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Metabolism and pharmacokinetics of the cardiotonic agent piroximone and of its major metabolite in dog

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1. Piroximone was administered orally (p.o.) and intravenously (i.v.) to male Beagle dog. *In vitro*, piroximone was incubated with dog liver microsomes.

2. Piroximone was metabolized *in vivo* to five metabolites (**1–5**) representing approximately 20% of the total administered dose.

3. The parent drug and its metabolites were totally eliminated in urine.

4. Reduced piroximone (piroximole), representing approximately 10% of the administered dose, was identified as the major metabolic product *in vivo*.

5. *In vitro*, piroximone was metabolized by dog liver microsomes to isonicotinic acid (**1**) and piroximole (**4**), with the same ratio as *in vivo* (**1**:**4** = 0·2). The Michaelis–Menten parameters were determined for piroximole formation and were: $K_{m\,app} = 733\mu M$ and $V_{max\,app} = 232$ pmol/mg protein/min.

6. Comparison of the pharmacokinetics of piroximone and piroximole revealed that both compounds were very well absorbed ($F = 93 \pm 7$ and $89 \pm 8\%$ respectively), slightly distributed ($V_{d\,app} = 0{\cdot}78 \pm 0{\cdot}04$ and $1{\cdot}02 \pm 0{\cdot}09$ l/kg p.o., and $0{\cdot}95 \pm 0{\cdot}05$ and $0{\cdot}76 \pm 0{\cdot}13$ l/kg i.v. respectively) and excreted into urine to the same extent ($U_{Ex} = 54{\cdot}7 \pm 1{\cdot}2$ and $53{\cdot}2 \pm 12{\cdot}6\%$ p.o., and $59{\cdot}1 \pm 5{\cdot}3$ and $51{\cdot}2 \pm 5{\cdot}7\%$ i.v. respectively), except that the clearance of piroximone was two-fold higher than that observed for piroximole ($Cl_T = 7{\cdot}77 \pm 1{\cdot}35$ and $4{\cdot}12 \pm 0{\cdot}44$ ml/min/kg p.o., and $7{\cdot}68 \pm 1{\cdot}25$ and $4{\cdot}06 \pm 0{\cdot}51$ ml/min/kg i.v. respectively).

Introduction

Piroximone [4-ethyl-1,3-dihydro-5(4-pyridinyl-carbonyl)-2H-imidazol-2-one; MDL 19205], is a specific inhibitor of a high-affinity cAMP phosphodiesterase type III (Kariya *et al.* 1984, Cheng *et al.* 1985). It is a cardiotonic agent with positive inotropic properties being developed for the treatment of congestive heart failure (Schnettler *et al.* 1982, 1986, Dage *et al.* 1984, Roebel *et al.* 1984). The pharmacokinetics of piroximone have been previously reported in dog (Keeley *et al.* 1983), the healthy volunteer (Haegele *et al.* 1991), and patients evaluated to have congestive heart failure (unpublished data). The metabolism of piroximone has been reported *in vivo* and *in vitro* in rat, with isonicotinic acid being identified as a major metabolic product (Berg-Candolfi *et al.* 1992).

This report describes the *in vivo* and *in vitro* metabolism of piroximone in dog as well as a comparison study of the pharmacokinetics of piroximone and its major metabolite in the same species.

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Materials and methods

Chemicals

Non-radioactive piroximone with a chemical purity of 99·9% (0·1% isonicotinic acid) was provided either as crystals (Marion Merrell Dow Research Institute, Cincinnati, OH, USA) or solubilized in ampoules containing 1·5 mg/ml in physiological saline (Marion Merrell Dow Research Institute, Winnersh, UK). [^{14}C]piroximone with a specific activity of 17·4 Ci/mol and a radiochemical purity of 99·8%, was synthesized by Dr Eugene R. Wagner (Marion Merrel Dow Research Institute, Indianapolis, IN, USA). The radiolabelled carbon atom was located on the inter-ring carbonyl (Berg-Candolfi *et al.* 1992).

Piroximole (MDL 20770) and 4-(3,4-dimethoxybenzoyl)-1,3-dihydro-5-methyl-2H-imidazol-2-one (MDL 82261), with a chemical purity of 99·9%, were obtained from Marion Merrell Dow Research Institute (Cincinnati).

Isonicotinic acid and ammonium acetate were purchased from Sigma (St Louis, MO, USA), methanol and dichloromethane (spectroscopic grade) from Merck (Darmstadt, Germany) and trifluoroacetic anhydride from Aldrich (Milwaukee, WI, USA).

Animals

Male Beagle dogs (11–12·2 kg) (Elevage Canin Dammann Lebeau, Gambais, France) were kept under controlled conditions with respect to temperature (21 ± 1°C), humidity (55 ± 10%) and a diurnal lighting cycle (17 h). They were fasted overnight (16 h) before dosing and for 4 h after dosing.

Dosage preparation and administration

[^{14}C]piroximone was diluted with non-radioactive piroximone to the following specific activities: 1·51 and 1·55 μCi/mg for oral (p.o) and intravenous (i.v.) studies respectively. To achieve complete solubilization, the dose solutions were acidified with 1M HCl to pH 3·5. The dogs were dosed p.o. and i.v. with [^{14}C]piroximone at a dose of 3 mg/kg. A washout period (drug free) of 1 week was maintained between the p.o. and i.v. dosing.

Piroximole was freshly prepared in acidified physiological saline at pH 4·5 and was administered (p.o. and i.v.), as the racemate, at a dose of 3 mg/kg.

Sample collection

Urine, faeces (only for piroximone administration) and blood samples were collected over 96 h, after p.o. and i.v. administration of piroximone. All samples were stored at − 20°C until analysis.

Measurement of radioactivity in biological samples

Radioactivity was measured using a liquid scintillation counter (LS 5000 CE, Beckman, San Ramon, CA, USA) and Aquasol-2 as scintillator (Dupont, Boston, MA, USA) as previously reported (Berg-Candolfi *et al.* 1992).

Urinary metabolite profile of piroximone

The samples were treated and analysed as reported previously (Berg-Candolfi *et al.* 1992), except for the use of a M680 gradient delivery module and a M486 variable-wavelength UV detector with a M746 integrator (all from Waters, Millipore Association, Milford, MA, USA). Tetrabutylammonium acetate was also absent from the mobile phase.

Radioactivity was detected in net counts with RUNF1B software and converted to cpm with A280 software (both from Radiomatic, Packard, Rungis, France). The amount of piroximone and of each metabolite excreted, expressed as % dose, was calculated by multiplying the fraction excreted in urine with the % recovered by hplc as a radioactive peak.

Identification of piroximone metabolites

Metabolites were isolated as reported previously (Berg-Candolfi *et al.* 1992). The separation of fractions **1–3** and **5** was achieved by hplc with an isocratic elution using 0·05 M ammonium acetate (pH 6·8) on a μBondapak NH_2 (10-μm particle size) column (30 cm × 3·9 mm i.d.) connected to a μBondapak NH_2 (Waters) guard column.

For the GC-MS analysis, **1** was derivatized with 100 μl dichloromethane and 20 μl trifluoroacetic anhydride (TFAA), for 30 min at 60°C. After evaporation to dryness under nitrogen, the fractions were solubilized in methanol:dichloromethane (1:1). Mass spectrometric analysis was carried out as previously reported (Berg-Candolfi *et al.* 1992).

The LC-MS analysis was carried out on underivatized isolated fractions, using a thermospray interface. LC was carried out using the conditions as described previously for the analysis of the urine samples by hplc, except for the use of a 600MS solvent delivery module (Waters), a Zorbax C_8 (5-μm particle size) column (25 cm × 4·6 mm i.d.) (SFCC, Neuilly-Plaisance, France) and a gradient elution period extended to 30 min. To obtain better ionization, a solvent of 40% methanol was added after the column, delivered by a 590MS solvent delivery module (Waters) at a flow-rate of 0·2 ml/min.

Thermospray MS was performed on a SSQ 70, single-stage, quadrupole mass spectrometer, equipped with a TSP2 thermospray interface (both from Finnigan MAT, San Jose, CA, USA). The ion source was operated in the discharge-on-and-filament-off mode. The ion source was maintained at 200°C and the vaporizer at 110°C. The discharge, repeller and photomultiplier voltages were set at 600, 40 and 1500 V respectively. Data were acquired and processed using a standard software (Finnigan MAT). Scanning data were acquired between 150 and 500 amu.

Metabolism of piroximone by liver microsomes

Microsomes were prepared as described previously (Berg-Candolfi *et al.* 1992), from a piece of a thawed adult male Beagle dog liver.

Microsomes were incubated with 8–4000 nmol (8–4000 μM) piroximone containing 0·14–6·96 μCi [^{14}C]piroximone by adjusting the microsomal protein concentration to 1·3 mg/ml (Lowry *et al.* 1951). The incubations were carried out at 37°C for 45 min in a shaking waterbath (100 oscillations/min), with a buffer system containing 81 mM disodium phosphate buffer (pH 7·4), 14·3 mM $MgCl_2$, 47·6 mM nicotinamide and a NADPH-generating system consisting of 0·9 mM $NADP^+$, 1·6 mM glucose-6-phosphate and 0·3 I.U./ml glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (Berg-Candolfi *et al.* 1992). The enzymatic reactions were terminated by protein precipitation with 50% methanol.

Linearity of enzyme kinetics with respect to time and protein concentration were verified by varying the incubation time from 10 to 80 min and the protein concentration from 0·3 to 5·2 mg/ml.

Michaelis–Menten parameters ($K_{m\,app}$ and $V_{max\,app}$) were determined by ENZFITTER software (Biosoft, Cambridge, UK). Hepatic intrinsic clearance (Cl_{int}) was estimated from the ratio $V_{max\,app}/K_{m\,app}$.

Pharmacokinetics of piroximone

Determination of the concentration of piroximone in urine and plasma was carried out following the procedure described by Keeley *et al.* (1983). The internal standard employed throughout the processing of the samples was 4-(3,4-dimethoxybenzoyl)-1,3-dihydro-5-methyl-2H-imidazol-2-one.

The recovery of piroximone from plasma was determined as described by Keeley *et al.* (1983), with a similar extent of approximately 60%. Calibration curves in urine and plasma were established from the peak area ratios, ranging from 2 to 625 and from 0·04 to 5 μg/ml respectively. Linear regression analyses yielded the following equations, for urine and plasma respectively: $y = 6{\cdot}44 \times 10^{-3}\,x + 4{\cdot}89$ ($r^2 = 0{\cdot}999$), and $y = 0{\cdot}496\,x + 0{\cdot}017$ ($r^2 = 0{\cdot}999$) (x is expressed in μg/ml urine or plasma, and y as mV).

SIPHAR software (Simed, Créteil, France) was used to process the data, with a single-compartment (urine) or a bi-compartment (plasma) pharmacokinetic model. The *sigma-minus* method was chosen for the evaluation of the pharmacokinetics in urine (Martin 1967, Gibaldi and Perrier 1982, Rowland and Tozer 1989). Peak plasma concentrations (C_{max}) and time to peak (t_{max}) were estimated using the bi-compartment pharmacokinetic model. The half-life was determined employing linear regression analysis for the log–linear terminal phase of the curve ($t_{1/2\beta} = 0{\cdot}693/\beta$). The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule and extrapolated to infinity according to $AUC^{0-\infty} = AUC^{0-t} + (C_t/\beta)$. The bioavailability ($F$) was determined as $(AUC_{p.o.} \times dose_{i.v.})/(AUC_{i.v.} \times dose_{p.o.})$. Total clearance ($Cl_T$) was calculated as $(F \times dose)/AUC$. The apparent volume of distribution ($V_{d\,app}$) was obtained according to $V_{d\,app} = Cl_T/\beta$. Hepatic systemic clearance (Cl_H) was evaluated using the well-stirred model (Rowland and Tozer 1989).

Pharmacokinetics of piroximole

The same internal standard was used as described for piroximone. Hplc analysis was achieved using a Zorbax C_8 (5 μm particle size) column (25 cm × 4·6 mm i.d.) (SFCC) at a flow rate of 1·5 ml/min, as follows: urine: 10-min isocratic elution with 0·05 M phosphate buffer and 10% (v/v) methanol (pH 7), 15-min linear gradient with 0·05 M phosphate buffer and 10–60% (v/v) methanol (pH 7), 5-min isocratic with 0·05 M phosphate buffer and 60% (v/v) methanol (pH 7); and plasma; isocratic elution with 0·05M phosphate buffer and 30% (v/v) methanol (pH 7). UV detection was carried out at 254 nm.

The recovery of piroximole from plasma was determined as described by Keeley *et al.* (1983), with a similar extent of approximately 60%. The calibration curves in urine and plasma were established as for piroximone, but using peak height instead of peak area and ranged from 5 to 625 and from 0·08 to 5 μg/ml for urine and plasma respectively. Linear regression analyses yielded the following equations for urine and plasma respectively: $y = 0{\cdot}011\,x + 0{\cdot}021$ ($r^2 = 0{\cdot}999$), and $y = 0{\cdot}636\,x - 0{\cdot}134$ ($r^2 = 0{\cdot}998$) (x is expressed in μg/ml urine or plasma, and y as mV).

The extraction of piroximole from plasma necessitated saturation of the aqueous phase with 2 ml sodium sulphate (Karoum *et al.* 1975).

Pharmacokinetics of piroximone metabolites in urine following piroximone administration

The *sigma-minus* method was used with the metabolites being detected on line by ^{14}C radioactivity measurements.

Results

Mass balance

Within 48 h after dosing, 104·8 ± 4·0% ($n = 3$) and 99·7 ± 13·3% ($n = 3$) of the total radioactivity administered were recovered in urine samples, after p.o. and i.v. administration respectively. 91·6 ± 10·9% ($n = 3$) (p.o.) and 90·9 ± 10·3% ($n = 3$) (i.v.) were excreted within 8 h after dosing.

Urinary metabolite profile of piroximone

Four metabolites (**1–4**) were detected in the 0–1-h urine samples and on further metabolite (**5**) in the 4–6-h urine samples (**1** at this time had disappeared). Within 24 h after dosing, the total metabolism of piroximone (**1–5**) accounted for about 20% of the administered dose. Metabolite **4** was the major metabolite and represented about 10% of the administered dose. Metabolite **1–3** and **5** accounted for approximately 2, 3, 2 and 3% of the administered dose respectively (figure 1).

Identification of piroximone metabolites

Isonicotinic acid has previously been identified as the predominant metabolite **1** by GC-MS (Berg-Candolfi *et al.* 1992). Fraction **1** contained another but minor metabolic product, tentatively identified as isonicotinyl formamide, whose mass spectrum exhibited pseudomolecular ions MH^+ at m/z 151 and MNH_4^+ at m/z 168 (data not shown). Since no synthetic standard was available, no absolute confirmation of the identity of this compound could be obtained. However, derivatization of the compound with TFAA at 60°C for 24-h yielded the expected di-trifluoroacetyl derivative, yielding an adduct ion MNH_4^+ at m/z 360 (data not shown).

In fraction **4,** the reduced compound, piroximole, was identified by combined LC-MS at a retention time of 13 min. The mass spectrum showed a protonated molecular ion MH^+ at m/z 220 and a fragment ion, corresponding to $[MH\text{-}H_2O]^+$ at m/z 202 (figure 2 (*a*)).

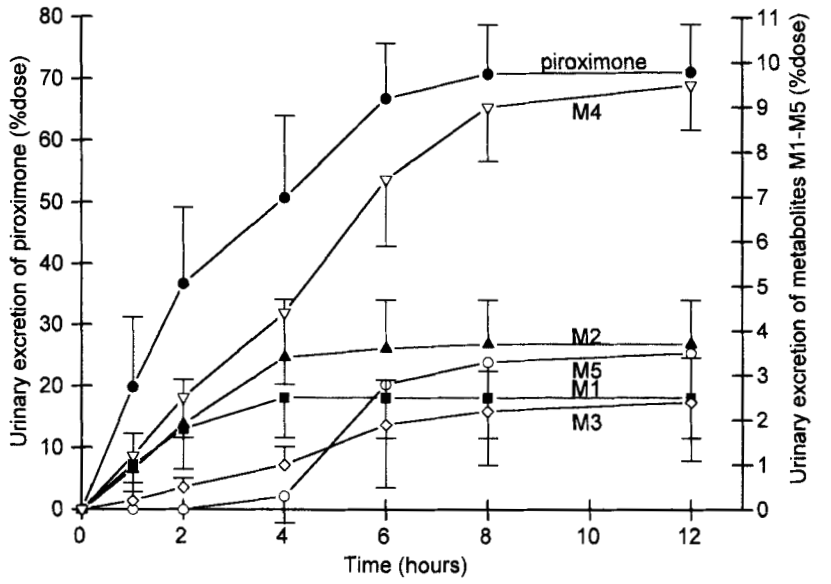
The impurities present in fractions **2, 3** and **5** hindered their identification, and were not further characterized.

Metabolism of piroximone by dog liver microsomes

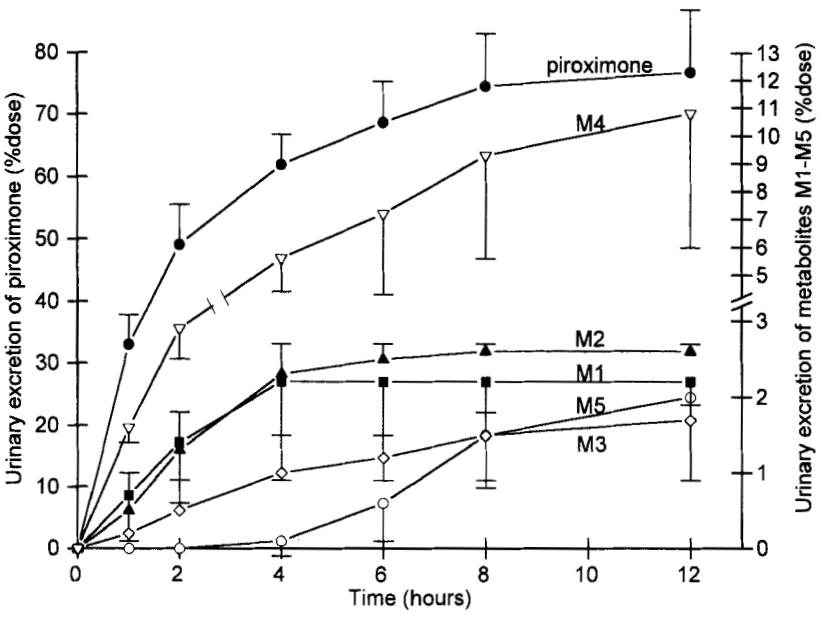
Metabolites **1** and **4** were formed at a rate of 5·1 ± 0·9 ($n = 3$) and 22·3 ± 1·2 ($n = 3$) pmol/mg protein/min respectively after incubation of piroximone with liver microsomes. Michaelis–Menten parameters were determined for **4** formation as follows: $K_{m\,app} = 733\ \mu M$, and $V_{max\,app} = 232$ pmol/mg protein/min. The correlation coefficients versus time and protein concentration for the formation of **4** were established as 0·998 and 0·993 respectively ranging over 10–80 min and 0·3–2·6 mg protein/ml.

Pharmacokinetics of piroximone and piroximole

The pharmacokinetic parameters are described in table 1. C_{max} of piroximone and piroximole were almost identical (12·56 ± 1·25 and 11·40 ± 0·67 nmol/ml respectively). t_{max} of piroximole showed more variability than for piroximole (0·75 ± 0·66 and 1·00 ± 0·00 h respectively). F for piroximone and piroximole were comparable (93 ± 7 and 89 ± 8% respectively), as well as the U_{Ex} (54·7 ± 1·2 and 53·2 ± 12·6% p.o., and 59·1 ± 5·3 and 51·2 ± 5·7% i.v. respectively) and $V_{d\,app}$ (0·78 ± 0·04 and



(*a*)



(*b*)

Figure 1. Cumulative urinary excretion of piroximone and metabolites after (*a*) p.o. and (*b*) i.v. administration of 3 mg/kg [^{14}C] piroximone. Data are mean ± sd ($n = 3$).

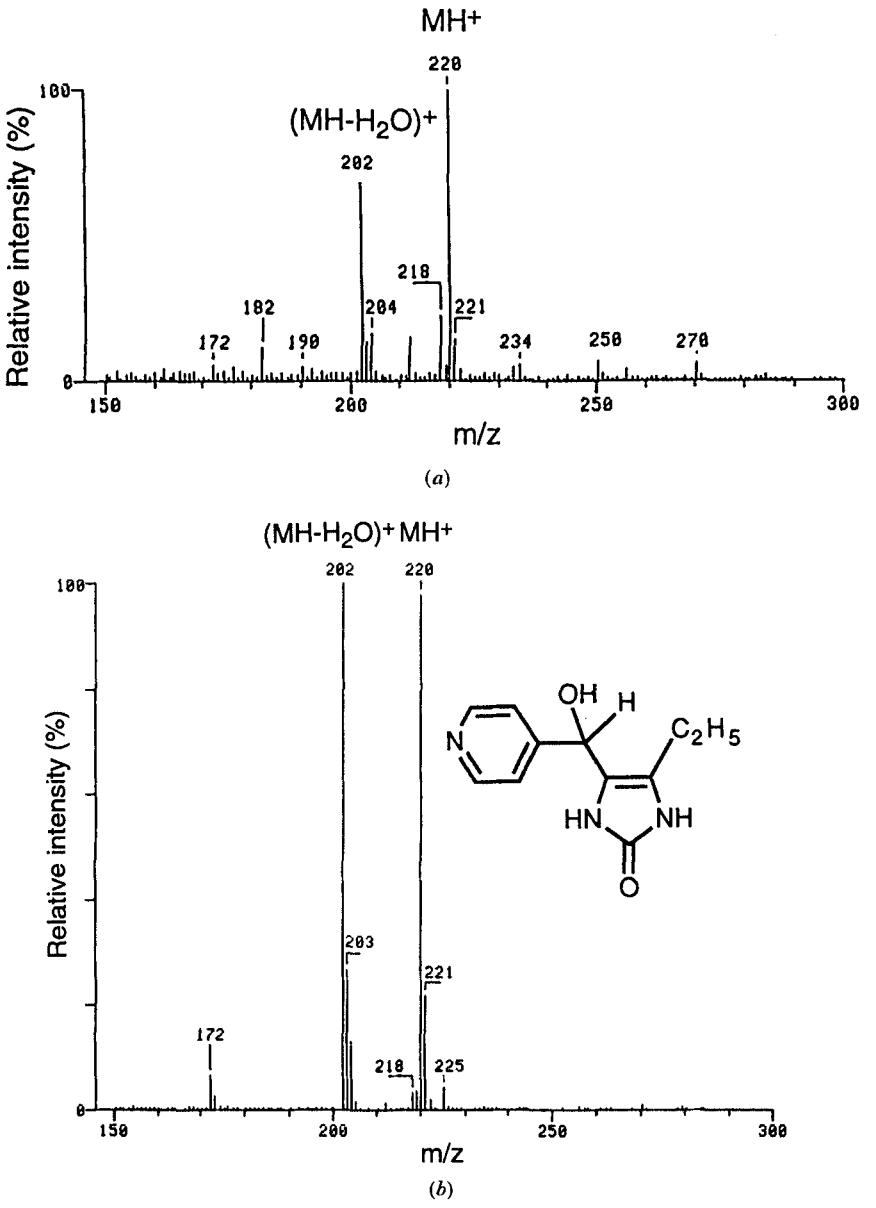


Figure 2. Positive ion thermospray mass spectra (LC-MS) of (*a*) **4** and (*b*) authentic piroximole.

1·02 ± 0·09 l/kg p.o., and 0·95 ± 0·05 and 0·76 ± 0·13 l/kg i.v. respectively). By contrast, $t_{1/2\beta}$ was about two-fold smaller for piroximone than for piroximole (1·18 ± 0·17 and 2·88 ± 0·49 h p.o., and 1·45 ± 0·25 and 2·29 ± 0·17 h i.v. respectively) as well as the $AUC^{0-\infty}$ (27·81 ± 5·18 and 49·12 ± 1·26 nmol.h/ml p.o., and 30·17 ± 4·88 and 56·86 ± 7·44 nmol.h/ml i.v. respectively). Total clearance (Cl_T), renal clearance (Cl_R) and extra-renal clearance (Cl_{ER}) were about two-fold higher for piroximone than for piroximole (Cl_T = 7·77 ± 1·35 and 4·12 ± 0·44 ml/min/kg p.o., and 7·68 ± 1·25 and 4·06 ± 0·51 ml/min/kg i.v., Cl_R = 4·62 ± 1·03 and 2·48 ± 0·61 ml/min/kg p.o., and 4·50 ± 0·42 and 2·08 ± 0·47 ml/min/kg i.v.,

Table 1. Pharmacokinetic parameters for piroximone and piroximole in dog plasma after p.o. and i.v. administration of 3 mg/kg.

	C_{max} (nmol/ml)	t_{max} (h)	$t_{1/2\beta}$ (h)	$AUC^{0-\infty}$ (nmol.h/ml)	F (%)	U_{Ex} (%)	Cl_T (ml/min kg)	Cl_R (ml/min/kg)	Cl_{ER} (ml/min/kg)	$V_{d\,app}$ (l/kg)
Piroximone (p.o.)	12·56 ± 1·25*	0·75 ± 0·66	1·18 ± 0·17	27·81 ± 5·18	93 ± 7	54·7 ± 1·2	7·77 ± 1·35**	4·62 ± 1·03**	3·14 ± 0·52**	0·78 ± 0·04**
Piroximole (p.o.)	11·40 ± 0·67	1·00 ± 0·00	2·88 ± 0·49	49·12 ± 1·26	89 ± 8	53·2 ± 12·6	4·12 ± 0·44**	2·48 ± 0·61**	1·64 ± 0·27**	1·02 ± 0·09**
Piroximone (i.v.)			1·45 ± 0·25	30·17 ± 4·88		59·1 ± 5·3	7·68 ± 1·25	4·50 ± 0·42	3·18 ± 0·87	0·95 ± 0·05
Piroximole (i.v.)			2·29 ± 0·17	56·86 ± 7·44		51·2 ± 5·7	4·06 ± 0·51	2·08 ± 0·47	1·97 ± 0·30	0·76 ± 0·13

* Mean ± SD ($n = 3$).
** Corrected for F.

$Cl_{ER} = 3{\cdot}14 \pm 0{\cdot}52$ and $1{\cdot}64 \pm 0{\cdot}27$ ml/min/kg p.o., and $3{\cdot}18 \pm 0{\cdot}87$ and $1{\cdot}97 \pm 0{\cdot}30$ ml/min/kg i.v. respectively).

Cl_{int} for piroximole formation was determined by extrapolation as 32×10^{-5} ml/min/mg protein or 2·2 ml/min/liver and the corresponding Cl_H as 2 ml/min/liver (dog hepatic blood flow of 387 ml/min, dog liver weight of 530 g) (Spector 1956) (protein concentration of 13 mg/g liver, fraction of piroximone unbound in blood of 0·9).

Pharmacokinetics of piroximone metabolites in urine following piroximone administration

Metabolite **1** ($1{\cdot}3\,h < t_{1/2} < 1{\cdot}5\,h$) is the most rapidly excreted, followed by **2** ($t_{1/2} = 1{\cdot}5$ h), **3** ($t_{1/2} = 3$ h), **4** ($t_{1/2} = 3$ h), **5** ($t_{1/2} > 3$ h).

Discussion

In a previous study, isonicotinic acid was identified as a major metabolite of piroximone in rat (Berg-Candolfi *et al.* 1992). In the current study, we have identified a reduction product of piroximone, piroximole, as a major metabolite in dog, and we have compared its pharmacokinetics to the pharmacokinetics of piroximone in the same species.

The *in vivo* metabolism of piroximone in dog has shown that piroximone is metabolized to five metabolites (**1–5**), accounting for approximately 20% of the administered dose, and that they are totally eliminated in the urine with the parent drug. This renal elimination was rapid because only a small amount of radioactivity remained in the body 8 h after dosing. Similar results were reported in rat (Berg-Candolfi *et al.* 1992). Metabolite **4** was the major metabolite in dog, whereas **1** and **2** were the major metabolites in rat (Berg-Candolfi *et al.* 1992). Metabolite **5** was detected at later time intervals than the other metabolites, suggesting a slower formation and/or renal elimination, as was observed in rat (Berg-Candolfi *et al.* 1992).

The *in vitro* metabolism of piroximone by dog liver microsomes has shown that, under the conditions described, piroximone is metabolized to only two metabolites (**1** and **4**), and similar results have been reported in rat (Berg-Candolfi *et al.* 1992). Metabolites **1** and **4** are the major metabolites formed *in vivo* in rat and dog, and the only ones detected *in vitro* in the liver microsomal systems from both species. The liver appears to be the major organ involved in the metabolism of piroximone. The **1**:**4** ratios observed *in vitro* in rat (3·3) and dog (0·2), agree with the *in vivo* ratios observed in the same species (2·1–3·3 in rat, and 0·2 in dog) (Berg-Candolfi *et al.* 1992).

As **2**, **3** and **5** are formed only *in vivo* in rat and dog, they may arise from cytosolic metabolism of piroximone in the liver or via extrahepatic metabolism of piroximone; however, in the latter case, the gastrointestinal mucosa may be excluded, since the metabolic ratio remains unchanged after either p.o. or i.v. dosing.

Isonicotinic acid (**1**) was identified *in vivo* and *in vitro* as a metabolite of piroximone in rat and dog, and has also been observed as a metabolite of isoniazid and iproniazid (Guzman *et al.* 1984). Similarly, a derivative of isonicotinic acid was identified as a metabolite of the cardiotonic agent enoximone (Dage and Okerholm 1990). Isonicotinic acid was also obtained as a chemical degradation product of piroximone at pH 13 and 60°C, and as the major degradation product when piroximone was treated with 1% hydrogen peroxide (Chen *et al.* 1987). These results

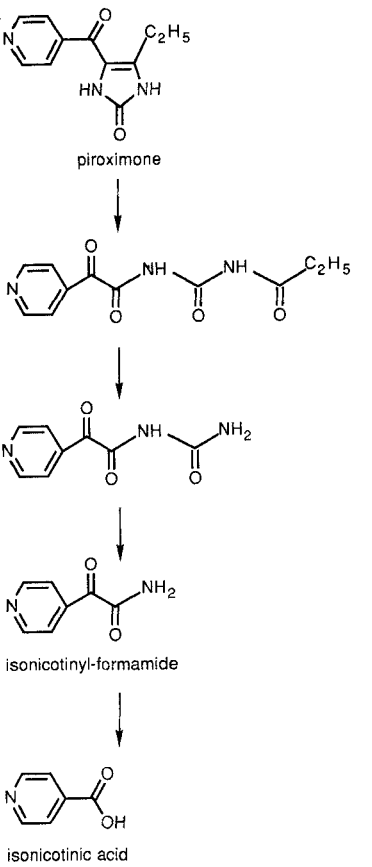


Figure 3. Hypothetical pathway for the metabolism of piroximone to isonicotinic acid.

suggest certain similarities in the chemical and enzymatic transformation of piroximone. Furthermore, Chen *et al.* (1987) reported a bis-acylurea structure, analogous to those resulting from the photo-oxidation of an imidazolone derivative (Tsuge *et al.* 1979), as a primary product of the formation of isonicotinic acid, isonicotinic acid being the final and major degradation product. Thus, based on these chemical results, we can hypothesize a pathway for the formation of isonicotinic acid via isonicotinyl formamide (figure 3), although the presence of isonicotinyl-formamide in fraction **1** was not fully established in our studies due to the unavailability of a synthetic standard. As discussed by Reed (1987), different systems may be involved in the peroxidation of drugs.

Piroximole (**4**) was identified as an *in vivo* and *in vitro* metabolite of piroximone in rat and dog. The possible involvement of a carbonyl reductase (Testa and Jenner 1976) or of a hydroxysteroid dehydrogenase (Bannenberg *et al.* 1992) in piroximole formation remains to be established.

The *in vivo* metabolic pathway of piroximone is summarized in figure 4. Metabolite **1** is isonicotinic acid, with probable trace amounts of isonicotinyl formamide, metabolite **4** is piroximole, and **2**, **3** and **5** are unidentified metabolites.

The glycine conjugate of isonicotinic acid, a metabolite of isoniazid, iproniazid (Guzman *et al.* 1984) and enoximone (Dage and Okerholm 1990), was not detected

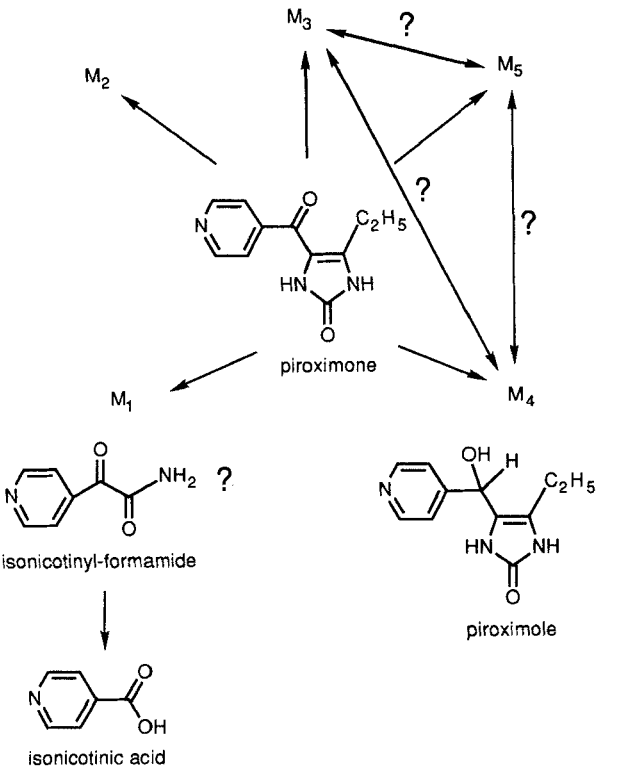


Figure 4. Metabolic pathway of piroximone in rat and dog.

in the case of piroximone. Piroximone is also not metabolized to an N-oxide on the phenyl ring (comparison of retention times with the synthetic standard), in contrast with enoximone, whose major metabolite was the sulphoxide (Dage and Okerholm 1990), probably because, in this case, the nitrogen atom is an inferior electron donor compared with the sulphur atom and the electron pair is partially delocalized.

The pharmacokinetic parameters of piroximone in dog were similar to those obtained by Keeley *et al.* (1983) in the same species. The pharmacokinetics of piroximone and piroximole, the major metabolite in dog, were compared. C_{max}, t_{max} and F for piroximone and piroximole determined to be approximately 12 nmol/ml, 0·9 h and 90% respectively, suggesting that both are very well and relatively rapidly absorbed through the gastrointestinal mucosa. The $V_{d\,app}$ of both molecules of approximately 0·9 1/kg being slightly higher than the total body water volume, which is considered as 0·7 1/kg for an adult dog, suggests some tissue distribution. U_{Ex} of piroximone and piroximole being $< 60\%$ suggests extensive metabolism for both. In contrast with man, no difference in U_{Ex} was observed between p.o. and i.v. administration (Haegele *et al.* 1991). A $t_{1/2\beta}$ of piroximone of approximately 1·3 h, suggests rapid elimination of this drug. In contrast with man, it was difficult to correlate this value to the $t_{1/2}$ calculated for urine, probably because of the fluctuations of urine flow and pH as well as of renal blood flow in these dogs (Trevor *et al.* 1971, Haegele *et al.* 1991) The same problem occurred with piroximole. Cl_R of piroximone (52 ml/min) is the same as the glomerular filtration rate of inulin, mannitol and creatinine (Trevor *et al.* 1971), suggesting that the major route of

elimination of piroximone is via glomerular filtration. $t_{1/2\beta}$ and $AUC^{0-\infty}$ being about two-fold greater for piroximole than for piroximone and Cl_T, Cl_R and Cl_{ER} being about two-fold lower for piroximole than for piroximone, suggesting that piroximole is eliminated more slowly than piroximone, probably due to tubular reabsorption. Since piroximone has a low hepatic extraction ratio, Cl_{int} is so small compared with hepatic blood flow that Cl_H calculated for the metabolism of piroximone to piroximole is approximately equal to Cl_{int} (Rowland and Tozer 1989). For this reason, an important difference is observed between this Cl_H (2 ml/min) and the Cl_{ER} (36 ml/min).

Metabolites **1** ($1{\cdot}3 < t_{1/2} < 1{\cdot}5$ h) and **2** ($t_{1/2} = 1{\cdot}5$ h) seem to be formation-rate limited, whereas **3** ($t_{1/2} = 3$ h), **4** ($t_{1/2} = 3$ h) and **5** ($t_{1/2} > 3$ h) seem to be elimination-rate limited (Rowland and Tozer 1989). Consequently, **3–5** appear to be sequential metabolites within the same metabolic pathway and the same rate-limiting step (figure 4).

Because piroximole has a chiral center and the two enantiomers may be considered as two different molecules, particularly on a pharmacokinetic basis (Evans *et al.* 1988, Jamali *et al.* 1989, Le Corre *et al.* 1991), direct and indirect methods were attempted to separate the two enantiomers for further stereoselective pharmacokinetic analyses (Krstulovic 1989, Hutt 1990, Metha 1990). Several chiral columns were therefore tested but without any success (data not shown). Diastereoisomers were also synthesized by esterification of the hydroxy group of piroximole with the chiral Mosher reagent (Dale *et al.* 1969, Dale and Mosher 1973), but it was impossible to separate them on a normal phase column.

Further studies of the metabolism of piroximone need to be undertaken in man, with particular emphasis on the elucidation of the enzymatic systems implicated in its metabolism.

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