

## Metronidazole and antipyrine metabolism in the rat: clearance determination from one saliva sample

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1. The applicability of a simple, non-invasive method for assessment of metronidazole and antipyrine metabolism in rats *in vivo* was investigated.

2. In 48 sample pairs of blood and pilocarpine-stimulated saliva from six rats the concentration of metronidazole was almost identical ( $r = 0.97$ ).

3. In 26 rats the clearance could be determined from one sample without loss of precision and accuracy compared with conventional determinations ( $r = 0.99$ ). If urine was collected for 24 h the fractional clearance representing each elimination pathway could be determined.

4. Pretreatment with phenobarbitone increased the fractional clearance of metronidazole by oxidation and glucuronidation 3.8-fold and 1.6-fold, respectively, whereas 3-methylcholanthrene pretreatment increased the rate of oxidation 10-fold and decreased the rate of glucuronidation 0.5-fold.

5. The clearance and fractional clearances of metronidazole and antipyrine administered in a mixture could be determined from the same saliva sample and urine collected for 24 h without drug-drug interactions.

6. Phenobarbitone pretreatment increased the formation rate of all metabolites of metronidazole and antipyrine administered in a mixture, whereas  $\beta$ -naphthoflavone increased the formation rates of only the oxidative metronidazole metabolites, norantipyrine and 4-hydroxyantipyrine, but not metronidazole glucuronide or 3-hydroxymethylantipyrine.

7. A mixture of metronidazole and antipyrine and non-invasive sampling are recommendable for the study of the differential metabolism of foreign compounds in rats *in vivo*.

Metronidazole has been suggested as a probe for the study of foreign compound metabolism (Loft *et al.* 1988, Loft and Poulsen 1989). Metronidazole is eliminated by cytochrome P-450-catalysed oxidations to metronidazole acetic acid and hydroxymetronidazole, by glucuronidation and by renal excretion of unchanged compound (Stambaugh *et al.* 1968, Loft *et al.* 1986). In isolated rat hepatocytes the formation rate of metronidazole metabolites enables the identification of 3-methylcholanthrene- and phenobarbitone-type of enzyme induction (Loft and Poulsen 1989). Moreover, formation of the oxidative metabolites of metronidazole and of the well-known probe drug, antipyrine, appears to be catalysed by different cytochrome P-450s and the use of both compounds in a mixture has been advocated (Loft *et al.* 1988, Loft and Poulsen 1989). Simultaneous administration of two or more drugs provides ideal conditions for the study of co-regulation of their metabolic rates and more information regarding possible differential regulation of the involved enzymes (Breimer 1983, Van der Graaff *et al.* 1983, Teunissen *et al.* 1986, Schellens *et al.* 1988).

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Clearance *in vivo* is the preferred term for expressing metabolite formation rates (Rowland 1972, Wilkinson 1987). The clearance of a compound is usually determined from a number of successive plasma samples after a single dose. However, if the volume of distribution can be independently estimated with sufficient accuracy the clearance can be determined from a single concentration-time point and that volume (Døssing *et al.* 1982). This one-sample method for clearance determination has been validated in man as well as in rat for a number of compounds, including antipyrine and other drugs, e.g. phenytoin and theophylline (Døssing *et al.* 1982, Pilgaard and Poulsen 1984, Bachmann *et al.* 1985a, b, 1988).

In rat, collection of blood implies stressful handling and/or indwelling catheters inserted under general anaesthesia, all of which may have considerable effects on the enzymes metabolizing foreign compounds (Capel *et al.* 1980, Wood and Wood 1984, Van Dyke *et al.* 1987, Chindavijac *et al.* 1988). In rat as well as in man the concentration of antipyrine is identical in blood, plasma and pilocarpine-stimulated saliva, allowing non-invasive collection of samples for clearance determination (Welch *et al.* 1975, Wilson *et al.* 1982). Similarly, in man saliva can replace plasma for determination of the pharmacokinetics of metronidazole. In fact, in man the clearance of both metronidazole and antipyrine ingested in a mixture can be determined from one saliva sample (Loft *et al.* 1988).

In the present work we investigated the applicability of a one-sample saliva method for clearance determination, combined with urine collection for the study of the metabolism of metronidazole and antipyrine after prototype induction in the rat *in vivo*.

## Materials and methods

### Chemicals

Metronidazole (5 mg/ml) and antipyrine (100 mg/ml) for injection, thiopentone, pilocarpine and phenobarbitone (PB) were obtained from the Danish Hospital Pharmacies, Leo Ltd and DAK (Copenhagen, Denmark). Reference compounds for analysis of metronidazole and metabolites were kindly donated by Rhône-Poulenc Pharma (Vitry, France) and Dumex Ltd (Copenhagen, Denmark). Reference compounds for analysis of antipyrine and metabolites, except 3-hydroxymethylantipyrine which was a kind gift from Leo Ltd (Copenhagen, Denmark), 3-methylcholanthrene (MC) and  $\beta$ -naphthoflavone were purchased from Aldrich (Steinheim, Federal Republic of Germany). Glusulase<sup>®</sup> was from Sigma (St Louis, MO). All solvents were of analytical or chromatographic grade and purchased from Merck (Darmstadt, FRG).

### Animals

Laboratory-bred male Wistar rats (225–275 g) were housed under constant temperature and humidity in a 12 h light cycle with free access to food (Altromin<sup>®</sup>) and tap water. When housed in metabolic cages the rats had free access to ground Altromin<sup>®</sup> and water. Different breeding batches of rats were used for the study of metabolism of metronidazole alone and metronidazole and antipyrine together—see below.

### Saliva-blood ratio of metronidazole

Six rats were dosed with thiopentone 9 mg/kg body weight *i.p.* for anaesthesia throughout the experiment. Catheters were placed in the carotid artery and the jugular vein. Metronidazole 10 mg/kg body weight was administered as a bolus in the jugular catheter. At timed intervals blood (50  $\mu$ l) was sampled from the carotid catheter for 5 h. Two minutes prior to blood sampling pilocarpine 0.2 mg was given subcutaneously for stimulation of saliva secretion and 75–100  $\mu$ l was collected from the sub-mandibular crevice in capillary tubes. Isotonic saline 0.4 ml/h was infused in the jugular catheter in order to conserve fluid balance.

### Pharmacokinetics of metronidazole

Twenty rats were divided into three groups. One group of seven rats was kept untreated, whereas seven rats were pretreated with PB at 1 mg/ml in the drinking water for 7 days, and six rats were treated with  $\beta$ -naphthoflavone 50 mg/kg body weight *i.p.* for 3 days. After pretreatment metronidazole 10 mg/kg was administered *i.p.* and pilocarpine (0.2 mg s.c.)-stimulated saliva samples of 100–150  $\mu$ l were collected at timed intervals for 6 h. The bioavailability of metronidazole administered *i.p.* was assumed to be unity.

The kinetics of metronidazole elimination from saliva were determined from the data obtained in the six anaesthetized rats and in the conscious 20 rats from the three groups with or without pretreatment. The clearance, volume of distribution and half-life were determined from the area under the curve and the slope of the log concentration-time curve. The one-sample clearance ( $CL$ ) was determined from the concentration ( $C$ ) in one sample collected after 3 or 4 h ( $t$ ), the dose ( $D$ ) and an assumed volume of distribution ( $V$ ) as:

$$CL = V \ln((D/V)/Ct)/t.$$

Since metronidazole like antipyrine is distributed in total body water  $V$  was assumed to be 0.67 l/kg body weight (Pilsgaard and Poulsen 1984).

#### Differential induction of metronidazole metabolism

Twenty-four rats were divided into groups of six. One group was kept untreated, whereas another was pretreated with PB at 1 mg/ml in the drinking water for 7 days and the remaining two groups with MC at 25 mg/kg body weight i.p. for 3 days. The rats were placed in metabolic cages and dosed with metronidazole 10 mg/kg body weight i.p. One of the groups pretreated with MC received antipyrine 14 mg/kg body weight i.p. concomitantly with metronidazole. Four hours (3 h in induced rats) later a saliva sample was collected after stimulation with pilocarpine 0.2 mg s.c. Urine was collected for 24 h, i.e. more than 10 half-lives, and sufficient for complete metabolism and excretion. The urine collection tubes connected to the metabolic cages were kept at 0°C.

#### Metronidazole-antipyrine interaction

Separated by 3 days and in random order, one group of six rats received three i.p. injections, consisting of either metronidazole 10 mg/kg body weight, antipyrine 14 mg/kg body weight, or both drugs in a mixture, respectively. Four hours after each administration pilocarpine-stimulated saliva was sampled and urine was collected for 24 h.

#### Differential induction of metronidazole and antipyrine metabolism

Eighteen rats were divided into 3 groups of six. One group was kept untreated, whereas the other two were pretreated with either PB at 1 mg/ml in the drinking water for 7 days or with  $\beta$ -naphthoflavone 50 mg/kg body weight i.p. for 3 days. A mixture of metronidazole 10 mg/kg and antipyrine 14 mg/kg body weight was administered i.p. Three hours (induced rats) or 4 h (control rats) later pilocarpine-induced saliva (300  $\mu$ l) was sampled and urine collected for 24 h.

The one-sample clearances of metronidazole and antipyrine were calculated as described above and elsewhere, respectively (Pilsgaard and Poulsen 1984, Poulsen and Pilsgaard 1985). The fractional clearance representing each elimination pathway was calculated as the product of the clearance and the fraction of the dose excreted as that particular metabolite.

#### Analytical procedures

Various h.p.l.c. analyses were used. For the assay of metronidazole in blood and saliva, samples of 50  $\mu$ l were immediately mixed with an equal amount of sterile water, causing haemolysis in the former. Acetonitrile 150  $\mu$ l containing the internal standard was added, and after centrifugation the supernatant was left to evaporate overnight before injection on a 15 cm Spherisorb ODS 5  $\mu$ m chromatographic column (Loft *et al.* 1986, 1988). The eluent was methanol (6% v/v) and acetonitrile (4% v/v) in phosphate buffer pH 4.0 monitored at 320 nm. Samples were analysed singly with a detection limit of less than 1  $\mu$ M. Urine samples were assayed in duplicate for metronidazole and metabolites before and after incubation for 16 h in acetate buffer pH 4.5, containing the internal standard and glucuronidase/arylsulphatase 200/5 IU/ml (Glutulas<sup>®</sup> Type H-2 from *Helix pomatia*). The samples were mixed with 4 vol. of cold 0.1 M-ZnSO<sub>4</sub> and after centrifugation an aliquot of the supernatant was injected under conditions as described above (Loft *et al.* 1986).

For the assay of antipyrine in saliva, single samples of 50  $\mu$ l were mixed with an equal amount of perchloric acid 2 N, containing phenacetin as internal standard. After centrifugation an aliquot of the supernatant was injected on a 15 cm Nucleosil ODS 5  $\mu$ m column eluted with methanol/water (45/55; v/v) monitored at 254 nm. Urine samples were assayed in duplicate for antipyrine and metabolites before and after incubation for 3 h in acetate buffer pH 4.5, containing the internal standard and glucuronidase/arylsulphatase 6000/150 IU/ml. After saturation with NaCl the samples were extracted with 9 volumes of chloroform/ethanol (9/1; v/v). After evaporation at reduced pressure and room temperature the residue was constituted in methanol and eluent, and aliquots were injected (Loft *et al.* 1987). The eluent was methanol/phosphate buffer pH 7.8 (35/65) and the remaining conditions as for the assay of saliva. Sodium pyrosulphite was added to all solvents.

For all the assays the calibration graphs were linear in the relevant concentration range and the interassay coefficient of variation did not exceed 8%. There was no analytical interference between metronidazole, antipyrine and their metabolites.

### Statistics

Regression and correlation analyses were done by the method of least-squares. The pharmacokinetic constants were compared between the groups by means of one-way analysis of variance with Duncan's multiple range test for *post-hoc* comparison of means. The interaction between metronidazole and antipyrine was investigated by a paired *t*-test. The level of statistical significance was set at 0.05.

### Results

In 48 simultaneous saliva and blood samples the concentrations of metronidazole were almost identical (figure 1). The slope and intercept of the regression were 1.06 (0.98–1.14; 95% confidence interval) and  $2 \mu\text{M}$  ( $-2.6$ ), respectively ( $r=0.97$ ;  $p<0.05$ ).

#### *Pharmacokinetics of metronidazole*

Under all experimental conditions the elimination of metronidazole from rat saliva could be described by an open one-compartment model (Fig. 2). The time zero intercept of the concentration–time curve and the calculated apparent volume of distribution after *i.v.* administration used for the anaesthetized rats and after *i.p.* administration in the conscious rats were identical, supporting the assumption of complete bioavailability (table 1; figure 2). However, during anaesthesia the clearance of metronidazole was decreased by 40% and the half-life prolonged by 70% ( $p<0.05$ ; table 1). After pretreatment with PB or  $\beta$ -naphthoflavone the clearance was increased by 70% and 30% and the half-life shortened by 40% and 20%, respectively ( $p<0.05$ ; table 1). The coefficient of variation between the determined volume of distribution and the assumed value of 0.67 l per kg body weight was 8.6%.

In 26 rats the metronidazole clearance calculated from one sample collected 3 or 4 h after dose was identical to that calculated from the area under the complete elimination curve (figure; table 1). The regression slope was 0.98 (0.92–1.04) and the intercept 0.2 ml/min per kg ( $-0.2$ –0.6), respectively ( $r=0.99$ ;  $p<0.05$ ).

#### *Differential induction of metronidazole metabolism*

Pretreatment with PB or MC increased the clearance of metronidazole to about the same extent, *i.e.* by 60% and 50%, respectively ( $p<0.05$ ; table 2). However, MC treatment increased the fractional clearances to the oxidative metabolites, metronidazole acetic acid and hydroxymetronidazole, 10-fold, whereas the rate of glucuronidation was decreased by 50% ( $p<0.05$ ; table 2). In contrast, PB treatment increased both the rates of oxidation and glucuronidation, by 400% and 60%, respectively ( $p<0.05$ ; table 2). PB, but not MC, increased the renal clearance of unchanged metronidazole, whereas none of the inducers altered the urinary recovery significantly (table 2).

#### *Metronidazole–antipyrine interaction*

In the six rats dosed on three occasions with metronidazole, antipyrine or both in a mixture, the drugs had no influence on the metabolism of each other. When administered alone and in mixture the total clearances of metronidazole and antipyrine were  $4.3 \pm 0.7$  versus  $4.3 \pm 1.9$  ml/min per kg and  $5.3 \pm 1.4$  versus  $5.1 \pm 1.4$  ml/min per kg, respectively ( $p>0.05$ ). The effects of MC pretreatment on the metabolism of metronidazole were not altered by coadministration of antipyrine (table 2).

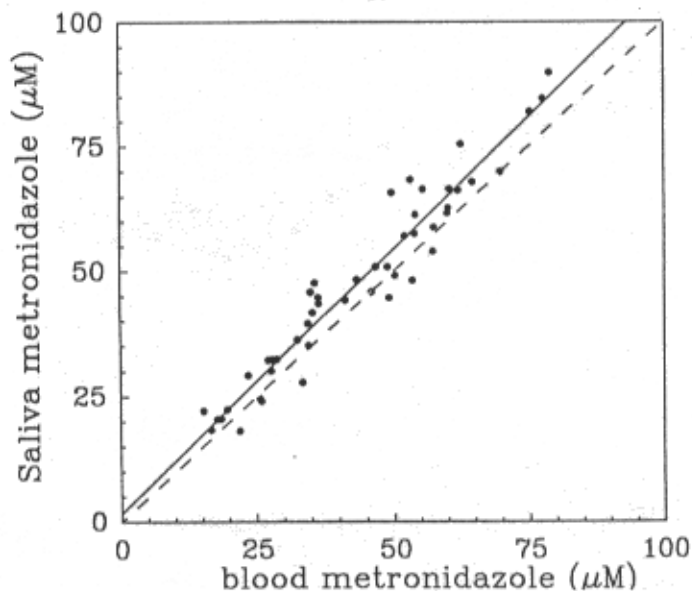


Figure 1. Saliva and blood concentrations of metronidazole in rats.

Plot of saliva concentration against blood concentration of metronidazole in 48 simultaneous samples from six anaesthetized rats. The lines of regression (solid) and identity (dashed) are shown.

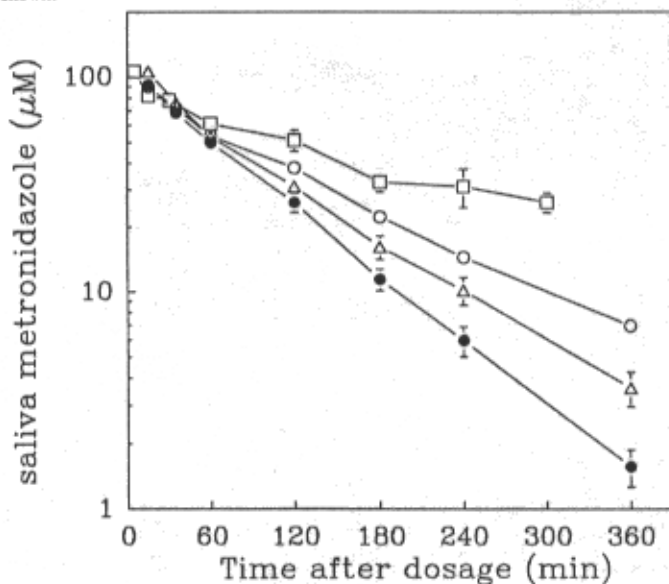


Figure 2. Concentration-time profiles of metronidazole in rats.

Saliva concentrations-time curves after administration of metronidazole 10 mg/kg body weight to control rats (○), to rats under thiopental anaesthesia (□) and to rats pretreated with phenobarbitone (●) or  $\beta$ -naphthoflavone ( $\Delta$ ). Values are means with SD of six or seven rats.

Table 1. Metronidazole pharmacokinetics in rats.

	Half-life (min)	Volume of distribution (l/kg)	Clearance from AUC (ml/min per kg)	One-sample clearance (ml/min per kg) from	
				3 h sample	4 h sample
Control	96 ± 11	0.67 ± 0.03	4.8 ± 0.4	4.9 ± 0.5	5.2 ± 0.6
Anaesthesia	159 ± 70*	0.62 ± 0.12	3.1 ± 1.3*	3.2 ± 1.4*	3.2 ± 1.2*
Phenobarbitone	58 ± 10*	0.67 ± 0.07	8.1 ± 1.6*	7.9 ± 1.6*	8.0 ± 1.6*
β-Naphthoflavone	73 ± 11*	0.64 ± 0.07	6.2 ± 1.1*	6.6 ± 1.1*	6.3 ± 1.2*

Control rats and rats under thiopental anaesthesia or pretreated with phenobarbitone or β-naphthoflavone received metronidazole 10 mg per kg i.p. or i.v. The pharmacokinetic parameters were determined from saliva collected at timed intervals.

\*  $p < 0.05$ ; values are means ± SD,  $n = 6$  or 7.

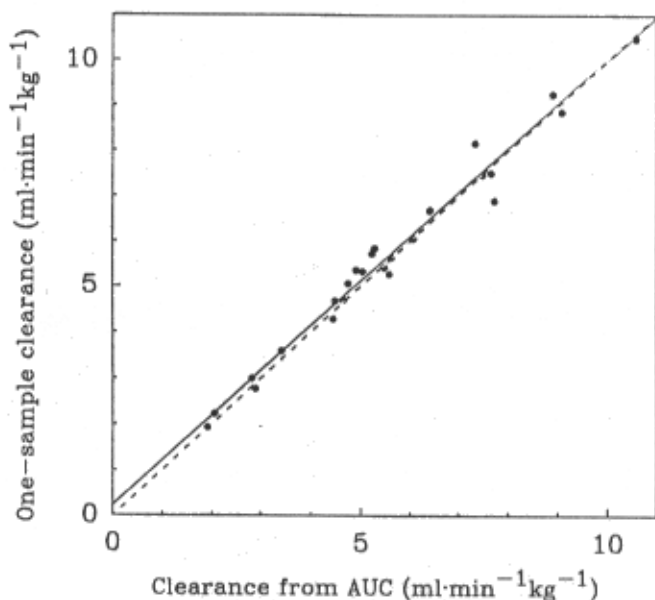


Figure 3. One-sample clearance of metronidazole in rats.

Plot of the clearance of metronidazole calculated from one sample collected 4 h after administration and from the area under the complete elimination curve (AUC) in 26 rats. The lines of identity (solid) and regression (dashed) are almost superimposed.

#### Differential induction of metronidazole and antipyrine metabolism

Pretreatment with PB and  $\beta$ -naphthoflavone increased the clearance of metronidazole by 130% and 100%, respectively ( $p < 0.05$ ; figure 4). PB increased the clearance to metronidazole acetic acid, hydroxymetronidazole and metronidazole glucuronide 7-fold, 4-fold and 3-fold, ( $p < 0.05$ ) respectively. After pretreatment with  $\beta$ -naphthoflavone the clearance of metronidazole to the acetic acid and hydroxy metabolite was approximately 5-fold increased ( $p < 0.05$ ), whereas the clearance by glucuronidation was not significantly changed. None of the inducers altered the renal clearance of unchanged metronidazole (figure 4). The urinary recovery of metronidazole and metabolites was  $44 \pm 5\%$ ,  $55 \pm 7\%$ , and  $55 \pm 15\%$  of dose in the control, PB-, and  $\beta$ -naphthoflavone-treated groups, respectively ( $p > 0.05$ ).

In the control group the total clearance of antipyrine was  $5.3 \pm 1.0$  ml/min per kg. PB pretreatment increased the antipyrine clearance and fractional clearance to its three metabolites to about the same extent, i.e. approximately 4-fold ( $p < 0.05$ ; figure 4). In contrast, induction with  $\beta$ -naphthoflavone increased the antipyrine clearance 4-fold ( $p < 0.05$ ), the fractional clearance to norantipyrine and 4-hydroxyantipyrine 10-fold ( $p < 0.05$ ), and left the clearance to 3-hydroxymethylantipyrine unchanged (figure 4). None of the inducers altered the renal clearance of unchanged antipyrine (figure 4). The urinary recovery of antipyrine and metabolites was  $61 \pm 12\%$ ,  $71 \pm 12\%$ , and  $50 \pm 17\%$  of dose in the control, PB-, and  $\beta$ -naphthoflavone-treated groups, respectively ( $p > 0.05$ ).

Table 2. Clearances of metronidazole in rats.

	Total clearance of metronidazole	Clearance to metronidazole acetic acid	Clearance to hydroxy-metronidazole (ml/min per kg)	Clearance to metronidazole glucuronide	Renal clearance	Recovery (percentage of dose)
Control	5.3 ± 1.4	0.17 ± 0.09	0.11 ± 0.05	1.39 ± 0.17	0.75 ± 0.23	48 ± 10
Phenobarbitone	8.3 ± 1.0*	0.81 ± 0.19*	0.53 ± 0.16*	2.18 ± 0.41*	1.15 ± 0.18*	62 ± 8
3-Methylcholanthrene	8.0 ± 1.4*	1.65 ± 0.57*†	1.07 ± 0.57*†	0.70 ± 0.09*†	0.74 ± 0.12†	51 ± 10
3-Methylcholanthrene + antipyrine†	9.1 ± 1.0*	1.87 ± 0.80*†	1.23 ± 0.51*†	0.84 ± 0.41*†	0.60 ± 0.24†	50 ± 17

Control rats and rats pretreated with phenobarbitone or 3-methylcholanthrene (3-MC) received metronidazole 10 mg/kg i.p. and the clearance and fractional clearances were determined from one saliva sample and urine collected for 24 h.

\*  $p < 0.05$  compared to control; †  $p < 0.05$  compared to phenobarbitone group. Values are means ± SD;  $n = 6$ .

† 14 mg/kg i.p. was administered concomitantly with metronidazole.



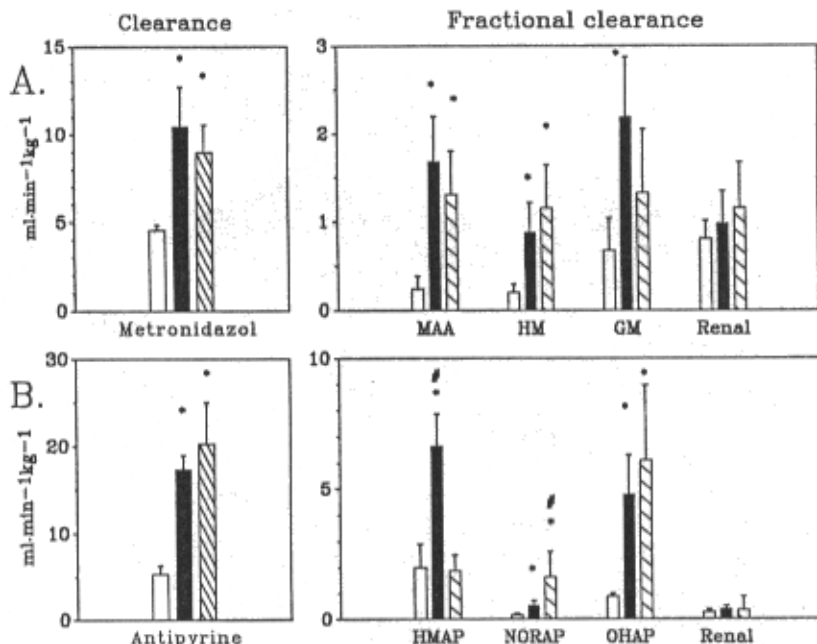


Figure 4. Differential induction of (A) metronidazole and (B) antipyrine metabolism in rats.

Clearance and fractional clearances representing each elimination pathway of metronidazole 10 mg/kg and antipyrine 14 mg/kg body weight in rats pretreated with phenobarbitone (■),  $\beta$ -naphthoflavone (▨) or nothing (□). Values are means with SD of six rats. MAA = metronidazole acetic acid; HM = hydroxymetronidazole; GM = metronidazole glucuronide; HMAP = 3-methylhydroxyantipyrine; NORAP = norantipyrine; OHAP = 4-hydroxyantipyrine; renal = clearance by renal excretion of unchanged compound. \* denotes  $p < 0.05$  versus control; # denotes  $p < 0.05$  versus the other pretreatment.

Table 3. Correlations between the clearances of metronidazole and antipyrine.

	Total clearance of metronidazole	Clearance to metronidazole acetic acid (ml/min per kg)	Clearance to hydroxymetronidazole	Clearance to metronidazole glucuronide
Total clearance of antipyrine	0.81*	0.81*	0.85*	0.55*
Clearance to 3-hydroxymethylantipyrine	0.63*	0.57*	0.19	0.75*
Clearance to norantipyrine	0.50*	0.38	0.64*	0.30
Clearance to 4-hydroxyantipyrine	0.77*	0.70*	0.76*	0.69*

Metronidazole 10 mg/kg and antipyrine 14 mg/kg were administered i.p. in a mixture to 18 rats, six of which were pretreated with phenobarbitone and six with  $\beta$ -naphthoflavone and the clearance and fractional clearances were determined from one saliva sample and urine collected for 24 h.

\*  $p < 0.05$ .

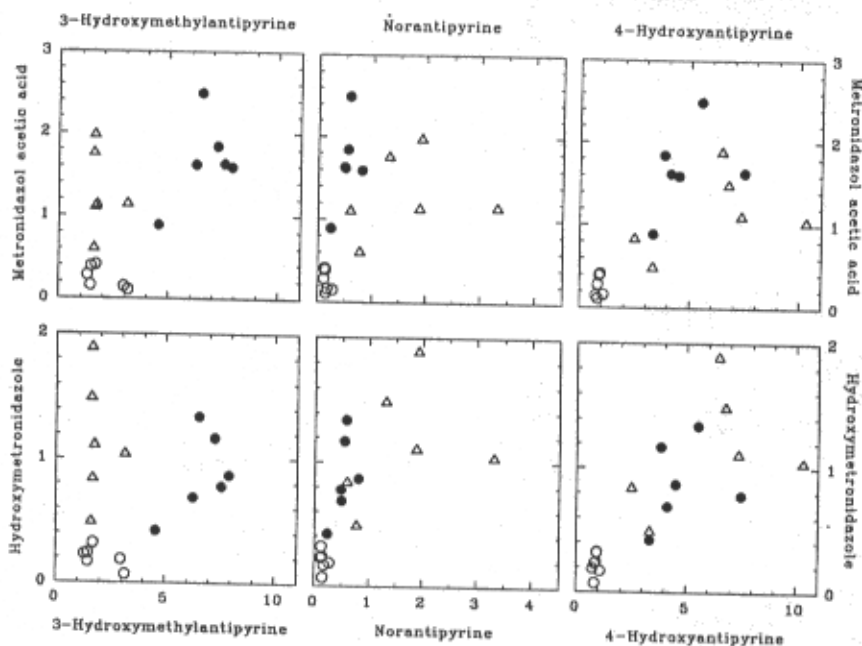


Figure 5. Covariation of metonidazole and antipyrine metabolism in rats.

Plots of the fractional clearances of metronidazole acetic acid and hydroxymetronidazole on the fractional clearances of antipyrine in control rats (○) and in rats pretreated with phenobarbitone (●) or  $\beta$ -naphthoflavone ( $\Delta$ ).

The formation rates of the three metronidazole metabolites correlated significantly with several of those of the antipyrine metabolites (table 3). However, the correlations between the metabolite formation rates and the clearance of the other drug, and between those of the antipyrine metabolites and the rate of glucuronidation of metronidazole, were as high or higher than the correlations between the formation rate of the oxidative metabolites of the two drugs. Moreover, as shown in figure 5 the latter correlations were generally related to the effects of the pretreatments and not apparent within each group of rats.

### Discussion

In the present study it was demonstrated that, in the rat, saliva may replace blood or plasma for pharmacokinetics studies of metronidazole (figure 1). This eliminates the need of anaesthesia, surgical stress and indwelling catheters, or possible stressful blood sampling, factors that may all affect foreign compound metabolism as also shown in the anaesthetized rats (Capel *et al.* 1980, Wood and Wood 1984, Van Dyke *et al.* 1987, Chindavijac *et al.* 1988). Moreover, the clearance could be determined from only one sample, minimizing work and animal handling. With collection of urine and/or combining metronidazole and antipyrine in a mixture, differential effects on various metabolic pathways could be studied.

#### Clearance determination from one saliva sample

The pharmacokinetics of metronidazole in saliva was in agreement with the previously reported plasma values for rats (Buttar and Siddiqui 1980, Jung and Shah 1986; table 1; figure 2). Administration i.p. was chosen as a simple method, not requiring fasted animals, for rapid and complete absorption. The latter was supported by the almost identical time zero intercept after i.v. and i.p. administration of metronidazole. The elimination could be described by a one-compartment kinetic model and the volume of distribution was equivalent to total body water, i.e. 0.67 times the body weight and equivalent to that of antipyrine (Pilsgaard and Poulsen 1984). Pilocarpine in the dose used for saliva stimulation has been shown not to alter the volume of distribution of antipyrine (Wilson *et al.* 1982).

The clearance of metronidazole could be determined from one sample without systematic deviation from that determined from the complete curve and with minimum random variation (figure 3). The ideal time for collection of the one sample for clearance determination is between 1 and 2 divided by the elimination rate constant, i.e. 1.4 to 2.8 times the half-life (Døssing *et al.* 1983). In this time window the random variation and effect of a bias in the estimation of the volume of distribution are minimal. With later sampling the accuracy of the one-sample method decreases slightly, whereas too early sampling may be deleterious (Døssing *et al.* 1982, 1983, Loft *et al.* 1988, Poulsen and Loft 1988). In uninduced rats the half-life of metronidazole was 1.4–1.9 h, and 4 h was chosen as the optimal saliva collection time. With a possible bias of 8.6% in the assumed volume of distribution, i.e. the coefficient of variation between that and the measured value, and the one sample collected after two, three and four half-lives, the resulting potential bias in the clearance determination can be calculated to 2%, 4% and 5%, respectively (Loft *et al.* 1988, Poulsen and Loft 1988). Nevertheless, in some induced rats with high clearance values the sample was taken after more than four half-lives without an apparent increase in the variation of the clearance determination (figure 3). Moreover, the clearance determined from the 3 and 4 h sampling time point did not differ in any treatment group (table 1). In uninduced rats the half-life of antipyrine was identical to that of metronidazole, and the ideal times for sample collection coincide. In the induced rats the clearance of antipyrine was increased twice as much as that of metronidazole with an expectedly corresponding decrease in half-life and the chosen sampling time of 3 h was probably later than the ideal time window.

#### Metabolism of metronidazole

In uninduced rats the urinary metabolite profile of metronidazole concurred with previous studies *in vivo* and in isolated hepatocytes (La Russo *et al.* 1978, Loft and Poulsen 1989). Accordingly, glucuronidation and excretion of unchanged compound were the dominant routes of elimination, whereas the oxidative metabolites, metronidazole acetic acid and hydroxymetronidazole, were of minor quantitative importance (table 2). The urinary recovery of metronidazole and metabolites ranged from 44% to 62% of the dose, in agreement with a study in rats dosed with the radiolabelled compound (La Russo *et al.* 1978). In that study approximately 25% of the dose was excreted in the bile, mainly as the glucuronide and to a lesser extent unchanged, irrespective of PB pretreatment (La Russo *et al.* 1978).

After pretreatment with PB and MC the modest increase in the clearance of metronidazole covered large alterations in the metabolite profile (table 2). Thus, in

agreement with previous results from isolated rat hepatocytes both pretreatments, MC in particular, increased the clearance by oxidation to metronidazole acetic acid and hydroxymetronidazole, whereas PB induced the rate of glucuronidation also (Loft and Poulsen 1989). The effect of PB treatment on metronidazole metabolism in mice and rats *in vivo* and in isolated rat liver has been reported to be an exclusive increase in the rate of glucuronidation, although quantitative data were not provided (Stambaugh *et al.* 1968, LaRusso *et al.* 1978, Allars *et al.* 1985). By contrast, in humans treated with PB the hydroxylation of metronidazole was the only route of elimination with an increased rate (Loft *et al.* 1987). The significant increase in the renal clearance of unchanged metronidazole after PB treatment is unexplained, but was not seen in the rats dosed with the mixture of metronidazole and antipyrine.

The two parallel study parts of the differential induction of metronidazole metabolism, one with the drug administered alone, and one concomitantly with antipyrine, appeared to yield slightly different results. Thus, in the latter part, the rate of glucuronidation was lower in the control group, PB pretreatment affected the metabolism more, whereas the effect of  $\beta$ -naphthoflavone was less pronounced than that of MC (table 2; figure 4). Several explanations may be offered for these differences. The rats used in the two parts of the study were from different breeding batches. Further, the dosage of PB depended on the intake of water, which may have differed. Finally, the potency of  $\beta$ -naphthoflavone and MC as polyaromatic hydrocarbon type inducers may differ more than the two-fold difference in the doses employed in the present study.

#### *Metabolism of antipyrine*

The metabolism of antipyrine as assessed by the clearance and the urinary metabolite profile was in agreement with previous studies on rats *in vivo*, including the effects of PB and polyaromatic hydrocarbon type induction (Danhof *et al.* 1979, Inaba *et al.* 1980, Rhodes and Houston 1983, Teunissen *et al.* 1983a, b; figure 5). In the uninduced and PB-pretreated rats the pattern of antipyrine metabolites agreed with the corresponding *in vitro* results from isolated rat hepatocytes and rat liver microsomes (Kahn *et al.* 1982, Loft and Poulsen 1989). So far unexplained, is the effect of polyaromatic hydrocarbon type induction on the formation of norantipyrine and 4-hydroxyantipyrine, which has not been found in the *in vitro* studies (Kahn *et al.* 1982, Loft and Poulsen 1989).

#### *Covariation of metronidazole and antipyrine metabolism*

Simultaneous administration of two or more drugs allows study of co-regulation of their metabolic rates without interference from time-dependent intra-individual variability in enzyme activities and liver blood flow (Breimer 1983, Van der Graaff *et al.* 1983, Teunissen *et al.* 1986, Schellens *et al.* 1988). Moreover, the use of the same samples for the kinetic estimations eliminates possible sampling variation. Nevertheless, the present correlations between the formation rates of metabolites from metronidazole and antipyrine were poorer than between the clearance values of the two drugs (table 3). Further, the correlation coefficients ( $r$ ) between the metabolite formation rates of the two drugs were lower than the 0.8 usually required as indications of shared metabolic pathways (Breimer 1983, Teunissen *et al.* 1986, Schellens *et al.* 1988). Finally, the correlations mainly related to the effects of the inducers and were not seen within the groups (figure 5). This lends further support

to the hypothesis that the oxidative metabolites of metronidazole and antipyrine are formed by differentially regulated enzymes (Loft *et al.* 1987, 1988, Loft and Poulsen 1989). It should be borne in mind, however, that the PB-induced cytochromes P-450 can form all metabolites of the drugs without particular preference, whereas the polyaromatic hydrocarbon-induced species preferentially form hydroxymetronidazole from metronidazole, and norantipyrine, and particularly 4-hydroxyantipyrine, from antipyrine (Loft and Poulsen 1989).

Metronidazole and antipyrine could be administered as a mixture, their clearances determined from the same saliva sample and the metabolite profiles from the urine collected for 24 h. The two drugs had no influence on the metabolism of either. In agreement, no interaction between single doses of the two drugs has been found in man (Van Staiger *et al.* 1984, Loft *et al.* 1987). In rats pretreated with MC the effect of antipyrine co-administration on metronidazole metabolism was also negligible. In isolated rat hepatocytes this pretreatment decreased the inhibition constant of antipyrine toward the hydroxylation of metronidazole to 300  $\mu\text{M}$  compared to peak antipyrine concentrations of 110–120  $\mu\text{M}$  in the present study (Loft and Poulsen 1989). In the hepatocyte study all other mutual metabolic inhibitions constants of the two drugs, irrespective of pretreatment, far exceeded the peak concentrations encountered in the present study (Loft and Poulsen 1989).

## Conclusion

With either metronidazole or antipyrine as probe, and collection of one saliva sample and urine for 24 h, it would be possible to identify PB and polyaromatic hydrocarbon-type induction in rats. By combining the two drugs in a mixture the same sampling would possibly reveal more differential effects on foreign compound metabolism. With this simple and largely non-invasive approach the animals could serve as their own controls in paired designs, allowing extensive time-course studies and/or be characterized regarding the various enzymic activities to relate to the toxicity and/or metabolism of other compounds.

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