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Metronidazole Bioassay

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Urine from patients receiving metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitromidazole] orally or per vagina were subjected to paper chromatographic fractionation and examined for anticlostridial activity. Unmodified metronidazole and several metabolites were detected. These findings indicate that the clostridial bioassay may be limited in its applicability to the study of the pharmacodynamics of metronidazole as it does not differentiate between the parent substance and some of its metabolites. Moreover, some of the latter, although they possess antibacterial activity, may not have antiprotozoan activity.

Metronidazole was initially described in 1959 as an agent with specific trichomonicidal activity (2). This compound has subsequently proven to be effective in amebiasis and in several other parasitic infections (3, 4, 6). Recently metronidazole was found to be active in vitro against anaerobic microorganisms, and experience is being accumulated on the use of this agent in anaerobic infections (1, 5, 10). The metabolism of metronidazole and the basis of its activity are still poorly understood. Recently a bioassay utilizing *Clostridium* was described as being useful for determining the concentration of metronidazole in body fluids (7). The purpose of this report is to present findings which suggest that this bioassay may be restricted in its usefulness.

MATERIALS AND METHODS

Urine specimens. The urine specimens were obtained from patients who had received metronidazole (Flagyl, Amersham/Searle, Arlington Heights, Ill.) for a period in excess of 24 h. First-voided morning specimens were obtained, sterile filtered, and stored in the cold. Ten specimens were used in this study, eight from patients with vaginitis receiving 250 mg orally three times a day, one from a vaginitis patient treated per vagina 250 mg three times a day, and one from a patient with an amebic abscess receiving 750 mg orally three times a day.

Bioassay. For bioassay only recent clinical isolates of *C. perfringens* were used. There were no differences among the clostridial strains with respect to susceptibility to metronidazole or its metabolites. Blood agar plates were inoculated with approximately 10^7 microorganisms in 0.1 ml. When the surface of the agar had dried, filter papers impregnated with urine were deposited on the surface of the plates, and these were incubated anaerobically (Gas-Pak system, BBL, Cockeysville, Md.) at 37 C for 18 h, whereupon zones of growth inhibition were measured.

Paper chromatography. Quantities (100 μl) of urine were applied across 15 cm of Whatman 3MM paper, and descending chromatography was carried out with 1-butanol saturated with water (86:14). Development was allowed to proceed for a minimum of 45 cm. The chromatogram was then dried and inspected under an ultraviolet lamp, and areas (2 by 2 cm) were excised. These were numbered and placed on the surface of blood agar plates inoculated with *C. perfringens*. The plates were handled as described above, and zones of growth inhibition were measured.

RESULTS

Anticlostridial activity could be demonstrated in each of the 10 urines, including the one from the patient receiving metronidazole per vagina. Paper chromatographic analysis, however, revealed that anticlostridial activity was associated not only with unmodified metronidazole (R_f = 0.77 to 0.91) but with several other substances differing in rates of migration (R_f = 0.10 to 0.18, 0.35 to 0.43, and 0.68 to 0.90). Some of the rates of migration are in the same range as those reported by others (9) for known urinary metabolites of metronidazole. However, the identity of these substances remains to be established. These activities were absent in "normal" urine.

DISCUSSION

Stambaugh et al. (8) demonstrated that the metabolism of metronidazole resulted primarily in the oxidation of the 2-methyl group to the corresponding hydroxymethyl derivative and that this substance together with unmodified metronidazole accounted for 60 to 70% of the

total nitro-containing compounds excreted in the urine of human subjects after oral administration. Subsequently four additional nitro-containing urinary metabolites were identified (9). Using paper chromatographic procedures, we were able to demonstrate anticlostridial activity corresponding to the rates of migration of at least three of the previously identified urinary metabolites. In addition, biological activity was also recovered in an area of the chromatogram which does not correspond to a previously identified metabolite of metronidazole.

The presence in urine and presumably other body fluids of more than one metabolite with biological activity limits the usefulness of the clostridial bioassay for metronidazole. This limitation must be considered when interpreting data on the pharmacokinetics of this drug when such information is based on the clostridial bioassay procedure. Moreover, it remains to be established whether these metabolites also possess antiparasitic activity before data obtained with this bioassay can be extrapolated to pharmacodynamics involving protozoan infection.

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