complete investigation or treatment usually results in failure, but in some cases spontaneous recovery will occur due to the causative strain of staphylococci disappearing from the family household.

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References


Mechanism of antimicrobial action of metronidazole
The spectrum of activity of metronidazole although wide is limited to those organisms whose metabolism is anaerobic or at least microaerophilic. This indicates that the drug affects a biochemical reaction unique to anaerobes and early studies identified this as the clostridium-type pyruvate phosphoroclastic reaction which was inhibited by metronidazole (Edwards & Mathison, 1970; Edwards, Dye & Carne, 1973). In this rather complex reaction pyruvate combines with phosphate to yield acetyl phosphate, H₄ and CO₂ and it is the evolution of H₂ which is specifically inhibited by metronidazole. The inference is, that the drug inhibits either the enzyme responsible for generating H₂ (hydrogenase) or the function of an electron transfer protein (ETP) which has not been characterized in Trichomonas vaginalis but which is ferredoxin in clostridia. That metronidazole acted as an electron sink accepting electrons from a reduced ETP or reduced ferredoxin in clostridia and reducing the drug via its nitro group was shown by Edwards et al. (1973) who also showed that tinidazole and dimetridazole behaved in a similar fashion indicating this to be a general property of biologically active 5-nitroimidazoles.

The inhibition of H₂ evolution and the concomitant reduction of the nitro group of metronidazole is not, however, the lethal event in susceptible microbes since the phosphoroclastic reaction recovers once all the drug is reduced (O'Brien & Morris, 1974). The basis for the selective toxicity of metronidazole towards anaerobes lies in the reduction potential at which the nitro group is reduced and which is thermodynamically compatible with that of the ferredoxins (ca —430-460 mV). The lowest redox potential obtainable by aerobes is about —350 mV so clearly, these organisms are unable to reduce metronidazole. Photosynthetic organisms however, including all higher plants contain ferredoxins which play a major role in the generation of NADPH₄ in photosystem 1. It is not surprising that metronidazole therefore inhibits photosynthesis and inhibits the growth of Rhodopseudomonas acidophila when grown anaerobically in the light, but not when it is grown aerobically in the dark (Edwards & Schoolar, 1971; Edwards, Mathison & Platt, 1974).

In susceptible microbes the reduction of metronidazole decreases its intracellular concentration thus providing a concentration...
gradient which favours uptake of the drug into anaerobes but not aerobes. It is clear from these observations that the microbicidal effect of the drug is due not to the parent molecule, but a reduction product or products—none of which have been isolated or characterized because of their inherent instability. Although some workers have postulated that the reduced drug may interact with protein on some workers have postulated that the microbicidal effect of the drug is due not to the parent molecule, but a reduction product or products—none of which have been isolated or characterized because of their inherent instability. Although some workers have postulated that the reduced drug may interact with protein on theoretical grounds (Ings, McFadzean & Ormerod, 1974) or that 30% of the labelled drug binds to intracellular components the distribution of which parallels that of protein (Muller, Lindmark & McLaughlin, 1977). It is now clear that the major site of action of metronidazole is DNA. The evidence for this comes from a number of sources including that of metronidazole inhibiting the uptake of labelled thymidine into the DNA of T. vaginalis and clostridia (Ings et al., 1974), the inability to extract DNA from metronidazole treated anaerobes, and the inhibition of DNA synthesis and degradation of existing DNA in clostridia (Plant & Edwards, 1976). Further studies from our laboratory showed that chemically reduced metronidazole caused a destabilization of the DNA helix and strand breakage as shown by spectrophotometric melting profiles and sucrose gradient sedimentation techniques (Edwards, 1977).

Recent studies in our laboratory using a variety of techniques including viscometry, spectrophotometric melting and renaturation profiles of DNA, sucrose gradient sedimentation ultracentrifugation, agarose gel electrophoresis and hydroxypatite chromatography have confirmed and extended the above results. Thus, metronidazole, reduced electrolytically (polarographically) under anaerobic conditions decreases the viscosity, hyperchromicity, renaturation, Tm value, molecular weight, helix content and sedimentation value of DNA, increases the single strand content of the DNA molecule and inhibits the action of DNA-ase 1 (Edwards, Knight & Kantor, 1978; Knight, Skolimowski & Edwards, 1978).

All this evidence indicates unambiguously that metronidazole causes extensive strand breakage of DNA which occurs only under anaerobic conditions and only with the reduced drug. The inhibition of DNA-ase 1 is interesting as this functions as a repair endonuclease in bacteria. Metronidazole may therefore exert a double blockade by inhibiting the mechanisms which usually repair strand breaks in DNA as well as causing them. How this occurs is not known—it may inhibit the enzyme directly or a (reduced) drug-base complex may be formed which the enzyme is unable to recognize. Preliminary results from our laboratory indicate that both mechanisms may occur. Such a mechanism of action appears to be typical of the antimicrobial 5-nitroimidazoles such as tinidazole, dimetridazole, ornidazole and sulindazole and is similar to that of the nitrofurans; but important differences exist. Nitrofurans are not reduced by ferredoxin-linked mechanisms because their redox potentials are more positive than the nitroimidazoles. Instead, specific nitro-reductase enzymes reduce these drugs and since these enzymes are present in aerobes nitrofurans do not have the selective toxicity exhibited by the 5-nitroimidazoles. The reduction products of nitrofurans do, however, have similar morphological effects to those of metronidazole (Buchner & Edwards, 1975) and other nitroimidazoles in that they cause strand breakage and fragmentation of DNA (McCalla, 1977, 1979).

There remains one aspect of the antimicrobial action of metronidazole which has yet to be explained adequately—the apparent inactivity of metronidazole which appears as clinical resistance in a few patients receiving the drug for trichomoniasis, but which always yields T. vaginalis which is fully sensitive to metronidazole in vitro. This effect (known as metronidazole inactivation) appears to be the result of metronidazole-insensitive organisms in the vagina absorbing the drug and, because they are unable to reduce it, their viability is not affected but the drug concentration in the vaginal fluid is thus decreased below that required to kill T. vaginalis. Several organisms commonly found in the vagina are able to inactivate metronidazole and these include Escherichia coli and Streptococcus faecalis, mimae, proteus and klebsiella (McFadzean, Pugh, Squires & Whelan, 1969; Nicol et al., 1966) but the event is rare. Some nitroimidazoles, for example misonidazole are inactivated three times faster than metronidazole under laboratory conditions (Edwards et al., 1979).

Finally, the reports of the effects of metronidazole as a mutagenic and carcinogenic drug—often couched in emotive terms should be put into perspective. There is substantial evidence that both metronidazole and some of its metabolites in humans are mutagenic in bacteria when the drug is reduced during the Ames test (Rosenkranz & Speck, 1977). It is not mutagenic under conditions where the drug is not reduced. Since
mammalian cells are unable to reduce the drug the significance of the mutagenicity data may well be irrelevant. There have also been some reports of weak tumourigenic or carcinogenic activity at lifetime doses 400 to 500 times that required for trichomoniadis therapy and several reports indicate no significant carcinogenic activity whatever (Roe, 1977a, b). The significance of these findings has not been established but must be related to the benefit-risk ratio which is highly in favour of the drug (Finegold, 1977).

In summary, metronidazole is selectively toxic to anaerobes alone because only anaerobes have electron transfer proteins of sufficiently low redox potential to reduce the nitrogroup. The reduced drug binds to DNA causing strand breakage, dissolution of helix formation and degradation of the macromolecule which may be exacerbated by a concomitant inhibition of a DNA repair mechanism. These effects lead to an overall disruption of DNA replication and transcription and leads to death of the cell within 2 to 3 generations depending on the drug concentration.

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