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Evaluation of absorbability of centpropazine in rats: in-situ and in-vivo approaches

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Abstract

Intestinal absorption of centpropazine was studied in rats by both in-situ (closed-loop method) and in-vivo (portal–venous difference) approaches. The drug was found to be well absorbed from solution in in-situ studies. However, the results obtained in-vivo suggested that very low amounts of drug reach the portal circulation after oral dosing. This could imply extensive binding to the mucosa or metabolism in the intestinal wall. The presence of higher amounts of metabolites in the portal vein compared with the inferior vena cava samples signal their formation in the gastrointestinal tract or enterohepatic recirculation. These findings will be useful in incorporating suitable structural and formulation modifications for enhancing the bioavailability of centpropazine and its analogues.

Introduction

Centpropazine (Figure 1) is a new antidepressant developed by CDRI (Central Drug Research Institute, Lucknow, India). The molecule showed a wider safety margin than imipramine in acute toxicity studies and could prove safer than tricyclics in overdosage by suicidal patients, a persistent risk in severely depressed patients (Gupta et al 1989). Its side-effects were also mild and transient (Srivatsava et al 1992). Considering the promising results of centpropazine, detailed pharmacokinetic and metabolism studies were conducted in rats and man. However, pharmacokinetic studies indicated a very low oral bioavailability of 0.19% in rats. Similar results were observed in man after single oral doses of 40 and 120 mg (unpublished data). The low bioavailability observed can be attributed to low aqueous solubility in the gastrointestinal fluids, poor permeability or extensive metabolism in the gastrointestinal tract. The physicochemical properties ($pK_a = 6.98 \pm 0.08$; $\log P = 3.15 \pm 0.013$; low aqueous solubility $< 2\ \mu g\ mL^{-1}$ at pH 7) of centpropazine are likely indicators of the gastrointestinal tract acting as a potential barrier for attainment of effective concentrations in serum. Evaluation of this barrier was performed using both in-situ and in-vivo approaches to assess the reasons for the low bioavailability with the aim of improving the molecular structure of centpropazine analogues and formulation variables.

Materials and Methods

Chemicals

Centpropazine (purity > 99%) was obtained from the Pharmaceutics Division of CDRI. All other chemicals were of analytical grade and were procured from local

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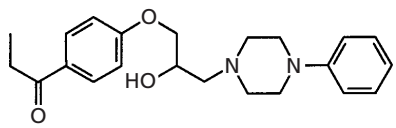

Figure 1 Chemical structure of centpropazine.

sources unless mentioned. The synthesis of the metabolite (centpropazine-OH) was carried out in this laboratory.

Chromatography

The concentrations of centpropazine and its metabolite were determined using a LC-10AT*vp* pump with low pressure gradient flow control valve (FCV-10AL*vp*), SIL-10AD*vp* autoinjector with 50 μL loop, SPD-M10A photodiode array detector, DGU-14A (degasser), CTO-10A*vp* (column oven set at 30°C) and RF-10Axl fluorescence detector set at 250 nm (excitation)/350 nm (emission). The flow line through valve A contained 100% KH_2PO_4 (25 mM, pH 3.5) buffer while in the line for valve B a 70:30 (% v/v) acetonitrile–KH_2PO_4 (25 mM, pH 3.5) buffer mixture was used. Chromatograms were recorded and integrated by Shimadzu Class LC10 (Version 1.61) software on a Compaq Deskpro Computer. Separations were achieved on a reverse-phase C_{18} cartridge column (Spheri-5, 5 μm, 220 × 4.6 mm i.d.) (Perkin Elmer, USA) preceded by a guard column (30 × 4.6 mm) packed with the same material. The mobile phase was pumped at 1.2 mL min^{-1}. The acetonitrile content was increased linearly from 35% to 45% over the first 20 min and then linearly from 45% to 65% in the next 25 min and then decreased linearly to 35% in the next 5 min. The retention times of M1 (unknown metabolite), centpropazine-OH and centpropazine were 6, 11 and 17 min, respectively (Figure 2). The longer run time with a higher percentage of acetonitrile was used to elute the endogenous components, thus avoiding the possibilities of interferences in the subsequent runs.

In-situ experiment

The experiment was carried out in young healthy male albino Sprague-Dawley rats (n = 5 for each experiment/time point), weighing 250 ± 25 g, obtained from the Laboratory Animal Division of CDRI. The animals were housed in plastic cages in standard laboratory conditions with a regular 12-h day–night cycle using a non-heat radiating lamp. Free access to standard pelleted laboratory chow (Goldmohar Laboratory Animal Feed, Lipton India Ltd, Chandigarh, India) and water was allowed. The rats were acclimatised to this environment for at least 2 days before conducting the experiment. In both the studies mentioned below the rats were fasted for 12–16 h. Blood samples were collected, serum was separated by centrifugation at 1200 rev min^{-1} for 10 min at 4°C and stored at –60°C until analysed. All surgical procedures were carried out under ether anaesthesia, taking suitable pre- and post-operative care. All experiments, euthanasia and disposal of carcasses were executed in accordance with the guidelines of the local ethical committee for animal experiments.

The surgical procedure was similar to that as previously described (Doluisio et al 1969). Briefly, the rats were anaesthetized by giving a 1 mg g^{-1} intraperitoneal injection of 25% w/v urethane solution 30 min before the surgery, and the small intestine of the anaesthetized rat was exposed by midline incision. A 30-cm intestinal loop was prepared by inserting two L-shaped glass cannulae, one isoperistaltically at the proximal end of the duodenum and the other antiperistatically at the distal end of the ileum. The loop was washed with 30 mL perfusion solution (NaCl, 1.45×10^{-1} M; KCl, 4.56×10^{-3} M; $CaCl_2$, 1.25×10^{-3} M; NaH_2PO_4, 5.00×10^{-3} M; Diamond et al 1970) to clear the intestinal contents. The perfusion solution was then expelled and 1.6 μg mL^{-1} solution ($\approx$ 12 mL) of centpropazine in Sorensen's buffer (19.2% of 0.067 M KH_2PO_4 and 80.8% of 0.067 M Na_2HPO_4 solution) was filled into the system ($\approx$ 12 mL). Samples of the intestinal solution (200 μL) were withdrawn at 5, 10, 15, 20, 25 and 30 min from alternate syringes and stored at −30°C till further analysis. The sampling procedure helped to mix the intestinal fluid thereby ensuring uniform drug concentration throughout the intestinal segment. A heating lamp was positioned to maintain the preparation at 37 ± 2°C while manipulating the absorption fluids.

In-vivo experiment

The local absorption kinetics of centpropazine from the intestinal tract was evaluated using portal–venous concentration difference (P-V difference) after oral administration of 40 mg kg^{-1} centpropazine (PEG 600–sodium acetate buffer 40%:60%) in conscious rats. The mean local absorption time (t_a) from the intestine into the portal system was estimated by simultaneously measuring the portal and venous concentrations (Tabata et al

1996). The total amount of centpropazine absorbed was estimated from the portal vein concentration using both CL (clearance) method (Kwon & Inskeep 1996) and Q method (Tabata et al 1995).

Erythrocyte distribution

The blood flow rate (Q_b) was not used in the calculations for estimating the drug absorbed in in-vivo experiments since the drug can be distributed in the red blood cells also. Since serum was the matrix analysed instead of blood concentration (C_b) and the levels of drug in serum and plasma (C_p) were similar, plasma flow rates (Q_p) were calculated and employed in the following equation (Yano et al 1991):

$$Q_p/Q_b = C_b/C_p = (1-H_t)(1+k_b) \quad (1)$$

where H_t is the haematocrit (0.45) and k_b is the partition ratio of the drug between the erythrocytes and plasma.

The partition ratio (k_b) of centpropazine was evaluated in heparinized whole blood obtained from rats dosed with 5 mg kg^{-1} intravenously. The blood sample was analysed by the protein precipitation method immediately. The remaining portion was centrifuged for 5 min at 2000 *g* and the plasma concentrations measured to estimate C_b/C_p. The k_b was accordingly calculated.

Analytical method

The portal and venous concentrations of centpropazine and its hydroxylated metabolite were determined from their calibration curves in serum. The concentration of an unknown metabolite, M1, was estimated from the calibration curve of centpropazine-OH, since both the analytes had similar UV spectral characteristics. The concentration of centpropazine in in-situ studies was determined after diluting the samples in a 1:2 ratio with acetonitrile and analysis by HPLC.

Data analysis

In in-situ experiments, log (%) remaining was plotted versus time on Microsoft Excel Ver 5.0 to calculate the first-order disappearance rate constants.

The amount of drug absorbed in the portal blood after oral dosing (D_{pog}) has been estimated using the clearance method (CL method):

$$D_{pog} = AUC_{pog} \times CL_s \quad (2)$$

where CL_s is the systemic clearance and AUC_{pog} is the area under the curve of centpropazine in the portal vein.

The total amount of drug absorbed was also calculated using the Q method:

$$D_{pog} = Q_p \times (AUC_p - AUC_v) \quad (3)$$

where subscripts p and v refer to portal and venous samples, respectively.

Since blood flow, Q_b, was given as 9.8 mL min^{-1} for a 250-g rat (Davies & Morris 1993), the effective plasma flow rate was calculated according to the equation:

$$Q_p = 9.8 \times (1-H_t) \times (1+k_b) \times W_t/250 \quad (4)$$

where W_t is the weight of the rat.

The local moments for the absorption rate time curve include the following:

$$t_a = \frac{(MRT_p \times AUC_p - MRT_v \times AUC_v)}{AUC_p - AUC_v} \quad (5)$$

where t_a is the mean local absorption time from gastrointestinal tract into the portal system.

Assuming that the absorption kinetics from the intestinal tract can be approximated by a one-compartment system, t_a can be correlated to absorption rate constant (k_a) by (Tabata et al 1996):

$$k_a = 1/t_a \quad (6)$$

AUC and MRT of serum concentration–time curve were calculated by Microsoft Excel without extrapolation because the time profile around the terminal phase was too unstable to extrapolate.

Results and Discussion

In-situ absorption studies

The logarithmic plot of mean concentration–time profile of centpropazine in rats is shown in Figure 3. The absorption rate constant of centpropazine was calculated from the slope of the logarithmic plots of mean concentration–time profile by the linear regression method. The absorption rate constant ($k_a = 0.04$ min^{-1} or 2.4 h^{-1}) is close to the k_a of metoprolol (3 h^{-1}), which is classified as a well-absorbed drug (Amidon et al 1995). About 70% of centpropazine was absorbed within the first half-hour of the experiment due to favourable higher percentage un-ionisation at pH 7.4 ($pK_a = 6.98 \pm 0.08$) and higher log P (3.15 ± 0.013).

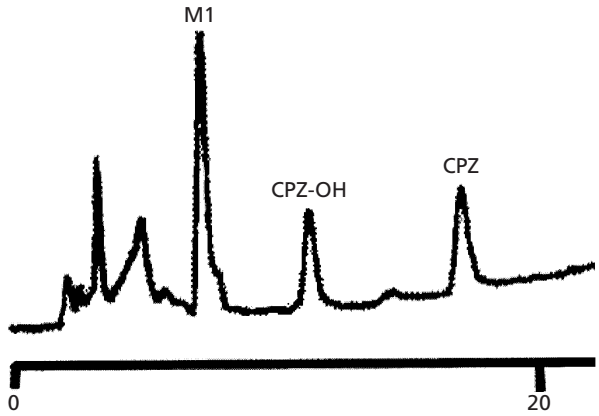

Figure 2 Representative chromatogram of 30 min serum sample after a single oral dose of 40 mg kg^{-1} in rats. CPZ, centpropazine.

In-vivo absorption studies

The serum levels of centpropazine, centpropazine-OH and metabolite M1 in the portal vein are shown in Figure 4. The portal level of centpropazine peaked at 10 min and was 671 ± 64 ng mL^{-1} as compared with a systemic level of 8.47 ± 3.94 ng mL^{-1}. The levels of the drug and metabolites were consistently higher in the portal vein at all time points in comparison with systemic concentrations. The venous and portal serum concentration–time profiles (Figure 5) had multiple peaks and had not merged till the last sampling point. It strongly suggests the presence of regional variations (i.e., duodenum vs jejunum vs ileum) in drug absorption. Centpropazine might be well absorbed from upper regions of the intestine where PEG will help to keep the drug in solution for longer period of time, thus enabling better absorption (Lin et al 1996).

The presence of centpropazine-OH in portal serum samples from the first sampling point (10 min) indicates that the drug may be metabolized in the stomach wall and in the upper regions of the intestine, possibly by reductase enzymes. The high levels of centpropazine-OH observed in the portal vein is attributed to metabolism of centpropazine during absorption, since circulating levels of the metabolite are very low and

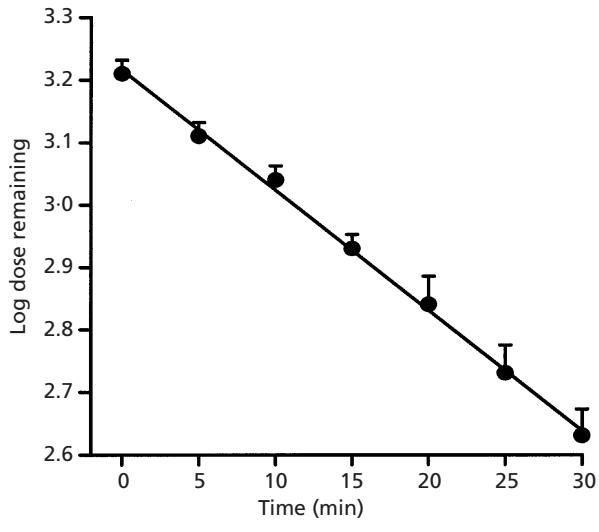

Figure 3 Time-course of centpropazine absorption at pH 7.4 from rat small intestine during in-situ experiment. Bars represent s.d.

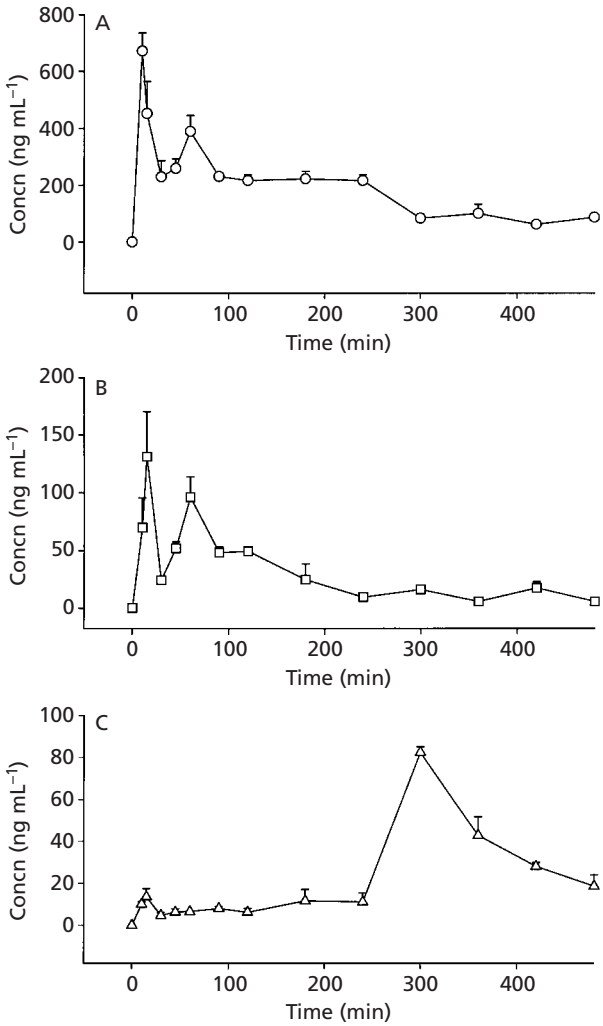

Figure 4 Plot of centpropazine (A), centpropazine-OH (B) and M1 (C) vs time, as observed in portal vein in rats after an oral dose of 40 mg kg^{-1} centpropazine. Bars represent s.d.

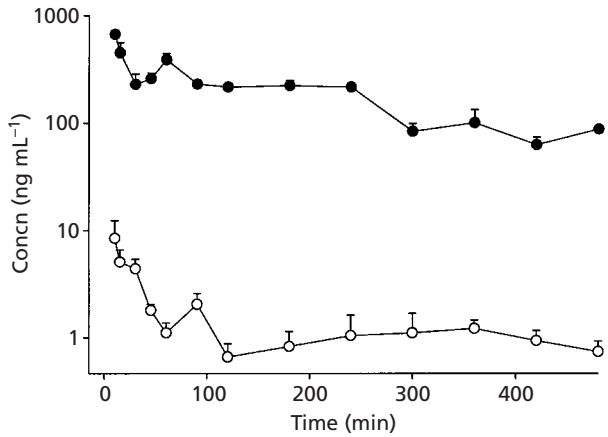

Figure 5 Overlay of portal (●) and venous (○) serum concentrations of centpropazine in rats after an oral dose of 40 mg kg^{-1} centpropazine. Bars represent s.d.

Table 1 Estimates of AUC, MRT and t_a of centpropazine in in-vivo absorption studies.

AUC (ng min mL^{-1})	
Portal	86190
Venous	643
MRT (min)	
Portal	171
Venous	185
t_a (min)	171

Where AUC is the area under the curve, MRT is the mean residence time and t_a is the local absorption time.

biliary excretion is negligible (Atul et al, unpublished data). The metabolite M1, however, was extensively excreted in the form of conjugates in bile and was present to a very much lesser extent in the circulation as the non-conjugated form (unpublished data). The portal serum levels of M1 were sustained until 4 h post dose. The presence of a secondary peak in the profile of M1 at 4 h is consistent with the 3–6-h transit time required for the conjugated form of drugs to reach the caecum after biliary excretion (Ouellet & Pollack 1995). The rate and extent of de-conjugation reactions in the large intestine is $>$ 2-fold higher than in the small intestine (Scheline 1973). Thus it is most likely that following hydrolysis of the glucuronide in the large intestine, the liberated M1 is reabsorbed into the portal system.

The partition ratio k_b of centpropazine was calculated to be 0.605 ± 0.02 (equation 1). The plasma flow rate used was determined to be 8.63 mL min^{-1} (equation 4). The amount of drug available before reaching the liver compartment (D_{pog}) was calculated to be 2.1 mg by the CL method (equation 2) and 0.6 mg by the Q method (equation 3). The difference in the calculations could be because the CL method assumes that only liver and intestine metabolise the drug. However, in in-vitro metabolism studies, it was found that red blood cells, lungs, etc., were found to metabolise centpropazine. Thus only 6% of the dose is absorbed into the portal system, by the last sampling time point (16 h), after a single oral dose of 40 mg kg^{-1}. A notable discrepancy in the % D_{pog} between the Q and CL methods might indicate that intestinal metabolism is an important systemic elimination pathway of the drug (Kwon & Inskeep 1996). The mean absorption time ($MAT = MRT_{oral} - MRT_{iv}$) was calculated to be 159 min. MAT is the sum of t_a (local absorption time) and t_H (hepatic transit time). The values of MRT_{oral} and MRT_{iv} were estimated to be 186 min and 27 min, respectively. The MAT, calculated to be 159 min, is not very significantly different from the t_a value of 171 min. This indicates that the mean transit time through the liver is negligible in calculated MAT.

The AUC and MRT of the time-courses of portal and venous concentration calculated by the trapezoidal rule are given in Table 1. The absorption rate constant (equation 6) was calculated to be 0.005 min^{-1}, which was 8 times lower than the rate constant (0.04 min^{-1}) observed in in-situ studies. However, the basic assumption in this model is that the intestinal tract behaves as a one-compartment system. This difference in observed k_a (in-vivo vs in-situ) could be as a result of precipitation of the drug in the intestine owing to the low aqueous solubility at this pH. The precipitated drug will further undergo dissolution slowly to be absorbed in the absorptive regions of the intestine. The difference between the in-situ and in-vivo results also may be due to the presence of intestinal contents in the in-vivo studies. Moreover, the in-situ model did not account for potential drug metabolism in the intestinal lumen or gastrointestinal-tract wall and drug binding to the mucosa, which would reduce the fraction available for absorption.

Conclusion

Overall, the data show that centpropazine, after a single oral dose of 40 mg kg^{-1} in the rat, is rapidly absorbed from the stomach (early C_{max} at 10 min) due to the presence of PEG in the drug formulation and probably precipitates in the intestine during its transit along the

gastrointestinal tract due to its low aqueous solubility of 2 μg mL^{-1}. The drug then most likely undergoes slow dissolution possibly due to the effect of bile and other physiological factors. The slow absorption rate constant observed in-vivo (0.005 min^{-1}), in comparison with the elimination rate constant, 0.06 min^{-1} (unpublished data), could lead to a so-called flip-flop situation and thus contribute to the low oral bioavailability of centpropazine. This could possibly be reversed by administering the drug as a more soluble derivative which would improve absorption and enable attainment of higher therapeutic levels. Although there is no data describing the relationship between pharmacokinetics and pharmacodynamics of the drug, if higher serum levels of the drug could be attained, a lower dose might be required to achieve the targeted therapeutic efficacy. The metabolite centpropazine-OH, observed in the portal vein, is suggestive of the role of the gastrointestinal tract in the metabolism of centpropazine, hence its low oral bioavailability. The data relating to M1 also strongly support the role of enterohepatic re-circulation in its residence in the body. However, other factors, such as formulation variables, bile, etc., need to be examined more closely for a better understanding of the absorption mechanisms of centpropazine and improvement of its bioavailability.

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