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Tissue Distribution and Excretion of CDRI-81/470 in Rats

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Abstract

Methyl-*N*[5[[4-(2-pyridinyl)-1-piperazinyl]carbonyl]-1*H*-benzimidazol-2-yl] carbamate (CDRI-81/470) is a broad spectrum anthelmintic agent, effective against both intestinal and systemic parasitism. Tissue distribution and excretion of CDRI-81/470 were studied in rats after a single oral dose of 100 mg kg^{-1} CDRI-81/470.

One of the metabolites was identified in pilot studies as its *N*-decarboxylate derivative and characterized by synthesis. HPLC assay methods for the simultaneous estimation of CDRI-81/470 and its *N*-decarboxylate derivative in tissues, bile, urine, and faeces were developed and validated. The parent compound was quantitated in all major tissues and organs up to 48 h post-dose. Among the tissues other than serum, the highest concentrations of CDRI-81/470 were found in liver, whereas only trace levels were found in brain. Approximately 3% of the administered dose was excreted unchanged in urine at 120 h post-dose, whereas approximately 7% was recovered in faeces. The contribution of the biliary route for the excretion of parent compound was less than 0.5%. The *N*-decarboxylate derivative was quantitated in faeces (1–4%) and bile ($< 0.1\%$) but was absent in serum, tissues, and urine. An additional metabolite was isolated from bile and characterized as the pyridinyl-5-hydroxy derivative of CDRI-81/470.

CDRI-81/470 showed rapid absorption and distribution into all major organs and tissues, and underwent extensive metabolism in rats. Two metabolites in bile were identified and characterized by synthesis.

Methyl-*N*[5[[4-(2-pyridinyl)-1-piperazinyl]carbonyl]-1*H*-benzimidazol-2-yl] carbamate (CDRI-81/470; Figure 1) is a broad spectrum anthelmintic agent (Katiyar et al 1984, 1987, 1988; Kumar et al 1984; Srivastava et al 1988; Gupta et al 1992), being developed for veterinary and clinical therapy. CDRI-81/470 has shown efficacy against both intestinal and tissue parasites (Katiyar et al 1987), dermal absorption (Gupta et al 1992), and chemoprophylactic action against nematodes (Srivastava et al 1988). The compound has recently been approved for veterinary use in India and is in phase-two clinical trials. Tissue distribution of CDRI-81/470 has been previously studied using ^{14}C and ^{3}H labels. However, the use of a labelled compound is of limited value in differentiating the pharmacokinetic profiles of a drug and its metabolites.

As a part of the drug development programme, this study was undertaken to characterize the distribution and excretion of unlabelled CDRI-81/470 in rats, using an HPLC analytical technique.

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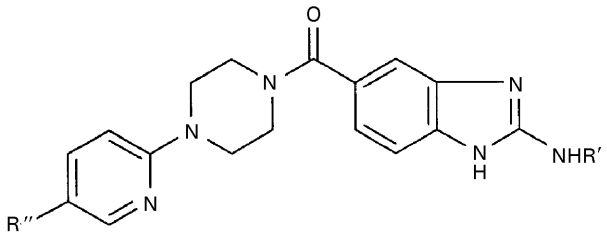
Materials and Methods

Chemicals and reagents

Reference standard CDRI-81/470 (purity $> 99\%$) and the solution formulation were obtained from the Pharmaceutics Division of the Central Drug Research Institute. Anaesthetic ether i.p. (T.K. Chemical Enterprises Pvt. Ltd, India) was used as received in animal experiments as an anaesthetic, but was purified by distillation for use as an extraction solvent in sample processing. Isopropanol, acetonitrile, chloroform and methanol were of HPLC grade (S.D. Chemicals, Boisar,



	R′	R″
I	$COOCH_3$	H
II	H	H
III	$COOCH_3$	OH

Figure 1. Chemical structures of methyl-*N*[5[[4-(2-pyridinyl)-1-piperazinyl]carbonyl]-1*H*-benzimidazol-2-yl] carbamate (CDRI-81/470; **I**), its decarboxylate metabolite (**II**), and hydroxy metabolite (**III**).

India). All other reagents were of analytical grade and were used without further purification.

One of the metabolites in the treated rat bile was identified by mass spectrometric analysis as the *N*-decarboxylate derivative of CDRI-81/470. This metabolite was synthesized as follows. To stirred 5-[4′-(2″-pyridinyl)-1′-piperazinyl]carbonyl-*o*-phenylenediamine (Kumar et al 1984) (0·297 g, 1 mmol) was added, in small portions, cyanogen bromide (0·106 g, 1 mmol) at room temperature (Leonard et al 1947). Sodium hydroxide (0·04 g, 1 mmol) in 2 mL water was added after 5 h to the reaction mixture. The solution was cooled in an ice-bath, the solid was filtered, washed with 2 × 5 mL water, air-dried, and recrystallized from $CHCl_3 : CH_3OH$ to yield 0·258 g (80%) of colorless product, 5-[4′-(2″-pyridinyl)-1′-piperazinyl]carbonyl]-2-amino-1*H*-benzimidazole, m.p. 260°C. ^{1}H NMR (d_6-DMSO), δ ppm, 60 MHz: 2·05 (s, 4H, -NCH_2-), 2·51 (m, 4H, -NCH_2-, 6·5–6·75 (m, 2H, -NH_2), 6·85 (s, 1H, -NH-), 7·00–7·25 (m, 2H), 7·40–7·70 (m, 2H), and 8·00–8·23 (m, 3H). MS (FAB) m/z: 323 ($M^+ + 1$), 322 (M^+). IR, ν_{max} (KBr) cm^{-1}: 3330 (NH-stretching, asym), 3210 (NH-stretching, sym), 2925 (CH-stretching), 1650 (conjugated > C=O), 1615 (C=C) and 1595 (C=N). Elemental analysis ($C_{17}H_{18}N_6O$) C, H, N, calculated: C, 63·35; H, 5·59; N, 26·09%; found: C, 62·96; H, 5·68; N, 25·66%.

Animals

Healthy, young, male albino Sprague-Dawley rats, 200 ± 20 g, were used in the studies. The rats were procured from the Laboratory Animal House of the Central Drug Research Institute, and acclimatized to a 12-h day–night cycle for at least 2 days before the study. The rats were fasted overnight with free access to water before dosing and food was allowed freely 2 h after the dosing.

Tissue distribution

A solution formulation containing 100 mg mL^{-1} CDRI-81/470 (Nagaraja et al 1995b) was administered as a single 100 mg kg^{-1} oral dose. Five rats were killed under ether anaesthesia at each time point (1, 2, 4, 8, 12, 24 and 48 h) post-dose. Blood was collected from the vena cava and serum was separated. After bleeding the rats through the vena cava, blood, skin, muscle, adipose tissue, heart, kidneys, lungs, liver, spleen, testes, brain, gastrointestinal tract and gastrointestinal contents were collected. Tissues were blotted and weighed and kept on a filter paper moistened with phosphate-buffered saline (0·146 M, pH 7·4). To study the distribution of CDRI-81/470 into red blood cells, blood, plasma and serum were separately collected from the rats killed at 4 h and analysed for CDRI-81/470 content. Urine and faeces samples were collected only at 0–24 and 24–48 h. The volume of urine output was measured and faeces samples were dried, weighed and powdered. Tissue samples were processed and analysed immediately. Serum, urine, and faeces samples were stored at −30°C until analysis.

Excretion

The excretion of CDRI-81/470 was studied in normal and bile-duct cannulated rats (n = 5). The bile duct was cannulated with PE-47 tubing under light ether anaesthesia and the rats were allowed to recover before dosing. After administering a single oral dose of 100 mg kg^{-1} CDRI-81/470, the rats were placed individually in Bollman cages (Waynforth 1980) with free access to food and water, for the collection of faeces, urine, and bile (bile-duct cannulated group). Urine and faeces samples were collected up to 120 h at 24-h intervals as described above. Bile was collected at 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–12, 12–24, 24–48, 48–72, 72–96, and 96–120 h post-dose. All the samples were stored at −30°C until analysis.

Sample analysis

Blood/serum was analysed for CDRI-81/470 by the protein precipitation method described for blood (Nagaraja et al 1995a). Briefly, 0·5 mL acetonitrile was added to 0·2 mL serum, and the sample was kept in the dark (4°C) for 30 min with occasional vortex-mixing. After centrifugation at

2000 *g* (0°C, 10 min), 0·5 mL of the supernatant was evaporated to dryness. The residue was reconstituted in 0·2 mL mobile phase for HPLC analysis.

Bile. Bile (0·2 mL) was vortex-mixed with 3 mL ether–isopropanol (90 : 10, v/v) solvent mixture for 1 min and then centrifuged at 2000 *g* (0°C, 10 min). The organic phase was separated after snap-freezing the aqueous layer in liquid nitrogen. Extraction was repeated with another 3 mL sample of the solvent. The combined organic layer was evaporated to dryness under reduced pressure in a Speed Vac Concentrator (Savant Instruments Inc., NY). The residue was reconstituted with 0·2 mL mobile phase for HPLC analysis.

Urine. Urine (0·2 mL) was acidified (pH < 2) with 0·05 mL 0·1 M orthophosphoric acid and extracted twice with 3 mL ethyl acetate as described above. The organic layer was discarded and the aqueous layer was basified (pH 8) with 0·05 mL 0·1 M aqueous potassium hydroxide. This layer was extracted twice with 3 mL ether–isopropanol (90 : 10, v/v). The combined organic layer was evaporated to dryness and the residue was reconstituted with 0·2 mL mobile phase for HPLC analysis.

Faeces. Faeces samples (0·1 g) were wetted with 0·1 mL water and sonicated in an ultrasonic bath for 15 min. Acetonitrile (2 mL) was added to the faecal sample, vortex-mixed for 1 min and sonicated for 30 min with occasional vortex-mixing. The sample was centrifuged at 2000 *g* (0°C, 10 min) and the supernatant was decanted. Extraction was repeated with another 2 mL acetonitrile. Combined acetonitrile was evaporated to dryness under reduced pressure and the residue was reconstituted in 1 mL mobile phase for HPLC analysis.

Tissues. Weighed tissue (0·1–0·2 g) or whole organ was homogenized in 5 mL phosphate-buffered saline using a Potter-Elvehjem type homogenizer (Top Syringe Manufacturing Company, Mumbai, India). Acetonitrile (0·5 mL) was added to a sample of tissue homogenate (0·2 mL), and left for 30 min with occasional vortex-mixing. The sample was centrifuged at 2000 *g* (0°C, 10 min), and 0·5 mL of the supernatant was evaporated to dryness under reduced pressure. The residue was reconstituted with 0·2 mL mobile phase for HPLC analysis.

Chromatographic conditions

Serum, tissues, bile, urine and faeces were assayed for CDRI-81/470 and its *N*-decarboxylate derivative content by an HPLC method (Nagaraja et al 1995a) with suitable modifications. The HPLC apparatus consisted of a pump (Kontron, Model 600; Zurich, Switzerland), coupled with a fluorescence detector (Shimadzu, Model RF-535; Kyoto, Japan) set at excitation and emission maxima of 295 and 375 nm, respectively. The samples were injected through a fixed 50-μL loop injector (Rheodyne, Model 7125; Cotati, CA). Mobile phase, at a flow-rate of 1 mL min^{-1}, consisted of acetonitrile and phosphate buffer (50 mM, pH 6) (25 : 75 v/v) for the simultaneous quantitation of CDRI-81/470 and its *N*-decarboxylate derivative in each of the biomatrices. Separations were achieved on a reversed-phase C_{18} column (Spheri-5, 5 μm, 220 × 4·6 mm i.d.) preceded by a guard column (30 × 4·6 mm i.d.) packed with the same material (Pierce Chemical Co., Rockford, IL).

Recovery, linearity and variations in intra- and inter-batch accuracy and precision were determined for bile and urine at low, medium and high concentrations (10, 100, and 1000 ng mL^{-1} CDRI-81/470 and 25, 100, and 1000 ng mL^{-1} *N*-decarboxylate derivative). Recovery of CDRI-81/470 and its *N*-decarboxylate derivative from faeces was assessed at 0·25, 5 and 10 μg mL^{-1} analyte concentrations and accuracy and precision were assessed at 10 μg g^{-1}. Recovery of the two analytes from tissue homogenates was studied at 10 μg g^{-1}. The number of samples in each of the above validation experiments was five except for tissues (n = 3).

Data analysis

Pharmacokinetic parameters of CDRI-81/470 in tissues were assessed by a non-compartmental approach using PCNONLIN software (ver. 4.2, Statistical Consultants Inc., KY). The area under the curve and area under the first moment curve were calculated from mean concentrations from five rats (0–48 h). Comparison between the groups was assessed by an unpaired Student's *t*-test with $P \leq 0{\cdot}05$ considered significant.

Results

Bioanalysis

The assay methods developed were specific and the chromatograms of blank matrices showed no interfering peaks in the eluting regions of either of the analytes. The standard curves in bile and urine were linear over the range 10–1000 ng mL^{-1} CDRI-81/470 and 25–1000 ng mL^{-1} *N*-decarboxylate derivative. The recovery of CDRI-81/470 and

its *N*-decarboxylate derivative, variations in intra- and inter-batch accuracy and precision in bile, urine, and faeces are summarized in Table 1. These variations were within acceptable limits (Shah et al 1992). Recovery of CDRI-81/470 and its *N*-decarboxylate derivative from the homogenates of each of the tissues (n = 3) was assessed at a concentration of 10 $\mu g\,g^{-1}$, and was between 87·5 and 96·8% for CDRI-81/470 and between 86·8 and 96·3% for the *N*-decarboxylate derivative with the coefficient of variation less than 10%. The retention times of CDRI-81/470 and its *N*-decarboxylate derivative under the modified conditions were 11·0 ± 0·2 and 7·0 ± 0·2 min, respectively.

Tissue distribution

After a single oral dose, CDRI-81/470 was widely distributed into tissues and organs of rats. The parent compound could be monitored in all tissues collected. Distribution is expressed as concentration, percentage of administered dose, and tissue to serum concentration ratio.

Peak levels of CDRI-81/470 were observed at 2 h in all tissues, with highest levels in serum and lowest levels in brain tissue (Table 2). The highest percentage of administered dose (calculated based on either actual weight of organ or standard weights of tissues; Davis & Morris 1993) was present in muscle followed by skin > adipose > serum > liver > kidney > lung > spleen > testes > heart > brain. The percentages of administered dose at t_{max} (2 h) are given in Table 2.

There was no difference between serum and plasma concentrations of CDRI-81/470 at 4 h post-dose. Fractions distributed into red blood cells were 1·78 ± 0·74 and 1·41 ± 0·56%, in plasma and serum, respectively, indicating that CDRI-81/470 is con-

Table 1. Assay validation parameters of CDRI-81/470 and its *N*-decarboxylate derivative in bile, urine and faeces of rats.

Concn ($ng\,mL^{-1}$)	Recovery (%) (mean ± s.d.)		Bias (%)		RSD (%) (intra-batch)		RSD (%) (inter-batch)	
(n = 5)	CDRI-81/470	*N*-Decarboxylate derivative	CDRI-81/470	*N*-Decarboxylate derivative	CDRI-81/470	*N*-Decarboxylate derivative	CDRI-81/470	*N*-Decarboxylate derivative
Bile								
10	89·0 ± 3·3	NA	+7·8	NA	13·6	NA	12·6	NA
25	NA	72·5 ± 4·5	NA	−0·3	NA	8·8	NA	10·6
100	94·8 ± 3·0	74·9 ± 2·9	−11·1	−2·6	2·2	3·8	5·3	6·8
1000	92·6 ± 2·1	78·9 ± 0·8	−12·0	+9·5	4·6	7·8	4·7	4·1
Urine								
10	109 ± 10·5	NA	+9·3	NA	9·6	NA	8·7	NA
25	NA	82·8 ± 12·8	NA	−5·9	NA	15·6	NA	9·7
100	91·1 ± 3·6	76·2 ± 6·4	−8·9	−10·9	4·0	8·4	9·5	9·0
1000	89·9 ± 3·3	75·2 ± 4·7	−10·2	+0·5	3·7	6·2	11·9	10·1
Faeces								
0·25	94·5 ± 2·6	60·3 ± 3·6	NA	NA	NA	NA	NA	NA
5	102 ± 1·3	60·5 ± 4·4	NA	NA	NA	NA	NA	NA
10	97·7 ± 12·4	59·6 ± 0·8	−6·1	−2·3	7·3	12·1	2·9	5·6

RSD, relative standard deviation; NA, not analysed.

Table 2. Pharmacokinetic parameters and distribution pattern of CDRI-81/470 in organs and tissues of rats at t_{max} (2 h) after a single oral dose of 100 $mg\,kg^{-1}$ solution.

Organ/tissue	C_{max}	MRT (h)	% Administered dose	Tissue to serum concentration ratio
Serum	75·1 ± 25·0 $\mu g\,mL^{-1}$	8·56	2·83 ± 1·06	–
Liver	65·1 ± 16·0 $\mu g\,g^{-1}$	9·30	2·11 ± 0·90	0·76 ± 0·16
Skin	58·1 ± 19·1 $\mu g\,g^{-1}$	11·55	12·4 ± 4·54	0·30 ± 0·06
Lung	37·9 ± 17·3 $\mu g\,g^{-1}$	10·75	0·28 ± 0·10	0·51 ± 0·18
Kidney	35·0 ± 6·9 $\mu g\,g^{-1}$	11·49	0·31 ± 0·08	0·5 ± 0·14
Heart	27·1 ± 7·0 $\mu g\,g^{-1}$	10·82	0·09 ± 0·03	0·38 ± 0·12
Spleen	31·0 ± 8·7 $\mu g\,g^{-1}$	11·79	0·14 ± 0·04	0·42 ± 0·07
Muscle	26·7 ± 6·8 $\mu g\,g^{-1}$	9·72	18·7 ± 10·4	0·17 ± 0·2
Adipose	25·9 ± 12·6 $\mu g\,g^{-1}$	11·59	3·00 ± 2·04	0·11 ± 0·04
Testes	8·94 ± 3·83 $\mu g\,g^{-1}$	12·35	0·12 ± 0·05	0·12 ± 0·04
Brain	3·64 ± 0·8 $\mu g\,g^{-1}$	12·54	0·03 ± 0·01	0·04 ± 0·02

fined to the plasma/serum compartment and not distributed into red blood cells.

The fractions of administered dose present in the intestinal and systemic compartments were compared. The relative distribution of the dose in the tissue (including blood) and the gastrointestinal compartments as a function of time is summarized in Figure 2. The fraction of the dose recovered from the gastrointestinal tract was higher than tissues except at t_{max} (2 h) where the recovery from the two compartments was equal. The total recovery of CDRI-81/470 (excluding urine and faeces) decreased steadily from 88% at 1 h to 0·5% at 48 h.

A minor fraction of the dose was excreted unchanged in both urine and faeces. The percentage of administered dose recovered at 24 and 48 h was $2{\cdot}49 \pm 0{\cdot}68$ and $2{\cdot}82 \pm 0{\cdot}5\%$ in urine, and $4{\cdot}97 \pm 1{\cdot}44$ and $6{\cdot}78 \pm 0{\cdot}4\%$ in faeces, respectively. The total recovery (gastrointestinal tract, tissues and excreta) of CDRI-81/470 at 48 h revealed that less than 10% of the administered dose could be accounted for as parent compound.

Tissue to serum ratio. The tissue to serum concentration ratios of CDRI-81/470 were calculated at each time point and the values at 2 h are given in Table 2. The ratio was generally less than unity and not more than 1·5 at any given time point. The highest and the lowest ratios were observed for liver and brain, respectively.

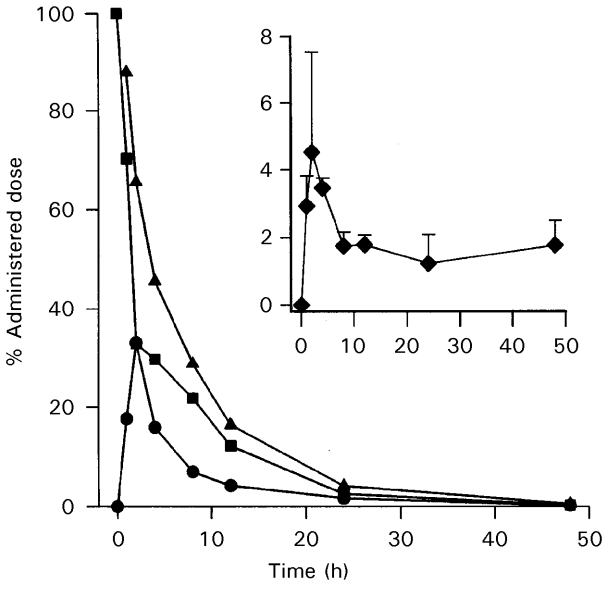


Figure 2. Recovery of methyl-*N*[5[[4-(2-pyridinyl)-1-piperazinyl]carbonyl]-1*H*-benzimidazol-2-yl] carbamate (CDRI-81/470) from tissues and gastrointestinal tract after a single oral dose of 100 mg kg^{-1} solution (●, tissues; ■, gastrointestinal tract; ▲, total recovery). The inset shows the recovery of the *N*-decarboxylate metabolite of CDRI-81/470.

Metabolism

The *N*-decarboxylate metabolite of CDRI-81/470 was detected in faeces, and in the contents of small and large intestines (total 1–5% of the administered dose, equivalent to CDRI-81/470). Traces of the metabolite were also detected in liver, kidney, gut wall and urine up to 8 h. Thereafter, the *N*-decarboxylate derivative could not be detected in any of these tissues or urine, although it could be quantitated in faeces samples at 24–48 h. It was not found in any other tissue or blood/serum up to 48 h. The amounts of *N*-decarboxylate derivative recovered in the gastrointestinal contents and faeces (expressed as percentage equivalents of CDRI-81/470) were less than 5% of the administered dose (Figure 2).

An additional metabolite of CDRI-81/470 in rat bile was also isolated and characterized. Bile, collected from five rats up to 48 h after oral dosing was pooled and exhaustively extracted with ethyl acetate. After evaporating the organic solvent, the residue was crystallized in a methanol–ether solvent system. Spectroscopic analyses showed that this metabolite was methyl-*N*[5[[4′-(5″-hydroxy-2″-pyridinyl)-1′-piperazinyl]carbonyl]-1*H*-benzimidazol-2-yl] carbamate (Figure 1), m.p. 238°C.^{1}H NMR: δ (d_6-DMSO), ppm, 300 MHz: 3·60 (br s, 8H, -N$(CH_2)_4$), 3·76 (s, 3H, -$COOCH_3$), 6·73–6·76 (d, 1H, 3″-Py H, J = 9·0 Hz), 7·05–7·09 (dd, 1H, 4″-Py H, J = 2·7 and 2·7 Hz), 7·14–7·17 (d, 1H, 7-Ar H, J = 8·4 Hz), 7·41–7·44 (d, 1H, 6-Ar H, J = 8·1 Hz), 7·47 (s, 1H, 4-Ar H), 7·73–7·74 (d, 1H, 6″-Py H, J = 2·7 Hz), 9·05 (s, 1H, Py-5″-OH), 11·5 (br s, 2H, 2 x -NH). MS (FAB) m/z: 397 ($M^+ + 1$), 218. FT-IR, ν_{max} (KBr) cm^{-1}: 3300–3400 (-OH); 3194 (NH-str); 2900–2800 (CH-str); 2336, 2365; 1730 (> C=O, ester); 1650 (conjugated > C=O); 1600 (-C=C- and -C=N- stretching); 1490, 1439 (C-O str). The hydroxy substitution was further confirmed by D_2O shake and two-dimensional NMR experiments. Chromatograms of urine and faeces samples from dosed rats also showed the presence of this metabolite, with a retention time of 5 min.

Excretion

Excretion of CDRI-81/470 and its *N*-decarboxylate derivative in faeces, urine, and bile of both normal and bile-duct cannulated rats is summarized in Table 3. In the faeces samples of normal rats, the maximum fraction of total CDRI-81/470 excreted ($7{\cdot}26 \pm 0{\cdot}5\%$ of dose) was observed within first 24 h. The *N*-decarboxylate derivative was also present in faeces and accounted for 3% of the administered dose (0–120 h). Similar to the parent compound, recovery of the *N*-decarboxylate deri-

vative was maximum at 0–24 h. Approximately 3% of the administered dose was excreted in urine up to 120 h and of this, 2·6% was recovered within the first 24 h. The *N*-decarboxylate derivative was detected only in trace quantities in a few urine samples and could not be quantitated. In bile-duct cannulated rats, the percentage of administered dose excreted in faeces (0–120 h) was 6·9 ± 2·91% which did not differ significantly from normal rats. However, the percentage of the decarboxylate metabolite in faeces was only 0·91 ± 0·33% compared with 2·93 ± 1·08% in normal rats. Approximately 1·7% of the administered dose was recovered as the parent compound in urine, and the *N*-decarboxylate derivative was not present in measurable amounts. The percentage of CDRI-81/470 and its *N*-decarboxylate derivative excreted in bile up to 120 h was 0·29 ± 0·09 and 0·06 ± 0·01%, respectively. Total recovery of CDRI-81/470 and its *N*-decarboxylate derivative up to 120 h in bile accounted for less than 0·5% of the administered dose.

Discussion

The distribution and excretion of unlabelled CDRI-81/470 was studied in rats to characterize the disposition of the parent compound. CDRI-81/470 was widely distributed into all the major tissues. C_{max} was attained in each of the tissues at 2 h, irrespective of perfusion and composition of the tissue. The highest levels of CDRI-81/470 were found in serum at all time points. In blood, the compound was not distributed into red blood cells and was confined to the plasma/serum compartment, of which, 96% was in the bound form (unpublished results). Tissue to serum concentration ratios were generally less than unity and did not change significantly during 0–48 h.

In the tissues, the highest levels of CDRI-81/470 were found in liver while only trace amounts were quantitated in brain. The presence of trace amounts of CDRI-81/470 (pKa 2·08 and log P 1·07; Monif 1993), in brain, was in agreement with a previous study using ^{14}C-labelled compound (Paliwal et al 1997). However, in another study (Srivastava et al 1994), using ^{3}H-labelled compound, higher radioactivity was reported in brain than in adipose and testis. Possible metabolites of CDRI-81/470, a benzimidazole carbamate with a keto substitution at the 5-position of the benzimidazole ring are likely to be more polar than the parent compound (McKellar & Scott 1990) and hence may not contribute to the radioactivity in brain.

The mean residence time of the parent compound in the tissues ranged from 8·6 to 12·5 h which was comparable with that found in blood (7·61 ± 0·86 and 8·06 ± 1·78 h for oral suspension and solution, respectively; Nagaraja et al 1995b).

After a 1 mg kg^{-1} oral dose of ^{14}C-labelled CDRI-81/470 to bile-duct intact rats, 22 and 65% was recovered in urine and faeces (0–96 h), respectively (Paliwal et al 1997). The majority of this fraction was recovered at 0–24 h as in this study. This pattern was different from that after an intravenous dose (1 mg kg^{-1}) where approximately 40 and 48% were recovered in urine and faeces, respectively, at 0–96 h. After a similar oral dose in bile-duct cannulated rats, 18 and 25% of the administered dose was recovered in urine and faeces, respectively, at 0–24 h, while 38% of the dose was recovered in bile during the same period (Paliwal et al 1997). In

Table 3. Excretion of CDRI-81/470 and its *N*-decarboxylate derivative in faeces, urine and bile of rats.

		% Administered dose		
	Compound	0–24 h	24–48 h	0–120 h
Faeces				
Normal	CDRI-81/470	5·41 ± 1·43	1·73 ± 1·09	7·26 ± 0·50
	N-Decarboxylate derivative	2·03 ± 0·72	0·74 ± 0·55	2·93 ± 1·08*
	Total	7·21 ± 1·11	2·58 ± 1·49	10·2 ± 1·5
Bile-duct cannulated	CDRI-81/470	4·80 ± 3·37	1·75 ± 0·87	6·9 ± 1·08
	N-Decarboxylate derivative	0·55 ± 0·42	0·31 ± 0·42	0·91 ± 0·33
	Total	5·35 ± 3·09	2·06 ± 0·80	7·81 ± 2·75
Urine				
Normal	CDRI-81/470	2·56 ± 0·07	0·25 ± 0·24	2·96 ± 0·61*
Bile-duct cannulated	CDRI-81/470	1·40 ± 0·57	0·27 ± 0·26	1·72 ± 0·54
Bile				
	CDRI-81/470	0·25 ± 0·10	0·03 ± 0·04	0·29 ± 0·09
	N-Decarboxylate derivative	0·05 ± 0·02	0·01 ± 0·00	0·06 ± 0·01
	Total	0·30 ± 0·01	0·04 ± 0·04	0·35 ± 0·09

Values are mean ± s.d., n = 5. *$P \leq 0{\cdot}05$ significant difference between normal and bile-duct cannulated rats.

addition, urine accounted for 35% of the administered dose (0–24 h) after an intravenous dose in bile-duct cannulated rats and no radioactivity was detected in faeces. Differences in the extent of urinary and fecal excretion of radioactivity between intravenous and oral routes indicate a possible first-pass metabolism by the liver. The biliary route contributed to the excretion of approximately 38% within the first 24 h, of which, an insignificant fraction, could be accounted for as CDRI-81/470 in this study. Of 22% in urine, approximately 3% could be accounted for as CDRI-81/470, whereas approximately 7% of 65% represented the parent compound in faeces. In contrast, after a 100 mg kg^{-1} oral dose of ^{3}H-labelled CDRI-81/470 (Srivastava et al 1994), 12% of the administered dose was recovered in urine while 86% was present in faeces (0–7 days). Unabsorbed residue might account for this higher faecal excretion since a suspension of ^{3}H-labelled compound was administered compared with a solution of the ^{14}C labelled compound. In this study, only 5–10% of the administered dose could be accounted for as CDRI-81/470 and *N*-decarboxylate derivative in faeces, while 1–4% was accounted for in urine in both bile-duct intact and bile-duct cannulated rats. The contribution of the biliary route for the excretion of the parent compound and *N*-decarboxylate derivative was not significant. An additional metabolite with a retention time of 5 min was isolated from bile and characterized as the pyridinyl-5-hydroxy derivative of CDRI-81/470.

In conclusion, bioanalytical methods for the determination of CDRI-81/470 in urine, bile, faeces, and tissues of rats were developed. Tissue distribution of unlabelled CDRI-81/470 was studied. The compound was well distributed into different tissues and underwent extensive metabolism. Two metabolites were identified, one of which was quantitated in bile, urine, and faeces. These results highlight the importance of using unlabelled rather than labelled compounds for tissue distribution studies.

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