Mechanism of Resistance in Metronidazole

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Chapter 19
Mechanism of Resistance in Metronidazole

Abhay Dhand and David R. Snydman

Metronidazole [1-(2 hydroxyethyl)-2-methyl-5-nitroimidazole] was introduced in the 1960s. Since then it has been drug of choice for human infections caused by various anaerobic and microaerophilic bacteria (Bacteroides, Clostridia, Helicobacter) and parasites (Trichomonas, Giardia, Entamoeba). Other Gram-positive anaerobes (e.g., lactobacilli, propionobacterium acnes, majority of the periodontal pathogens, peptostreptococci) are known to be inherently resistant to metronidazole. Virtually all the anaerobic Gram-negative rods are known to be susceptible to metronidazole.

Sensitivity testing for anaerobes is not performed routinely. Therefore, resistance to metronidazole is under-reported. With improvements in molecular detection, increasing resistance rates are being noted. This emerging resistance to metronidazole poses various diagnostic and therapeutic dilemmas. Mechanisms of resistance are being defined, and a better understanding is the key for prevention of resistance and improved management of these infections.

1 Antimicrobial Mechanism of Action

5-Nitroimidazole is administered as a prodrug. It enters the cell by passive diffusion and is activated in either the cytoplasm in bacteria, Entamoeba, and Giardia, or in a specialized organelle called hydrogenosome in Trichomonas. Activation to its cytotoxic form occurs via transfer of an electron from various donors to the nitro group, which converts it to a nitroso free-radical form. This toxic metabolite interacts primarily with DNA, RNA, or intracellular proteins, leading to DNA strand breakage, inhibited repair, and disrupted transcription. If the disruption of DNA is faster than its repair, it ultimately leads to cell death.

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The selective toxicity and effectiveness of metronidazole depend on the cytoplasmic environment in the anaerobic and microaerophilic organisms, which provides a sufficiently low redox potential environment required for the activation of the drug. Metronidazole has very low reduction potential (E_{17} – 486 mV) and will be activated only in conditions where low redox status in maintained. Oxygen has higher affinity for an electron than metronidazole (E_{17} –150 mV). Therefore, oxygen can either successfully compete with 5-nitroimidazole for the electron from the electron carrier or be able to remove the electron from the activated nitroso group, thereby re-forming the parent drug – a phenomenon known as *futile cycling*. Similarly, downregulation of various intracellular electron donors may prevent activation of the prodrug, and therefore the lack of efficacy.

2 Mechanism of Resistance

The proposed mechanisms of resistance are

1. Decreased drug uptake or increased efflux
2. Decreased drug activation/change in the biological target
3. Increased oxygen scavenging capabilities (SOD/catalase/peroxidase)
4. Enhanced activity of DNA repair enzymes

2.1 Bacteroides

Metronidazole resistant (MTZ-R) *Bacteroides fragilis* was first reported in a patient with Crohn's disease after long-term therapy with metronidazole (1). Metronidazole resistance in *Bacteroides* spp. is quite rare but has been reported in several countries (2, 3). Metronidazole resistance among *Bacteroides* spp. is of concern, as these species can also be resistant to a wide variety of antimicrobial agents including β-lactams, tetracycline, clindamycin, cefoxitin, and imipenem (4).

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Breuil et al. and Reysset et al. showed that all Bacteroides strains that were resistant to 5-nitroimidazole harbored a genetic determinant, which was either plasmid borne or on the chromosome (5, 6). This resistance was shown to be transferable by a conjugation-like process to susceptible strains with a frequency ranging from 10^{-3} to 10^{-7} per donor. These genetic determinants have been shown to be specific nitroimidazole-resistant genes (*nim*), presumably encoding a nitroimidazole reductase that converted nitroimidazole to aminoimidazole, thereby avoiding the formation of toxic nitroso radicals that are essential for antimicrobial activity. So far seven *nim* genes (*nim* A,B,C,D,E,F,G) have been described. These genes are commonly transcribed from promoters located within different insertion elements. Gal and Brazier studied 50 resistant isolates and found the *nim*A gene was the most common, followed by *nim*B and *nim*E (7). Although the presence of a *nim* gene does not always equate to therapeutic resistance, prolonged exposure of *nim*-gene carrying *Bacteroides* spp. to metronidazole can select therapeutic resistance. Diniz et al. used a combination of proteomics for identification of differentially expressed proteins and other genes involved in the adaptive response to metronidazole (8). Protein profile of resistant strains showed upregulation of lactate dehydrogenase and downregulation of flavodoxin and impaired enzymatic activity of pyruvate–ferrodoxin oxidase reducatse. They also suggested that multiple enzymes involved in oxidation/reduction and electron transfer reactions may be important in activation of MTZ and possible mechanisms of inducing resistance. This supports the idea that there is no one specific gene for MTZ resistance and multiple possible pathways for resistance exist.

2.2 Helicobacter pylori

High rates of metronidazole resistance in *H. pylori* have also been reported worldwide. In Western Europe 20–45% isolates of *H. pylori* have been reported as MTZ-R. This rate is higher in developing countries, within immigrant populations, and in young women who may have received this agent in the past for parasitic infections or gynecologic infections (9–11). Thompson and Blaser showed that inactivation of *recA* (a gene needed for generalized DNA repair and recombination) severely impaired the ability of *H. pylori* mutants to survive treatment with UV light, ciprofloxacin, and metronidazole (12). Expression of a cloned *recA* gene obtained from a resistant strain of *H. pylori* in *E. coli* raised its level of resistance (12). Smith and Edwards showed that a relationship existed between the intracellular oxygen-scavenging ability of *H. pylori* and sensitivity of the bacterium to metronidazole. MTZ-R strains of *H. pylori* possessed considerably lower soluble cytosolic NADH oxidase activity than MTZ-S strains (13). Goodwin et al. first demonstrated that a major mechanism of MTZ resistance in *H. pylori* is due to null mutations in the *rdxA* gene, which encodes an oxygen-insensitive NAD(P)H nitroreductase (14). Using a cosmid cloning approach in MTZ-R strains, they identified an open reading frame (ORF) that had protein level homology to classical oxygen-insensitive NAD(P)H nitroreductases. An *H. pylori* gene corresponding to this ORF was designated *rdxA*. In a series of elegant experiments Goodwin et al. also showed that *E. coli* (normally MTZ-R) was rendered MTZ-S by a functional *rdxA* gene, introduction of *rdxA* on a shuttle vector plasmid into formerly MTZ-R *H. pylori* rendered it MTZ-S, and replacement of *rdxA* in MTZ-S *H. pylori* with a rdxA::camR null insertion allele resulted in MTZ-R phenotype (14). Kwon et al. reported role of an additional gene *frxA*, which encodes NAD(P)H flavin oxidoreductase, in MTZ resistance in *H pylori* (15). Using a lambda phage genomic library, they identified an MTZ nitroreductase encoding gene, NAD(P)H flavinoxidoreductase (*frxA*). Frame shift mutations leading to premature termination of frxA protein were associated with metronidazole resistance in *H. pylori*. This was further confirmed by insertion activation of *frxA* and/or *rdxA* genes. In addition, cloned *frxA* gene expressed in *E. coli* showed nitroreductase activity and rendered normally metronidazole-resistant *E. coli* sensitive. Strains carrying *frxA* null alleles enhanced MTZ resistance in *rdxA* deficient cells. Also, inactivation of genes that encode ferridoxin-like protein (fdxB) along with previously described *frxA* and *rdxA* genes increased the MIC of MTZ-S strains (16). This suggested that multiple possible factors might be involved in high-level resistance to MTZ. Jeong et al. suggested two types of MTZ-S strains by genetic (mutational) and molecular tests on the basis of the need for inactivation of *rdxA* alone or along with *frxA* gene to render *H. pylori* resistant (17). Subsequent work suggested that *rdxA* gene might play a major role in the high-level resistance to metronidazole (18).

2.3 Trichomonas

The first report of resistance appeared in *Trichomonas vaginalis* about two years after introduction of metronidazole (19). Recently, there has been an increase in the recognition of metronidazole-resistant trichomoniasis associated with increase in therapeutic failures (20). In trichomonads, activation of MTZ occurs within specialized organelles, the hydrogenosome, which contains pyruvate:ferrodoxin oxidoreductase (PFO) and ferrodoxin. PFO catalyzes the decarboxylation of pyruvate to acetyl CoA, transferring the electron to ferrodoxin. MTZ replaces protons as the acceptor of electrons donated by ferrodoxin. In the absence of the drug, these protons would normally be reduced to molecular hydrogen

by hydrogenase. Yarlett, Yarlett, and Lloyd provided evidence that the reductive activation of metronidazole is diminished in resistant strains relative to drug-sensitive strains (21, 22). Quon et al. examined the intracellular levels of ferrodoxin and its mRNA in four clinically resistant strains and demonstrated decreased levels of ferrodoxin and its mRNA. This was attributed to reduced transcription of the ferrodoxin gene as determined by nuclear run-on assays (23). Cerkasovova et al. noted that Tritrichomonas foetus strains that are highly resistant to MTZ lack detectable enzymatic activity for pyruvate:ferrodoxin oxidoredutase and hydrogenase (24). The molecular basis for these altered enzyme activities has not been established.

2.4 *Clostridium* spp.

Clostridium species are usually sensitive but *C. ramnosum* may require higher concentrations for inhibition (25, 26). There are reports of high-level resistance to metronidazole in *C. difficile* isolates from horses (27). There is one report of documented resistance (high minimum inhibitory concentration (MIC) with therapeutic failure) in a *C. difficile* isolate in a patient with *C. difficle*-associated diarrhea. (28)

Santengelo et al. developed *E. coli* F19recA, nitrate reductase-deficient mutant that was rendered MTZ-S by isolating and expressing *C. acetobutylicum* genes on recombinant plasmids. Further tests on these isolates revealed that flavodoxin and hydrogenase genes were responsible for electron transfer system, suggesting its possible role in metronidazole resistance (29). Church et al. provided biochemical evidence that hydrogenase 1 of *C. pasteuranicum* plays a critical enzymatic role in the reduction of metronidazole via a ferrodoxin-linked mechanism (30, 31).

2.5 Entamoeba and Giardia

Drug-resistant Giardia isolates have been grown from patients with therapeutic failure with metronidazole. There is no reported clinical resistance in Entamoeba, but resistant strains have been generated in vitro in various laboratories.

Purified PFOR and ferrodoxin have been shown to activate MTZ in vitro. Upcroft and Upcroft characterized biochemical markers in a clinically resistant isolate and showed that PFOR is downregulated up to fivefold. Ferrodoxin 1, which is the next electron acceptor in the transport chain, is also downregulated about seven times (32). Increased efflux of the drug also might be responsible in protecting the parasite.

Entamoeba produces superoxide dismutase (SOD), catalase, and peroxidase for detoxification of oxygen and its breakdown products. Only one 2-oxoacid oxidoreductase, PFOR, has been detected in Entamoeba and it is predominantly membrane bound. Upcroft and Upcroft showed marked increase in superoxide dismutase activity in MTZ-resistant *E. histolytica*, while PFOR activity remained constant (32). Wassmann et al. confirmed the lack of change in PFOR activity in resistant strains. They also showed increased expression of iron-containing FE-SOD and peroxiredoxin, while the expression of flavin reductase and ferrodoxin1 was decreased (33).

3 Cross-Resistance

There is documented cross-resistance between all the currently used 5-nitroimidazole drugs and their worldwide availability (32, 34).

4 Mechanism of Spread of Resistance

Although both plasmid-mediated and chromosomally mediated resistance has been described, the transfer to metronidazole-sensitive Bacteroides species does not yet appear to be a problem. Also, a combination of several mechanisms may be required for emergence of high-level resistance in various organisms that might lead to therapeutic failures.

5 Alternative Agents

5.1 Helicobacter Pylori

Virtually all *H. pylori* isolates are susceptible in vitro to a variety of antimicrobial agents, including bismuth salts, amoxicillin, macrolides, nitrofurans, tetracyclines, and aminoglycosides. Combination therapy with a bismuth salt and two antibiotics has been widely used. After treatment failure, a second course of triple therapy may still be effective; alternatively, a regimen not including imidazoles may be used.

5.2 Trichomonas Vaginalis

If infection persists in a patient treated with a 7-day regimen and re-infection can be ruled out, other options include treating with 2 g of metronidazole orally daily for 3–5 days; 1–2 g of metronidazole daily for 14 days along with 500 mg intravaginally

daily; high-dose intravenous metronidazole (35), intravaginal paromomycin (36, 37), and tinidazole, which has recently been approved by the FDA. Tinidazole has been shown to be effective in some cases of metronidazole-resistant *T. vaginalis* infection (38). Crowell et al. found that although in vitro activities of metronidazole and tinidazole against the parasite are highly correlated, the tinidazole does have lower MICs than metronidazole (34).

5.3 Giardia

Some success has been noticed with quinacrine and albendazole in combination with metronidazole in cases of giardiasis treatment failures (39).

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