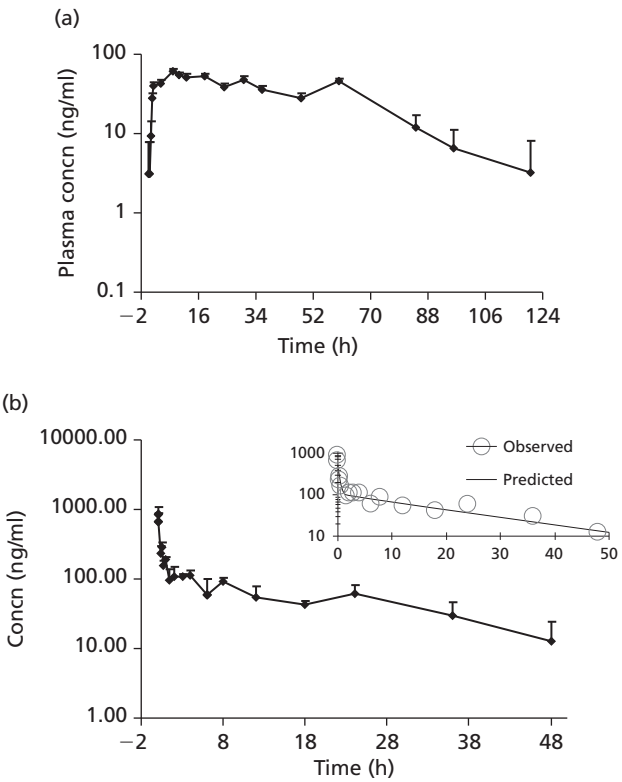

Figure 5 Plasma concentration–time profile of S002-853 after (a) oral at 40 mg/kg and (b) intravenous at 10 mg/kg dose administration in male rats. Inset shows two-compartment data-fitting curve in WinNonlin. Data are means ± SD, $n = 3$

Table 1 Pharmacokinetic parameters of S002-853 in male rats after oral or intravenous administration

Pharmacokinetic parameters	Oral	Intravenous
T_{max} (h)	8.0 ± 0.0	n.a.
C_0 (ng/ml)	n.a.	998.21 ± 322.3
C_{max}(ng/ml)	60.93 ± 13.7	n.a.
t½α (h)	n.a.	0.161 ± 0.04
t½β (h)	19.56 ± 2.9	16.30 ± 2.1
AUC_{0-t} (ng h/ml)	3378.42 ± 861.8	n.a.
AUC_{0-inf} (ng h/ml)	3469.28 ± 934.1	2716.92 ± 244.1
Vd (l/kg)	96.73 ± 25.1	69.41 ± 8.06
Vss (l/kg)	n.a.	79.43 ± 8.4
CL (l/h per kg)	3.42 ± 0.89	3.65 ± 0.33
MRT (h)	43.20 ± 3.5	22.77 ± 2.8
K_a (h^{-1})	0.049 ± 0.0	n.a.
Bioavailability (%)	29.48 ± 8.5	n.a.

n.a., not applicable. Data are presented as means ± SD, $n = 3$.

Unlike the oral dose, the concentration–time profile after an intravenous dose could be fitted assuming conventional compartmental kinetics (Figure 5b). The pharmacokinetic models were compared according to Akaike information criteria and Schwarz value criteria, with minimum values regarded as best representation of plasma concentration–time data. A two-compartmental model with a first-order elimination process could best describe the concentration–time data for S002-853. On intravenous dosing the levels of S002-853 decreased biexponentially with rapid distribution (t½α) and slow elimination half-lives (t½β) of 0.161 h and 16.30 h, respectively. The levels of S002-853 were monitored up to 48 h post dose. The pharmacokinetic parameters of post-intravenous dose to rats are listed in Table 1.

Discussion

S002-853 showed less than 5% adsorption to the silicone cannulae over a 90-min period, hence, it was appropriate to employ it in the in-situ recirculation perfusion method for measurement of absorption. Data from the in-situ study depict the S002-853 disappearance rate from the gut lumen fluid. The term disappearance and absorption will be used interchangeably since absorption is the driving force for the disappearance process in the gut just as it is for the appearance process in the rising blood concentration curve. S002-853 was slowly absorbed from the intestine and 36.88% of the compound was absorbed within 1.5 h of the commencement of the experiment.

Low plasma protein binding (26.37 ± 2.58%) of S002-853 may lead to an unbound drug concentration that favours tissue redistribution or clearance of drug from the body. The low protein binding of S002-853 found in the in-vitro study reflects the in-vivo results for high volume of distribution after oral and intravenous administration. The high volume of distribution obtained suggests distribution of S002-853 outside the vascular compartment.[18]

After oral dosing with S002-853, it appears that the absorption was slow, as plasma concentrations peaked at 8 h

post-dose. An irregular concentration–time profile was observed for S002-853. At present it is difficult to give any conclusive explanation for the appearance of multiple peaks after oral dosing. Another contributing factor may be the non-aqueous solubility that might have resulted in precipitation of the dose in the intestine and did not redissolve in the absorptive regions of the gastrointestinal tract.[19] Other possible mechanisms of the phenomena include storage and subsequent release of drug from a post-absorptive depot site (possibly liver parenchymal cells), entero-hepatic recycling and variable absorption rates along the gastrointestinal tract.[20] The above explanation may also account for the difference in absorption rate constant values between the in-situ and oral dose pharmacokinetic studies (K_a 0.274/h and 0.049/h, respectively).

The MRT value of 22.77 h after an intravenous dose indicates that S002-853 is retained in the system for longer periods of time due to slow elimination from the body. The volume of distribution at steady state (Vss) was 79.43 l/kg, which is much higher than the total body water (0.6 l/kg), suggesting that S002-853 is well distributed outside the vascular compartment. The systemic bioavailability of the compound was only 29.48% after oral administration. The large clearance or low oral bioavailability of the compound indicates a high extraction ratio across the eliminating organs.

Conclusions

This study reports the absorption kinetics, protein binding, pharmacokinetics and oral bioavailability of S002-853. The absolute bioavailability of S002-853 by the oral route was found to be 29.48%. The terminal half-life of S002-853 after oral and intravenous doses was 19.56 and 16.30 h, respectively. The MRT values were high after both oral and intravenous doses. The absorption rate constant (K_a) of S002-853 was found to be 0.274/h in the in-situ absorption study but 0.049/h in the oral-dose pharmacokinetic study. Plasma protein binding of S002-853 was found to be $26.37 \pm 2.58\%$ by the charcoal adsorption method. This study will prove useful in predicting the pharmacokinetic outcome during tissue distribution, excretion and multiple drug therapy studies of S002-853.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Siddharth NS. Containing the global epidemic of diabetes. *J Diabetol* 2001; 3: 11.
2. Larmer J. Insulin and oral hypoglycemic drugs, glucagon. In: Gilman AG *et al*. eds. *The Pharmacological Basis of Therapeutics*, 7th edn. New York: Macmillan Publishing 1985: 1490.
3. Belinky PA *et al*. Structural aspects of the inhibitory effect of glabridin on LDL oxidation. *Free Radic Biol Med* 1998: 24: 1419–1429.
4. Sharma B *et al*. Effects of flavonoid-rich extract from seeds of Eugenia jambolana (L.) on carbohydrate and lipid metabolism in diabetic mice. *Food Chem* 2008; 110: 3697–3705.
5. Takashi S *et al*. Oxazolidinedione derivatives, their production and use. US Patent, 5932601, 1999.
6. Manach C, Donovan JL. Pharmacokinetics and metabolism of dietary flavonoids in humans. *Free Radic Res* 2004; 38: 771–785.
7. Rowland M, Tozer TN. *Clinical Pharmacokinetics: Concepts and Applications*. Philadelphia: Lea and Febiger, 1980: 20.
8. Gautam N *et al*. Liquid chromatographic-tandem mass spectrometry assay for quantitation of a novel antidiabetic S002-853 in rat plasma and its application to pharmacokinetic study. *Biomed Chromatogr* 29 Oct 2009, epub ahead of print.
9. Doluisio JT *et al*. Drug absorption I: An *in-situ* rat gut technique yielding realistic absorption rates. *J Pharm Sci* 1969; 58: 1196–1201.
10. Schurgers N *et al*. Comparison of four experimental techniques for studying drug absorption kinetics in the anaesthetized rat *in-situ*. *J Pharm Sci* 1986; 75: 117–119.
11. Issar M *et al*. Pharmacokinetics, *in-situ* absorption and protein binding studies of new neuroleptic agent centbutindole in rats. *Eur J Pharm Sci* 2003; 19: 105–113.
12. Srivastava P, Gupta RC. In-situ absorption and protein binding characteristics of CDRI-85/92, an antiulcer phamacophore. *Int J Pharm* 2003; 257: 97–102.
13. Khurana M *et al*. Binding of centchroman with human serum as determined by charcoal adsorption method. *Int J Pharm* 1999; 192: 109–114.
14. Yamaoka K *et al*. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J Pharmacokinet Biopharm* 1978; 6: 165–175.
15. Schwarz G. Estimating the dimension of a model. *Ann Stat* 1978; 6: 461–465.
16. Robbie G *et al*. Poor and unusually prolonged oral absorption of amphotericin B in rats. *Pharm Res* 1999; 16: 455–458.
17. Polema FGJ *et al*. Intestinal absorption of drugs I: the influence of taurocholate on the absorption of dantrolene in the small intestine of the rat. *J Pharm Sci* 1989; 78: 285–289.
18. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993; 10: 1093–1095.
19. Wang YX *et al*. A double peak phenomenon in the pharmacokinetics of alprazolam after oral administration. *Drug Metab Dispos* 1999; 27: 855–859.
20. Lin JH. Species similarities and differences in pharmacokinetics. *Drug Metab Dispos* 1995; 23: 1008–1021.