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THE METABOLISM OF METRONIDAZOLE (1-2′-HYDROXYETHYL-2-METHYL-5-NITROIMIDAZOLE)

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Abstract—The metabolism of metronidazole has been studied in man and dog, and was similar in both species. The urine contained unchanged metronidazole (I), the corresponding acid, 2-methyl-5-nitroimidazole-1-ylacetic acid (II), and a second metabolite which has been tentatively identified as the ether glucuronide of metronidazole (III).

METRONIDAZOLE (1-2′-hydroxyethyl-2-methyl-5-nitroimidazole) shows a wide range of anti-protozoal activity and in particular has found widespread use in the oral treatment of trichomoniasis.[1–5] We thought it worthwhile to study the metabolism of this drug in man and dog with the hope of throwing some light on its mode of action.

EXPERIMENTAL

Administration of the drug

Beagle dogs were dosed by stomach tube with 100 mg/kg of the drug. A human volunteer was given a single oral dose of 1 g.

Collection of samples

Urine samples were taken from dogs by catheterization 6 hr after dosage and combined with urine eliminated during this period collected in a metabolism cage. The urine excreted by the human volunteer was collected during the 9-hr period following dosage. Control urine samples were collected prior to administration.

Estimation of nitroimidazoles

The Bratton and Marshall[6] colorimetric reaction was used to estimate nitroimidazoles, after reduction to the corresponding amines, in urine and eluates from chromatograms. A sample of suitable volume was diluted with hydrochloric acid to give 20 ml 1·0 N solution, and the nitro compound was reduced by the addition of 1 g of zinc dust. After 30 min the mixture was filtered and the colormetric reaction carried out on 10 ml of the filtrate. The optical densities were measured on a Unicam S.P.500 spectrometer at 510 mμ.

Chromatography and detection of metabolites

Chromatography was carried out on urine samples which had been concentrated to a small volume at 30° under reduced pressure. Chromatograms were developed on

Whatman No. 1 paper using the descending technique with *n*-propanol–0·88 ammonia (7:3) as the solvent.

The nitroimidazoles were conveniently detected by viewing the chromatograms under u.v. light of wavelength 254 mμ when the compounds quenched the background fluorescence of the paper. They could also be detected by a diazotisation reaction after reduction of the nitro group. The following reagents were used in succession: (a) 15% titanous chloride in N-hydrochloric acid, diluted 1:10, (b) amyl nitrite: *n*-butanol:acetic acid (10:5:1) and (c) 0·1% N-(1-naphthyl)ethylene diamine dihydrochloride in *n*-butanol:2N-hydrochloric acid (95:5). Nitroimidazoles gave pink spots.

Identification and estimation of metabolites

The metabolites were eluted from the chromatograms with methanol and the eluates were concentrated and re-chromatographed in several solvent systems in comparison with reference compounds (see Table 1). For quantitative estimations methanol eluates were evaporated to dryness, the residue was dissolved in a suitable amount of water and the colormetric reaction was carried out as described above.

Semi-quantitative estimations of glucuronides were carried out on eluates from chromatograms using the method of Paul.[7]

Isolation of metabolites

The urine from a dog dosed with metronidazole was passed through an ion-exchange column of 800 ml Amberlite resin IR45 (OH^- form) and the column was washed with 800 ml of water. The column was then eluted with 2000 ml of 0·5 N hydrochloric acid and finally washed with 400 ml of water. The major part of the nitroimidazoles was contained in the latter fractions and washings which were evaporated to dryness to give 1·6 g of a gummy residue. This was resolved by dry column chromatography.[8] The gum was dissolved in 15 ml of a mixture of equal parts of pyridine, *n*-butanol and water, and absorbed on to 15 g of micro-crystalline cellulose (Avicel). This was applied to the top of a column of 320 g of dry cellulose contained in a glass tube of 8·0 cm diameter lined with polythene sheet, and the column developed with the pyridine:*n*-butanol:water mixture until the solvent front reached the bottom of the column. The column was extruded by sliding out the polythene liner, and the metabolites detected by viewing under u.v. light.

Compounds

2-Methyl-5-nitroimidazol-1-ylacetic acid was synthesized as follows: a solution of 3·4 ml of chromic acid solution (prepared from 54 g of chromium trioxide and 47 ml of concentrated sulphuric acid and made up with water to 200 ml) was added drop by drop with stirring to a suspension of 1·51 g of 1-2′-hydroxyethyl-2-methyl-5-nitroimidazole in 15 ml of hot water. An exothermic reaction occurred and the solution boiled. When this had subsided, the green solution was heated for five minutes on a steam-bath, cooled and repeatedly extracted with boiling ethyl acetate. The combined extracts were dried and evaporated giving 0·9 g of the crude acid which was recrystallized from ethanol–light petroleum (b.p. 40–60°) affording 0·5 g of the pure acid, m.p. 179–180°. (Found: C, 39·2; H, 4·2; N, 23·1. $C_6H_7N_3O_4$ requires C, 38·9; H, 3·78; N, 22·7.)

The acid on reaction with thionyl chloride in benzene solution, followed by treatment of the crude acid chloride with aniline, yielded an anilide which crystallized from ethanol with m.p. 186–187°. (Found: C, 55·6; H, 4·9; N, 22·1. $C_{12}H_{12}N_4O_3$ requires C, 55·35; H, 4·6; N, 21·5).

RESULTS

Excretion of metronidazole

In two dogs receiving 100 mg/kg of the drug, recoveries of nitroimidazoles (calculated as metronidazole) were 38 and 31 per cent of the administered dose in the first 6 hr. In a human volunteer receiving 1 g of the drug, the recovery was 17 per cent in the 9 hr after administration.

Metabolism of metronidazole

Chromatography of the 6-hr urine of dogs receiving the drug revealed three compounds which were not present in the control urine sample. The R_f values of these compounds in *n*-propanol:ammonia (7:3) were 0·36, 0·53, and 0·80 and these were designated metabolites A, B and C respectively. Chromatography of the 9-hr urine from the human volunteer gave identical results. Re-chromatography of the metabolites B and C in several solvent systems in comparison with reference compounds (see Table 1) showed that they were chromatographically identical with 2-methyl-5-

TABLE 1. CHROMATOGRAPHY OF METABOLITES OF METRONIDAZOLE AND REFERENCE COMPOUNDS

Solvent systems used were (1) *n*-propanol:ammonia (7:3), (2) iso-propanol:ammonia:water (200:10:20), (3) *n*-butanol:pyridine:water (1:1:1), (4) t-butanol:water:methyl ethyl ketone:diethylamine (80:80:40:8), (5) *n*-butanol:formic acid:water (77:10:13).

Compound	R_f values in solvent					Detection	
	1	2	3	4	5	u.v. light	diazo reaction
Metabolites							
A	0·36	—	—	—	—	quench	pink
B	0·53	0·31	0·59	0·78	0·78	quench	pink
C	0·80	0·82	0·89	—	0·77	quench	pink
Reference Compounds							
Metronidazole (I)	0·80	0·82	0·89	—	0·77	quench	pink
2-methyl-5-nitro-imidazol-1-yl acetic acid (II)	0·53	0·31	0·59	0·75	0·78	quench	pink

nitroimidazol-1-ylacetic acid (II) and unchanged drug (I) respectively. Eluates of metabolite A gave strongly positive reactions for glucuronide while those prepared from chromatograms of control urine at the same R_f value as that of metabolite A gave negative results.

Viewing of the dry column chromatogram of the purified urine under u.v. light revealed three bands corresponding to metabolites A, B and C. The band containing metabolite C was broken up and extracted with boiling methanol. The combined extracts were evaporated and the residue was recrystallized from ethyl acetate and finally from water to give a substance m.p. 152° undepressed on admixture with

authentic metronidazole (I), and having an i.r. spectrum identical with that of the latter. The band containing metabolite B was extracted with methanol in a similar manner. The combined extracts were evaporated to dryness, the residual gum was dissolved in 20 ml of water and passed through a column of 20 ml of Amberlite resin IR50 (H^+ form). The column was washed with 50 ml of water, the combined eluates were evaporated to dryness and the residue was dissolved in 5 ml of water. The aqueous solution was extracted with 6 × 5 ml of ethyl acetate. The dried combined extracts were evaporated to 2 ml, clarified with charcoal and evaporated to the point of incipient crystallization. After ice-cooling the crystals m.p. 173·5–175° were filtered off and re-crystallized from ethyl acetate to give a substance m.p. 178·5–179° identical with that of, and undepressed by, 2-methyl-5-nitroimidazol-1-yl acetic acid (II); the i.r. spectra were also identical. All efforts to isolate metabolite A from the third u.v. absorbing band failed and invariably resulted in formation of metronidazole.

Quantitative estimations of nitroimidazoles in the eluates from paper chromatograms demonstrated that in the dog 61 per cent of the excreted drug was unchanged, 28 per cent was the acid (II), and 11 per cent was eliminated as a glucuronide conjugate (III). In man the comparable figures were 69, 26 and 5 per cent.

DISCUSSION

Metronidazole exhibited identical metabolic patterns in dog and man, and in both cases 60–70 per cent of the excreted drug was unchanged. The major metabolic pathway was one involving oxidation of the hydroxy group, leading to the carboxylic acid. A conjugate of glucuronic acid was also formed which is believed to be the ether conjugate of metronidazole, 2-(2-methyl-5-nitroimidazol-1-yl)ethyl-α-D-glucopyranosiduronic acid (III), because of its facile breakdown to metronidazole. The metabolism of metronidazole is summarized in the Fig. 1.

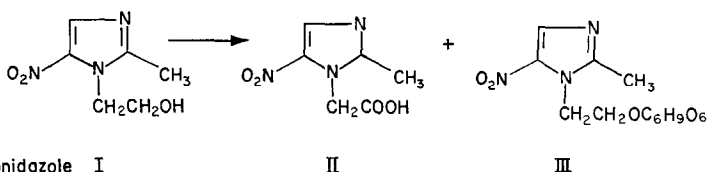

FIG. 1. Urinary excretion of metronidazole in dog and man.

Proportion in urine			
Dog*	61%	28%	11%
Man	69%	26%	5%

Although nitro compounds are frequently reduced in mammals, it is of interest to note that we did not find any evidence of reduction of the nitro group in metronidazole.

Manthei[9–11] and his co-workers in their work on the metabolism of metronidazole claimed to have detected chromatographically six metabolites in the urine of patients receiving the drug. These included (a) unchanged metronidazole, (b) a glucuronide, (c) an acid "probably that derived by oxidation of metronidazole" (d) the glycine conjugate of (c), (e) an azobis derivative of metronidazole, and (f) a non-nitro compound formed by the cyclization of the acidic side-chain of (c). There seemed to be

* Mean percentages for 2 dogs.

considerable variation in the excretion pattern from patient to patient; not all of the metabolites appeared in each case. These workers did not isolate any of the metabolites in a pure state and the identification rested mainly on colour reactions. Two of the putative metabolites (c) and (e) were, however, shown to be chromatographically identical with products present in crude reaction mixtures. We have positively identified the putative metabolites (a) and (c), and have also detected the glucuronide (b). No evidence was obtained for the presence of the other substances but this may be because we did not examine enough samples.

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