

Metronidazole clearance: A one-sample method and influencing factors

After 96 administrations of metronidazole to 36 subjects, it was found that the clearance could be determined from one plasma sample, the dose, and a volume of distribution estimated from sex, age, body weight, and height, without loss of precision and accuracy compared with conventional clearance determinations ($r > 0.97$). In 230 sample pairs the plasma and saliva concentrations of metronidazole were identical ($r = 0.99$). In 119 subjects the one-sample clearance of metronidazole was unimodally distributed. Body weight ($r = 0.28$) and the alcohol consumption ($r = 0.23$) correlated with the metronidazole clearance. In the same subjects the consumption of tobacco ($r = 0.28$), alcohol ($r = -0.19$), coffee/tea ($r = 0.27$), age ($r = -0.24$), and sex ($r = 0.28$) correlated with the antipyrine clearance. The clearances of metronidazole and antipyrine were correlated ($r = 0.34$). The differential influence of the environmental factors on the elimination rates supports differential metabolism of metronidazole and antipyrine. (CLIN PHARMACOL THER 1988;43:420-8.)

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The clearance of a drug is considered the most useful pharmacokinetic parameter in the evaluation of first-order elimination capacity.¹ It is usually determined from the concentration of the drug in a large number (frequently two digit) of successive plasma samples after a single dose. However, if the volume of distribution (V_{area}) can be estimated with sufficient accuracy (e.g., from simple demographic data), the clearance can be determined from a single concentration-time point, the dose, and that V_{area} .² The one-sample method for determination of clearance (OSCL) has been validated for antipyrine in adults,² children,³ and rats⁴ and for other drugs such as theophylline, phenytoin, and amobarbital.⁵⁻⁷ The interchangeability of saliva and plasma samples has further facilitated the one-sample antipyrine test, allowing self-administration and self-sampling in subjects instructed in writing.

Metronidazole is an antimicrobial drug with a number of kinetic similarities to antipyrine. It is rapidly and completely absorbed after oral administration, distributed in total body water, and eliminated with log-linear

kinetics, mainly by oxidative metabolism.⁸⁻¹⁰ The concentration of metronidazole has been reported to be equal in saliva and plasma, although this has not been demonstrated systematically.¹¹⁻¹⁴ The hydroxylation of metronidazole, the main elimination pathway, is phenobarbital inducible, but the involved enzyme(s) appears different from those responsible for the oxidation of antipyrine.¹⁵ Thus metronidazole is a candidate for the increasing list of probe drugs for hepatic drug metabolism with a potential for opportunistic use. A one-sample method with an optional use of saliva for clearance determination would further enhance its usefulness.

In the present study we investigated the applicability of an OSCL of metronidazole, the relation between concentrations of metronidazole in plasma and saliva, and factors influencing the clearance of metronidazole and antipyrine.

MATERIAL AND METHODS

OSCL of metronidazole. The clearance of metronidazole (CL) was determined 96 times in 36 subjects in the age range 18 to 91 years. Eight subjects had liver cirrhosis and encephalopathy. Seven healthy volunteers were also studied during microsomal enzyme inhibition with cimetidine or induction with antipyrine or phenobarbital. The dose of metronidazole was 0.5 gm by intravenous infusion (36 times) or orally (44 times), but eight volunteers also received 2.0 gm by both routes of administration. The oral 0.5 gm metronidazole dose

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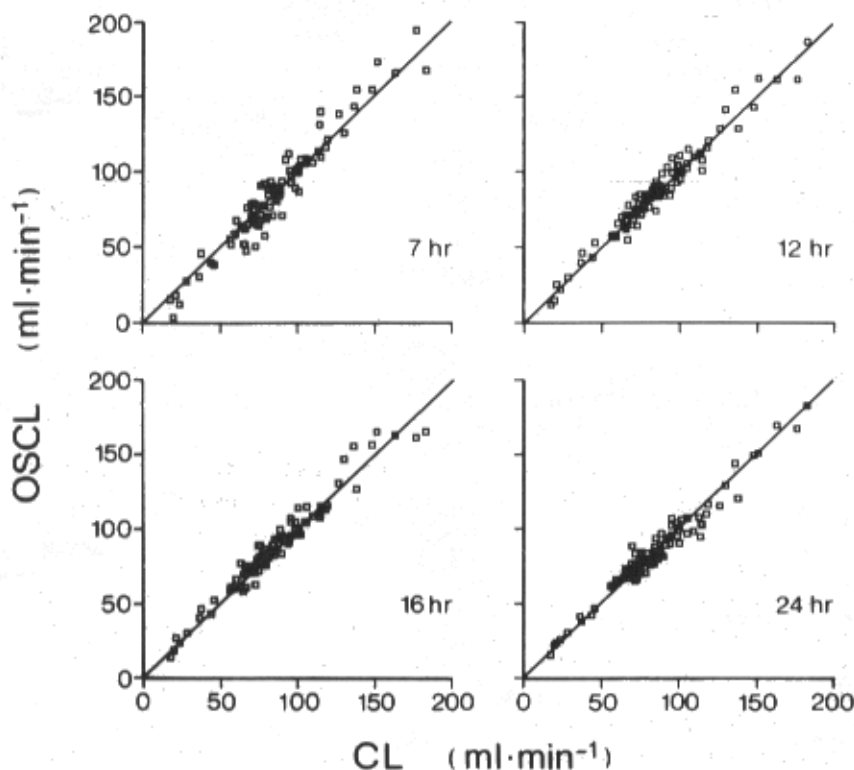


Fig. 1. Plots of the CL of metronidazole determined from a single plasma sample taken 7, 12, 16, or 24 hours after administration (OSCL) on the CL determined from the complete elimination curve. The lines of identity are shown.

was administered during treatment with cimetidine, 1.0 gm per day, six times and concomitantly with antipyrine, 1.0 gm, five times, with oxazepam, 15 mg, 14 times, and with both drugs 12 times. Details of the pharmacokinetics of metronidazole in the patients with liver disease and in the volunteers studied during enzyme inhibition and induction and after administration of 2.0 gm have been reported elsewhere.^{10,15,16}

Between 10 minutes and 60 hours after administration, six to 16 plasma samples were collected. In 90 of the 96 cases urine was collected for at least 48 hours. Two hundred thirty saliva samples were collected simultaneously with plasma samples. Plasma and urine samples were assayed for metronidazole and its hydroxymetabolite by HPLC as described previously.¹⁰ Saliva was assayed as plasma with a 5% day-to-day coefficient of variation.

After 60 intravenous and oral administrations, early concentration-time points were available and the CL was calculated as the dose divided by the AUC determined according to the trapezoidal rule and extrapolated to infinity. Ignoring concentration-time points earlier than 3 hours after administration, CL was also calcu-

lated as the product of the V_{area} and the elimination rate constant (k) determined from the time zero intercept and slope of the log-concentration time curve estimated by linear regression, respectively. After 36 oral 0.5 gm administrations, only postabsorption data were available and CL was calculated by the latter method. The CL of metronidazole by hydroxylation (CL_{H_1}) was calculated as the excreted amount plus the plasma concentration of the hydroxymetabolite (if detectable in plasma at the end of urine collection) times the steady-state volume of distribution of metronidazole (V_{ss}) divided by the AUC ($n = 60$) or as the product of the fraction of the dose excreted as metabolite and CL ($n = 36$). Metronidazole and its hydroxymetabolite were assumed to have identical V_{areas} .¹⁶

OSCL was determined from plasma samples taken about 7, 12, 16, or 24 hours after administration as:

$$OSCL = \frac{\ln(D/V_{est}) - \ln(C)}{t} \cdot V_{est}$$

where D is the dose, C is the concentration at time t , and V_{est} is the volume of distribution estimated from body weight (BW) in kilograms, height (BH) in cen-

Table I. Results of linear regression analysis of the CL of metronidazole determined from a single concentration time point (OSCL) on the CL determined from the complete elimination curve

Mean sampling time (hr)	Slope	Intercept	r	s ²
7.2 (6.9-7.5)	1.06 (1.00-1.12)*	-6.3 (-11.5-1.1)†	0.97	75
11.9 (11.8-12.0)	1.00 (0.98-1.02)	1.2 (-2.3-4.7)	0.98	35
15.6 (15.4-15.8)	0.99 (0.97-1.01)	3.4 (-0.1-6.8)	0.98	33
23.8 (23.3-24.3)	0.98 (0.93-1.03)	2.0 (-2.4-6.3)	0.97	53

s², residual variance.

Ninety-five percent confidence intervals are shown in brackets.

*P < 0.05 vs. 1; †P < 0.05 vs. 0.

timeters, age in years, and sex: $V_{\text{est}} = 0.297 \times \text{BW} + 0.273 \times \text{BH} - 0.1 \times \text{age} - S$, where S is 19.2 and 14.6 for women and men, respectively. This equation was developed by multiple linear regression of the V_{area} of metronidazole determined from the time zero intercept of the elimination phase of the log-concentration time curve after the 96 administrations on BW, BH, age, and sex.

Variation and bias of the OSCL of metronidazole. Separated by 2 to 4 weeks, the OSCL of metronidazole after oral administration of 0.5 gm was determined twice in 68 subjects. The coefficient of variation was determined as the SD of the difference between the two determinations divided by the combined mean.

If the V_{area} estimate is different from the true value, the OSCL may also be different from the true CL. The biased one-sample clearance (BOSCL) will be:

$$\text{BOSCL} = \frac{\ln(D/(B \cdot V_{\text{area}})) - \ln(C)}{t} \cdot B \cdot V_{\text{area}}$$

where B is the ratio between the biased and the true V_{area} . $C = D/V_{\text{area}} \cdot e^{-kt}$ may be inserted and the equation rearranged to: $\text{BOSCL} = (k - \ln(B)/t) \cdot B \cdot V_{\text{area}}$. The ratio between the biased and the true clearance appears after division and rearrangement:

$$\frac{\text{BOSCL}}{\text{CL}} = B - \frac{B \cdot \ln(B)}{\ln(2) \cdot t/t_{1/2}}$$

where $t_{1/2} (= \ln(2)/k)$ is the half-life.

Factors influencing the OSCL of metronidazole and antipyrine. The CL of metronidazole and antipyrine was determined by the one-sample method in 119 (seven women) healthy subjects in the age range 18 to 62 years. Metronidazole, 0.5 gm, and antipyrine, 1.0 gm orally, was administered concomitantly to 105 subjects and separated by 2 days to 14 subjects. Oxazepam, 15 to 30 mg orally, was administered as the second or third drug in a cocktail to 91 of the subjects. The phar-

macokinetics of oxazepam are to be reported elsewhere. A plasma or saliva sample collected 12 to 24 hours after administration was used for determination of the CL of both metronidazole as described above and antipyrine as described previously³ with HPLC determination of antipyrine.¹⁵ All administrations took place at the end of a vacation to avoid influence from the working environment. The average daily consumption of tobacco as number of cigarettes, alcohol as number of drink equivalents, and caffeine as number of cups of coffee plus 0.6 times number of cups of tea was recorded on a questionnaire.

Antipyrine-oxazepam-metronidazole interaction. Separated by 1 to 2 weeks and in random order, six healthy, male subjects took metronidazole, 0.5 gm orally, alone, antipyrine, 1.0 gm, alone, and the two drugs in a cocktail with oxazepam, 30 mg. The CL of metronidazole was determined from a single plasma sample taken after 16 hours as described above. The CL of antipyrine was determined from a single saliva sample.^{3,15}

Statistics. Regression analysis was done by the method of least squares. The population distribution of the metronidazole CL was investigated by probit analysis. The influence of the recorded factors on the metronidazole and antipyrine CL was investigated by multivariate regression and covariate analysis by means of the BMDP 6R program. The drug interaction was analyzed by a paired t test. The level of statistical significance was set at 0.05. When appropriate, values are presented with 95% confidence limits in brackets.

RESULTS

After all metronidazole administrations the terminal elimination curve was log linear. After the 60 administrations in which early data were available the ratio between the clearance estimated from the extrapolated time zero intercept and slope of the postabsorption/postdistribution concentration-time points and that

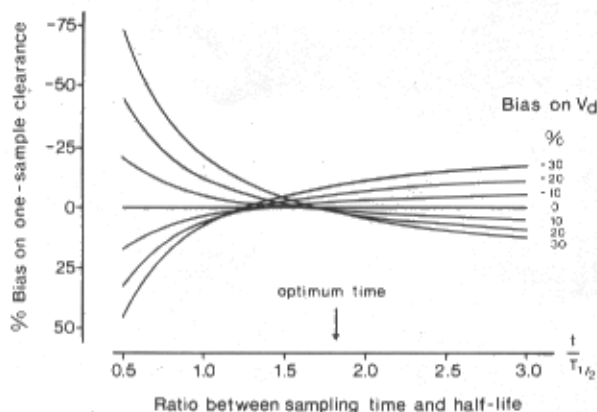


Fig. 2. Plots of the OSCL bias on the sampling time related to the $t_{1/2}$ for various bias on the V_{area} (V_d).

determined from the total AUC was 1.03 (1.01 to 1.05; 95% confidence interval).

Plots of the OSCL on the metronidazole CL determined from the complete elimination curve are shown in Fig. 1, and the corresponding regression analysis is summarized in Table I. At all sampling time points from about 7 to 24 hours after administration, correlations were high ($r > 0.96$). At the 7-hour sampling time point only there was systematic deviation between OSCL and CL reflected by intercept and slope of the regression curve significantly different from 0 and 1, respectively. At the 12-, 16-, and 24-hour sampling time points the residual SD was 7%, 7%, and 9% of the grand mean of 82 ml/min, respectively.

The coefficient of variation between V_{est} and V_{area} of metronidazole was 13%. The intraindividual coefficient of variation of V_{area} determined in 22 subjects receiving metronidazole more than once was 12%.

The intraindividual coefficient of variation of the OSCL of metronidazole determined from duplicate measurements in 68 subjects was 10%.

The influence of a biased estimate of V_{area} on OSCL is shown in Fig. 2. If the sample is taken between 1 and 3 half-lives after drug administration the OSCL bias is much less than the bias on V_{area} . At sampling time $t = 1/k$, a bias on the V_{area} estimate has minimal influence. At the previously determined optimum sampling time ($t = 1.25/k$) for minimum random variation,¹⁷ the bias on the clearance ranges from -3% to +10% with a V_{area} bias of $\pm 30\%$. If the sample is taken earlier than 0.5 times the half-life after administration, even a small V_{area} bias may be deleterious for the determination of OSCL.

The OSCL and the CL of metronidazole by hydrox-

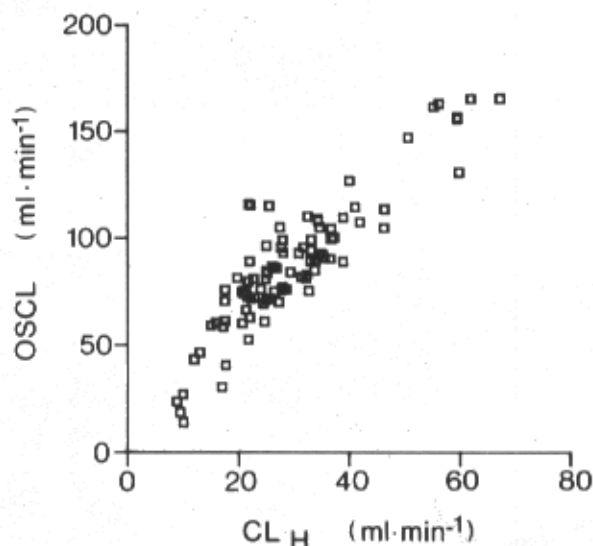


Fig. 3. Plot of the CL of metronidazole determined from one plasma sample taken 16 hours after administration (OSCL) on the CL of metronidazole by hydroxylation (CL_H).

ylation were significantly correlated ($r = 0.89$; Fig. 3). The CL by hydroxylation was 0.35 times (0.31 to 0.39; 95% confidence interval) the total CL.

The close correlation ($r = 0.99$) without systematic deviation between plasma and saliva concentrations of metronidazole is shown in Fig. 4. The slope of the regression curve was 1.00 (0.98 to 1.02) and the intercept 0.0 (-0.9 to 0.1). The residual SD after regression was 9% of the mean. For the plasma and saliva concentrations of the hydroxymetabolite the slope and intercept were 0.84 (0.78 to 0.90) and 0.0 (-0.8 to 0.2), respectively, and the residual variance after regression was 19% of the mean ($r = 0.94$; Fig. 1). Metronidazole and its hydroxymetabolite were stable in saliva for 14 days at room temperature, provided the sample was protected from direct light.

The distribution of the OSCL in 119 healthy subjects is shown in Fig. 5. The distribution was unimodal by visual inspection and according to probit analysis.

The OSCL correlated significantly with the sex, body weight, height, and alcohol consumption but not with age or consumption of tobacco or coffee/tea (Table II). After analysis of each independent variable removing the effect of the other independent variables by means of multivariate analysis, only body weight and alcohol consumption correlated significantly with OSCL. The multiple regression equation was: $OSCL = 38 + 0.58 \times BW + 2.8 \times \text{daily number of drinks}$.

The antipyrine CL correlated significantly with sex,

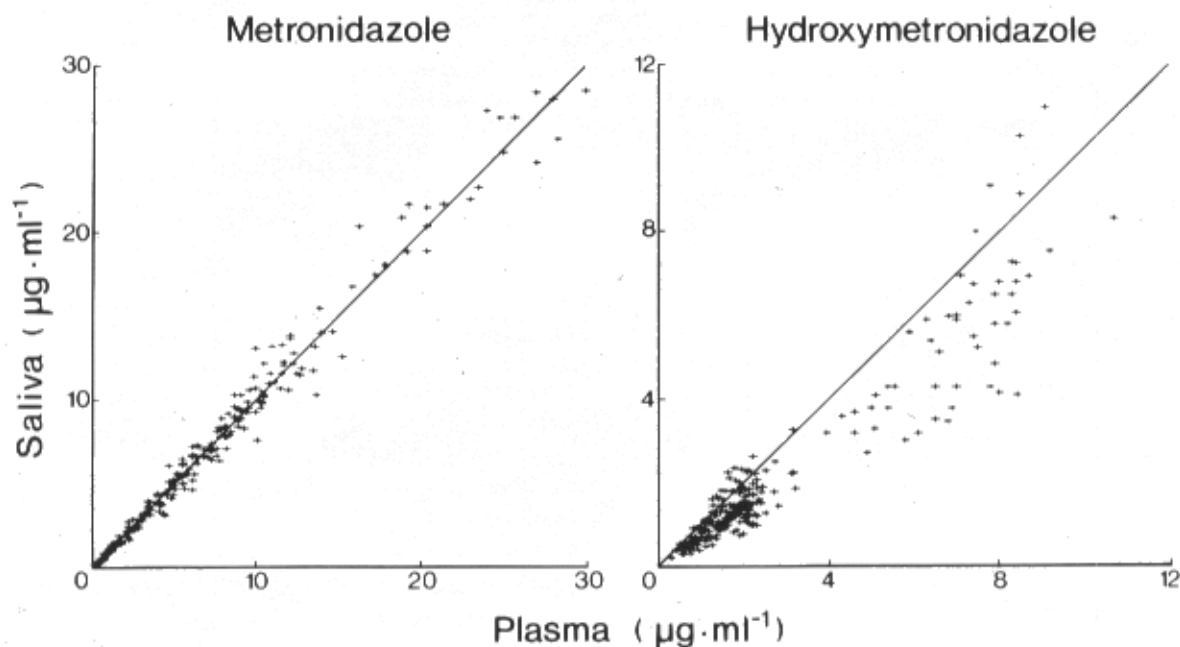


Fig. 4. Plots of saliva on plasma concentrations of metronidazole and its hydroxymetabolite in 230 simultaneous samples. The lines of identity are shown.

the consumption of tobacco, and the coffee/tea index. According to multivariate analysis, age and alcohol consumption also correlated significantly with the antipyrine CL. The regression equation was: $CL_{AP} = 48 + 0.52 \times \text{daily number of cigarettes} + 1.2 \times \text{coffee/tea index} - 2.1 \times \text{daily number of drinks} - 0.43 \times \text{age} (-19 \text{ for women})$.

The CL of metronidazole and antipyrine correlated significantly with or without removal of the effect of the independent variables (Table II). The correlation coefficient between the two CLs was 0.40 vs. 0.33 (Z test; $P > 0.05$), if the effect of tobacco, alcohol, and caffeine consumption respective of all other independent variables was removed.

The OSCLs of metronidazole and antipyrine administered in a triple cocktail with oxazepam were 0.98 times (0.92 to 1.04) and 1.04 times (0.97 to 1.10) the respective control values.

DISCUSSION

In the present study we demonstrated the applicability of an OSCL of metronidazole, the interchangeability of plasma and saliva for studies of metronidazole kinetics, that the population distribution of the CL of metronidazole was unimodal, and that environmental factors such as tobacco, alcohol, and coffee/tea may

influence the CL of metronidazole and antipyrine differently.

The elimination kinetics of metronidazole are log linear, the oral bioavailability is complete, and the duration of absorption and distribution is negligible compared with that of elimination.⁸⁻¹⁰ Thus, for practical purposes, metronidazole obeys single-compartment kinetics, and the CL determined from postabsorption/postdistribution data was almost identical to that determined from the complete AUC in the present study. Moreover, it was demonstrated that the CL of metronidazole could be determined from an assumed V_{area} , and the dose, and a single sample taken later than 12 hours after administration, without systematic deviation from the CL determined from the complete elimination curve and with very little random variation. The OSCL of metronidazole was applicable in the dose range 0.5 to 2.0 gm and irrespective of route of administration, concomitant administration of other drugs, or severely reduced hepatic function covering most clinical settings. The intraindividual coefficient of variation of the OSCL of metronidazole was 10% as predicted from theoretical considerations.¹⁷

Theoretically, the random variation of an OSCL is minimal if the sample is taken between $1/k$ and $2/k$, usually $1.25/k$ ¹⁷ (i.e., between 12 and 24 hours in

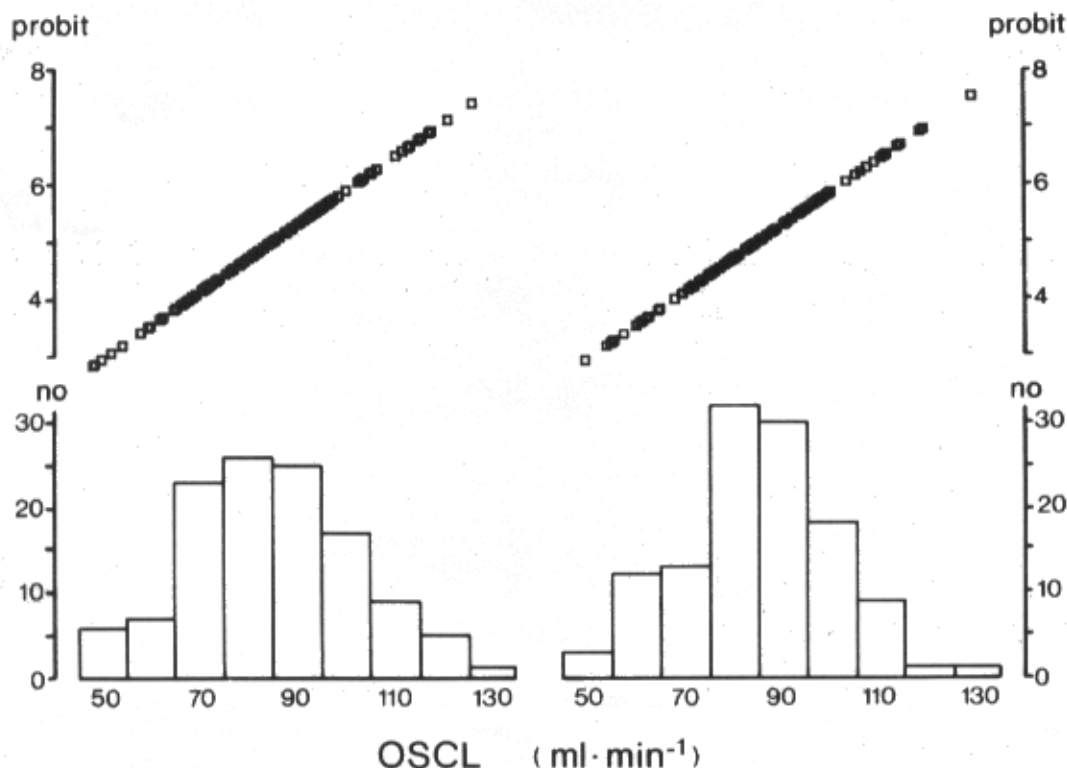


Fig. 5. Frequency distribution and probit plot of the CL of metronidazole determined from a single sample of saliva or plasma (OSCL) in 119 healthy subjects. In the left panel are the crude values and in the right panel are the values after correction for influencing factors.

healthy subjects receiving metronidazole).⁸⁻¹⁰ In the present study it was confirmed that the influence of a bias in the estimation of the V_{area} has little influence on the OSCL if the sample is taken from 1 to 3 half-lives after administration with a minimum at $1/k$. At sampling time point $1.25/k$ the OSCL bias is $<10\%$ for V_{area} estimated with a 30% bias.

The coefficient of variation between the V_{area} estimated from demographic variables, V_{est} , and the V_{area} determined from the decay of metronidazole in plasma was 12%, equivalent to the 13% intraindividual variation in this V_{area} determination. Thus the true V_{area} of metronidazole appears to be estimated as accurately from the demographic variables as from the plasma disappearance of the drug. This is further supported by the minimal random variation between OSCL and the CL determined from the complete elimination curve.

The OSCL was highly correlated to the CL by hydroxylation, which on average represented 35% of the total CL. Thus the total elimination rate of metronidazole estimated as OSCL is a measure of its rate of hydroxylation.

The concentration of metronidazole in saliva predicted that of plasma without systematic deviation and with a 9% residual SD compared with the interassay analytic variation of about 5%.¹⁰ Thus, suggestions in previous reports¹²⁻¹⁴ of identical concentrations in these two body fluids have been confirmed systematically throughout the concentration range. In future studies of metronidazole pharmacokinetics, saliva may replace plasma samples, although the plasma concentration of the hydroxymetabolite will be underestimated.

Factors influencing the rate of metronidazole elimination estimated as the OSCL were studied in a healthy population sample of seven women and 112 men. Two other model drugs, antipyrine and oxazepam, were administered concomitantly. Previously it had been demonstrated that single doses of antipyrine and metronidazole have no influence on the pharmacokinetics of each other.^{15,18} In the present study the OSCLs of the two drugs were not influenced when they were administered in a cocktail with oxazepam.

In the 119 healthy subjects the distribution of OSCL was unimodal, even when adjusted for the modifying

Table II. Correlation matrix of various factors and the CL of metronidazole (OSCL) and antipyrine in 119 subjects

	Age (yr)	Sex (F/M)	Body weight (kg)	Height (cm)	Daily consumption of			CL _{AP} (ml/min)
		7:112 F: 0 M: 1			Tobacco (No. of cigarettes)*	Alcohol (No. of drinks)	Coffee/tea (index)†	
Mean	38	—	76	178	13.7	1.2	6.5	58
SD	13	—	10	7	6.4	1.3	3.6	16
Sex	0.32‡							
Body weight	0.22‡	0.38‡						
Height	-0.38	0.19‡	0.36‡					
Tobacco	0.29‡	0.17‡	0.002	-0.14				
Alcohol	0.19‡	0.20‡	0.17‡	-0.01	0.28‡			
Coffee/tea	0.32‡	0.13	0.07	-0.06	0.29‡	0.14		
CL _{AP}	-0.13	0.22‡	0.05	0.12	0.25‡	-0.08	0.24‡	
CL _{AP} §	-0.24‡	0.28‡	0.06	-0.02	0.28‡	-0.19‡	0.27‡	
OSCL	0.001	0.23‡	0.37‡	0.29‡	0.13	0.28‡	0.13	0.36‡
OSCL§	-0.04	0.11	0.28‡	0.15	0.09	0.23‡	0.10	0.34‡

*Mean and SD of 45 smokers; 74 subjects were nonsmokers.

†Index = No. of cups of coffee + 0.6 × No. of cups of tea.

‡Denotes $P < 0.05$.

§Correlation coefficients after removing the effect of the other independent variables by means of multivariate analysis.

factors, suggesting that hydroxylation of metronidazole is not subject to an important genetic polymorphism.

The OSCL correlated significantly with body weight, height, sex, and the self-reported average daily consumption of alcohol. Because several of these variables correlated with each other, the independent contribution of each had to be estimated. Multivariate analysis revealed that only body weight and alcohol consumption were significant independent predictors of the rate of metronidazole elimination. Although unlikely, it cannot be excluded that the association with body weight is a result of its use for the determination of OSCL. The association between alcohol consumption and OSCL suggests that the metabolism of metronidazole, like that of many other drugs, is induced by alcohol ingestion.¹⁹ The proof awaits a longitudinal study.²⁰

According to multivariate analysis, the significant independent predictors of antipyrine CL were sex and the self-reported average daily consumption of tobacco and coffee/tea with positive correlation coefficients and age and the consumption of alcohol with negative correlation coefficients. The positive effect of smoking and the negative effect of age on the rate of antipyrine elimination are well known,²¹⁻²³ and a higher CL in men than in women has been found in some studies,^{24,25} whereas most previous cross-sectional studies have failed to show significant associations between the consumption of coffee and alcohol and the antipyrine CL.^{21,26} The negative correlation between alcohol con-

sumption and antipyrine CL is particularly intriguing because studies with subjects serving as their own controls have shown either no effect on the CL²⁷ or a reducing effect on the $t_{1/2}$ ²⁸ of alcohol ingestion for 7 days or more. Thus it appears that more studies concerning the influence of daily use of stimulants on the metabolism of antipyrine are needed. Moreover, the CL of antipyrine reflects the aggregate activity of at least three cytochrome P-450 isozymes, each of which may be affected differently by alcohol.

The differential influence of the three stimulants and the three demographic variables on the CLs of metronidazole and antipyrine support our previously posed hypothesis that the two drugs are metabolized by different cytochrome P-450 isozymes.¹⁵ This was founded on the lack of effect of cimetidine on the rate of hydroxylation of metronidazole and the lack of correlation between that rate and the rate of formation of the major metabolites from antipyrine.¹⁵ The lack of association between OSCL and smoking in the present study suggests that the enzyme(s) responsible for hydroxylation of metronidazole does not belong to the types inducible by polyaromatic hydrocarbons.²⁹

In small groups of young, healthy, nonsmoking volunteers and patients with liver disease,^{15,16} we have previously found much stronger correlations ($r > 0.8$) between the CLs of metronidazole and antipyrine than in the present study ($r = 0.34$). The apparent discrepancy may be explained by differences in the influencing

factors between the present material, which included a number of smokers and consumers of significant amounts of alcohol and coffee, and the small volunteer groups, which did not. Because the CLs of metronidazole and antipyrine are influenced by different factors, the correlation between them will diminish with increasing appearance of these factors in the population sample. However, the correlation coefficient between the two CLs was not significantly higher when the effect of the consumption of tobacco, alcohol, and coffee/tea were removed by means of multivariate analysis. Because antipyrine is metabolized by at least three cytochrome P-450 isozymes and metronidazole is eliminated by routes other than hydroxylation, correlations between the total CLs of the two drugs do not indicate shared enzymatic pathways but may suggest common regulatory mechanisms.

The differential effects on the CLs of metronidazole and antipyrine demonstrate the increased yield of information when two or more drugs are administered concomitantly for studies of environmental influences on pharmacokinetics.³⁰ Metronidazole and antipyrine are particularly well suited for coadministration in a cocktail design, because the CL of both can be determined from a single saliva or plasma sample.

In conclusion, we have demonstrated that the CL of metronidazole can be determined from a single sample taken 12 to 24 hours after administration, the dose, and an estimated V_{area} and that saliva can replace plasma for studies of metronidazole kinetics. The CL of metronidazole, which mainly depends on hydroxylation, was positively correlated to body weight and height and consumption of alcohol in contrast to the CL of antipyrine, which was positively correlated to sex and consumption of tobacco and coffee and negatively to age and consumption of alcohol, supporting that the two drugs are metabolized by different microsomal enzymes.

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