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Identification of lactams as in vitro metabolites of piperidine-type phenothiazine antipsychotic drugs

Ge Lin[a,*], Ka-Wing Chu[b], Lyaquatali A. Damani[b], Edward M. Hawes[c]

[a]*Department of Pharmacology, Faculty of Medicine, Chinese University of Hong Kong, Shatin, N.T., Hong Kong*
[b]*Department of Pharmacy, Faculty of Medicine, Chinese University of Hong Kong, Shatin, N.T., Hong Kong*
[c]*College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5C9, Canada*

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Abstract

The metabolism of the piperidine-type phenothiazine antipsychotic agents thioridazine, mesoridazine and sulforidazine was studied in vitro with 10 000*g* liver supernatants obtained from rats and dogs. After incubations at 37°C for different time intervals, the incubates were extracted with dichloromethane and the isolated compounds analyzed by HPLC, direct probe MS and on-line HPLC-MS. Five lactam metabolites of these three drugs were unequivocally identified in the rat in vitro system, but none was found in dog preparations; at least one lactam metabolite was identified for each drug in the rat. The lactams of thioridazine and thioridazine ring sulfoxide were characterized as metabolites of thioridazine for the first time in any system. The other three lactam metabolites, namely the lactams of mesoridazine, sulforidazine and mesoridazine ring sulfoxide, were found in vitro for the first time, although they have been previously reported as in vivo metabolites of these drugs. The results indicate that rat would be a more suitable animal model than dog for further studies on the formation of lactam metabolites of these drugs.

Keywords: Lactam metabolites; In vitro metabolism; Thioridazine; Mesoridazine; Sulforidazine

1. Introduction

Thioridazine (**Ia**, Fig. 1), mesoridazine (**Ib**) and sulforidazine (**Ic**) are piperidine-type phenothiazine antipsychotic drugs that differ from each other only in the oxidative state of the sulfur atom in the ring 2-substituent. The metabolism of the phenothiazine ring, side-chain *S*-oxidation and *N*-demethylation and *N*-oxidation of the side-chain nitrogen of these drugs have been demonstrated in vivo [1–7]. There are few reports on the metabolites resulting from these routes in vitro [7–9]. However, until recently there was little definitive information about the metabolism of the piperidine ring of this class of drugs. The lactam metabolites of mesoridazine ring sulfoxide (**IIIb**) and sulforidazine ring sulfoxide (**IIIc**) were tentatively identified in urinary extracts of medicated patients [6]. For drugs containing saturated nitrogen-containing heterocyclic ring systems, it is well established that in various species metabolism may result in the formation of lactam

* Corresponding author.

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metabolites. During the formation of these metabolites, an electrophilic iminium ion has been postulated as an intermediate, and this may be responsible for some toxic effects of such drugs [10,11].

Recent in vivo metabolic studies of **Ia**, **Ib** and **Ic** in human, rat and dog [12–15] have demonstrated the presence of lactam metabolites in the urine of all three species examined. When compared with the amounts of other metabolites, lactam metabolites were found in appreciable amounts in the cases of human and rat. However, there are no reports on formation of such metabolites of these drugs in vitro. This paper describes the biotransformation of these drugs in vitro using 10 000*g* liver homogenate obtained from rat and dog, with a focus on the identification of lactam metabolites. The structures of the parent drugs and their metabolites are shown in Fig. 1.

2. Experimental

2.1. Materials

Thioridazine, mesoridazine and sulforidazine were gifts from Sandoz Pharmaceuticals (Basle, Switzerland). All other reference standards were synthesized in the authors' laboratories [16–18]. Solvents used for extraction and for the preparation of the HPLC mobile phase were of HPLC grade (BDH Laboratory Supplies). All other chemicals were of reagent grade (Aldrich Chemical).

2.2. HPLC conditions

The HPLC system consisted of a liquid chromatograph (Hewlett-Packard HP 1050) equipped with a 20 μl sample loop injector (Rheodye Model 7125) and a multiple-wavelength UV spectrophotometer. Data were recorded and analyzed by a HP Vectra VL2 486 computer using ChemStation software. The chromatographic column used in the normal-phase mode was an Isco Spherisorb cyano (5 μm) column (250 × 4.6 mm i.d.), and that utilized in the reversed-phase mode was an Alltech Alltima C-8 (5μm) column (250 × 4.6 mm i.d.). The guard column for normal-phase operation was packed with 10 μm Spherisorb cyano packing (packed in-house). A guard column cartridge (HP MOS Hypersil (5 μm), 20 × 4 mm i.d.) was used for reversed phase HPLC analysis. The mobile phases were degassed before use by filtration (Millipore HVLP-type membrane filters) and sonification. HPLC was performed at ambient temperature. Three mobile phase systems were utilized. Mobile phase 1 consisted of 2,2,4-trimethylpentane–dichloromethane–methanol–diethylamine (68.0:19.0:12.9:0.1, v/v/v/v), with a flow rate of 0.8 ml min^{-1}. Mobile phase 2 contained the same components but in the ratio 68.0:19.0:12.7:0.3 (v/v/v/v). Mobile phase 3 consisted of methanol–0.05 M ammonium acetate–diethylamine (70.00:29.99:0.01, v/v/v), with a flow rate of 1.0 ml min. Mobile phases 1 and 2 were used for normal-phase chromatography and mobile phase 3 was used for reversed-phase HPLC analysis. The multiple-wavelength UV detector was set at 254 nm with a detector sensitivity of 0.05 absorbance units full-scale.

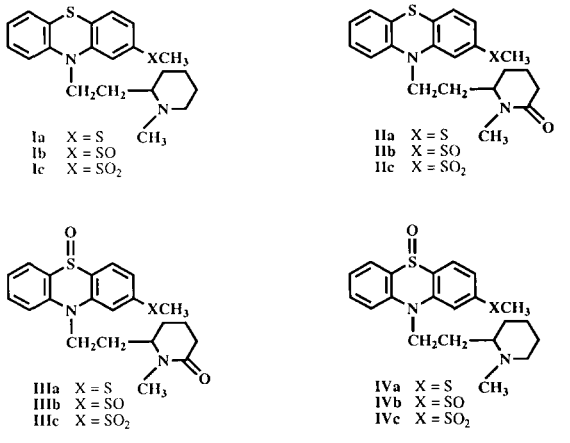

Fig. 1. Structures of thioridazine (**Ia**), mesoridazine (**Ib**) and sulforidazine (**Ic**), and their metabolites. **IIa**, Lactam of thioridazine; **IIb**, lactam of mesoridazine; **IIc** lactam of sulforidazine; **IIIa**, lactam of thioridazine ring sulfoxide; **IIIb**, lactam of mesoridazine ring sulfoxide; **IIIc**, lactam of sulforidazine ring sulfoxide; **IVa**, thioridazine ring sulfoxide; **IVb**, mesoridazine ring sulfoxide; **IVc**, sulforidazine ring sulfoxide.

2.3. HPLC–MS conditions

HPLC–MS was carried out on a Finnigan Mat RTSQ 7000 mass spectrometer equipped with an electrospray interface. The column was Ultrasphere C-8 (250 × 4.6 mm i.d.). The mobile phase consisted of methanol–0.05 M ammonium acetate (70:30, v/v), and was filtered and degassed before use. The column was maintained at ambient temperature with a flow rate of 0.4 ml min^{-1}. Full-scan mass spectra from the HPLC–MS system were obtained in the peak centroid mode over the mass range m/z 350–500.

2.4. Mass spectrometry

Mass spectra of samples were obtained in the electron impact (EI) mode using a direct insertion probe technique on a VG Micromass 7070F instrument coupled to a VG analytical 11-250J data system operating at an ionizing potential of 70 eV and a source temperature of 180°C.

2.5. Liver homogenate preparation

All operations were conducted below 4°C. Liver from female and male rats (Sprague–Dawley) or from female dogs (beagle) were homogenized in Tris buffer (pH 7.4; 0.05 M) and centrifuged at 10 000*g* for 25 min. The 10 000*g* supernatant was stored at −70°C until required. Protein concentration was determined by a modified Lowry method [19].

2.6. Incubation system

A typical incubation system contained 2 μmol of substrate in 0.5 ml of potassium phosphate buffer (pH 7.4; 0.1 M), 0.5 ml of 10 000*g* liver supernatant (ca. 25 mg protein ml^{-1}) and 1 ml of an NADPH-regenerating cofactor solution (2 μmol of $NADP^+$, 10 μmol of glucose 6-phosphate, 1 unit of glucose 6-phosphate dehydrogenase and 10 μmol of $MgCl_2$). Incubations were performed at 37°C for different time intervals (from 15 min to 12 h) and reaction was quenched by addition of dichloromethane.

2.7. Characterization of in vitro metabolites

The incubation mixtures were extracted with dichloromethane (2 × 3.5 ml). The combined organic extracts were dried with Na_2SO_4 and evaporated to dryness and the residue was reconstituted in 100 μl of acetonitrile. Aliquots of the acetonitrile solution were directly analyzed by both HPLC and HPLC–MS analysis.

The lactam metabolites were isolated by normal-phase HPLC. The portions of mobile phase corresponding to the appropriate chromatographic peaks were collected. The collected fractions were further purified by re-injecting them into the HPLC system and recollecting the appropriate portions of mobile phase. Mobile phase 1 was used for the isolation of metabolites of sulforidazine and mobile phase 2 was used for metabolites of mesoridazine and thioridazine. The collected fractions were evaporated to dryness and analyzed by mass spectrometry as direct insertion probe samples.

3. Results

3.1. Characterization of in vitro metabolites of sulforidazine in rat liver homogenates

Sulforidazine metabolism was initially investigated in rat liver homogenates, since the 2-substituent of this drug is fully oxidized and its metabolic profile was presumed to be less complex than those of the other two drugs. The organic extracts of incubations with sulforidazine were first analyzed by normal-phase HPLC using mobile phase 1. Chromatograms obtained from studies using increasing incubation times exhibited similar peak patterns, and the areas of the peaks apparently corresponding to metabolites increased over the first 4 h of incubation. A typical chromatogram of the extract of an incubation of sulforidazine is shown in Fig. 2. Four peaks were present in the extracts of test incubates (Fig. 2C) which were not present in the chromatogram of a control extract (Fig. 2A). The retention times of these peaks were compared with those obtained from the similarly extracted and analyzed stan-

Fig. 2. Normal-phase HPLC traces of extracts of rat liver homogenates of (A) control incubation without substrate, (B) authentic standards spiked into the incubation mixture and (C) incubation with sulforidazine for 4 h. Mobile phase 1. **Ic**, sulforidazine; **IIc**, lactam of sulforidazine; **IIIc**, lactam of sulforidazine ring sulfoxide; **IVc**, sulforidazine ring sulfoxide; **V**, unknown.

dards spiked in the in vitro system without incubation (Fig. 2B). Three of the peaks in the test incubates were co-chromatographed with sulforidazine (**Ic**), the lactam of sulforidazine (**IIc**) and sulforidazine ring sulfoxide (**IVc**). The identity of each of these three analytes was further investigated by comparison of their chromatographic properties with those of authentic standards using reversed-phase HPLC analysis utilising mobile phase 3. The chromatographic data are summarized in Table 1.

The lactam of sulforidazine was isolated by collection of the appropriate fractions from normal-phase HPLC and then analyzed by direct probe EI-MS. The mass spectrum of the isolated lactam of sulforidazine was compared with that of an authentic standard. Both spectra showed a molecular ion at m/z 416 [M + ·] (% intensity, standard/metabolite = 66/47), a characteristic ion at m/z 112 (100/100) corresponding to the 1,6-dehydro-1-methylpiperidin-2-one fragment ion and other diagnostic ions at m/z 290 (22/18), 277 (17/20), 211 (11/10) and 140 (23/22). Based on the above evidence, the lactam of sulforidazine was unequivocally identified as an in vitro metabolite of sulforidazine from rat liver homogenate.

The retention times of the fourth peak at 6.4 min in normal-phase (Fig. 2, **V**) and 8.7 min in reversed-phase HPLC analysis differed from those of the available reference standards. Direct probe EI-MS of the isolated fraction did not allow the identification of this metabolite. Further studies on the characterization of this metabolite are in progress.

3.2. Characterization of in vitro metabolites of mesoridazine in rat liver homogenates

Organic extracts derived from incubation of mesoridazine with rat liver homogenates were analyzed by both normal-phase (mobile phase 2) and reversed-phase (mobile phase 3) HPLC. As in the case of sulforidazine, the qualitative metabolic profile of mesoridazine did not change with incubation time, but the amounts of the metabolites as evidenced by peak areas increased as the incubation time increased over the first 4 h. The HPLC peak patterns and retention times were compared with those obtained from authentic standards (Table 1). Seven compounds tentatively identified were as follows: mesoridazine (**Ib**), sulforidazine (**Ic**), the lactam of mesoridazine (**IIb**), the lactam of sulforidazine (**IIc**), the lactam of mesoridazine ring sulfoxide (**IIIb**), mesoridazine ring sulfoxide (**IVb**) and sulforidazine ring sulfoxide (**IVc**). In the cases of sulfoxide metabolites **IIIb** and **IVb**, each of them showed two separated peaks in the HPLC traces. It is well established that such metabolites of piperidine-type phenothiazine antipsychotic agents can be chromatographically separated into their diastereomers [6,7,20–23]. Whereas the diastereomers of sulfoxide **IVc** were not separated by this HPLC system, they were separated in HPLC–MS analysis, which utilized reversed-phase conditions (Fig. 3).

Among the three tentatively identified lactam metabolites, only the lactam **IIb** could be isolated

Table 1
Chromatographic data for standards and analytes isolated from rat liver homogenate incubations ($n = 3$)

Analyte	Retention time (min)					
	Thioridazine incubation		Mesoridazine incubation		Sulforidazine incubation	
	Standard	Isolated	Standard	Isolated	Standard	Isolated
Thioridazine (**Ia**)	4.4[a]	4.4[a]				
Mesoridazine (**Ib**)			6.0[a]	6.0[a,c]		
			22.2[b]	22.2[b]		
Sulforidazine (**Ic**)			6.0[a]	6.0[a,c]	4.3[a]	4.3[a]
			17.6[b]	17.7[b]	18.8[b]	18.7[b]
Lactam of thioridazine (**IIa**)	5.2[a]	5.1[a]				
Lactam of mesoridazine (**IIb**)			7.0[a]	7.0[a]		
			7.2[b]	7.1[a]		
Lactam of sulforidazine (**IIc**)			7.8[a]	7.5[a]	5.4[a]	5.3[a]
			6.7[b]	6.7[b]	6.7[b]	6.9[b,c]
Lactam of thioridazine ring sulfoxide (**IIIa**)	8.4[a]	8.3[a]				
Lactam of mesoridazine ring sulfoxide (**IIb**)[d]			11.2[a]	11.1[a]		
			11.6[a]	11.7[a]		
			3.4[b]	3.4[b]		
Thioridazine ring sulfoxide (**IVa**)[d]	6.4[a]	6.5[a]				
	6.7[a]	7.0[a]				
Mesoridazine ring sulfoxide (**IVb**)[d]			8.5[a]	8.5[a]		
			9.3[a]	9.1[a]		
			7.2[b]	7.1[b,c]		
Sulforidazine ring sulfoxide (**IVc**)			9.5[a]	9.3[a]	7.7[a]	7.4[a]
			6.7[b]	7.1[b,c]	6.7[b]	6.9[b,c]

[a] Data obtained from normal-phase HPLC (mobile phase 1 for sulforidazine incubations, mobile phase 2 for mesoridazine and thioridazine incubations).
[b] Data obtained from reversed-phase HPLC.
[c] Analytes were eluted together.
[d] Diastereomers of ring sulfoxides were separated by the normal-phase HPLC analysis.

in an adequate amount for analysis by direct insertion probe EI-MS. The EI mass spectrum of the isolated compound was compared with that obtained from the authentic standard. Both spectra exhibited the molecular ion at m/z 400 [M + ·] with a relative intensity of 50% (metabolite) and 100% (standard), and other diagnostic ions, such as those at m/z 385 (% intensity, standard/metabolite = 44/24), 260 (25/15), 245 (27/22), 140 (20/13) and 112 (94/100). Therefore, the identity of the lactam of mesoridazine was confirmed.

In order to characterize unequivocally the identities of the other lactam metabolites of mesoridazine, the extracts were further directly analyzed by on-line HPLC–MS. Typical chromatograms obtained from such HPLC–MS analysis are shown in Fig. 3A. Although the reconstructed total ion chromatogram (RTIC, Fig. 3A) does not clearly show resolved peaks, except for **Ic** and **IVb**, a separated peak was obtained when the appropriate quasi-molecular ion [MH^+] of each suspected analyte was used to outline each individual reconstructed ion chromatogram (Fig. 3A). Each peak in the reconstructed ion chromatograms was then analyzed by electrospray mass spectrometry, and all mass spectra showed the quasi-molecular ion as the base peak (Fig. 3B). In the cases of **Ic** and **IVb**, they eluted together under the HPLC–MS conditions, but were separated by both normal- and reversed-

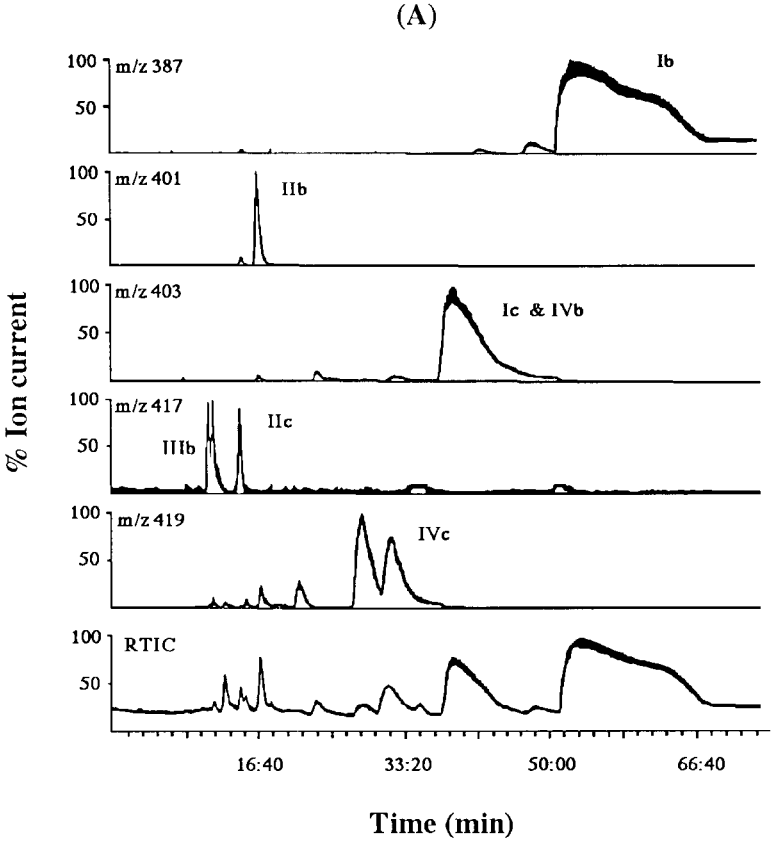

Fig. 3(a)

phase conventional HPLC analyses (Table 1). The identity of each analyte was confirmed by comparison of both the chromatographic behavior and mass spectral data with those for an authentic reference sample.

The chromatograms obtained from HPLC–MS analysis exhibited separation of the diastereomers of sulforidazine ring sulfoxide, whereas the diastereomers of mesoridazine ring sulfoxide were not resolved. In the case of the lactam of mesoridazine ring sulfoxide, the chromatogram showed a splitting peak, indicating that the diastereomers were not completely separated (Fig. 3A). These observations contrast with the above-noted resolution of the diastereomers of **IIIb** and **IVb** but not **IVc** under the conventional HPLC conditions (Table 1), and thus illustrate the importance of selection of appropriate HPLC conditions in the separation of diastereomers of a specific compound.

3.3. Characterization of in vitro metabolites of thioridazine in rat liver homogenates

The same analytical methods (normal-phase HPLC and on-line HPLC–MS) were employed to characterize the in vitro metabolites of thioridazine. The identity of each metabolite was characterized by the comparison of the chromatographic behaviours and mass spectral data of each analyte with those for an authentic standard. Two lactam metabolites, namely the lactam of thioridazine (**IIa**) and the lactam of thioridazine ring sulfoxide (**IIIa**), in addition to thioridazine, mesoridazine, sulforidazine and thioridazine ring sulfoxide, were identified in organic extracts of

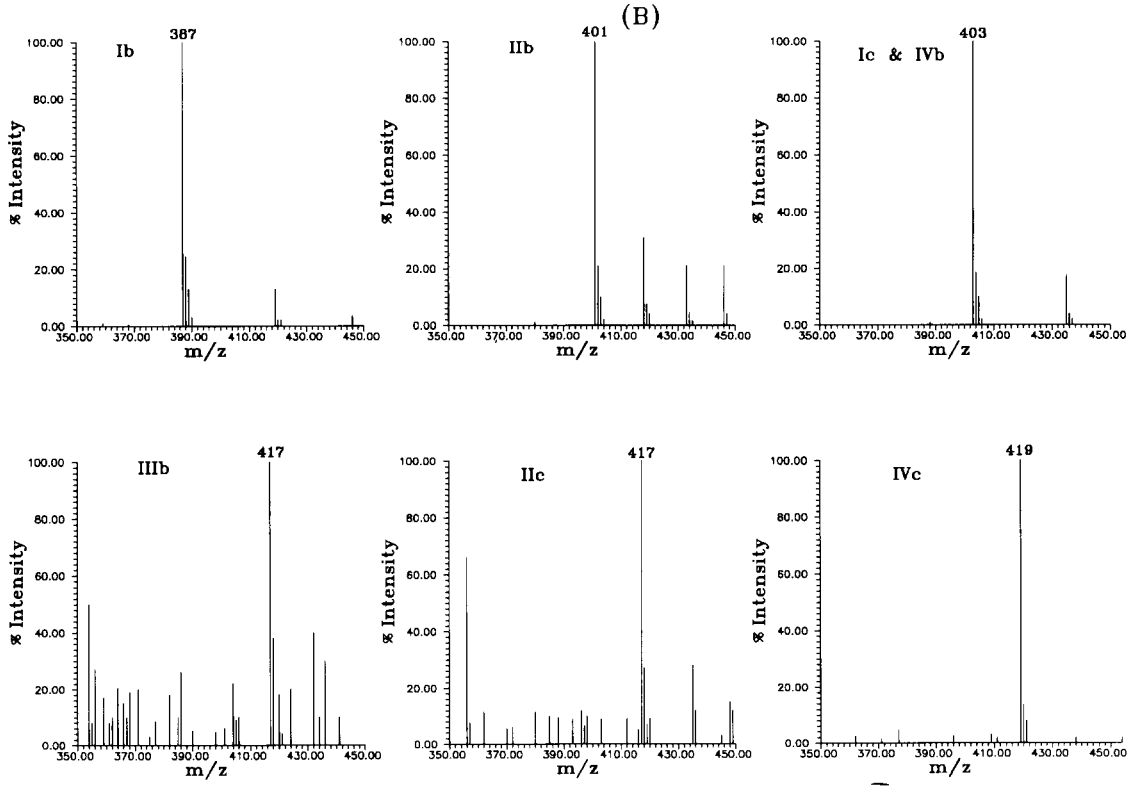

Fig. 3(b)

Fig. 3. Reconstructed total ion chromatogram and reconstructed ion chromatograms of HPLC–electrospray MS analysis of the extract from rat liver homogenate incubation with mesoridazine (A) and electrospray mass spectra of each individual analyte corresponding to the appropriate reconstructed ion chromatogram (B). RTIC, reconstructed total ion current. **Ib**, mesoridazine; **Ic**, sulforidazine; **IIb**, lactam of mesoridazine; **IIc**, lactam of sulforidazine; **IIIb**, lactam of mesoridazine ring sulfoxide; **IVb**, mesoridazine ring sulfoxide; **IVc**, sulforidazine ring sulfoxide.

incubations carried out for different time intervals. Typical chromatograms and the mass spectra for the two lactam metabolites as obtained by HPLC–MS are shown in Fig. 4. The lactam of thioridazine, but not the lactam of thioridazine ring sulfoxide, was present at a high enough concentration in the extracts of the incubation mixtures to allow its isolation using HPLC and its subsequent analysis by direct probe EI-MS. The EI mass spectral data for **IIa** were consistent with its structure by comparison of the authentic standard. Both spectra obtained form the isolated and standard **IIa** exhibited the molecular ion at m/z 384 [M + ·] (% intensity, standard/metabolite = 31/7) and other diagnostic fragement ions at m/z 254 (20/13), 112 (34/33), 96 (20/5) and 83 (100/100).

3.4. Characterization of in vitro metabolites of mesoridazine, sulforidazine and thioridazine in dog liver homogenates

The identification of the metabolites of mesoridazine, sulforidazine and thioridazine from dog liver homogenates was carried out by the same methods as described for the analysis of rat samples. The compounds identified from the extracts of dog liver incubation with sulforidazine were the intact sulforidazine and sulforidazine ring sulfoxide. The corresponding compounds for mesori-

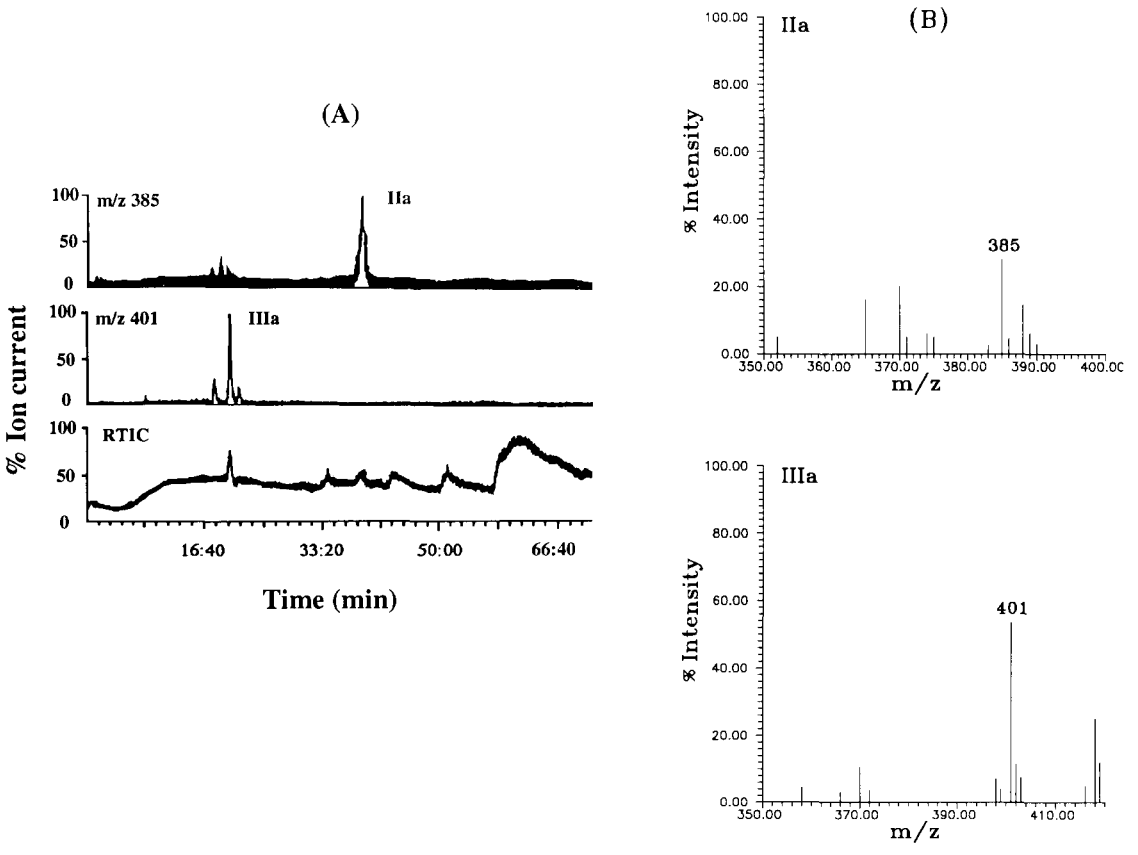

Fig. 4. HPLC–electrospray MS analysis of the lactam metabolites from the extract of rat liver homogenate incubation with thioridazine. (A) Reconstructed total ion chromatogram and reconstructed ion chromatograms for *m/z* 385 (**IIa**, lactam of thioridazine) and *m/z* 401 (**IIIa**, lactam of thioridazine ring sulfoxide; (B) electrospray mass spectra for **IIa** and **IIIa**.

dazine were mesoridazine, sulforidazine and mesoridazine ring sulfoxide, while thioridazine, mesoridazine, sulforidazine and thioridazine ring sulfoxide were found in the qualitative metabolic studies with thioridazine (Table 2). No lactam metabolites were found in any of the incubations.

4. Discussion

The metabolites of piperidine-type phenothiazine antipsychotic drugs present in the organic extracts of in vitro incubation mixtures were investigated. Therefore, aqueous soluble metabolites such as conjugates and phenols were not detected. Except for one metabolite produced from the incubation of sulforidazine in rat liver homogenate, the analytes were unequivocally identified by comparison of both HPLC properties and mass spectral data with those for reference standards. Since the present study mainly focused on lactam metabolites, the various HPLC conditions were opitmized to separate the lactams from other metabolites. In some cases, two non-lactam metabolites co-eluted with each other, such as **Ic** and **IVb** in the HPLC–MS analysis and **Ib** and **Ic** under conventional HPLC conditions (Table 1).

4.1. Metabolic profiles in rat

The metabolites unequivocally identified in in vitro incubations with sulforidazine were the lac-

Table 2
Comparison of identified metabolites of thioridazine, mesoridazine and sulforidazine in rat and dog in in vivo[a] and in vitro studies

Compound	No.	Thioridazine				Mesoridazine				Sulforidazine			
		Rat		Dog		Rat		Dog		Rat		Dog	
		In vivo	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro
Thioridazine	**Ia**	+	+	+	+								
Lactam of thioridazine	**IIa**	nd	+										
Lactam of thioridazine ring sulfoxide	**IIIa**	nd	+										
Thioridazine ring sulfoxide	**IVa**	+	+	+	+								
N-Desmethyl-thioridazine ring sulfoxide		+	nd	+	nd								
Mesoridazine	**Ib**	+	+	+	+	+	+	+	+				
Lactam of mesoridazine	**IIb**					+	+						
Lactam of mesoridazine ring sulfoxide	**IIIb**	+	nd	+	nd	+	+						
Mesoridazine ring sulfoxide	**IVb**	+	nd	+	nd	+	+	+	+				
N-Desmethylmesoridazine ring sulfoxide		+	nd	+	nd	+	nd	+	nd				
Sulforidazine	**Ic**	+	+	+	+	+	+	+	+	+	+	+	+
Lactam of sulforidazine	**IIc**					nd	+			+	+		
Lactam of sulforidazine ring sulfoxide	**IIIc**	+	nd			+	nd	+	nd	+	nd	+	nd
Sulforidazine ring sulfoxide	**IVc**	+	nd	+	nd	+	+	+	nd	+	+	+	+
N-Desmethylsulforidazine ring sulfoxide										+	nd		
Sulforidazine *N*-oxide								+	nd				

+ = Detected; nd = not detected

[a] The data for in vivo studies in the urine of rat and dog were reported previously [12–15].

tam of sulforidazine and sulforidazine ring sulfoxide; these metabolites have previously been reported as in vivo urinary metabolites in rats administered sulforidazine [12]. The in vitro metabolic pathways in rat was less complex than that in vivo in rat urine as summarized in Table 2.

In the case of mesoridazine, six metabolites were identified in the extracts of rat liver homogenates as compared with the seven metabolites found in rat urine [14]. The metabolic profile of thioridazine in rat liver homogenate was much simpler than in vivo in rat urine (Table 2). Only five metabolites were definitively identified in vitro, whereas nine urinary metabolites have been found in vivo [15].

The in vitro metabolic profiles of each of these three drugs at different incubation time intervals were qualitatively similar. However, based on chromatographic peak areas, the amounts of metabolites produced by each drug in vitro reached a maximum at about 4 h incubation. Overall, the metabolic profiles of these three drugs in the rat in vitro system were much simpler than those produced in vivo in the urine of rat since in the latter case primary metabolites are more prone to further metabolism.

4.2. Metabolic profile in dog

The metabolism of each of these three drugs using liver homogenates of dog was observed to be much simpler than that observed in vivo in the urine of dog [13–15] (Table 2). Whereas the lactam metabolites were not found in vitro in the present study, one lactam metabolite for each drug was detected in trace amounts in vivo in dog urine [13–15]. In addition, metabolites resulting from *N*-oxidation, which were identified in vivo, could not be detected in vitro. In particular, in the cases of mesoridazine and thioridazine far fewer metabolites were identified in vitro as compared with in vivo. Moreover, whereas the metabolism of the piperidine-type phenothiazine antipsychotic drugs was limited to ring *S*-oxidation and side chain *S*-oxidation in vitro, urinary metabolites that additionally resulted from *N*-demethylation, *N*-oxidation and lactam formation were found in vivo.

4.3. Lactam metabolites

Five lactam metabolites of thes drugs were identified in rat liver homogenates but none in dog liver preparations. In the rat in vitro system, at least one lactam metabolite was found for each drug. To the best of our knowledge, the lactam of thioridazine and the lactam of thioridazine ring sulfoxide were characterized as the metabolites of thioridazine for the first time in any system. The other three lactams, namely the lactams of mesoridazine, sulforidazine and mesoridazine ring sulfoxide, were identified as in vitro metabolites in this study, although they have previously been identified as in vivo metabolites of mesoridazine and sulforidazine [12–15].

Only the lactam of sulforidazine was found as a metabolite of sulforidazine in vitro in rat, in contrast to in vivo where the lactam of sulforidazine ring sulfoxide was found also. In the case of mesoridazine, three lactam metabolites were identified in vitro (**IIb**, **IIc** and **IIIb**) and two of these were also among the lactam metabolites found in vivo (**IIb** and **IIIb**). The two lactam metabolites of thioridazine found in vitro in rat, namely the lactam of thioridazine and the lactam of thioridazine ring sulfoxide, were not identified in vivo in any species. Whereas these two metabolites result from lactam formation and in one case additional ring *S*-oxidation, the two lactam metabolites (**IIIb** and **IIIc**) found in urine of rats admisistered thioridazine result from these two routes of metabolism plus side-chain *S*-oxidation.

5. Conclusions

Lactam formation has been identified previously to be one of the multiple pathways in the metabolism of piperidine-type phenothiazine antipsychotic drugs in vivo in human, rat and dog [12–15]. The present work is the first report on qualitative investigations of lactam metabolites of this class of drugs in vitro. Five lactam metabolites of these three drugs were unequivocally determined in rat liver homogenate incubations. Two of them, the lactam of thioridazine and the lactam of thioridazine ring sulfoxide, were iden-

tified for the first time in any systems. Since lactam metabolites could be found in the extracts of liver homogenate incubations from rat but not dog, the rat may be a more suitable species than dog for further investigations of the mechanism of such biotransformation. This observation is consistent with data from previous in vivo studies, since lactam formation of these drugs in vivo was more pronounced in rat than in dog [12–15].

Lactam formation has been investigated in various drugs containing saturated nitrogen-containing heterocyclic ring system(s) [10,11]. The lactam metabolite is formed via two steps; initial hepatic cytochrome P450 monooxygenase-mediated hydroxylation of the carbon atom alpha to the ring nitrogen leads to the unstable carbinolamine or iminium ion intermediate, which is then further oxidized by cytosolic molybdenum hydroxylases to produce the lactam metabolite [10,11,24–26]. Biological distribution and enzyme activities of molybdenum hydroxylases, especially hepatic aldehyde oxidase, have been extensively investigated by Beedham and co-workers, and the enzyme activity of aldehyde oxidase has been shown to be low in both rat and dog liver [27–29]. It is interesting that although the liver 10 000*g* supernatant fractions utilized in the present study contain both cytochrome P450 oxidases and molybdenum hydroxylases, only the rat liver preparation produced the lactam metabolites. This observation may indicate that the species difference in the lactam formation might be due to differences in the species-specific isoform composition and/or activities of either P450 oxidases or xanthine oxidases in rat and dog. Further in vitro studies are being performed to determine the mechanism of lactam formation of this class of drug.

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