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Absolute oral bioavailability of ditekiren, a renin inhibitor peptide, in conscious rats

B.D. Rush [1], K.F. Wilkinson [1], W.Z. Zhong [2], S.K. Closson [2], D.B. Lakings [2,*] and M.J. Ruwart [1]

[1] *Drug Delivery Systems Research and* [2] *Drug Metabolism Research, The Upjohn Co, Kalamazoo, MI 49001 (U.S.A.)*

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Summary

Absolute oral bioavailability of ditekiren, a renin inhibitor peptide, was determined in rats. Unanesthetized rats which had been fitted with chronic biliary and superior vena cava cannulae were dosed either orally (p.o.) at 50 mg/kg or intravenously (i.v.) at 10 mg/kg with ditekiren. Serum ditekiren levels were measured with an activity assay and biliary ditekiren was quantitated by both activity assay and HPLC assay. Cumulative biliary elimination (B_T) indicated that oral bioavailability was 1.3%. The disposition kinetics of ditekiren were independent of route of administration. These data indicate that ditekiren is poorly absorbed across the gut and that, once in the systemic circulation, ditekiren is rapidly cleared. Even though oral bioavailability was low, serum ditekiren levels were equivalent to 4–8 times the in vitro IC_{50} for renin inhibition for this peptide throughout the 2.5 h after oral administration that blood levels were monitored.

Introduction

The therapeutic success of the angiotensin converting enzyme (ACE) inhibitors for treatment of renin-dependent hypertension and congestive heart failure (Ferguson et al., 1984) has stimulated research efforts with renin inhibitory peptides (RIPs), which are more specific inhibitors of the renin-angiotensin cascade (Paulsen et al., 1973; Burton et al., 1980; Haber, 1983). However, because oral administration is the most accepted means of drug therapy for chronic diseases such as hypertension, the development of the RIPs as viable therapeutic entities has been impeded by their low oral bioavailability.

Ditekiren (bocPro-Phe-NMeHis-LeuΨ($CHOCH_2$)Val-IleAmp), a potent inhibitor of both human and monkey renin in vitro (Pals et al., 1986; Thaisrivongs et al., 1986) is currently being clinically evaluated as an intravenous (i.v.) infusion to assess its hypotensive response in man. This RIP has been shown to decrease the blood pressure component attributed to renin after i.v. or oral (p.o.) administration in the renin-infused

Correspondence: B. Rush, The Upjohn Co., Unit 7271-267-6, 301 Henrietta St., Kalamazoo, MI 49001, U.S.A.
* *Present address:* Bristol Myers-Squibb, P.O. Box 4000, Princeton, NJ 08543-4000, U.S.A.

rat and the sodium-depleted monkey models (Pals et al., 1986). While the pharmacodynamic response after oral administration in these animal models is evidence for ditekiren absorption from the gastrointestinal tract, the extent of oral absorption has not been determined by quantitative methods. In the present study, serum and biliary ditekiren levels were measured in unanesthetized rats after p.o. or i.v. administration, and the absolute oral bioavailability and disposition of ditekiren were determined.

Materials and Methods

Formulations

The oral (p.o.) solution of ditekiren was prepared at a concentration of 12.5 mg/ml in 0.1 M citric acid and the intravenous (i.v.) formulation was 2.5 mg/ml in 0.006 M citric acid/saline solution. The concentration of ditekiren in these solutions was confirmed by a stability-indicating assay (Lakings et al., 1989).

Animal preparation

Upjohn strain, male, Sprague Dawley rats (presurgical weight 220–240 g) were used. Bile duct, duodenum, and superior vena cava (SVC) cannulae were implanted aseptically under metofane (R) anesthesia. The cannula construction (Weeks, 1972) and the surgical techniques (Weeks, 1972; Ruwart et al., 1979) are modifications of techniques which have been previously described. The cannulae were exteriorized behind the head and secured there with stainless-steel autoclips. When the animals were not involved in tests, enterohepatic recirculation was maintained via connection of the bile duct and duodenum cannulae with a 'U'-shaped 20 mm length of 21 gauge stainless-steel tubing, and patency of the SVC cannula was maintained by filling the cannula with heparin (100 U/cm^3) and plugging the tip with a stainless-steel stylet wire.

Experimental design

The animals were allowed 4–5 days to recover from surgery, then were fasted overnight with access to water. The following morning, the rats were placed in Bowman restrainers (Scholting, Inc., Chicago, IL) and the tubing connector between the bile duct and duodenum cannulae was removed. A tubing extension was spliced onto the bile cannula and bile collection was begun into calibrated tubes which were placed below the level of the rats. A continuous infusion of sodium taurocholate (100 mM at 0.84 cm^3/h) was then started immediately through the duodenum cannulae and this supplementation was maintained throughout the 6 h of bile collection to compensate for the loss of bile salt and to prevent dehydration. Ditekiren was administered as an i.v. bolus (at 10 mg/kg by hypodermic injection through the tail vein) or p.o. (at 50 mg/kg by gastric intubation). Because patency of the chronic cannulas could not be maintained through an adequate washout period, i.v./p.o. cross-over studies were not possible. Bile was collected in hourly fractions for 6 h and blood was sampled from the SVC cannula at 0, 30, 60, 90, and 150 min after dosing.

Assays

Bile samples were assayed for the presence of a renin inhibitor using an activity assay which has been previously described (Ruwart et al., 1990). As a means of confirming the activity assay results, the biliary concentration of ditekiren was also determined by HPLC-UV using a modification of a method which has been previously described (Lakings et al., 1989). The HPLC assay lacked adequate sensitivity to be useful for the quantitation of ditekiren in serum, therefore sera were analyzed by activity assay only.

Pharmacokinetics and statistics

The serum and biliary results for ditekiren were evaluated pharmacokinetically using noncompartmental techniques (Gibaldi and Perrier, 1982). The biliary excretion results (activity assay and HPLC) from i.v. and p.o. dosed rats were evaluated to determine the apparent terminal disposition rate constant (β), the disposition rate half-life ($t_{1/2}\beta$), and the cumulative biliary excretion (B_T). The slope of the linear regression equation of the rate of change in biliary elimination (dB/dT) vs the midpoint of each collection

interval (t_{mid}) was used to calculate β. The biliary clearance (CL_B or B_T/AUC) was calculated for the i.v. dosed rats. In the equation $CL_B = B_T/AUC$, B_T represents biliary elimination of ditekiren for 0–2.5 h rather than total elimination for 0–6 h and AUC represents serum AUC from 0–2.5 h. Total oral absorption of ditekiren was determined from the B_T data using the following formula (Gibaldi and Perrier, 1982):

$$\text{Oral bioavailability} = (\text{p.o. } B_T)/(\text{i.v. } B_T) \times (\text{dose}_{\text{i.v.}}/\text{dose}_{\text{p.o.}}) \times 100\% \quad (1)$$

B_T represents the cumulative biliary elimination (0–6 h) and for i.v. B_T and $\text{dose}_{\text{i.v.}}$ the group mean for i.v.-dosed rats is used. The activity assay and HPLC results were statistically evaluated using the paired t-test (Goodnight, 1982) to determine if the assays yielded similar values.

Results

Serum analysis (activity assay)

Four of five i.v.-dosed rats and five of eight p.o.-dosed rats had SVC cannula from which serum samples could be collected. Ditekiren concentrations were determined by activity assay and the values are illustrated in Fig. 1. Three of the four i.v.-dosed rats had similar profiles (Fig. 1a), while one animal (rat 11) exhibited somewhat higher concentrations at each collection time. Bile flow appeared to be somewhat diminished in rat 11 which is possibly indicative of a partial or temporary obstruction of the bile duct cannula which may have affected serum clearance. However, total biliary excretion of ditekiren for this animal was comparable to the group mean, therefore, the data from this rat were not excluded from the evaluation.

Four of the five p.o.-dosed rats had similar profiles (Fig. 1b), while one animal (rat 17) exhibited a higher concentration (155 ng/ml) at the earliest time point. However, cumulative biliary elimination indicated that ditekiren bioavailability for rat 17 was not improved in comparison to other p.o.-dosed rats. These observations suggest that the maximum blood levels may have been attained prior to 30 min in the p.o.-dosed rats, with the possible exception of rat 17. Serum ditekiren concentrations remained relatively unchanged from 60 min to the time of the last blood sample at 150 min, suggesting that absorption of ditekiren was not complete at 150 min after oral dosing.

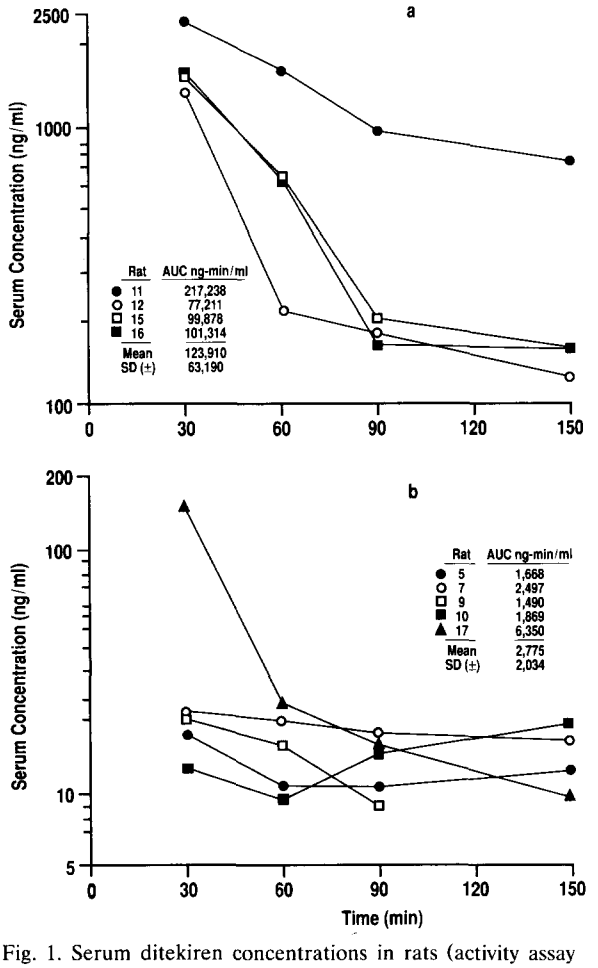

Fig. 1. Serum ditekiren concentrations in rats (activity assay results) after i.v. administration of 10 mg/kg (a) or oral administration of 50 mg/kg (b).

Biliary analyses (activity assay and HPLC)

Bile was collected in hourly fractions for 6 h. Biliary concentrations of ditekiren were mea-

sured by activity assay and by HPLC (Fig. 2). In i.v.-dosed rats, 77% of the administered dose of ditekiren was recovered in the bile during the 6 h following administration, with 94% of this quantity found in the first two hourly collections. These data indicate that ditekiren is rapidly cleared from systemic circulation.

In orally dosed rats, 1.05% of the total administered dose of ditekiren was recovered during the 6 h of bile collection. Although biliary ditekiren recovery diminished over time, 40% of the recoverable ditekiren was present in the 2–6 h bile collections in p.o.-dosed rats. In i.v.-dosed rats, 94% of the recoverable ditekiren was eliminated in bile during the first 2 h and only 6% of the recoverable ditekiren was eliminated in the 2–4 h bile collections.

The activity assay and HPLC assay yielded very similar results (Fig. 2); however, the results of the two assays differed statistically ($p < 0.05$, paired t-test) for the 0–1 and 5–6 h collection intervals. The agreement between the HPLC and activity assay results suggests that ditekiren is either eliminated without substantial metabolism or, if degradation product(s) were present in bile, they had little or no renin inhibitory properties. This finding is consistent with earlier studies in both monkey (Lakings et al., 1989) and rat

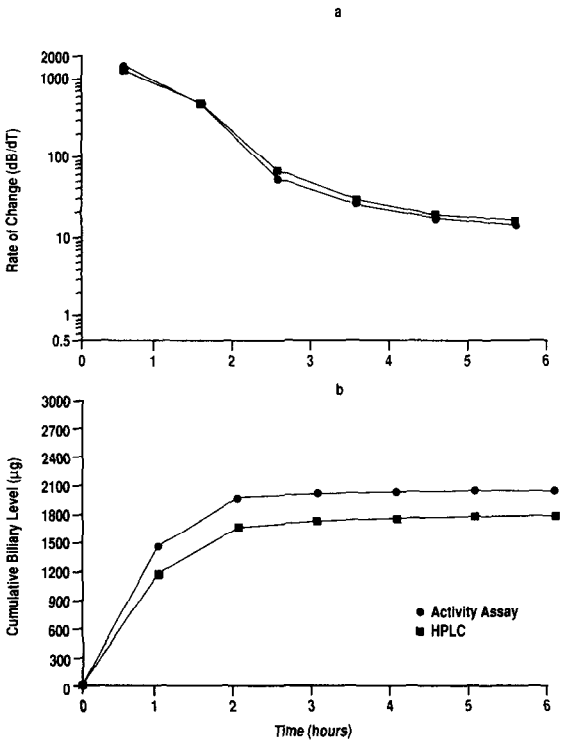

Fig. 3. Biliary elimination kinetics of ditekiren in i.v.-dosed rats. Panel a shows dB/dT vs t_{mid} and panel b shows cumulative biliary ditekiren levels (B_T). Each point represents the mean of five rats; the results of the activity assay and HPLC determinations are plotted separately.

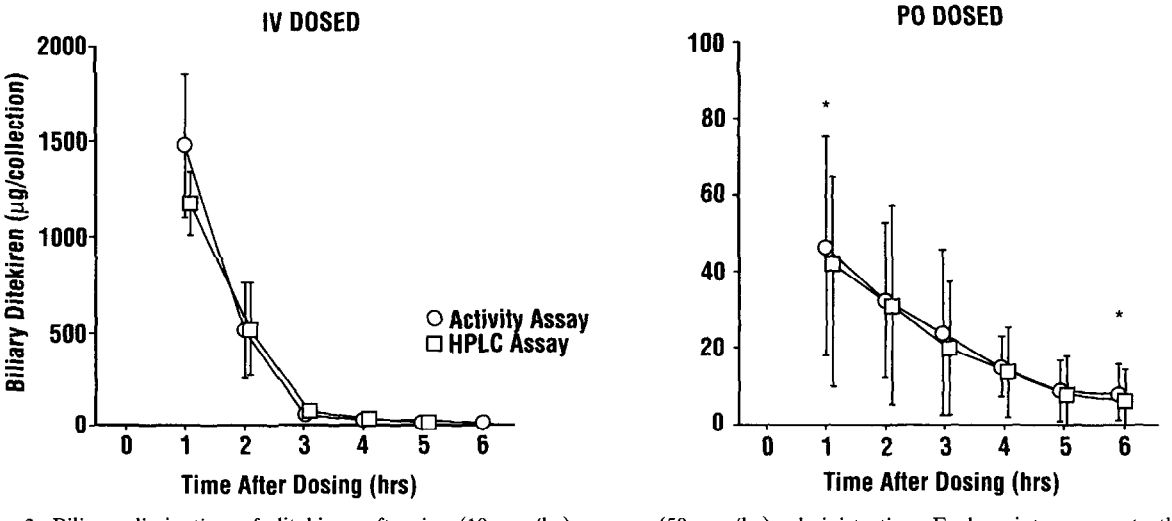

Fig. 2. Biliary elimination of ditekiren after i.v. (10 mg/kg) or p.o. (50 mg/kg) administration. Each point represents the mean ± SD of five rats (i.v.-dosed) and 8 rats (p.o.-dosed). The results of the activity assay and HPLC determinations are plotted separately and an asterisk indicates difference, $p < 0.05$ paired t-test, between assay results.

(Greenfield et al., 1989) which indicated that i.v. ditekiren is excreted, as parent compound, primarily by the liver via the bile.

The mean biliary excretion profiles are illustrated in Figs. 3 (i.v.-dosed rats) and 4 (p.o.-dosed rats) as the rate of change per collection interval (dB/dT) vs t_{mid} and B_T vs time. Pharmacokinetic evaluation of the biliary results gave β (mean $\pm$ SD) from the i.v.-dosed rats of 0.61 ± 0.21 h^{-1} (activity assay), and 0.58 ± 0.28 h^{-1} (HPLC). The (population) $t_{1/2}\beta$ for activity assay and HPLC was 1.1 and 1.2 h, respectively. For p.o.-dosed rats, the β values (mean $\pm$ SD) were 0.49 ± 0.14 h^{-1} (activity assay) and 0.49 ± 0.24 h^{-1} (HPLC) and the (population) $t_{1/2}\beta$ was 1.4 h for both assay methods. These results suggest that the disposition kinetics of ditekiren after i.v. and p.o. administration were similar and independent of the assay technique. Biliary clearance

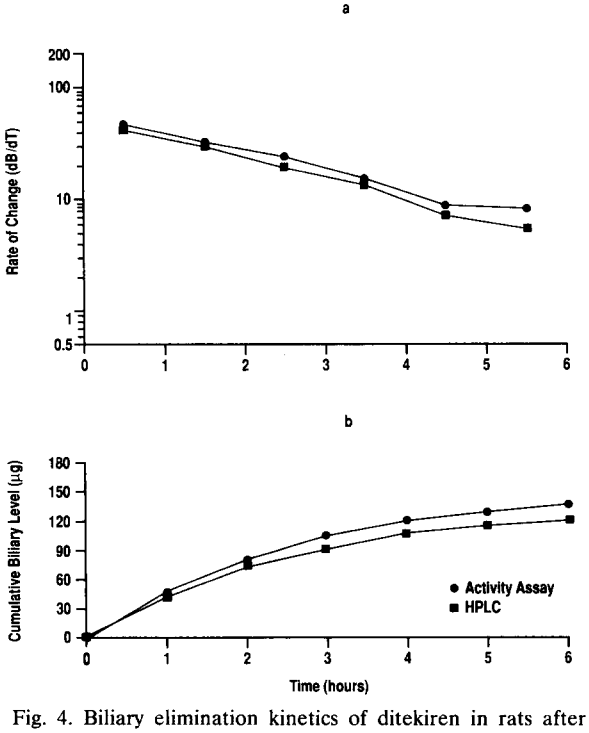

Fig. 4. Biliary elimination kinetics of ditekiren in rats after p.o. administration of 50 mg/kg ditekiren. Panel a shows the dB/dT vs t_{mid} and panel b shows B_T levels. Each point represents the mean of eight rats and the results of the activity assay and HPLC assay determinations are plotted separately.

(CL_B) of ditekiren, determined using the mean results of each assay, was 17 ml/min (activity assay) and 15 ml/min (HPLC), indicating that this RIP is cleared rapidly from systemic circulation.

The B_T results indicated that absolute oral bioavailability of ditekiren in the rat, in 0.1 M citric acid, was $1.3 \pm 0.9\%$ (mean $\pm$ SD of activity assay and HPLC).

Discussion

Oral bioavailability of ditekiren in the rat was determined from biliary elimination of the peptide to be $1.3 \pm 0.9\%$ (activity assay and HPLC mean). In i.v.-dosed rats, 94% of the recoverable ditekiren was eliminated into bile during the first 2 h after dosing. These data suggest relatively rapid clearance of ditekiren from systemic circulation. This observation also suggests that serum ditekiren concentrations, shown in Fig. 1a, represent only the terminal phase of the kinetic profile. This conclusion is consistent with the findings of Greenfield et al. who reported a very rapid serum clearance during the first 30 min after i.v. administration of radiolabeled ditekiren followed by a slower rate of clearance thereafter. Due to this probable truncation of the serum AUC, pharmacokinetic analysis of the serum data was not possible. Fig. 1b shows that serum ditekiren concentrations were relatively unchanged between 60 and 150 min after oral dosing, which suggests continued exposure of the gut lumen to ditekiren, and although the extent of absorption during this time was conspicuously low, absorption may not have been complete at 2.5 h after dosing. In spite of apparent poor oral absorption and rapid serum clearance, the serum concentration of ditekiren in orally dosed rats remained 4–8 times the in vitro IC_{50} for renin inhibition (Pals et al., 1986) throughout the 2.5 h that blood levels were monitored.

Poor oral bioavailability, such as the 1.3% exhibited by ditekiren, is characteristic of large and moderate-sized peptides. It is therefore encouraging that some reduction in the molecular size and peptidic nature of renin inhibitors has been

achieved without appreciable loss of renin-binding potential (Kleinert et al., 1988; DeGasparo et al., 1989; Morishima et al., 1989). However, initial reports suggest that the oral bioavailability of these smaller or nonpeptidic renin inhibitors is, thus far, unremarkable. For instance, a nonpeptidic renin inhibitor was reported to be 2.8–9.7% orally bioavailable in rats (Morishima et al., 1989) and a tetrapeptide length renin inhibitor with 10^{-9} M in vitro renin inhibitory potency was shown by another group to be less than 1% bioavailable in normal human volunteers after oral administration (DeGasparo et al., 1989).

Other investigators have proposed that transcellular transport across the gut epithelium might be controlled by the energy required (desolvation energy) to free the solute from water in the gut lumen (Stein, 1967; Diamond and Wright, 1969). Since increased hydrogen-bonding potential would raise desolvation energy (Stein, 1967), transcellular transport could possibly be improved by systematic modifications of the molecular structure which result in reduction in hydrogen-bonding potential of the solute molecule. The results of in vitro (Conradi et al., 1991) and in vivo (Karls et al., 1990) investigations using a series of model peptides indicate that the application of this theory to peptide absorption may be feasible. However, the extent that the molecular structure of biologically active peptides can be modified without loss of biological activity is obviously limited; therefore, further investigations are necessary to ascertain the practical limits of application to enhancement of oral peptide absorption.

Poor oral bioavailability continues to be an imposing obstacle to development of stable peptide-based drugs as useful agents for treatment of chronic diseases. This complex problem of achieving adequate systemic blood levels of peptidemetic molecules has stimulated interest in intrapulmonary delivery as a viable alternative route of administration of peptide-based drugs. The terminal airways of the lung not only represent a tremendous surface area for absorption, but additionally, intrapulmonary delivery offers a means of possibly averting 'first pass' clearance by the liver. A nonapeptide with luteinizing hormone releasing agonist activity was shown to be 4–18% bioavailable in human volunteers after intrapulmonary dosing (Adjei and Garren, 1990), and when corrected for respirable fraction, bioavailability reportedly ranged from 35 to 55%. Also, a renin inhibitor peptide, A-64662, which was absorbed poorly by the oral route, was reported to be 100% bioavailable in the dog after intrapulmonary administration (Garren et al., 1990). Evidence indicates that even relatively large molecular weight proteins like insulin can be transported to some degree across the lung and inhalation therapy is apparently well tolerated by patients (Wigley et al., 1971). However, there are also indications which suggest that transfer of peptides across the lung may be dose-dependent (Garren et al., 1990; Niven et al., 1990). Therefore, additional work will be required before the potential of intrapulmonary delivery of peptides for chronic therapy can be fully assessed.

In conclusion, although ditekiren is a potent inhibitor of primate renin and possesses good stability against proteolytic enzymes, poor oral bioavailability impedes the development of this peptide renin inhibitor as a therapeutically useful oral agent for use in the treatment of renin-dependent hypertension.

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