

Pharmacokinetics of metronidazole and its principal metabolites and their activity against *Gardnerella vaginalis*

C S F EASMON,* C A ISON,* C M KAYE,‡ R M TIMEWELL,‡ AND S G DAWSON†
From the Departments of *Medical Microbiology and †Genitourinary Medicine, St Mary's Hospital Medical School, London, and the ‡Clinical Research Department, May & Baker Ltd, Dagenham, Essex

SUMMARY The hydroxy metabolite of metronidazole was found to be more active against 21 strains of *Gardnerella vaginalis* than the parent compound and less affected by culture in carbon dioxide. After 400 mg oral metronidazole (Flagyl) plasma concentrations of the two agents were below the minimum inhibitory concentrations (MICs) for most *G vaginalis* strains tested. With 2 g metronidazole the plasma concentrations exceeded the MICs of the more sensitive strains. Even with the lower dose of metronidazole clinically useful concentrations of metronidazole and its hydroxy metabolite were present in the urine. Urinary excretion of these compounds may contribute to the efficacy of metronidazole in the treatment of vaginitis associated with *G vaginalis*.

Introduction

Although metronidazole is active against most obligate anaerobes it has little useful clinical activity against facultative bacteria. One exception is *Gardnerella vaginalis*, a facultative Gram-variable bacillus strongly associated with non-specific vaginitis.¹ While metronidazole is only moderately active against *G vaginalis* in vitro (reported minimum inhibitory concentrations (MICs) ranging from 2 mg/l to >50 mg/l) it is very effective in vivo against vaginitis associated with *G vaginalis*.^{2,3} Several hypotheses have been put forward to explain this discrepancy. Obligate anaerobes may play some part, indeed possibly the major part, in the aetiology of gardnerella-associated vaginitis, either by providing essential nutrients or by inhibition of host defence mechanisms. The efficacy of metronidazole is, therefore, related to its effect on these organisms rather than on *G vaginalis*.⁴ Furthermore, metronidazole may accumulate in the vagina and reach clinically useful concentrations, or the conditions of in-vitro sensitivity testing may not truly reflect those in vivo.³ Another very attractive theory is based on the finding that the 2-hydroxy-methyl metabolite of metronidazole was very much more

active against *G vaginalis* than the parent compound.⁵⁻⁷ This hydroxy methyl metabolite is a major breakdown product of metronidazole in man and the two compounds have been detected in vaginal secretions.⁸

In this study we have examined the sensitivity of 20 clinical isolates and one stock strain of *G vaginalis* to metronidazole and its two major metabolites in vitro. We have also determined by high-performance liquid chromatography the concentrations of these compounds in the plasma of volunteers after 400-mg and 2-g doses of oral metronidazole and have examined the inhibitory and bactericidal activity of the same plasma samples against *G vaginalis* isolates of varying in-vitro sensitivity.

Materials and methods

BACTERIA

G vaginalis NCTC 10915 was used as the control strain. Twenty-one strains from patients with vaginitis were isolated on Columbia agar supplemented with human blood (5% v/v) amphotericin B 2 mg/l, gentamicin sulphate 4 mg/l, and nalidixic acid 30 mg/l.⁹ They were identified by differential haemolysis (β -haemolysis on human but not on horse blood agar), Gram-stain morphology, oxidase and catalase reactions, and fermentation of starch, maltose, and glucose.⁹ Strains were stored in liquid nitrogen.

Address for reprints: Dr C S F Easmon, Department of Medical Microbiology, St Mary's Hospital Medical School, London W2 1PG

Accepted for publication 29 March 1982

The activity of the hydroxy metabolite of metronidazole against *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus*, and group B streptococci was also investigated.

ANTIMICROBIAL AGENTS

Metronidazole and its 2-hydroxymethyl and 1-acetic acid metabolites were provided as pure powder by May & Baker Ltd.

Minimum inhibitory concentrations (MICs) were determined by the agar dilution techniques using human blood agar and a multipoint inoculator (Denley). The inoculum size was 10^6 colony-forming units (cfu)/ml. Plates were incubated at 37°C for 48 hours, both in 7% CO₂ and anaerobically using the Gaspak system (BBL).

PHARMACOKINETIC STUDIES

Four volunteers were given a single oral dose of 400 mg metronidazole. Plasma concentrations of metronidazole and its two principal metabolites were measured three, six, and 24 hours later and urine concentrations 24 hours later by specific high-performance liquid chromatography.¹⁰ One patient undergoing treatment for giardiasis (metronidazole 2 g daily for three days) also provided blood for plasma concentrations of the three compounds three and 24 hours after each dose.

The inhibitory and bactericidal activity of the plasma samples was determined against six *G vaginalis* isolates of different sensitivity. Doubling dilutions of plasma were made in Isosensitest broth supplemented with 10% heat-inactivated horse serum. The bacterial inoculum was 10^5 cfu/tube. After incubation at 37°C for 48 hours, tubes showing no visible growth were subcultured on human blood agar for a further 48 hours at 37°C in 7% CO₂.

Results

SUSCEPTIBILITY OF *G VAGINALIS* TO METRONIDAZOLE AND ITS METABOLITES

Figs 1 and 2 show the cumulative MICs of metronidazole and its hydroxy metabolite for *G vaginalis* NCTC 10915 and 20 strains grown anaerobically or in 7% CO₂. For all strains the MIC of the acid metabolite was >64 mg/l. The hydroxy metabolite was consistently more active against *G vaginalis* than metronidazole itself and its activity less affected by culture in CO₂ rather than by anaerobic culture.

Table I shows that the hydroxy metabolite of metronidazole was not active against *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus*, or group B streptococci.

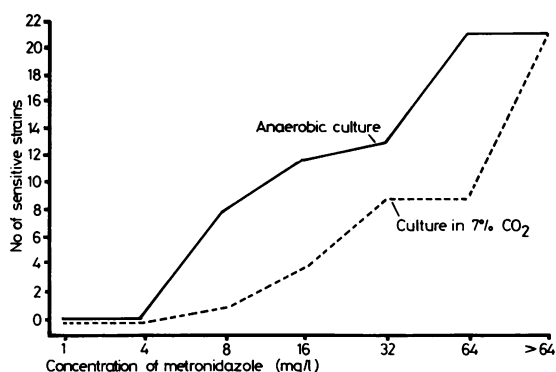


FIG 1 Cumulative MIC of metronidazole for 21 isolates of *G vaginalis* cultured anaerobically and in 7% CO₂.

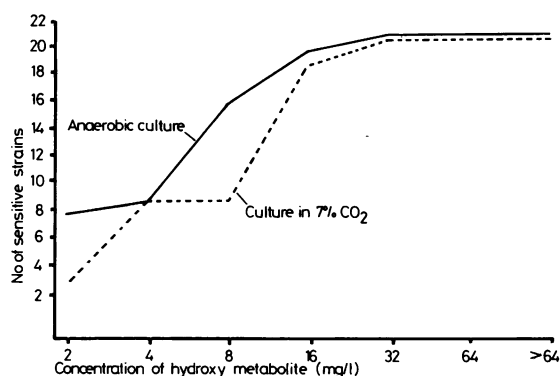


FIG 2 Cumulative MIC of hydroxy metabolite of metronidazole for 21 isolates of *G vaginalis* cultured anaerobically and in 7% CO₂.

TABLE I Susceptibility of facultative bacteria to metronidazole and hydroxy metabolite

Organism	Minimum inhibitory concentration (mg/l)			
	Metronidazole		Hydroxy metabolite	
	AnO ₂	CO ₂	AnO ₂	CO ₂
Group B streptococcus	>256	>256	>256	>256
<i>Staphylococcus aureus</i>	>256	>256	>256	>256
<i>Escherichia coli</i>	128	>256	128	>256
<i>Klebsiella aerogenes</i>	128	>256	128	>256

AnO₂ = anaerobic

PLASMA AND URINARY CONCENTRATIONS

Four volunteers each received 400 mg metronidazole orally. Plasma concentrations of metronidazole and its hydroxy metabolite three, six, and 24 hours later are shown in table II. No acid metabolite was detected in any of the plasma samples. The plasma

TABLE II *Plasma concentrations of metronidazole and its hydroxy metabolite in four subjects three, six, and 24 hours after 400 mg oral metronidazole. (No acid metabolite detected.)*

Subject No	Plasma concentrations (mg/l) at (hours):					
	Metronidazole			Hydroxy metabolite		
	3	6	24	3	6	24
1	7.0	5.2	1.0	1.0	1.3	0.7
2	8.1	6.0	1.1	0.7	0.8	0.8
3	8.3	6.4	1.3	0.6	0.9	0.7
4	13.0	9.6		1.0	1.4	

concentration of metronidazole fell steadily over 24 hours, but the hydroxy metabolite remained roughly constant. At three hours the hydroxy metabolite represented 9% of the total drug; at 24 hours it represented nearly 40%. The urinary concentrations of the parent compound and its major metabolites in samples obtained 24 hours after are shown in table III. The urinary concentrations of the hydroxy and acid metabolites were greater than those of metronidazole.

Table IV shows the plasma concentrations over four days in a patient taking 2 g metronidazole a day orally for three days. No acid metabolite was detected in the plasma. Three hours after the antibiotic had been taken plasma concentrations of

TABLE III *Urine concentrations of metronidazole and its principal metabolites 24 hours after 400 mg oral metronidazole*

Subject No	Urine concentrations (mg/l)		
	Metronidazole	Hydroxy metabolite	Acid metabolite
1	4.3	10.8	6.0
2	7.9	48.0	33.6
3	6.1	35.0	24.4
4	7.3	38.0	57.0

TABLE IV *Plasma concentrations of metronidazole and its hydroxy metabolite during a three-day course of oral metronidazole 2 g daily. (No acid metabolite detected.)*

Day	Plasma concentrations (mg/l)	
	Metronidazole	Hydroxy metabolite
1		
Three hours after antibiotic	50.4	4.3
2		
Before antibiotic	12.7	4.5
Three hours after antibiotic	48.4	6.8
3		
Before antibiotic	10.2	5.0
Three hours after antibiotic	47.0	8.1
4		
	11.8	6.5

metronidazole were between 47 mg/l and 50 mg/l falling to between 10.2 mg/l and 12.7 mg/l by 24 hours. The plasma concentration of hydroxy metabolite, although much lower than that of metronidazole, showed little variation between the concentrations at three hours and 24 hours. Multiple dosages gave rise to a steady increase in metabolite concentrations over four days, comprising about 10% of total drug at three hours rising to about 30% at 24 hours. These results parallel those seen after a 400-mg dose of metronidazole.

INHIBITORY AND BACTERICIDAL EFFECTS ON *G VAGINALIS*

None of the plasma samples taken after the 400-mg dose of metronidazole either killed or inhibited the growth of any of the *G vaginalis* isolates or strains tested. Plasma samples taken three hours after the 2-g dose inhibited and killed two isolates, with MICs of 2 mg/l for the hydroxy metabolite and 8 mg/l for metronidazole, at a dilution of 1/8. Plasma taken 24 hours after the 2-g dose killed one of these two isolates at a dilution of 1/8 but neither inhibited nor killed the other strain. The other four strains, including the NCTC control, were unaffected by any of the plasma samples.

Agglutination was seen with higher concentrations of some plasma samples and *G vaginalis*.

Discussion

We agree with other workers,⁵⁻⁷ who found that while metronidazole was moderately active against *G vaginalis* its hydroxy metabolite was far more so. Gardnerellas are alone among facultative bacteria in being so sensitive to these agents. Whether *G vaginalis* shares with obligate anaerobes the capacity to reduce metronidazole¹¹ and whether the greater activity of the hydroxy metabolite is related to greater uptake or more effective killing remains to be determined. It does seem likely that the greater activity of the hydroxy metabolite against *G vaginalis* is specific for this organism as we did not find it to be any more active than metronidazole against other facultative bacteria. Ralph and Amatnieks⁵ found it to be less active than metronidazole against *Bacteroides* spp.

MICs of metronidazole were generally lower when tested anaerobically than in CO₂ but this difference was less noticeable with the hydroxy metabolite. Our MICs are somewhat higher than those reported by Ralph and Amatnieks.⁵

Three hours after ingestion the metabolite accounted for about 10% of total drug in the plasma rising to 30-40% at 24 hours. This increase in the proportion of the metabolite compared with a steady

fall in the concentration of metronidazole over the same time.¹⁰ There was some evidence of a steady rise in concentrations of hydroxy metabolite in the patient receiving metronidazole for three days. The hydroxy metabolite was the major product in the urine, with high concentrations being reached 24 hours after a 400-mg dose of metronidazole. After 400 mg metronidazole the plasma concentrations of both compounds were lower than the MICs for most *G vaginalis* strains. With the 2-g dose, however, plasma concentrations of these agents were above the MICs for the more sensitive strains. The urine concentrations of the hydroxy metabolite were far in excess of the MICs of most strains tested. The effect of plasma samples on *G vaginalis* was that expected from these results. It is possible that a five-day course of metronidazole 400 mg twice daily might have a cumulative effect resulting in concentrations rather higher than we obtained. If the sensitivities of our 20 clinical isolates are representative and if the concentrations in vaginal secretions equal those in serum, however, it must be doubtful whether this would be effective directly against many strains of *G vaginalis*.

A treatment regimen based on 500 mg metronidazole twice daily does appear to be effective in *G vaginalis*-associated vaginitis.⁴ If *G vaginalis* is the primary pathogen and if the success of metronidazole treatment is not based on its effect on accompanying anaerobes, how then can this success be explained? There are three possibilities. Firstly, the antibiotic could be concentrated within vaginal secretions to a much higher degree than in serum. Secondly, antibiotic excreted in the urine may play a major part in killing vaginal pathogens. Even 24 hours after a single 400-mg dose of metronidazole the urinary concentrations of hydroxy metabolite in three of the four subjects were above the MICs of all *G vaginalis* strains tested. The fourth subject had lower concentrations, but as total urine volumes were not measured the amount excreted is not known. Thirdly, concentrations of antibiotic below the MIC may still affect the pathogenesis of the infective process.

G vaginalis appears to be primarily a pathogen of mucosal surfaces. Adhesion to vaginal epithelium is likely to play an important part in the infection; indeed, the clue cell, an epithelial cell covered with adherent gardnerellas, is a diagnostic feature of the condition. Relatively low concentrations of metronidazole or its hydroxy metabolite could affect adherence to cells. A similar phenomenon has been observed with other bacteria¹² and deserves further investigation. Although we have not measured drug

concentrations after an 800-mg dose of metronidazole, it is likely that those reached on a regimen of 800 mg twice daily would directly inhibit many *G vaginalis* strains.

The higher dosage of 2 g does appear to give serum concentrations of the hydroxy metabolite which are directly active against many strains of *G vaginalis*. Single-dose treatment is more acceptable to patients because of the bitter taste of metronidazole and its possible effects when combined with alcohol.

Urethral carriage of *G vaginalis* in male partners may be important in the epidemiology of vaginitis.¹³ The high concentrations of hydroxy metabolite in the urine suggest that metronidazole should be very effective in eradicating such carriage possibly after only one or two doses.

We thank Mr M G Sankey and Mrs J E Holt for excellent technical assistance.

References

1. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis: a newly defined specific infection previously classified "non-specific" vaginitis. *Am J Obstet Gynecol* 1955;69:962-76.
2. Pfeifer TA, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Role of *Haemophilus vaginalis* and treatment with metronidazole. *N Engl J Med* 1978;298:1429-34.
3. Balsdon J, Taylor GE, Pead L, Maskell R. *Corynebacterium vaginale* and vaginitis: a controlled trial of treatment. *Lancet* 1980;i:501-4.
4. Spiegel CA, Amsel R, Eschenbach D, Schoenkecht F, Holmes KK. Anaerobic bacteria in non-specific vaginitis. *N Engl J Med* 1980;303:601-7.
5. Ralph ED, Amatnieks YE. Relative susceptibilities of *Gardnerella vaginalis* (*Haemophilus vaginalis*), *Neisseria gonorrhoeae* and *Bacteroides fragilis* to metronidazole and its two major metabolites. *Sex Transm Dis* 1980;7:157-60.
6. Balsdon J, Jackson D. Metronidazole metabolite and *Gardnerella vaginalis* (*Corynebacterium vaginale*). *Lancet* 1981;i:1112.
7. Shanker S, Munro R. Sensitivity of *Gardnerella vaginalis* to metabolites of metronidazole and tinidazole. *Lancet* 1982;ii:167.
8. Mathei RW, Feo LG, Stambaugh JE. Identification of the metabolites of metronidazole in the human vagina. *Wiad Parazytol* 1969;15:403-5.
9. Ison CA, Dawson SG, Hilton J, Csonka G, Easmon CSF. A comparison of culture and microscopy in the diagnosis of *Gardnerella vaginalis* infection. *J Clin Pathol* 1981; in press.
10. Kaye CM, Sankey MG, Thomas LA. A rapid and specific semi-micro method involving high-pressure liquid chromatography for the assay of metronidazole in plasma, saliva, serum, urine and whole blood. *Br J Clin Pharmacol* 1980;9:528-9.
11. Muller M. Mode of action of metronidazole on anaerobic micro-organisms. In: Phillips I, Collier J, eds. *Metronidazole. Proceedings of the 2nd International Symposium on Anaerobic Infections*. London: Royal Society of Medicine Academic Press, Grune & Stratton, 1979.
12. Alkan ML, Beachey EH. Excretion of lipoteichoic acid by group A streptococci. Influence of penicillin on excretion and loss of ability to adhere to human oral mucosal cells. *J Clin Invest* 1978;61:671-7.
13. Dawson SG, Ison CA, Easmon CSF. Vaginitis revisited. *Br Med J* 1981;ii:1333-4.