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Plasma hydroxy-metronidazole/metronidazole ratio in patients with liver disease and in healthy volunteers

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Metronidazole pharmacokinetics were studied in patients with different degrees of liver cirrhosis, classified according to the Child-Pugh algorithm (A, B or C, as liver disease severity increases) and in schistosomic patients. Metronidazole (500 mg) was administered i.v. as a slow infusion over 20 min, and blood samples were collected at set intervals after the end of the infusion. The plasma concentrations of metronidazole and its main metabolite hydroxy-metronidazole were quantified by reversed-phase h.p.l.c. with u.v. detection. The metronidazole and hydroxy-metronidazole areas under the curve from 0 to 24 h (AUC0,24h), the metronidazole terminal elimination half-life ($t_{1/2}$), the total clearance (CL), the metronidazole volume of distribution (V) values and the hydroxy-metronidazole/metronidazole concentration ratios as a function of time were calculated for each group. Comparison of the metronidazole AUC0,24h, $t_{1/2}$ and CL values revealed that metronidazole metabolism is progressively impaired as the severity of liver disease increases. There were no variations in these parameters between the schistosomic and Child-Pugh A groups. In addition, there were no differences in the V and hydroxy-metronidazole AUC0,24h among the various groups studied. However, metronidazole metabolism was delayed in patients with hepatic disease, as illustrated by the hydroxy-metronidazole/metronidazole ratio 10 min after the end of metronidazole infusion. These results indicate that the clinical assessment of liver disease is paralleled by an impairment of metronidazole metabolism. Of the studied variables, we propose the hydroxy-metronidazole/metronidazole ratio 10 min after metronidazole infusion as a suitable and practical index for liver function evaluation.

Keywords metronidazole metabolism pharmacokinetics cirrhosis schistosomiasis

Introduction

The need for suitable tests to assess the functional state of the liver has increased as a result of the successful development of hepatic transplant techniques. Several tests, such as lignocaine metabolite formation [1, 2], indocyanine green clearance [3], caffeine elimination [4] and metronidazole kinetics [5–7], have recently been used in order to determine which alterations correlated with the short term prognosis for patients with liver disease.

In humans, metronidazole is extensively metabolized primarily by as yet not fully identified cytochrome(s) P450 [8], giving rise to two principal metabolites: the hydroxy metabolite (having about 65% of the pharmacological activity of metronidazole) and the inactive acetic acid metabolite [9, 10]. Glucuronidation and renal excretion of the unchanged compound are minor elimination pathways. Elimination occurs mainly by renal excretion of the metabolites (60–80% of total dose); faecal excretion accounts for only 6 to 15% of total dose [11]. Since metronidazole lacks cardiovascular or central effects, it presents certain clinical advantages as an agent for screening patients with liver disease.

In this study, we have compared metronidazole pharmacokinetics and metabolism in healthy volunteers, in patients with various degrees of chronic

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liver disease (CLD) and in patients with mansonic schistosomiasis.

Methods

Volunteer and patient selection

Seven healthy volunteers (three females; 43.4 ± 5.7 years, 69 ± 12 kg, 165 ± 9 cm), as determined by their medical history, physical examination and laboratory tests, ten schistosomic patients (five females; 49.7 ± 12.9 years, 59 ± 13 kg, 163 ± 6 cm), as determined by their medical history, the absence of alcoholism, negative serology for hepatitis B and C and the presence of periportal fibrosis detected by ultrasonography, and 35 cirrhotic patients, as determined by their medical history, laboratory tests and the presence of diffuse alterations in the liver parenchyma detected by ultrasonography, were selected for the study. The Child-Pugh criteria [3] grade cirrhotic patients on the basis of serum albumin and bilirubin, prothrombin time, severity of ascitis and encephalopathy, each one on a 3-point scale. According to this algorithm, 14 cirrhotic patients (five females; 45.2 ± 10.5 years, 70 ± 13 kg, 166 ± 9 cm) were classified as A (score 5–6), nine (three females; 48.2 ± 11.7 years, 67 ± 11 kg, 165 ± 7 cm) were classified as B (score 7–9) and 12 (three females; 46.1 ± 11.2 years, 58 ± 12 kg, 163 ± 7 cm) were classified as C (score 10–15). Ten Child-Pugh C patients were positive for any history of encephalopathy, while all Child-Pugh A or B patients were negative. No patients diagnosed with liver tumours (primary or metastic) were included. All subjects gave their written informed consent to participate in the study and the clinical protocol was approved by the University Hospital Ethics Committee.

Protocol

The patients and volunteers were admitted to the Clinical Pharmacology Unit wards early in the morning and remained there until the last blood sample was collected. The study consisted of a single intravenous administration of metronidazole (Flagyl®, 500 mg in 100 ml) over 20 min. At the end of the infusion, the cannula was washed with 20 ml of sterile saline. Blood samples (5 ml) from an antecubital vein were collected into EDTA-containing tubes before and 5, 10, 20, 30, 60 and 90 min and 2, 3, 4, 8, 12, 18 and 24 h after the infusion. The blood samples were centrifuged at 2000 *g* for 5 min and the plasma decanted and stored at –20° C until assayed.

Drug analysis

Plasma metronidazole and hydroxy-metronidazole concentrations were measured by reverse-phase h.p.l.c. with u.v. detection, as previously described [12, 13]. This method has a sensitivity of 100 ng ml^{-1} and the mean intra-assay coefficient of variation (up to 25 μg ml^{-1} of both compounds) is 4%.

Pharmacokinetic and statistical anlaysis

A first-order terminal elimination rate constant (k_{el}) for metronidazole was derived by log-linear regression of selected data points describing a terminal log-linear decaying phase from the concentration *vs* time curves. Metronidazole half-life ($t_{1/2}$) was estimated from this rate constant ($t_{1/2} = \ln 2/k_{el}$). The areas under the plasma metronidazole and hydroxy-metronidazole concentrations *vs* time curves (AUC0,24h) were calculated by the trapezoidal rule method. Extrapolation of metronidazole AUC to infinity (AUC) was done by addition of the value C_{24}/k_{el} (where C_{24} = plasma metronidazole concentration at 24 h post dose). Metronidazole total systemic clearance (CL) was calculated as the dose divided by the AUC. The volume of distribution of metronidazole (*V*) was calculated by dividing CL by k_{el}. Hydroxy-metronidazole/

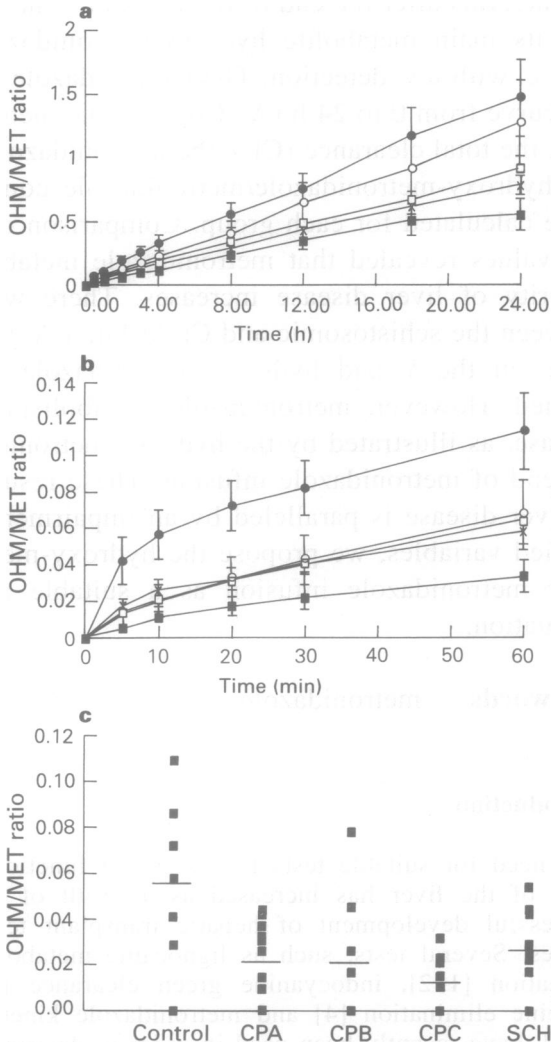

Figure 1 Panels a and b: mean plasma hydroxy-metronidazole/metronidazole ratio time-course obtained after the i.v. administration of 500 mg metronidazole in the control (●), Child-Pugh A (□), Child-Pugh B (○), Child-Pugh C (■) and schistosomic (×) groups. Panel c: individual and mean plasma hydroxy-metronidazole/metronidazole ratios obtained 10 min after the administration of metronidazole in the control, Child-Pugh A (CPA), Child-Pugh B (CPB), Child-Pugh C (CPC) and schistosomic (SCH) groups.

metronidazole ratios at each time point were calculated and plotted as a function of time. Differences between groups were analyzed by one-way ANOVA and Student's *t*-test for unpaired data. All values are expressed as mean ± s.d. unless otherwise stated.

Results

Panels a and b of Figure 1 show the time course of the mean plasma hydroxy-metronidazole/metronidazole ratio. The scattergram (panel c) shows individual and mean plasma hydroxy-metronidazole/metronidazole ratios obtained 10 min after the end of metronidazole infusion in the five groups of subjects. Table 1 summarizes the metronidazole pharmacokinetic parameters and the hydroxy-metronidazole/metronidazole ratios 10 min after the end of metronidazole infusion for the five groups of subjects. One-way ANOVA showed no difference among the groups for metronidazole *V* and hydroxy-metronidazole AUC0,24h. The other pharmacokinetic parameters evaluated were significantly different among the five groups.

Discussion

Although it has been reported that the disposition of oral metronidazole in hepatic cirrhosis and in hepatosplenic schistosomiasis patients is not significantly different from normal controls [5], we have observed several pharmacokinetic differences in patients with either CLD or schistosomiasis compared with healthy controls following a single metronidazole (500 mg) infusion. Metronidazole pharmacokinetics are also altered in patients with liver encephalopathy [7]. Our findings that metronidazole CL decreases and $t_{1/2}$ increases were reasonably well related to the severity of liver disease, reflected as an impairment of metronidazole metabolism in these patients, probably mediated by a lower activity of the specific cytochrome P450 system involved. Furthermore, *in vitro* experiments have shown that the activities of some members of the cytochrome P450 family from a group of mixed liver diseases were significantly impaired when compared with those from normal individuals [14].

Interestingly, schistosomic patients also presented an increased $t_{1/2}$ when compared with healthy volunteers. Since this disease is characterized by the presence of *Schistosoma mansoni* and its eggs in the portal system [15], the difference observed is more likely related to a decreased hepatic flow secondary to portosystemic shunting rather than cytochrome P450 dysfunction. Since metronidazole metabolism is unlikely to be limited by hepatic blood flow (it has a low intrinsic hepatic clearance relative to blood flow and low plasma protein binding), a limitation on the reflex compensatory increase in arterial flow from the hepatic artery or concurrent sinusoidal capillarisation could account for the impaired metronidazole metabolism [16].

The finding that *V* is not altered, as previously observed [7], indicates that ascites, generally present in severe liver disease, does not significantly affect metronidazole distribution.

All the previously described pharmacokinetic parameters, although important for the detection of liver

Table 1 Pharmacokinetic parameters obtained from the four groups of patients and control group after the i.v. administration of 500 mg metronidazole (as mean ± s.d.)

Group	n	*MET* $t_{1/2}$ *(h)*	*MET CL* *(ml min^{-1} kg^{-1})*	*MET* V *(l kg^{-1})*	*MET AUC0,24h* *(mg ml^{-1} h)*	*OHM AUC0,24h* *(mg ml^{-1} h)*	*[OHM]/[MET]* *(at 10 min)*
Control	7	7.4 ± 2.2	1.53 ± 0.37	0.80 ± 0.32	81.4 ± 27.0	50.6 ± 30.2	0.056 ± 0.037
Child-Pugh A	14	10.7 ± 2.3** [a](1.0/5.4)	0.85 ± 0.26*** [a](−0.97/−0.39)	0.74 ± 0.11	124.9 ± 42.3* [a](6.7/80.4)	39.6 ± 10.5	0.021 ± 0.017** [a](−0.059/−0.011)
Child-Pugh B	9	13.5 ± 5.1* [a](1.6/10.5)	0.79 ± 0.36** [a](−1.13/−0.35)	0.79 ± 0.12	124.4 ± 25.8** [a](14.3/71.8)	50.5 ± 22.8	0.020 ± 0.027** [a](−0.063/−0.009)
Child-Pugh C	12	21.5 ± 12.7*‡‡† [a](3.7/24.4) [b](3.7/18.0) [d](2.8/19.8)	0.56 ± 0.28***‡†† [a](−1.29/−0.65) [b](−0.51/−0.07) [d](−0.59/−0.15)	0.81 ± 0.14	174.1 ± 52.0***‡§† [a](50.1/135.4) [b](11.0/87.3) [c](10.3/89.0) [d](0.24/77.8)	42.5 ± 38.1	0.011 ± 0.010**† [a](−0.071/−0.020) [d](−0.027/−0.001)
Schistosomic	10	10.2 ± 2.1* [a](0.5/5.0)	0.93 ± 0.19*** [a](−0.89/−0.31)	0.79 ± 0.09	135.0 ± 33.8** [a](20.7/86.7)	40.4 ± 14.5	0.026 ± 0.018* [a](−0.058/−0.004)

MET: metronidazole; OHM: hydroxy-metronidazole.
Numbers in brackets indicate lower and upper limits of the 95% confidence intervals of significant differences with respect to [a]Control, [b]Child-Pugh A, [c]Child-Pugh B and [d]schistosomic groups.
*: $P < 0.05$, ***$P < 0.01$ and ***: $P < 0.001$ *vs* Control group.
‡: $P < 0.05$ and ‡‡: $P < 0.01$ *vs* Child-Pugh A group.
§: $P < 0.05$ *vs* Child-Pugh B group.
†: $P < 0.05$ and ††: $P < 0.01$ *vs* schistosomic group.

function impairment, are not practical for clinical purposes, since they require blood sampling over at least 24 h and hence internment of the patients. Our results demonstrate a clear significant difference in the hydroxy-metronidazole/metronidazole ratio as early as 10 min after the infusion of 500 mg metronidazole, thus providing a rapid test for liver function that could be easily performed at an ambulatory level. The main advantage of this test is its sensitivity in allowing significant distinction between healthy individuals and patients with Child-Pugh A cirrhosis or with schistosomiasis, particularly since the latter two groups do not generally present any alterations in the clinical laboratory tests. A further advantage is the fact that metronidazole depicts linear pharmacokinetics within the normally used dosing range [13], thus allowing a reduction of the intravenous dose administered, e.g. in paediatric patients. Whether this test would be useful for liver disease prognosis is currently under investigation.

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