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STEADY-STATE PHARMACOKINETICS OF METRONIDAZOLE IN CROHN'S DISEASE

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ABSTRACT

The pharmacokinetics of metronidazole (MTZ) were studied in six Crohn's disease patients after multiple oral daily doses of 250, 500, 750, and 1000 mg day^{-1}. Pharmacokinetic indices were found to be independent of the dose administered. The half-life, volume of distribution and oral clearance of metronidazole were 9·5 ± 2·1 h, 0·732 ± 0·094 l kg^{-1} and 0·921 ± 0·175 (ml min^{-1}) kg^{-1} (mean ± SD), respectively. A strong linear correlation ($r = 0.95$) was found between the volume of distribution of MTZ and the patients' total body weight. The percentage of dose of metronidazole excreted in urine as the intact drug and metabolites as well as glucuronic acid conjugates ranged from 34·7 ± 7·4 to 58·9 ± 5·2. Both plasma and urine data exhibited very large inter-patient variations. However, intra-patient variations were negligible. Strong positive linear correlations were observed between the dose and the areas under the plasma concentration versus time curves, peak plasma concentrations as well as cumulative urinary excretion of the drug and its metabolites. It is concluded that in Crohn's disease, the pharmacokinetics of MTZ and its metabolites are linear and that the drug concentrations are dependent on the total body weight.

KEY WORDS Metronidazole Crohn's disease Linear pharmacokinetics

INTRODUCTION

The treatment of anaerobic infections, particularly trichomoniasis, improved markedly with the introduction of the prototype nitroimidazole, metronidazole (MTZ), into the market in 1960. Recently, the effectiveness of the drug in the treatment of Crohn's disease has been demonstrated.[1–4] Although in Crohn's disease a wide range of doses of MTZ are administered chronically, only limited information on pharmacokinetics of the drug in this disease condition is available. Following single doses, Melander *et al.*[5] reported a reduced and more variable absorption of the drug in Crohn's patients while Bergan *et al.*[6] observed a 50 per cent greater bioavailability compared to

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normal subjects. The discrepancy in the two studies may, at least in part, be attributed to the non-specific methods of assay used.[7] Very recently, Shaffer *et al.*[8] reported a complete absorption of MTZ in Crohn's disease. Following the administration of repeated doses to healthy subjects[9] changes were observed in the pattern of urinary excretion of MTZ and its metabolites. The half-life ($t_{½}$) and volume of distribution (V_d) of the intact drug remained unchanged.

This study was carried out to assess whether the observed changes in the urinary excretion of the drug and its metabolites observed following repeated administration to healthy subjects also occur in Crohn's patients and also if the changes influence the steady-state pharmacokinetics of MTZ at different dose levels. This is important as the reported side-effects of the drug are reversible and dose-dependent.[10–13] As a result of the reported wide intra- and inter-patient variations in pharmacokinetics of MTZ,[7] it was found necessary for each patient to serve as his or her own control.

METHODS

Materials

Flagyl® tablets and standard laboratory powder of MTZ, hydroxymetronidazole (HM) and metronidazole-1-acetic acid (MAA) were gifts from Rhône-Poulenc, Montreal, Canada. HPLC grade acetonitrile and tetrahydrofuran were purchased from Fisher Scientific Ltd. Tinidazole, antipyrine, ethanol, zinc sulphate, sodium acetate and triethylamine were of analytical grade.

Patients

Approval from the Ethics Review Committee of the University of Alberta Hospitals and patients' written consents were obtained. Six patients (Table 1)

Table 1. Characteristics of patients on first day of study

Patient	Sex	Age (years)	Weight (kg)	Height (cm)	CL_{cr}* (ml min^{-1})	CDAI†
1	M	45	101·5	182·7	124	125
2	F	62	55·7	167·0	40	183
3	M	25	71·0	179·0	130	35
4	M	28	53·7	169·0	96	141
5	M	39	79·4	183·5	119	239
6	F	30	65·0	166·9	78	124

* Creatinine clearance.
† Crohn's disease activity index.

volunteered for the study. Their Crohn's disease activity indices (CDAI) were calculated[14] on each day of sampling whereas creatinine clearance (CL_r) values were estimated on the first day of study only. They had active (CDAI > 150) or inactive (CDAI < 150) Crohn's disease involving the terminal ileum or terminal ileum plus colon. Liver function tests indicated that all patients had normal liver function. No other treatments were allowed during the study.

Study protocol

After a wash-out period of 4 days during which patients did not take any medications and following an overnight fast of at least 8 h each patient ingested one 250 mg MTZ tablet every 24 h for 7 days. Thereafter, the oral dose of MTZ was progressively increased to 500 mg, 750 mg, and 1000 mg per day, the period of each dosage regimen being 7 days. On the 7th day of each dosage regimen, blood was sampled (3 to 5 ml) via an indwelling catheter before dosing and at 0·25, 0·50, 0·75, 1·00, 1·50, 2·00, 3·00, 4·00, 5·00, 6·00, 7·00, 8·00, 9·00, 10·00, 12·00, 15·00, 16·00, and 24·00 h post-dosing. Total urine output was collected for 24 h. Blood samples were centrifuged immediately after collection, and the plasma portion as well as urine samples were kept frozen at −20° until analysis.

HPLC assay

Plasma samples were analysed for MTZ and its two principal metabolites, HM and MAA, by a selective high-performance liquid chromatography (HPLC) method, modified from that described by Jensen and Gugler.[15] To 100 µl aliquot in a 1·5 ml disposable microcentrifuge tube (Eppendorf Sybron/Brinkmann, Rexdale, Canada) was added 50 µl ethanol, 50 µl 0·1 M $ZnSO_4$ and 50 µl internal standard (6·24 mg ml^{-1} Antipyrine in water). The tubes were vortexed for 20 s, centrifuged for 10 min and 20–50 µl of the supernatant injected into an HPLC (Waters, Mississauga, Ontario, Canada) equipped with two pumps (Model 45) an auto-sampler (Model 710B), a variable UV detector (Model 481) set at 313 nm, an integrator (Model 730), a system controller (Model 721), and a reversed phase Nova-pak C-18, 5-micron, radial-pak column of 10 cm length and 8 mm internal diameter. The mobile phase contained tetrahydrofuran (THF) and a solution of 1 per cent acetic acid and 0·1 per cent triethylamine in water. The content of THF was gradually increased from 1 to 4 per cent, 3 min after injection of a sample. It was kept constant for another 3 min and then abruptly reduced to 1 per cent. Under these conditions the retention times of MAA, HM, MTZ and antipyrine were 3·0, 3·9, 5·3, and 9·8 min, respectively. A 5-min re-equilibration period was allowed before the next injection. Standard curves for HM and MTZ, constructed using peak area ratios, were linear ($r > 0·99$) throughout the period of study and the coefficients of variation of the slopes were 6·5 per cent and 6·7 per cent, respectively. The Y-intercept of the lines

were always between −0·047 and 0·065. The detection limit was 0·05 μg ml^{-1} for both compounds.

During the analysis of urine samples, it was observed that the solvent front of some patients' samples seemed to interfere with the MAA peak, thereby making quantitation difficult. A different HPLC method, modified from that reported by Salvesen *et al.*,[16] was therefore used for analysis of those urine samples. It was later decided that all urine samples should be analysed with the same HPLC procedure, resulting in some patients' urine being analysed with both methods. However, the same sample preparation procedure and HPLC system as described above were used except that urine samples (0·2 ml) were diluted six times with 0·075 M phosphate buffer, pH 6·8, prior to preparation for injection and a programmable multi-channel UV detector (Waters, M490) was used in place of the M481 detector. The mobile phase, acetonitrile dissolved in 0·02 M acetate buffer (pH 6·5), was pumped at a flow rate of 1·2 ml min^{-1} and tinidazole (12·5 μg ml^{-1}) was used as internal standard. The content of acetonitrile was increased from 3 per cent to 13·2 per cent 3 min after injection of a sample, kept constant for 7 min and then gradually reduced to 3 per cent in 5 min. The retention times of MAA, HM, MTZ, and tinidazole were 4·0, 8·7, 13·8, and 20·7 min, respectively. The coefficient of variation for slopes of standard curves were 7·2 per cent for MAA, 5·4 per cent for HM and 2·4 per cent for MTZ with the Y-intercepts lying between −0·06 and 0·056. The limit of detection was 0·1 μg ml^{-1} for all three compounds.

For the determination of glucuronides, urine samples were analysed before and after enzymatic hydrolysis of the conjugates. In a preliminary experiment, pure β-glucuronidase and a β-glucuronidase-sulphatase combination (Sigma Chemical Company, St. Louis, USA) were separately incubated with urine at 37° for 24 h. As no significant amount of sulphate conjugates were observed, pure β-glucuronidase was used for hydrolysis of the urinary conjugates. The activities of the enzymes were measured using the procedures recommended by the supplier. An enzyme concentration of 208 units per ml of incubate and an incubation period of 24 h at 37° were found to be the optimum conditions for hydrolysis of the glucuronic acid conjugates.

Treatment of data

The model-independent approach[17] was utilized for calculation of pharmacokinetic parameters. The area under the plasma concentration–time curves (AUCs) were computed from time zero to 24 h (one dosing interval at steady-state) using the linear trapezoidal rule. The volumes of distribution expressed as V_d/F (F, the extent of absorption) were determined by the area method. The oral clearance (CL_o) was computed by dividing the dose by the corresponding AUC. The overall elimination rate constant (β) was calculated from the terminal log-linear portion of the plasma concentration–time curve by linear regression using the least squares method. Renal clearances (CL_r)

were estimated by dividing the cumulative amounts excreted within a dosing interval by the corresponding AUCs.

Statistical analysis of the data was performed utilizing two-way ANOVA and linear regression[18] at the 0·05 level of significance. The peak plasma concentrations (C_{max}) and AUCs were corrected to the first dose (250 mg) before being subjected to two-way ANOVA. Standard deviations were computed as a measure of spread of pharmacokinetic indices about their respective means.

RESULTS AND DISCUSSION

The steady-state plasma concentration–time curves of MTZ and HM, in patient 3, are illustrated in Figure 1 as representative of the sample population. MTZ was absorbed rapidly and peak plasma levels were attained in 2·0 ± 0·7 h. HM was also detectable throughout the 24 h sample collection period but peaked later than the parent drug (t_{max} 7·0 ± 1·7 h). The C_{max} of MTZ ranged from 6·7 ± 1·2 μg ml^{-1} for the 250 mg day^{-1} dose to 23·9 ± 5·1 μg ml^{-1} for 1000 mg day^{-1}. The corresponding values for HM are 1·5 ± 0·4 μg ml^{-1} and 5·4 ± 1·8 μg ml^{-1}, respectively. Schneider *et al.*[19] have reported a positive correlation between serum concentrations of MTZ and CDAI. This relationship was not observed in this study. However, their mean MTZ C_{max} values are comparable to those observed by us. The acid metabolite, MAA, was only detectable in plasma in trace amounts 2 h after administration of 1000 mg MTZ per day.

The V_d/F varied significantly among patients, ranging in value from 39·91 to 78·26 (coefficient of variation, CV = 30·2 per cent). However, after correction for body weight, the CV was reduced to 12·8 per cent (Table 2). Interestingly, a strong linear positive correlation (r = 0·95) between the uncorrected V_d/F and total body weight of the patients was observed (Figure 2). This implies that if the dose of MTZ is based on body weight instead of a fixed amount, more consistent and predictable blood levels will be achieved in patients.

The $t_{1/2}$s of HM were longer and more variable than those of MTZ (23·3 ± 7·0 h vs 9·5 ± 2·1 h). We observed longer $t_{1/2}$ for HM in our patients than those reported in normal subjects.[9,20,21] The sampling period of 24 h used in our study was perhaps not long enough to accurately characterize the β-phase. That not withstanding, the computed $t_{1/2}$s did not show any statistically significant intra-patient variations.

Less than 20 per cent of the dose of MTZ was excreted as the intact drug and less than 10 per cent as its glucuronic acid conjugate (Table 3). The percent of the dose excreted as MAA and unconjugated HM averaged 12·1 ± 4·5 per cent and 17·8 ± 3·9 per cent, respectively. In healthy subjects, however, Jensen and Gugler[9] reported a decrease in urinary excretion of HM

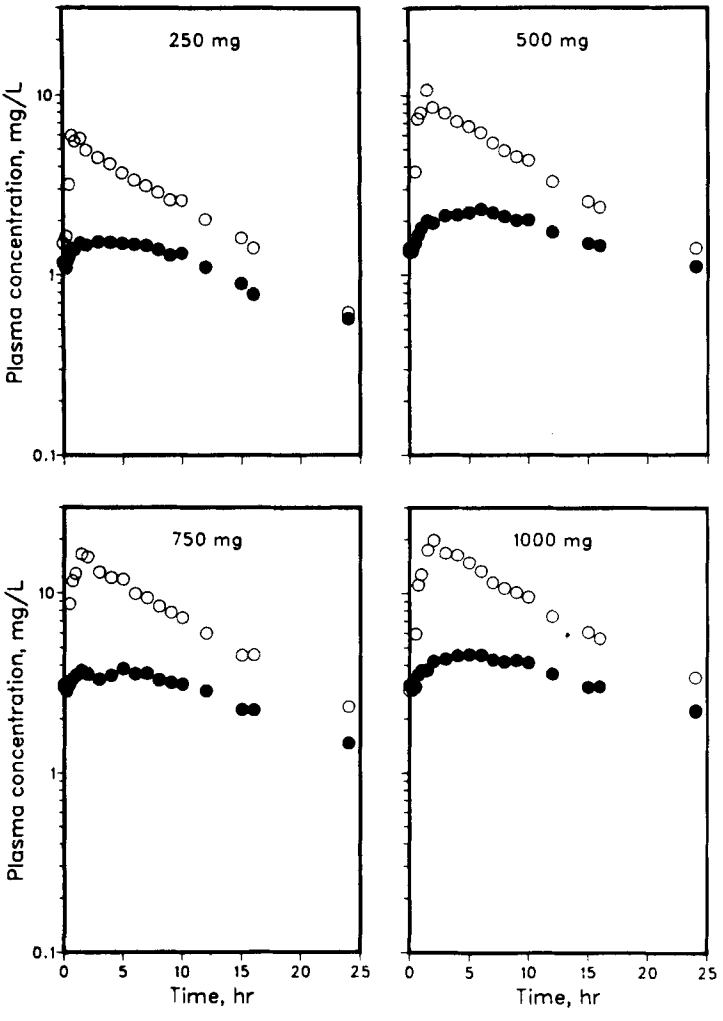

Figure 1. Steady state plasma concentration–time profiles of metronidazole (○) and hydroxy-metronidazole (●) in patient 3

(statistical methods not stated) when the single dose regimen was changed to multiple dosing. They associated this with a decreased metabolic conversion of MTZ to HM. If in fact HM is not further metabolized, their finding may indicate that the MTZ to HM metabolic pathway is saturable. Our results are in disagreement with this finding. Patients were taking progressively higher doses of MTZ for a period of one month and changes in urinary excretion of the drug or its metabolites were not significant.

Table 2. Mean steady-state pharmacokinetic parameters of metronidazole (MTZ) and hydroxy-metronidazole (HM)

Patient	MTZ					HM	
	Vd/F (l kg^{-1})	$t_{½}$ (h)	AUC* (mg l^{-1})h	CL_o (ml min^{-1}) kg^{-1}	CL_r (ml min^{-1}) kg^{-1}	$t_{½}$ (h)	AUC* (mg l^{-1})h
1	0·771	9·6	44·64	0·927	0·113	20·7	21·54
	(0·055)	(0·7)	(2·79)	(0·051)	(0·036)	(4·1)	(3·22)
2	0·645	9·4	96·57	0·794	0·069	21·4	38·96
	(0·069)	(0·7)	(15·01)	(0·114)	(0·016)	(3·5)	(4·00)
3	0·783	8·2	53·60	1·107	0·122	14·3	22·28
	(0·083)	(0·5)	(3·80)	(0·084)	(0·013)	(2·1)	(2·47)
4	0·815	11·5	97·37	0·823	0·094	31·6	25·75
	(0·072)	(1·1)	(2·73)	(0·023)	(0·027)	(17·3)	(2·87)
5	0·765	12·1	74·55	0·733	0·111	32·0	18·98
	(0·044)	(0·7)	(5·70)	(0·055)	(0·021)	(3·1)	(3·10)
6	0·614	6·3	56·57	1·139	0·108	20·0	29·03
	(0·030)	(0·9)	(6·19)	(0·128)	(0·010)	(4·0)	(3·90)
Grand mean	0·732	9·5	70·55	0·921	0·101	23·3	26·09
	(0·094)	(2·1)	(22·11)	(0·175)	(0·025)	(9·5)	(7·33)

* AUCs corrected to 250 mg dose.
SD in parenthesis.

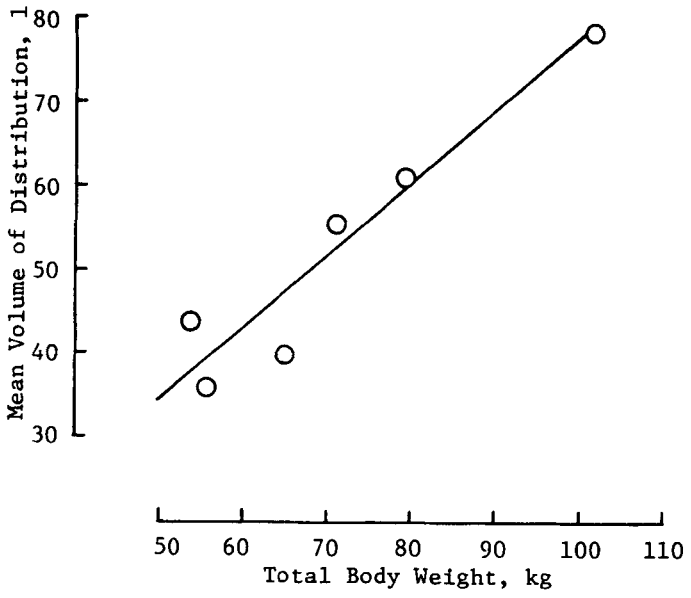

Figure 2. Correlation between the mean steady-state volume of distribution (Vd/F) and total body weight of patients

Table 3. Mean steady-state urinary excretion (expressed as per cent of dose) of metronidazole (MTZ), hydroxy-metronidazole (HM) and metronidazole-1-acetic acid (MAA)

Patient	MTZ		HM		MAA	Total
	Intact	*Glucur*	*Intact*	*Glucur*		
1	12·27	5·54	22·45	3·12	15·14	58·51
	(1·52)	(2·00)	(3·12)	(0·88)	(2·20)	(8·09)
2	10·23	6·03	12·75	3·25	15·66	47·92
	(1·88)	(1·36)	(1·23)	(0·91)	(1·27)	(4·06)
3	13·72	5·82	22·32	1·70	15·30	58·86
	(1·45)	(0·60)	(1·99)	(0·57)	(1·94)	(5·21)
4	11·80	5·88	17·12	2·22	13·95	50·97
	(2·48)	(1·85)	(1·58)	(0·82)	(1·20)	(6·31)
5	18·55	3·94	17·14	1·54	5·75	47·50
	(3·03)	(1·62)	(1·15)	(0·52)	(0·71)	(3·25)
6	9·42	2·29	14·76	1·00	6·92	34·65
	(1·91)	(0·63)	(3·93)	(0·55)	(2·13)	(7·36)
Grand mean	12·66	4·92	17·76	2·13	12·12	49·73
	(3·57)	(1·90)	(4·24)	(1·06)	(4·48)	(9·85)

SD in parentheses.

We found only small amounts of HM as glucuronide in our patients (0·5 to 4·3 per cent of dose). This is in contrast to the observations of Jensen and Gugler[9] in healthy subjects. They reported that up to 12 per cent of the dose was excreted as HM glucuronic acid and sulphate conjugates in 48 h. However, these workers used a combination of β-glucuronidase and sulphatase for the hydrolysis of urinary conjugates, and, therefore, were not able to differentiate between the two conjugates. The presence of sulphate conjugates of HM have been demonstrated only in the urine of mouse, and of both MTZ and HM in urine of rats by Stambaugh *et al.*[22] and Ings and McFadzean,[23] respectively. These workers did not find any sulphate conjugates in urine in man. In this study we used relatively pure sulphatase and also found neither the sulphate conjugates of the intact drug nor of its hydroxymetabolite in urine of the patients.

Pharmacokinetic indices calculated in our patients seem to be close to those reported in normal subjects.[9,20]. However, the results indicate substantial inter-patient variation for all pharmacokinetic indices of MTZ and HM. Greatest inter-patient variations were observed in the $t_{½}$ of HM (CV, 41 per cent) and t_{max} of MTZ (CV, 34 per cent). With respect to $t_{½}$ of MTZ, patient 6 has a relatively small value and can be regarded as an outlier. This patient is a smoker and was not prevented from smoking during the study. It is therefore likely that induction of MTZ-metabolizing enzymes may have occurred in this patient. This is also made manifest in the relatively large CL_o in this patient (Table 2).

Strong positive linear correlations between plasma concentration (C_{max} and AUC) and the dose of MTZ were observed for both MTZ ($r \geq 0{\cdot}98$ and $r \geq 0{\cdot}98$, respectively) and HM ($r \geq 0{\cdot}96$ and $r \geq 0{\cdot}97$, respectively). Plots of the AUCs of MTZ and HM versus orally administered dose of the drug are shown for all six patients in Figure 3. As depicted in Figure 4 the cumulative amounts excreted in urine in one dosing interval at the steady-state also correlated well with the dose of MTZ administered ($r \geq 0{\cdot}97$ for MTZ, $\geq 0{\cdot}91$ for HM, $\geq 0{\cdot}97$ for MAA). These strong linear correlations and the non-significant intra-patient variation of all pharmacokinetic parameters as was demonstrated by two-way ANOVA indicate linearity in pharmacokinetics of MTZ and its metabolites. Our finding that the kinetics of MTZ are linear in these patients also agrees with that reported in normal subjects.[7] Amon *et al.*,[24] using a non-specific polarographic assay, also reported this linearity in female patients infected with *Trichomonas vaginalis* in the dosage range of 250 mg to 2 g.

In conclusion, the pharmacokinetics of MTZ and HM in Crohn's patients is dose-independent within the 250–1000 mg day^{-1} dosage regimen range. Consequently, the dose of the drug may be altered in direct proportion to the desired plasma concentration. Due to the linear relationship between V_d/F and total body weight, it would be proper to administer MTZ to Crohn's patients on a mg kg^{-1} basis.

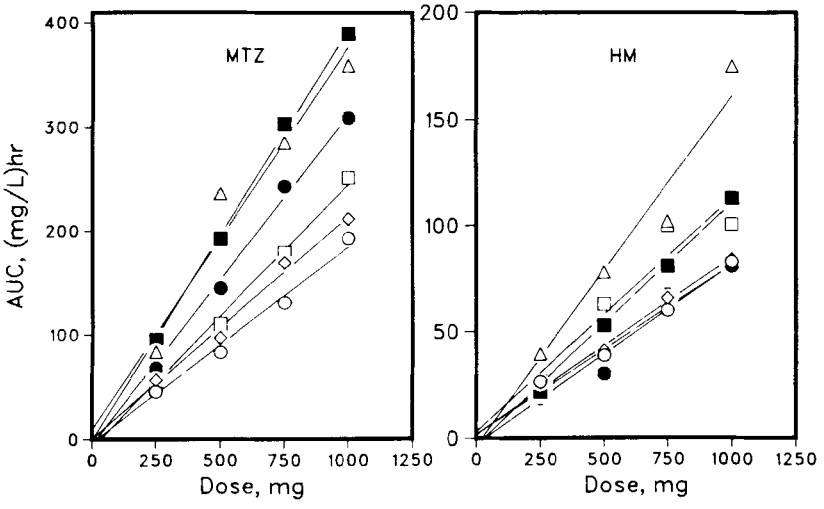

Figure 3. Regression plots of area under plasma concentration–time curves of metronidazole and hydroxy-metronidazole versus dose of metronidazole for patients 1 (○), 2 (△), 3 (◇), 4 (■), 5 (●), and 6 (□)

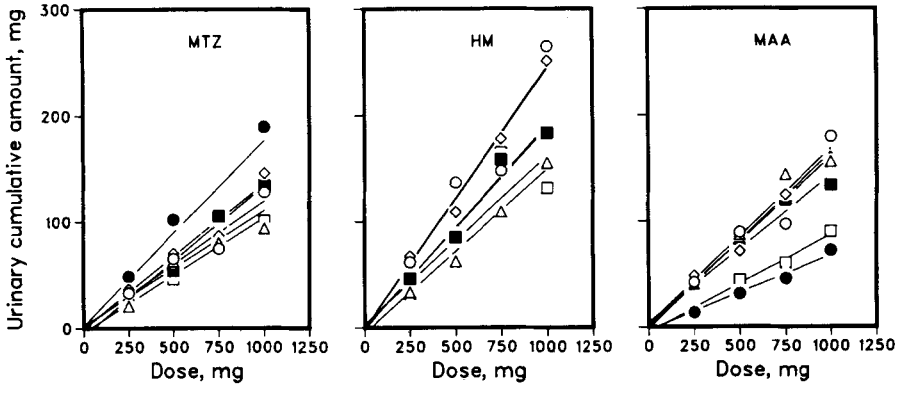

Figure 4. Regression plots of steady-state cumulative urinary excretion of metronidazole, hydroxy-metronidazole and metronidazole-1-acetic acid versus dose of metronidazole for patients 1 (○), 2 (△), 3 (◇), 4 (■), 5 (●), and 6 (□)

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