Pharmacokinetics of Metronidazole as Determined by Bioassay

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The pharmacokinetics of metronidazole, a drug effective in vitro against most anaerobic bacteria and promising in treating anaerobic infections, are described. Serum and urine levels after single and multiple doses in 10 adult male volunteers were measured by an agar well diffusion bioassay using clostridial species as the test organisms under anaerobic conditions. Peak serum levels averaged 11.5 μg/ml and 6.2 μg/ml after single 500-mg and 250-mg doses, respectively. Renal clearance was only 10.2 ml/min per 1.73 m², and less than 20% of the administered dose was recovered in the urine as active drug in 24 h. The average serum half-life was 8.7 h, and there was no protein binding as determined by an ultrafiltration method. With multiple doses of metronidazole (500 mg four times a day and 250 mg three times a day), blood levels increased progressively for the first few doses and then leveled off, with no significant accumulation occurring between 3 and 7 days. On 250 mg three times a day, serum levels just before the 8 a.m. dose (12 h after the preceding dose) on the third day averaged 3.9 μg/ml, and before the 8 p.m. dose, 5.7 μg/ml. For the higher, 500-mg dose (four times a day) regimen, the corresponding minimum serum levels were 13.1 μg/ml at 8 a.m. and 21.3 μg/ml at 8 p.m. Peak levels would have been about 10 μg/ml higher, and since the minimum inhibitory concentrations of most anaerobes including Bacteroides fragilis are less than 6 μg/ml, these concentrations should be highly effective therapeutically, even for severe infections.

With the increasing awareness of the pathogenicity of anaerobic bacteria, antimicrobial agents which were effective against these organisms are receiving greater attention. Metronidazole [1-(β-hydroxyethyl)-2-methyl-5-nitroimidazole], which has been used effectively for many years against certain protozoal infections, has been shown to be active against most anaerobic bacteria in vitro, and to be promising clinically in treating anaerobic infections (13). Except for one recent report (6), previous studies of blood and urine levels have relied upon nonbiological assay methods in which metronidazole and its metabolites are measured without regard to their antibacterial action (5, 14, 16). The present study was designed to measure biologically active metronidazole in the serum and urine after single and multiple doses and to determine its pharmacokinetics. Doses were chosen which have been used in treating diseases such as trichomoniase and amebiasis.

Protein binding was also studied since this property may be important in tissue penetration and renal clearance of the drug.

MATERIALS AND METHODS

Single-dose study. Ten healthy male volunteers weighing between 140 and 200 pounds were divided into two groups of five each. Group I received 250 mg of metronidazole (supplied as 250-mg tablets by G. D. Searle and Co., Chicago) (1 tablet) and group II, 500 mg (2 tablets). The volunteers had fasted for at least 10 h and were allowed food 2 h after taking the drug. No restriction on water intake before or during the study was imposed. Blood specimens were drawn through a scalp vein needle placed in a forearm vein and kept patent by a heparin lock. Samples were drawn at frequent intervals for 2.5 h and then hourly until 8 h after the dose was administered. A final specimen was drawn at 25 h. After centrifugation of the blood, serum was removed and kept frozen at −20°C until assayed. Urine specimens collected during the time intervals 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 h were measured and portions were frozen at −20°C for assay.

Multiple-dose study. The same volunteers as in the single-dose study participated in this study. Group 1 received 500 mg four times a day (0800, 1200, 1600, 2000 h) and group 2, 250 mg three times a day (0800, 1400, 2000 h) for 1 week. No dietary restrictions were imposed. Blood specimens were drawn on days 1, 3, and 7 prior to each oral dose to determine minimum
serum metronidazole levels and to see if cumulation occurred. Twenty-four-hour urine collections were made on days 3 and 7. To record the duration of measurable blood levels after a multiple-dose regimen, in one volunteer in each group (no. 4 and 8), the drug was stopped on day 6 and blood specimens were drawn on days 7, 8, and 9.

Assay. Serum and urine metronidazole levels were measured by an anaerobic modification of the agar well diffusion technique of Bennett and Kirby (1) and the details of this assay method will be described separately. Three types of clostridia were used as the test organisms and assay plates were incubated in a Gas Pak system (Baltimore Biological Laboratories). For drug levels above 2 \( \mu g/ml \), a Clostridium perfringens or C. capitis was used, and for levels between 0.25 \( \mu g/ml \) and 2 \( \mu g/ml \) a highly sensitive strain of Clostridium sporogenes was employed. Each serum and urine specimen was measured in quadruplicate (i.e., in four different wells) and the results were averaged. For the standard curves, metronidazole was added to normal pooled serum or phosphate-buffered saline (for urine specimens) with carefully assayed laboratory standard powder supplied by Searle and Co., Puerto Rico. Accuracy of the assay was determined to be \( \pm 10\% \).

The serum half-life was determined from the terminal portion (4 to 25 h) of the serum concentration versus time curve, by the formula \( T_{1/2} = \ln 2/K_e \), where \( \ln 2 \) is the natural logarithm of 2 and \( K_e \) (elimination constant) is the slope of the regression line as determined by the method of least squares from 5 or 6 points. The relationship of the regression line to a straight line was expressed as the correlation coefficient, \( r \).

The renal clearance of metronidazole was measured during the 4- to 8-h interval after single doses with the formula \( C = (U \cdot V/P) \cdot (1.73/BSA) \), where \( U \) is the urine concentration of metronidazole in micrograms per milliliter, \( V \) is the volume of urine (4- to 8-h interval) in milliliters per minute, \( P \) is the serum concentration at 6 h in micrograms per milliliter (midpoint of urine collection), and BSA is the body surface area.

Protein binding was measured in 100% serum at a concentration of 8 \( \mu g/ml \) by the modified ultrafiltration method of Bennett and Kirby (2) with six separate determinations, and the results were averaged.

RESULTS

Single-dose study. (i) Serum levels. The results are shown in Fig. 1 and 2, and peak, 4-, 8-, and 25-h levels and serum half-lives (\( T_{1/2} \)) for each volunteer after the single 250-mg and 500-mg oral doses are shown in Table 1.

Absorption of the 500-mg dose (2 tablets) was quite variable as shown by the peak levels which occurred within 15 to 45 min in three volunteers, but not until 2 and 4 h in the other two. The mean peak serum level was 11.5 \( \pm 5.58 \) (standard deviation [SD]) \( \mu g/ml \). However, the amount of drug present was quite uniform after about 3 h with very similar blood levels for all the volunteers, 7.52 \( \pm 0.68 \) (SD) \( \mu g/ml \), which declined slowly thereafter.

After the 250-mg dose, absorption was less variable and peak serum levels occurred within 75 min in all five volunteers. The mean peak serum level was 6.22 \( \pm 1.09 \) (SD) \( \mu g/ml \). As in the 500-mg dose study, levels after 3 h were very similar in all volunteers (3.82 \( \pm 0.20 \) [SD] \( \mu g/ml \)).

Absorption was very rapid in four volunteers (no. 1, 3, 8, 9), peak levels occurring within 15 min in two and by 30 min in the other two.

Blood levels declined in a slow and uniform manner after 3 to 4 h, and regression curves had a high degree of correlation with a straight line \((r = 0.99)\). Measurable blood levels were present 25 h after both the 250- and 500-mg doses and averaged 0.65 \( \pm 0.10 \) (SD) \( \mu g/ml \) and 1.36 \( \pm 0.12 \) (SD) \( \mu g/ml \), respectively. This represented slow, steady elimination since the regression...
not differ the average those using metronidazole 8.7 of value each dose ter of this results on than that the suggests 12 h. 24 the same in lines drawn from the average 4- to 8-h levels did not differ significantly \( P > 0.90 \) by \( t \) test from those using the 4- to 25-h levels.

The average serum half-life for the 4- to 25-h period was \( 8.3 \pm 0.4 \) (SD) h and \( 9.1 \pm 0.54 \) (SD) h for groups 1 and 2, respectively, with a figure of 8.7 when the two groups were averaged.

(ii) Urinary excretion. Figure 3 illustrates the average cumulative urinary excretion of metronidazole as percentage of the administered dose up to 48 h. Excretion was essentially the same in both groups and averaged 14.6% in 24 h. Proportionally more was excreted in the first 12 h (9.9%) than the second (4.7%). From 24 to 48 h, only an additional 2.8% was recovered, giving a total of 17.4% for the 48-h period. The concentration of metronidazole in the urine during the first 12 h averaged 88 and 35 \( \mu g/ml \) for the 500- and 250-mg doses, respectively.

(iii) Renal clearance. The average renal clearance for nine volunteers during the 4- to 8-h interval (Table 2) was found to be only \( 10.2 \pm 2.33 \) (SD) ml/min per 1.73 m\(^2\). One volunteer was excluded because of a urine output of only 55 ml during this interval. The creatinine clearance was \( >110 \) ml/min per 1.73 m\(^2\) in all volunteers.

Multiple-dose study. (i) Serum levels. The results of this study are presented in Fig. 4. All values represent the minimum serum levels after each dose since the specimens were obtained just prior to the next dose. For group 1 (500 mg four times daily), the levels increased to \( 14.6 \pm 1.76 \) (SD) \( \mu g/ml \) prior to the final dose on day 1 at 2000 h. Inspection of Fig. 4 suggests that the levels on day 7 were no higher than on day 3 and this is supported by a \( t \)-test value of \( P > 0.40 \). For these 2 days, metronida-
Table 2. Renal clearance of metronidazole as determined from the serum level at 6 h, and the urine concentration and volume during the 4- to 8-h interval

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Dose (mg)</th>
<th>Serum concn (μg/ml)</th>
<th>Urine Concén (μg/ml)</th>
<th>Volume (ml)</th>
<th>Renal clearance (mL/min per 1.73 m²)</th>
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<tbody>
<tr>
<td>2</td>
<td>250</td>
<td>3.12</td>
<td>67.0</td>
<td>150</td>
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<tr>
<td>3</td>
<td>250</td>
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<td>310</td>
<td>13.80</td>
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<tr>
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<td>3.23</td>
<td>15.7</td>
<td>550</td>
<td>9.79</td>
</tr>
<tr>
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<td>34.6</td>
<td>210</td>
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<tr>
<td>6</td>
<td>500</td>
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<tr>
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<tr>
<td>8</td>
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<tr>
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<td>76.4</td>
<td>185</td>
<td>10.00</td>
</tr>
<tr>
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<td>500</td>
<td>5.43</td>
<td>91.4</td>
<td>110</td>
<td>6.42</td>
</tr>
</tbody>
</table>

Mean ± SD 10.20 ± 2.34

Fig. 4. Average minimum (determined just prior to each succeeding dose) serum metronidazole concentrations (μg/ml) on days 1, 3, and 7 with the multiple-dose regimens.

were all minimum blood levels. The peaks after each dose might have been up to 100% higher by extrapolation from our single-dose studies.

Measurable serum levels (0.41 μg/ml and 0.54 μg/ml) were still present as long as 60 and 36 h, respectively, in the two volunteers who discontinued 2-g and 750-mg daily doses after 6 days. Serum half-lives for these two volunteers were 8.5 and 7.1 h, in the same range as the values observed in the single-dose studies.

(ii) Urinary excretion. Urinary excretion on days 3 and 7 is shown in Fig. 5. Average excretion of metronidazole and its biologically active metabolites was less than 20% of the administered dose in both groups. Slightly more was recovered in the urine in the high dose group, 19.1% (average of days 3 and 7), than in the low dose group, 16.6%. As expected, urinary excretion of metronidazole was somewhat higher in the multiple-dose than in the single-dose studies: 17.8% (average of groups 1 and 2, multiple dose) versus 14.6% (average of groups 1 and 2, single dose) because some antibiotic was in the first urine collected with the multiple-dose study.

(iii) Protein binding. Metronidazole was found to be essentially non-protein bound (0.92 ± 1.76%) when tested by ultrafiltration.

(iv) Side effects. No significant biochemical abnormalities were detected before or after the multiple-dose regimens as determined by serum electrolytes, total serum protein, serum albumin, serum calcium, serum bilirubin, blood urea nitrogen, serum creatinine, or serum alkaline phosphatase. In addition, no significant changes occurred in the hematocrit or leukocyte count.

No serious side effects occurred in any of the volunteers. Three experienced headaches of mild to moderate severity, for 3 to 4 h on the first day of the multiple-dose study, that did not
Other side effects noted included a mild gastric upset and a feeling of fatigue and mild diarrhea, in one volunteer each. None of the side effects were severe enough to necessitate cessation of the drug in any volunteer, and they are compatible with those described in the past in patients taking metronidazole.

DISCUSSION

As compared with previous studies, both similarities and some striking differences are apparent from these results. In general, peak blood levels as determined by bioassay with single 250- and 500-mg doses are in the same range as those reported previously with chemical methods (5, 16, 17), i.e., 5 to 6 \( \mu \)g/ml and 10 to 12 \( \mu \)g/ml, respectively. As in most previous studies, the rate of absorption was quite variable in our volunteers, with high blood levels occurring in some within 15 min after ingestion, and peaks as late as 2 to 4 h in others. Surprisingly, the one other study in which blood levels were determined by bioassay showed remarkably uniform absorption, with a peak level of 16 or 17 \( \mu \)g/ml in all volunteers at 3 h with a 500-mg dose (6). An explanation for these differences is not apparent.

In the multiple-dose studies, the blood levels increased progressively for the first several doses but leveled off by the third day, and no further accumulation occurred between 3 and 7 days. Particularly noteworthy was the fact that when 500 mg was given four times daily from 8 a.m. to 8 p.m., the blood level at 8 a.m. on the following day (12 h after the preceding dose) averaged 14 \( \mu \)g/ml and rose to 21 \( \mu \)g/ml just prior to the last dose that day. Maximum blood levels would be about 10 \( \mu \)g/ml higher for each dose. Thus, since most anaerobic bacteria, including \textit{Bacteroides fragilis}, have minimum inhibitory concentrations of 6 \( \mu \)g/ml or lower for metronidazole (7, 13, 17), a 2-\( g \) daily dose should give very favorable blood levels even for severe infections.

From our experiments, there appears to be no binding to serum proteins, although Taylor et al. (14), using a centrifuge-type ultrafiltration method, found metronidazole to be 20% bound. The absence of significant protein binding, and the small size of the molecule, would favor good distribution throughout the various tissue and fluid compartments, and evidence for this has been shown in several studies (3, 8, 9, 15). Furthermore, we assayed cerebrospinal fluid levels of 13.9 and 11.0 \( \mu \)g/ml with simultaneous serum levels of 15.4 and 8.34 \( \mu \)g/ml, respectively, 2 and 8 h after a 500-mg dose in a patient receiving 1 g of metronidazole daily for \textit{Bacteroides} meningitis. Metronidazole was started after 2 weeks of chloramphenicol, which had led to marked improvement. This is very promising therapeutically in view of the number of anaerobes isolated from central nervous system infections (12).

The prolonged blood levels of metronidazole, such as those noted in the morning 12 h after the last dose, are clearly due to the prolonged serum half-life of the drug, averaging 8.7 h in our volunteers. Previous half-life measurements using chemical methods of differing specificity for metronidazole and its metabolites have varied from 6.2 to 13.8 h (14, 16). The low renal clearance of only 10.2 ml/min per 1.73 \( m^2 \), a value not previously determined for this drug to our knowledge, is undoubtedly responsible for this prolonged half-life. Since metronidazole is a small molecule, not protein bound, it would be expected to be filtered freely through the glomeruli, and the low renal clearance may well represent a significant amount of tubular reabsorption. However, since we have not had a parenteral preparation available to measure serum clearance, we cannot be certain how much of the drug is disposed of by some mechanism other than renal clearance.

Of interest in this regard was the recovery of only 15 to 20% of the administered dose in the urine as measured by bioassay. This is in contrast to values of 35 to 65% (5, 11) with chemical methods, indicating that significant portions of the drug are excreted as biologically inactive metabolites. Chemical studies have indicated that approximately 15% of the administered dose appears unchanged in the urine (14), and this further supports the probability that metabolites of the drug are relatively inactive.

The pharmacokinetics of metronidazole are not unlike those of chloramphenicol, a drug used extensively in anaerobic infections (4). Absorption is similar and peak serum levels are slightly lower with chloramphenicol, and both drugs undergo significant biotransformation with excretion of inactive metabolites in the urine. Metronidazole, however, shows no significant protein binding versus 60% for chloramphenicol (10) and has higher sustained blood levels on account of its longer half-life, 8.7 h versus 3 h as determined by bioassay (4).

LITERATURE CITED


2. Bennett, J. V., and W. M. M. Kirby. 1965. A rapid,


