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In situ absorption and protein binding characteristics of CDRI-85/92, an antiulcer pharmacophore☆

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Abstract

CDRI-85/92, a new antiulcer drug, acts as a proton pump inhibitor arresting the secretion of acid in the stomach. The absorption kinetics of CDRI-85/92 was evaluated in situ using rat intestinal recirculation perfusion method. The experiment was conducted at pH 2.6 and 7.4 representing the acidic and the mild alkaline environment, which the drug experiences through the GIT during oral treatment. The rate of absorption was the same ($0.12\,h^{-1}$) at pH 2.6 and 7.4, thus suggesting equal absorption profile of the CDRI-85/92 throughout the GIT irrespective of the pH. Equal rates of absorption can also be correlated with the presence of acidic and basic groups in the structure of CDRI-85/92.

Protein binding studies of CDRI-85/92 using ultrafiltration were conducted in vitro and in vivo. Protein binding was found to be in the range of 31.49–32.91% both in in vitro and in vivo (employing 5-min post dose samples of rat serum after 20 mg kg^{-1} i.v. treatment of CDRI-85/92). The binding was found to be linear in the concentration range of 156.25–2000 ng ml^{-1} ($r^2 > 0.99$).

Keywords: Reversed phase chromatography; CDRI-85/92; Anti-ulcer agent; In situ absorption; Protein binding

1. Introduction

Omeprazole, rabeprazole and lansoprazole are the known antiulcer agents, which are classified as proton pump inhibitors and act by suppressing the gastric acid secretion by their ability to inhibit proton pump H^+, K^+-ATPase activity. The drugs are used for treatment of antiulcer. However, there have been reports of cases where blood disorders had occurred by the treatment (Carpio, 1990). In this context CDRI-85/92 (5-styryl-4, 5-*cis*-1,3-oxazl-2-one-4-carboxylic acid (Fig. 1) was synthesized in house. It is an oxazole derivative which acts as an antiulcer agent through its proton pump inhibitor nature. It was therefore essential to characterize CDRI-85/92 in terms of its absorption profile in GIT and protein binding capacity. Characterization of the in situ absorption and protein binding in model animals is required for toxicological and preclinical studies and also for extrapolation of the pharmacokinetics and pharmacodynamics in human (Rowland and Tozer, 1980). The present findings report the in situ absorption capacity and protein binding of CDRI-85/92, an antiulcer pharmacophore.

2. Materials and methods

2.1. Chemicals and reagents

CDRI-85/92 was synthesized in the Medicinal Chemistry Division of the Institute. HPLC grade solvents (methanol and acetonitrile) were procured from

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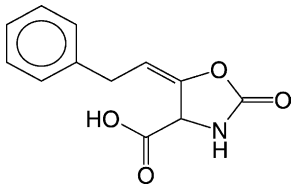

Fig. 1. Chemical structure of CDRI-85/92.

Ranbaxy Laboratories, New Delhi, India. All the other chemicals used in the experiment were of analytical grade. Triple distilled water was prepared from a quartz apparatus. Centrifree micropartition capillaries were obtained from Amicon Inc., MA, USA. A centrifuge (Research Centrifuge, Indian Equipment Corporation, Mumbai, India) containing a fixed angle rotor was employed in the study. All the buffers were filtered through 0.22 μm cellulose membranes (Whatman International Ltd., Mailstone, UK) prior to use. Drug free serum was obtained from the blood of normal *Sprague-Dawley* rats.

2.2. Stock and standard solutions of CDRI-85/92

Stock solution of CDRI-85/92 was prepared by dissolving 10 mg of the compound in 100 ml of methanol. Analytical standards and serum calibration standards of CDRI-85/92 were prepared in the range of 12.5–2500 and 2.5–1000 ng ml^{-1} respectively by serial dilution method.

2.3. Perfusion solution and drug formulation preparation

2.3.1. Perfusion solution

The perfusion solution was prepared by mixing 0.145 M NaCl, 4.56×10^{-3} M KCl, 1.25×10^{-3} M $CaCl_2$ and 5.0×10^{-3} M NaH_2PO_4 as mentioned by Doluisio et al. (1969).

2.3.2. Sorenson buffer

Sodium phosphate (0.067 M) buffer pH 7.4.

2.3.3. Isotonic citrate buffer pH 2.6

Prepared by dissolving 0.68 g of NaCl in 100 ml of citrate buffer containing 44.6 ml of 0.1 M citric acid solution and 5.4 ml of 0.2 M Na_2HPO_4.

2.3.4. Formulation A

In all 20 mg of CDRI-85/92 was transferred to a 100 ml of conical flask containing 20 ml of the Sorenson buffer pH 7.4. The contents were slowly agitated in a thermostatic incubator shaker for 1 h at 37 °C. The suspended drug particles (if any) were filtered off and the filtrate was analyzed for drug concentration by HPLC.

2.3.5. Formulation B

In all 20 mg of CDRI-85/92 was transferred to a 100 ml of conical flask containing 20 ml of the isotonic citrate buffer pH 2.6.

2.3.6. Formulation C

25 mg of CDRI-85/92 was dissolved in 2.0 ml of water, mixed properly and used (0.4 ml per 250 g of rat) as the intravenous administration through tail vein.

2.4. Animal experiments

2.4.1. Animals

Young, healthy, male, albino *Sprague-Dawley* rats ($n = 5$ for each experiment) weighing 250 ± 25 g, were obtained from Laboratory Animal Division of the Institute. The animals were housed in plastic cages at standard laboratory conditions with a regular 12 h day–night cycle using non-heat radiating lamp. Standard pellet laboratory chow (Goldmohar Laboratory animal feed, Lipton India Ltd., Chandigarh, India) and the water was allowed ad libitum. The rats were acclimatized to this environment for at least 2 days prior to start of the experiments. The rats were fasted overnight (12–16 h) prior to experiments, however, water was freely provided.

2.4.2. In situ studies using rat intestinal lumen

Rats were anaesthesized by administering urethane solution intraperitoneally (1 mg g^{-1} body weight) 30 min prior to the surgery (Doluisio et al., 1969). The small intestine of anaesthetized rat was exposed by midline incision on the abdominal region. A 10 cm intestinal loop was prepared 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 (Schurgers et al., 1986). The loop was washed with 30 ml of the perfusion solution to clear the intestinal

contents. The free ends of the canula were dipped into the reservoir. The perfusion solution was then replaced with 10 ml of formulation A or B. The drug solution was recirculated with the help of the peristaltic pump at the flow rate of 0.9 ml min^{-1}. Samples were withdrawn from the solution coming out of the intestine and were stored at −60 °C for further analyses. The entire experiment was performed under a closed hood in which heating lamps were used to maintain the body temperature of the rat at 37 ± 2 °C.

2.4.3. Protein binding studies

2.4.3.1. Non-specific binding of CDRI-85/92 with ultrafiltration device. Binding of CDRI-85/92 to the ultrafiltration membrane and receiving cell was determined separately. For binding of drug to the membrane, rat serum samples ($n = 3$) containing 10 μg ml^{-1} of CDRI-85/92 were placed in the loading cell of the Centrifree device. The aliquots of the spiked samples were analyzed for CDRI-85/92 prior to and 10 min after filling the loading cells. To detect the binding of the drug to the receiving cell, drug free serum was centrifuged at 1500 × *g* for 10 min at 37 °C in the Centrifree micropartition system and the ultrafiltrate was spiked with CDRI-85/92 (50 ng ml^{-1}). Spiked ultrafiltrate samples ($n = 3$) were placed in the receiving cell and analyzed prior to and 10 min after keeping in the cell. The difference in the concentrations indicated the extent of the non-specific binding of CDRI-85/92 with the loading/receiving cell.

2.4.3.2. In vitro protein binding studies. Normal rat serum was spiked with different concentrations of CDRI-85/92 ranging from 156.25 to 2500 ng ml^{-1}. The bound (loading cell) and unbound (receiving cell) fractions of CDRI-85/92 were separated from the serum samples by ultrafiltration through Centrifuge micropartition system (Amicon Inc., MA, USA). Centrifuge containing a fixed angle rotor was used for effective ultrafiltration. Rotor, centrifuge and samples were equilibrated at 37 °C prior to centrifugation. Samples in triplicates (1 ml) were placed in centrifree devices and centrifuged at 1500 × *g* for 10 min to collect the ultrafiltrates. The samples were stored at −60 °C till analyses.

2.4.3.3. In vivo protein binding studies. Protein binding in in vivo conditions was also evaluated. The rats were dosed 20 mg kg^{-1} i.v.; CDRI-85/92 (formulation C) and the blood samples were collected 5-min post dose. Blood was allowed to clot for 30 min and centrifuged at 1500 × *g* for 10 min to separate the serum. The samples were processed as mentioned in in vitro.

2.5. HPLC analyses

2.5.1. Instrumentation

The HPLC system consisted of a solvent delivery system with a controller (Kontron HPLC System, Unicam, Cambridge, UK) equipped with a 7125 injector (Rheodyne, Berkely, USA) fitted with a fixed 20 μl loop and a Kontron UV Spectrophotometer detector (Uvikon 730SLC set at 250 nm). The samples were injected with a 100 μl syringe. The eluants were monitored at wavelength 250 nm and chromatograms were integrated using C-R1B Chromatopac integrator (Shimadzu, Kyoto, Japan). Separation was achieved on a C-18 column (5 μm, 220 × 4.6 mm, i.d.), coupled with a guard column packed with the same material (5 μm, 30 × 4.6 mm, i.d.) (E-merck, Darmstadt, FR Germany, No. 619429). The HPLC system was equilibrated for approximately 30 min at a flow rate of 1 ml min^{-1} before analysis commenced. A vortex-mixer (Thermolyne, India), ultrasonic bath (Bransonic, Shelto, CY), a Model SVC-220H Speed vac concentrator (Savant, Nyor Heto/Maxi Dry Plus Germany) and a Model C-30 centrifuge (Remi, India) were used for sample preparations.

2.5.2. Chromatographic conditions

The mobile phase was prepared by mixing methanol, acetonitrile and 10 mM ammonium acetate buffer (pH 4.0) (29:1:70, v/v). The mobile phase was degassed for 15 min in the sonicator before use and was pumped at a flow rate of 1 ml min^{-1}. The chromatography was performed at ambient temperature.

2.5.3. Sample preparation and analyses

2.5.3.1. In situ absorption studies. The luminal samples (100 μl) were diluted with 300 μl of acetonitrile, vortex-mixed, centrifuged at 5000 rpm for 5 min. The supernatant was injected directly onto the HPLC. CDRI-85/92 was monitored on UV-Vis Uvikon detector set at wavelength of 250 nm. The unknown

concentrations of CDRI-85/92 were read from the standard curves in mobile phase.

2.5.3.2. In vitro protein binding. CDRI-85/92 was estimated in serum by protein precipitation method. In brief, 300 μl of acetonitrile was added to 100 μl of spiked/treated rat serum. The contents were vortex-mixed and centrifuged at 10,000 rpm for 5 min in a microcentrifuge (TOMY microcentrifuge, USA) at 4 °C. The supernatant was injected directly onto the HPLC for analyses. CDRI-85/92 was monitored in serum as well as in ultrafiltrate using UV-Vis spectrophotometer detector set at λ 250 nm. The unknown concentrations of CDRI-85/92 in the serum and ultrafiltrates were read from their respective standard curves in serum and mobile phase, respectively.

2.5.4. Calibration graph

Peak areas/heights of the analytical standard solutions of CDRI-85/92 were used to plot the calibration curve of the compound. The standard curve was used to calculate the concentrations of CDRI-85/92 present in the unknown solutions (luminal solution). Calibration curve using spiked serum standards were also plotted after processing the serum samples by linear regression of the data and used to read the unknown concentrations of CDRI-85/92 in serum.

2.5.5. Data analyses

2.5.5.1. In situ absorption studies. During recirculating perfusion, the absorption of CDRI-85/92 was evaluated by measuring the disappearance from the perfusate, which followed first-order kinetics. The time dependence of the luminal concentration C can be written as:

$$\ln\frac{C_t}{C_0} = -K_{dis}.t$$

where C_0 and C_t are the luminal concentrations of the model compound at 0 and t min, respectively and K_{dis} is the first-order disappearance rate constant (min^{-1}). The disappearance rate constant K_{dis} was calculated from the plots of $\ln(C_t/C_0)$ versus t by linear regression (Polema et al., 1989).

2.5.5.2. In vitro protein binding studies. CDRI-85/92 concentrations in spiked/in vivo serum samples and their respective ultrafiltrates were calculated to detect the percent binding of CDRI-85/92 with serum. The calculations were performed using linear regression application of Microsoft Excel software (version 5.1). Percent binding of CDRI-85/92 was calculated using the formulae:

$$\%\,\text{binding} = \left[1 - \frac{\text{Concentration of CDRI-85/92 in ultrafiltrate}}{\text{Concentration of CDRI-85/92 in spiked/unknown samples}}\right] \times 100$$

3. Results and discussion

3.1. Effect of pH on in situ absorption

CDRI-85/92 did not show any adsorption to the silicone cannulae over 30-min time period, hence, it was appropriate to employ it in the in situ recirculation perfusion method for measurement of absorption.

In order to simulate absorption of CDRI-85/92 from the stomach, formulation B was recirculated in the intestine and the absorption rate constant determined. The effect of pH on the absorption rate of CDRI-85/92 was also studied. Typical plots showing the disappearance of CDRI-85/92 from the intestinal lumen solutions are shown in Fig. 2. The absorption rate constant was calculated from the slope of the logarithmic plots of mean $[C_t/C_0]$-time profile by linear regression method (Swintowsky and Pogonowskawala, 1982). CDRI-85/92 was slowly 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 CDRI-85/92 at pH 7.4 was calculated to be 0.002 min^{-1} or $K_a = 0.12\ h^{-1}$. Only 6.7–8.8% of the compound was absorbed in 30 min at pH 2.6 and 7.4. This indicates that there is an equal absorption rate of CDRI-85/92 in the stomach and intestine although the rate of absorption (0.002 min^{-1}) appears to be very slow. This is evident by the equal percentage of the unionized specie of CDRI-85/92 at pH 2.6 and 7.4. This is also supported by the fact that the apparent absorption rate constant obtained at pH 2.6 and 7.4 is the same. It appears that the absorption of CDRI-85/92 is not pH dependent along the gastrointestinal

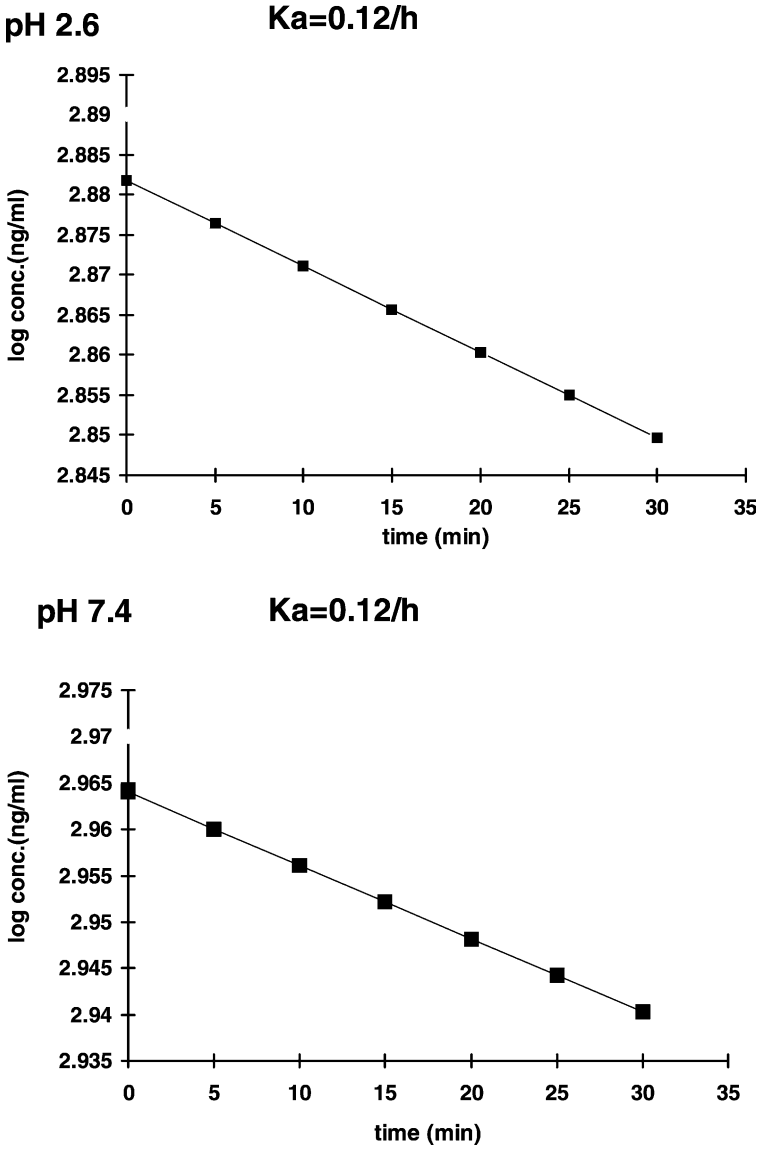

Fig. 2. Absorption constants of CDRI-85/92 at pH 2.6 and 7.4.

tract. The absorption rate constants at pH 7.4 in both the studies was found to be similar and showed no significant difference ($P > 0.05$) between the two. The absorption half-life was found to be 4.88 and 5.78 h at pH 2.6 and 7.4, respectively (Fig. 2). The results of the absorption pattern of CDRI-85/92 matched with its chemical structure i.e. the presence of acidic and basic groups, which is responsible for the equalized absorption of CDRI-85/92 throughout the GIT.

3.2. In vitro/in vivo protein binding studies

In vitro and in vivo studies were carried out to determine the extent of serum protein binding of CDRI-85/92. Unbound and bound drug from spiked/treated serum was separated by ultrafiltration technique. Ultrafiltration method was chosen, as it is comparable to the in vivo processes, like the ultrafiltration in the kidneys (Koike et al., 1985).

Table 1
In vitro and in vivo protein binding capacity of CDRI-85/92 in rat serum

Biomatrix	Concentration of CDRI-85/92 ($ng\,ml^{-1}$)	Percent binding	Mean ± S.D.	Percent unbound
Spiked normal rat serum	156.25	36.89		63.11
	312.50	30.17		69.83
	625.00	29.54	32.91 ± 3.13	70.46
	1250.00	35.02		64.98
	2500.00	32.93		67.07
Treated rat serum (i.v. dose $20\,mg\,kg^{-1}$)		32.45		67.55
		30.89	31.49 ± 0.84	69.11
		31.13		68.78

The non-specific binding of the drug was not associated with the material of the filtration device. In vitro binding of the CDRI-85/92 in the concentration range of 156.25–2000 $ng\,ml^{-1}$ with serum proteins was linear and concentration dependent (32.91 ± 3.13). Similarly, binding was also determined in vivo, where the drug was found to be 31.49 ± 0.84% in the bound form and well correlated with the in vitro binding data (Table 1). The percent binding of CDRI-85/92 concomitantly increased with the protein contents of the samples. The similarity in the protein binding capacity in in vitro and in vivo indicate that there is no effect of the metabolism of CDRI-85/92 on the protein binding phenomenon.

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References

Carpio, R.E., 1990. Clinical efficacy of low dose antacids in the treatment of peptic ulcer. Do we have anything better? Drug Invest. 2 (Suppl. 1), 18–21.

Doluisio, J.T., Billups, N.F., Dittert, L.W., Sugita, E.T., Swintosky, J.W., 1969. Drug absorption I: an in situ rat gut technique yielding realistic absorption rates. J. Pharm. Sci. 58, 1196–1201.

Koike, Y., Magnusson, A., Steiner, E., Rane, A., Sjogvist, F., 1985. Ultrafiltration compared with equilibrium dialysis in the determination of unbound phenytoin in plasma. Ther. Drug Monit. 7, 461–465.

Polema, F.G.J., Tucker, J.J., Chrommelin, D.J.A., 1989. Intestinal absorption of drugs I: the influence of taurocholate on the absorption of dantrolene in the small intestine of the rat. J. Pharm. Sci. 78, 285–289.

Rowland M., Tozer T.N., 1980. Clinical Pharmacokinetics: Concepts and Applications, Lea and Febiger, Philadelphia, p. 20.

Schurgers, N., Bijdendijk, J., Tukker, J.J., Crommelin, D.J.A., 1986. Comparison of four experimental techniques for studying drug absorption kinetics in the anaesthetized rat in situ. J. Pharm. Sci. 75, 117–119.

Swintowsky, J.V., Pogonowskawala, E., 1982. The in situ rat gut technique. Pharm. Ind. 3, 163–167.