Inhibition and Induction of Metronidazole and Antipyrine Metabolism

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Summary. The effect of cimetidine, antipyrine and phenobarbital on the pharmacokinetics of intravenous metronidazole and oral antipyrine has been examined in 7 healthy volunteers. The administration of cimetidine for 24 h before and throughout the sampling period failed to alter the total clearance of metronidazole or the rate of formation of the hydroxy metabolite, whereas the total and partial clearances of antipyrine were decreased 0.74 and 0.6-0.7-fold, respectively. Seven days of phenobarbital or antipyrine administration increased the total clearance of metronidazole 1.51- and 1.86-fold, respectively, and the total antipyrine clearance was 1.22 or 1.46-fold increased, respectively. The rate of metronidazole hydroxylation was significantly enhanced by both enzyme inducers. The partial clearance of antipyrine to the normetabolite was significantly increased by both inducers, whereas the rate of 4-hydroxylation was significantly increased only by prior antipyrine administration. The results indicate that the hydroxylation of metronidazole is not inhibited by cimetidine, but that it is inducible by phenobarbital or antipyrine. It is suggested that metronidazole and antipyrine are metabolized by different enzymatic pathways.

Keywords: metronidazole, antipyrine: cimetidine, phenobarbital, drug interaction, drug metabolism, pharmacokinetics

Metronidazole is a widely used antimicrobial, mainly eliminated by microsomal oxidation in the liver [1-3]. Interactions can be expected, therefore, between inducers and inhibitors of hepatic drug metabolism and metronidazole, possibly with therapeutic or toxicological implications. There are reports of therapeutic failure and/or a shortened half-life of metronidazole in patients treated with phenobarbital [4-6]. After administration for 6 days of the mono-oxygenase inhibitor, cimetidine, a reduction in metronidazole clearance by about 30% has been reported [7].

The effect of enzyme induction on metronidazole metabolism has not so far been studied systematically, and the effect of cimetidine on the formation of metabolites of metronidazole is unknown. In addition, a comparison within individuals of the formation of metabolites from metronidazole and the well known probe drug for mono-oxygenase activity, antipyrine, might indicate whether the two drugs share metabolic pathways [8].

In the present study, the effects of the enzyme inhibitor, cimetidine, and the enzyme inducers, phenobarbital and antipyrine, on the metabolism of metronidazole and antipyrine have been investigated.

Materials and Methods

Protocol

All the subjects studied were healthy volunteers, who participated after having given informed consent. The investigation protocol was approved by the Ethics Committee of Copenhagen County. The subjects took alcohol socially, but none smoked or took any drugs, except those under investigation, for at least one month before and throughout the study. Dietary habits and physical activity were constant.

Metronidazole Metabolism. Seven subjects (2 women and 5 men; age 24 to 37 years; weight 57 to 90 kg) were given metronidazole 500 mg (DAK) as an intravenous infusion over 20 min in a control experiment, and after each of the following pretreatments: cimet-
idine 1000 mg/day for 1 day continued throughout
the sampling period (7 subjects), phenobarbitone
100 mg/day for 7 days (4 subjects), and antipyrine
1000 mg/day for 7 days (5 subjects). The metronida-
zole treatments were separated by at least 4 weeks
and the order of the experiments was randomised.
Only 2 subjects were given metronidazole after both
the phenobarbitone and antipyrine pretreatments.
Blood was sampled in heparinised tubes before
and 10, 20, 30, 40, 60 and 80 min and 2, 3, 5, 8, 12, 16,
24, 36 and 48 h, and urine was collected at intervals
for 48 h after metronidazole administration. Plasma
samples and aliquots of the urine samples were
stored at −20 °C until analysed. Immediately follow-
ing metronidazole administration after phenobarbi-
tone pretreatment, one subject suffered neurotoxic
symptoms with nystagmus for a few minutes, fol-
lowed by a depressed level of consciousness and hy-
potension for about 1 h. After another hour he had
recovered completely. Kinetic characteristics in him
did not appear different from those in the other sub-
jects.

Antipyrine Metabolism. After an overnight fast, anti-
pyrine 1000 mg in aqueous solution was ingested,
once on the day after each metronidazole infusion
following a pretreatment, and once alone, 4 weeks
before or after the metronidazole administration.
About 24 h later a saliva sample was obtained and
urine was collected for 48 h. Following the antipy-
rine pretreatment, saliva samples were collected be-
fore and 3, 6, 9, 12, 16 and 24 h after the antipyrine
dose for measurement of its clearance. In one subject
only antipyrine metabolism was studied after pheno-
barbitone pretreatment.

Antipyrine – Metronidazole Interaction. In random
order and at intervals of at least one week, 10 sub-
jects (5 women and 5 men; age 18 to 37 years; weight,
54 to 85 kg) ingested metronidazole 500 mg (DAK)
as tablets, or antipyrine 1000 mg in aqueous solution,
or both drugs together. Plasma and urine were sam-
ped at regular intervals for 48 h following metronida-
zole administration. About 24 h after antipyrine ad-
ministration a saliva sample was collected.

Analytical Procedures
The plasma and urine concentrations of metronida-
zole and its major metabolites, hydroxymetronida-
zole (HM) and metronidazole acetic acid (MAA),
were determined by HPLC, as previously described
[3]. In the chromatograms of urine an unknown peak
suggestive of 1-(2-hydroxyethyl)-5-nitroimidazole-
2-carboxylic acid, a further oxidation product of
HM, elutes before the MAA peak [3, 9]. A reference
compound is not available to confirm its identity.
The urinary content of glucuronide conjugates was
estimated as the difference between determinations
with and without prior enzymatic hydrolysis.

For the assay of saliva and urine for antipyrine
and its metabolites the HPLC system was fitted with
a Waters Novapak column and the effluent moni-
tored at 244 nm. Saliva 200 μl was mixed with an
equal amount of acetonitrile containing phenacetin
as internal standard. After centrifugation, 5 μl of the
supernatant was injected. The urine samples were ass-
sayed after hydrolysis with glucuronidase/aryl sul-
phatase (Boehringer) at 37 °C for 3 h. After addition
of phenacetin as internal standard and saturation
with NaCl the sample was extracted with chloro-
form/ethanol (9/1 v/v), as described by Teunissen et
al. [10]. The organic phase was evaporated to dryness
under reduced pressure at room temperature. The
residue was dissolved in 100 μl methanol, diluted
with 250 μl mobile phase and 5 μl was injected. The
mobile phase was delivered at 0.5 ml/min and for
the assay of saliva and urine, respectively, consisted
of water/methanol (55/45 v/v) and phosphate buff-
er 0.01 M pH 6.8/methanol (60/40 v/v) containing
sodium pyrosulphite. Reference metabolites used for
the standard curves were norantipyrine and 4-hy-
droxyantipyrine (EGA-Chemie) and 3-hydroxy-
methylantipyrine (kindly donated by Drs. Danhof,
Eichelbaum and Yoshimura).

Pharmacokinetic Calculations
When appropriate, the ESTRIP computer program
[11] was used for the pharmacokinetic calculations.
The area under the plasma concentration versus time
curve of metronidazole or its metabolite (AUC) was
calculated according to the trapezoidal rule with ex-
trapolation to infinity. The total clearance of metro-
nidazole was calculated as the ratio between the dose
and the AUC. The other kinetic characteristics were
calculated according to an open two-compartment
model for intravenous administration [12]. The ab-
sorption of orally administered metronidazole was
assumed to be complete [2, 3] and the half-life and
apparent volume of distribution were calculated
from the slope of the terminal log concentration ver-
sus time curve and the AUC. The partial clearances
of metronidazole or its hydroxy metabolite were cal-
culated as the amount excreted divided by the
AUC[0.48] during urine collection.

The clearance of antipyrine was calculated from
the concentration in the single saliva sample and an
assumed volume of distribution estimated from body
weight, height and age, as previously described [13].
the dose, or by multiplying the disposition rate constant (slope of the log concentration-time curve) by the apparent volume of distribution (dose divided by time zero intercept minus predose concentration). A partial clearance was estimated for each of the major elimination pathways of antipyrine as the fraction of the dose excreted as the particular metabolite multiplied by the total clearance.

**Statistical Analysis**

The data from the intravenous metronidazole treatments were analysed by two-way analysis of variance with substitution of missing values [16]. Duncan's multiple range test was used for the post hoc comparison of means. The data from the two oral treatments were compared by a paired Student's t-test. A stepwise backward regression analysis was done by the method of least squares. P-values less than 0.05 were considered statistically significant.

**Results**

The administration of cimetidine for 24 h before and throughout the sampling period did not significantly alter the plasma kinetics of metronidazole (Figs. 1 and 2, Table 1). During cimetidine treatment the mean metronidazole clearance was 1.04 – times (0.90 to 1.18; 95% confidence limits) the control value, whereas the total saliva clearance of antipyrine was 0.74 – fold (0.58 to 0.90) reduced (Fig. 2).

After the administration of phenobarbitone or antipyrine for 1 week, the plasma concentrations of
metronidazole were decreased throughout the post-infusion period as compared to the control measurements (Fig 1). The total clearance of metronidazole on average was 1.51 - (1.37 to 1.65) and 1.86 - (1.75 to 2.03) fold increased and the elimination half-life was 0.67 - (0.52 to 0.82) and 0.60 - (0.45 to 0.75) fold decreased by the phenobarbionate and antipyrine pretreatments, respectively (Table 1, Fig. 2). The apparent volume of distribution at steady state was not significantly altered by the enzyme inducers. The mean phenobarbionate and antipyrine - mediated enhancement of the total antipyrine clearance was to 1.22 - (1.06 to 1.38) and 1.46 - (1.30 to 1.62) times the control value, respectively (Fig. 2).

The clearance of metronidazole by hydroxylation (CL\textsubscript{HM}) was not significantly altered, i.e. 0.90 - (0.70 to 1.10) times the control value, during cimetidine coadministration, but it was increased on average 1.49 - (1.29 to 1.69) and 1.73 - (1.5 to 1.93) fold after pretreatment with phenobarbionate and antipyrine, respectively (Table 2, Fig. 3). Coadministration of cimetidine decreased the partial clearance of antipyrine to the normetabolite 0.59 - (0.30 to 0.88) fold, whereas enzyme induction with phenobarbionate and antipyrine increased it to 1.32 - (1.03 to 1.61) and 1.76 - (1.47 to 2.05) times the control value, respectively (Fig. 3). On average the partial clearance of antipyrine by 4-hydroxylation was 0.68 - (0.38 to 0.98) fold decreased and 1.51 - (1.21 to 1.81) fold increased during cimetidine and after antipyrine administration, respectively, but it was not significantly affected by pretreatment with phenobarbionate, i.e. it was 1.22 - (0.92 to 1.52) times the control value (Fig. 3). The partial clearance of antipyrine to 3-hy-

### Table 1. Kinetics (mean ± SD) of metronidazole in a control experiment (Con) and after pretreatment with cimetidine (Cim), phenobarbionate (PB) or antipyrine (AP).

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>t\textsubscript{1/2} (h)</th>
<th>V\textsubscript{ss} (l)</th>
<th>CL (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con (n=7)</td>
<td>7.3 ± 1.0</td>
<td>50.0 ± 7.3</td>
<td>83 ± 15</td>
</tr>
<tr>
<td>Cim (n=7)</td>
<td>7.4 ± 1.7</td>
<td>49.2 ± 6.3</td>
<td>87 ± 22</td>
</tr>
<tr>
<td>PB (n=4)</td>
<td>4.9 ± 1.3\textsuperscript{*}</td>
<td>30.3 ± 4.1</td>
<td>130 ± 25\textsuperscript{*}</td>
</tr>
<tr>
<td>AP (n=5)</td>
<td>4.4 ± 0.4\textsuperscript{*}</td>
<td>55.1 ± 7.6</td>
<td>157 ± 21\textsuperscript{*}</td>
</tr>
</tbody>
</table>

\(t_{1/2}\) is the elimination half-life, \(V_{ss}\) is the volume of distribution at steady state, and CL is the clearance. \(\textsuperscript{*}\) denotes \(p<0.05\) vs control.

### Table 2. Clearance (mean ± SD) of metronidazole by the 4 major elimination pathways, renal excretion (CL\textsubscript{R}), glucuronidation (CL\textsubscript{G}), oxidation to the acetic acid metabolite (CL\textsubscript{AMA}) and hydroxylation (CL\textsubscript{HM}) in a control experiment (Con) and after pretreatment with cimetidine (Cim), phenobarbionate (PB) or antipyrine (AP). Values are in ml/min.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>CL\textsubscript{R}</th>
<th>CL\textsubscript{G}</th>
<th>CL\textsubscript{AMA}</th>
<th>CL\textsubscript{HM}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con (n=7)</td>
<td>12.6 ± 2.1</td>
<td>5.2 ± 2.3</td>
<td>13.1 ± 3.8</td>
<td>33.0 ± 8.9</td>
</tr>
<tr>
<td>Cim (n=7)</td>
<td>7.8 ± 1.7</td>
<td>3.6 ± 0.8</td>
<td>11.3 ± 4.0</td>
<td>29.8 ± 11.7</td>
</tr>
<tr>
<td>PB (n=4)</td>
<td>9.4 ± 2.0</td>
<td>4.1 ± 0.8</td>
<td>14.7 ± 3.2</td>
<td>53.2 ± 12.3\textsuperscript{*}</td>
</tr>
<tr>
<td>AP (n=5)</td>
<td>10.7 ± 1.9</td>
<td>4.2 ± 0.6</td>
<td>15.3 ± 4.2</td>
<td>57.0 ± 10.8\textsuperscript{*}</td>
</tr>
</tbody>
</table>

\(\textsuperscript{*}\) denotes \(p<0.05\) vs control.

### Fig. 3. The clearance of metronidazole by hydroxylation (CL\textsubscript{HM}) and the partial clearances of antipyrine to the nor- (CL\textsubscript{NORM}), 4-hydroxy (CL\textsubscript{O4HAP}) and 3-hydroxymethyl (CL\textsubscript{O3HAP}) metabolites of in a control experiment (Con) and after pretreatment with cimetidine (Cim), phenobarbionate (PB) or antipyrine (AP). The bars represent mean values.
Droxymethylantipyrine was not significantly altered by any of the pretreatments, i.e. it was 0.63 - (0.07 to 1.19), 1.35 - (0.79 to 1.91), and 1.38 - (0.82 to 1.94) times the control value with cimetidine, phenobarbital, and antipyrine respectively (Fig. 3). A backward stepwise multiple regression analysis excluded each of the partial clearances of antipyrine to the three main metabolites as a significant predictor of $\text{CL}_{\text{HM}}$ and left the total antipyrine clearance as an important predictor ($r = 0.84, p < 0.001$). The linear slope of the regression of $\text{CL}_{\text{HM}}$ on the total antipyrine clearance was 0.73 - (0.52 to 0.94) and the intercept -0.21 (-12.9 to 12.5).

During cimetidine coadministration, the renal clearance of metronidazole was significantly decreased, whereas the rate of metronidazole elimination by oxidation to the acetic metabolite and glucuronidation was unchanged (Table 2). Pretreatment with phenobarbital and antipyrine had no significant effect on the renal clearance of metronidazole, on its clearance by oxidation to the acetic acid metabolite or on glucuronidation (Table 2). The area under the plasma concentration versus time curve, the renal clearance and the fraction excreted as glucuronide conjugates of the hydroxy-metabolite of metronidazole were not significantly changed by any of the pretreatments (Table 3).

The coadministration of a single dose of antipyrine without pretreatment did not in any way alter the metabolism of orally administered metronidazole (Table 4).

### Table 3. Kinetics (mean ± SD) of the hydroxy metabolite of metronidazole in a control experiment (Con) and after pretreatment with cimetidine (Cim), phenobarbital (PB) and antipyrine (AP)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>AUC (μg h/ml)</th>
<th>CL_R (ml/min)</th>
<th>HM-gluc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con ($n=7$)</td>
<td>79 ± 10</td>
<td>50 ± 9</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Cim ($n=7$)</td>
<td>65 ± 14</td>
<td>45 ± 10</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>PB ($n=4$)</td>
<td>67 ± 9</td>
<td>50 ± 5</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>AP ($n=5$)</td>
<td>58 ± 7</td>
<td>55 ± 11</td>
<td>15 ± 4</td>
</tr>
</tbody>
</table>

AUC is the area under the plasma concentration time curve, CL is the clearance and HM-gluc is the fraction excreted as glucuronide conjugates.

### Table 4. Characteristic of the metabolism of metronidazole 500 mg orally and the clearance of antipyrine 1000 mg (CL_{AP}), each administered alone (control) and concomitantly (MET + AP). Mean ± SD for 10 subjects

<table>
<thead>
<tr>
<th>t_1 (h)</th>
<th>V (l)</th>
<th>CL (ml/min)</th>
<th>CL_R (ml/min)</th>
<th>CL_{CL} (ml/min)</th>
<th>CL_{MAA} (ml/min)</th>
<th>CL_{HM} (ml/min)</th>
<th>CL_{AP} (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.7 ± 0.6</td>
<td>51 ± 4</td>
<td>89 ± 13</td>
<td>8.8 ± 1.3</td>
<td>43.2 ± 1.3</td>
<td>13.6 ± 3.1</td>
<td>31.5 ± 8.7</td>
</tr>
<tr>
<td>MET + AP</td>
<td>7.0 ± 0.6</td>
<td>54 ± 6</td>
<td>90 ± 15</td>
<td>8.3 ± 1.0</td>
<td>3.8 ± 1.2</td>
<td>12.9 ± 3.3</td>
<td>30.4 ± 8.4</td>
</tr>
</tbody>
</table>

$t_1$ is the half-life, V the volume of distribution and CL is the total clearance and by renal excretion, glucuronidation (CL), oxidation to the acetic acid metabolite (MAA) and hydroxylation (HM).
shortened half-life of metronidazole in patients treated with phenobarbital are readily explained by the induction of metronidazole hydroxylation [4–6].

The increase in antipyrine clearance to 1.2–times its control value by phenobarbital pretreatment was somewhat less than the 1.5-fold or greater increase reported by others [14, 21, 22]. However, in most of those studies the pretreatment was given for more than 7 days, and since phenobarbital has a long half-life, its steady-state level and therefore maximal induction might not have been reached here. In contrast, the autoinduction of antipyrine metabolism after 7 days of administration was of the expected magnitude [21]. Accordingly, the recorded 60% increase in metronidazole clearance after phenobarbital treatment for 7 days might have been even greater if the exposure period had been longer. The failure of the changes in the clearance of antipyrine to the 3-hydroxymethyl metabolite and in the effect of phenobarbital on 4-hydroxylation to reach statistical significance may very well have been due to the small number of subjects and considerable interindividual variation. In agreement with the results it has previously been reported that N-demethylation followed by 4-hydroxylation of antipyrine are most sensitive to enzyme induction by phenobarbital and antipyrine [22], whereas cimetidine has been shown also to inhibit 3-methylhydroxylation [14, 23].

Coadministration of a single dose of antipyrine did not affect metronidazole metabolism, and other investigators have shown that a single dose of metronidazole does not alter the microsomal oxidation of antipyrine [23].

The results indicate that the cytochrome P-450 isozyme(s) responsible for the hydroxylation of metronidazole, like those responsible for N-demethylation and hydroxylation antipyrine, is/are inducible by phenobarbital and antipyrine. However, unlike those responsible for the oxidation of antipyrine, the isozyme(s) hydroxylating metronidazole do(es) not seem to be inhibited by cimetidine. Unlike antipyrine, metronidazole does not induce the metabolism of itself or other microsomal oxidized drugs [2, 24]. In addition, multiple regression analysis of the data excluded the partial clearances of antipyrine to all three major metabolites as significant predictors of the rate of metronidazole hydroxylation. If metronidazole and antipyrine shared a metabolic pathway, a strong correlation between the formation rates of the metabolites involved would have been expected [8]. Accordingly, it seems that metronidazole and antipyrine are metabolized by different cytochrome P-450 isozymes. The significant correlation between the total clearances of metronidazole and antipyrine does not necessarily suggest more than a similar regulatory mechanism.

It was previously suggested that a compound eluting in the chromatograms of urine was a further oxidation product of hydroxymetronidazole [3, 11]. Unfortunately, a reference compound is not available for its identification and quantification. However, judged from the chromatograms the amount of the unidentified compound excreted did not change much during the present study. In agreement with this, the kinetic characteristics of hydroxymetronidazole, i.e. the area under the plasma concentration versus time curve, the renal clearance, and the fraction excreted as glucuronide conjugates, were not significantly altered by the pretreatments.

In conclusion, the results indicate that the microsomal enzyme(s) responsible for hydroxylation of metronidazole is(are) distinct from those responsible for the oxidation of antipyrine. They are not, at least immediately, inhibited by cimetidine, but nevertheless are highly inducible by phenobarbital and antipyrine. The clinical implication of the study is that an increased therapeutic dosage of metronidazole should be considered if there is concomitant administration of an enzyme inducer.

Acknowledgement. The study was supported by the University of Copenhagen and Rhône Poulenc Pharma Norden A/S. Funds for purchase of the HPLC equipment were kindly supplied by the Lundbeck Foundation. We thank Ms S. Hjerpsted for her expert technical assistance.

References


Received: April 24, 1986 accepted in revised form: October 14, 1986
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