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Review article

Treatment failure in *Trichomonas vaginalis* vaginitis

The clinical introduction in 1960 of the 5-nitroimidazole, metronidazole, the first compound specifically and systemically effective against *Trichomonas vaginalis*,[1] led to spectacular 86–100% cure rates in patients with trichomonal vaginitis, instead of the uncertain results previously obtained with various locally administered substances. Other nitroimidazoles—nimorazole, tinidazole, ornidazole, secnidazole, carnidazole—have since been introduced into clinical practice. Not all are equally available, however, and a review did not show a clear advantage for any particular one, as cure rates for all were in the range of 85–95% and symptoms were relieved within a few days.[2] Metronidazole is the most extensively used and, although the others differ somewhat in pharmacological properties, all have similar modes of antimicrobial activity.

The improvement in treatment was nearly universal, and the normal oral dosage of metronidazole (200 mg three times a day for seven days) eliminated the organism in 90% of infected women. Even since very early in the use of these drugs, however, there have been occasional reports of failure to cure with repeated courses and with enhanced dosage.[3] Such failures have very distressing consequences for patients, as there is no certainly effective alternative treatment and topical palliatives are unsatisfactory. Setting aside non-compliance by and reinfection of the patient, failures have been attributed variously to: inability to absorb the drug or to transfer it to the vaginal content; inactivation of the drug by the vaginal flora; or evolution by the trichomonads of populations able to evade the action of the drug.

Individual idiosyncracies in absorbing metronidazole from the gut lumen or in its transfer into the vagina are unlikely to be the cause of treatment failure.[4] In a study in Edinburgh the concentrations of metronidazole were estimated simultaneously, by the highly specific and sensitive method of high pressure liquid chromatography, in the plasma and vaginal content of 11 "treatment failure" patients who had received the usual dosage (200 mg every eight hours by mouth) for two days and subsequently an increased dosage of 800 mg every eight hours for five days, both administered under supervision in hospital.[4] The concentrations of metronidazole in plasma and in vaginal secretions were found to be closely correlated with each other and with the dose of the drug. Mean plasma metronidazole concentrations were 8·3 mg/l with the 200 mg dosage, and with the higher dosage a mean of 36·7 mg/l was quickly reached and maintained. Mean metronidazole concentrations in vaginal secretions were closely related to the plasma concentrations, averaging 80% of them. The hydroxy metabolite of metronidazole, although it has less antitrichomonal activity, was also present in all plasma and vaginal samples.

The suggestion that metronidazole is inactivated by the vaginal bacterial flora is supported only by experiments in which the drug was exposed to large numbers of organisms in laboratory culture.

The evidence that other protozoal pathogens, such as those causing malaria and sleeping sickness, can readily evolve populations resistant to therapeutic drugs suggested that the protozoon *T vaginalis* could do so also. Of some 35 papers published, however, at least 12 discounted this as the cause, one as recently as 1982.[5] It is now, however, indisputable that *T vaginalis* stocks resistant to metronidazole do occur and that they are a main reason for "treatment failure".

The sensitivity of *T vaginalis* to metronidazole can be estimated in vitro but, although the experiments reported are all of generally similar design, they differ greatly in detail. Inocula are sometimes undefined or are standardised in different ways, sometimes in terms of the volume of the culture medium, sometimes in terms of the numbers of organisms inoculated; test media are diverse in constitution, particularly regarding the content of redox agents; often the constitution of the atmosphere superjacent to the cultures is not mentioned; or anaerobic or indefinitely defined aerobic cultures are used. Progress has been made recently in controlling the oxygenation of a test medium free of redox agent, to find a level of oxygen tension at which the best compromise could be reached between good growth conditions for the organisms and measuring their susceptibility to the drug.[6] Using atmospheres of pure nitrogen and of a mixture of 99% nitrogen and 1% oxygen, and setting up tests from pre-titrated cryopreserved stabilates of organisms, three categories of resistance were distinguished. Organisms isolated from "random" patients were all very sensitive. Organisms from patients who were not cured by several courses of enhanced dosages

were clearly resistant in both atmospheres. Organisms from patients who were cured by enhanced dosage were intermediate in sensitivity. Although occasional discordant results occurred, and the sample studied was small, the method offered the possibility of distinguishing "treatment-failure" patients whose infections are likely to be eliminated by enhanced dosage from those that are not.

The 5-nitroimidazoles are active against anaerobic organisms. Most of the clinically important anaerobic bacteria, such as *Bacteroides* spp, *Fusobacterium* spp, *Clostridium* spp bearing spores, and the protozoa susceptible to metronidazole, are inhibited by concentrations of about 3 mg/l, and anerobic Gram positive cocci and non-sporing bacilli are inhibited by about 6 mg/l.[7] The action of the 5-nitroimidazoles on anaerobic organisms is believed to be in four successive steps: the entry of the drug into the organism; the activation of the drug by its being reduced to cytotoxic intermediates, a process that is inhibited by oxygen; the toxic effect of these short lived intermediates, killing the organism; and the release of biologically inactive end products.[8] The main thrust of this hypothesis is that the nitro group has to be reduced in the target cell to form the toxic derivative; it is ineffective without such reduction. Thus the striking feature of metronidazole's activity against anaerobic organisms, both prokaryote and eukaryote, is its reduction, which is achieved by mechanisms that are important in the metabolism of anaerobes but have only a minor role for aerobic organisms.

Uptake of metronidazole in both aerobic and anaerobic organisms appears to be passive, simply by diffusion. In aerobic organisms the intracellular and extracellular concentrations of the drug equilibrate because the drug is not metabolised, and uptake is halted. In anaerobic organisms, on the other hand, it is metabolised; its intracellular concentration is thereby decreased, so promoting its continuous uptake and the intracellular accumulation of its derivatives.

Trichomonads are peculiar in possessing membrane bound organelles, called hydrogenosomes, in their cytoplasm. These are of unknown origin and perhaps evolved from clostridia. All the enzymes responsible for the conversion of pyruvate into acetate, carbon dioxide, and also hydrogen (hence their name), via acetyl coenzyme A, appear to be located in these bodies, which also carry out the reductive activation of the 5-nitroimidazoles using electrons produced in this enzyme pathway. The electron donors are thought to be electron transport proteins of low redox potential (doxins). The nitro group of the drug is reduced in these organelles under anaerobic conditions to form nitro radical anions that are cytotoxic. Oxygen is an important competitor for the available electrons, hence the reduced trichomonicidal activity of metronidazole under aerobic conditions. Some metronidazole resistant strains of *T vaginalis* have lowered oxygen affinities,[9] and one strain showed a ferredoxin with altered redox properties.[10] For one resistant strain it was suggested that low activity of NADH (reduced nicotinamide-adenine dinucleotide) oxidase in the cytoplasm allowed oxygen to penetrate the hydrogenosomes and interfere with their reducing the drug.[11] Another study, however, failed to show any correlation between this enzyme and resistance to metronidazole.[12] Thus, though drug resistant strains appear to be able to permit oxygen to penetrate the hydrogenosomes so as to compete for electrons there, the exact mechanisms are not yet understood.

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References

1 Durel P, Roiron V, Siboulet A, Borel LJ. Systemic treatment of human trichomoniasis with a derivative of nitro-imidazole. 8823 R.P. *British Journal of Venereal Diseases* 1960;**36**:21–6.
2 Meingassner JG, Heyworth PG. Intestinal and urogenital flagellates. *Antibiot Chemother* 1981;**30**:163–202.
3 Robinson SC. Trichomonal vaginitis resistant to metronidazole. *Can Med Assoc J* 1962;**86**:665.
4 Robertson DHH, Heyworth R, Harrison C, Lumsden WHR. Treatment failure in *Trichomonas vaginalis* infections in females. 1. Concentrations of metronidazole in plasma and vaginal content during normal and high dosage. *J Antimicrob Chemother* 1988;**21**:373–8.
5 Goulton J, Squires S. Metronidazole-resistant *Trichomonas vaginalis*. *Lancet* 1982;i:42.
6 Lumsden WHR, Harrison C, Robertson DHH. Treatment failure in *Trichomonas vaginalis* in females. 2. *In vitro* estimation of the sensitivity of the organism to metronidazole. *J Antimicrob Chemother* 1988;**21**:555–64.
7 Ralph ED. Clinical pharmacokinetics of metronidazole. *Clin Pharmacokinet* 1983;**8**:43–62.
8 Müller M. Mode of action of metronidazole on anaerobic bacteria and protozoa. *Surgery* 1983;**93**:165–71.
9 Yarlett N, Yarlett NC, Lloyd D. Metronidazole-resistant clinical isolates of *Trichomonas vaginalis* have lowered oxygen affinities. *Mol Biochem Parasitol* 1986;**19**:111–6.
10 Yarlett N, Yarlett NC, Lloyd D. Ferredoxin-dependent reduction of nitroimidazole derivatives in drug-resistant and susceptible strains of *Trichomonas vaginalis*. *Biochem Pharmacol* 1986;**35**:1703–8.
11 Clackson TE, Coombs GH. Factors affecting the trichomonicidal activity of metronidazole. *J Protozool* 1982;**29**:636.
12 Müller M, Gorrell TE. Metabolism and metronidazole uptake in *Trichomonas vaginalis* isolates with different metronidazole susceptibilities. *Antimicrob Agents Chemother* 1983;**24**:667–73.