Treatment of Infections Caused by Metronidazole-Resistant *Trichomonas vaginalis*

Sarah L. Cudmore, Kiera L. Delgaty, Shannon F. Hayward-McClelland,
Dino P. Petrin, and Gary E. Garber*

*Department of Biochemistry, Microbiology, and Immunology and Department of Medicine, Faculty of Medicine,
University of Ottawa, Ottawa, Ontario, Canada*

INTRODUCTION

Trichomoniasis is a sexually transmitted disease caused by the parasitic protozoan *Trichomonas vaginalis*. It is the most common nonviral sexually transmitted disease, with an estimated 170 million cases occurring worldwide each year (38). This estimate may well be low, however, since inapparent infection rates are as high as 50% in women and even higher in men (35). Trichomoniasis has been implicated in causing adverse pregnancy outcomes (43, 107, 108) and has been associated with an increased risk of human immunodeficiency virus (HIV) transmission (15, 61, 62, 89, 115).

Standard treatment for trichomoniasis is commonly with metronidazole, a 5-nitroimidazole used to treat infections caused by certain parasitic protozoa and anaerobic gram-negative bacilli. Unfortunately, metronidazole-resistant *T. vaginalis* has been implicated in an increasing number of refractory cases. The failure of metronidazole regimens to cure *T. vaginalis* infection is of concern because metronidazole is currently the only drug approved for the treatment of trichomoniasis in the United States. Refractory cases are therefore usually treated with increased doses of the drug, which leads to an increase in the occurrence of side effects. Clearly, alternative curative therapies are needed, and a vaccine would be desirable, given the widespread occurrence and impact of the disease.

Trichomonas vaginalis

T. vaginalis is a flagellated protozoan that can assume an ameboid form, usually on contact with other cells. Adherence to the epithelial cells of the urogenital tract is an essential step in pathogenesis. One of the most ancient eukaryotes, *T. vaginalis* possesses no mitochondria, instead producing some of its ATP in hydrogen-producing organelles called hydrogenosomes. Carbohydrate metabolism is fermentative, producing acidic end products. The metabolic pathways of *T. vaginalis* share characteristics with both eukaryotes and anaerobic prokaryotes. The activities of these pathways are critical to both *T. vaginalis* susceptibility to metronidazole and the processes by which the parasite develops resistance. *T. vaginalis* lacks the enzymes to synthesize de novo nucleotides and lipids, making it an obligate parasite reliant on salvage pathways. Coupled

* Corresponding author. Mailing address: Division of Infectious Diseases, Ottawa Hospital, General Campus, 501 Smyth Rd., Ottawa, ON, Canada K1H 8L6. Phone: (613) 737-8173. Fax: (613) 737-8099. E-mail: ggarber@ohri.ca.

with the fact that the fermentative metabolism of the protozoan is not a high-efficiency process, this means that *T. vaginalis* is a fastidious organism, requiring an environment rich in nutrients to survive (101).

Trichomoniasis

T. vaginalis infection in males is generally mild or asymptomatic. Asymptomatic carriers can serve as vectors for the disease, making it important to treat male partners of infected women to avoid reinfection. Trichomoniasis in men usually manifests as urethritis clinically similar to other nongonococcal infections, which generally resolves in 10 days or less. Symptomatic men present with a clear or mucopurulent discharge and dysuria (57, 58). Complications associated with *T. vaginalis* infection include prostatitis, balanoposthitis, epididymitis, and infertility (56, 82).

Trichomoniasis in women usually occurs during the reproductive years. Infection before menarche or after menopause is generally rare, and symptoms are mild and transient (108). The incubation period ranges from 3 to 28 days. Clinical presentation of trichomoniasis in women can include a wide array of symptoms, from asymptomatic to acute inflammatory disease. Unlike in men, where spontaneous resolution of the disease is common, *T. vaginalis* infection can persist for long periods in the female urogenital tract. Up to one-third of asymptomatic women will develop symptomatic infection within 6 months (43).

Mild trichomoniasis usually presents with pruritus and dyspareunia. Vaginal secretion may be present and is generally scanty and mixed with mucus. Acute infection with *T. vaginalis* is characterized by severe pruritus, vaginitis, and vulvitis accompanied by dysuria and dyspareunia. Colpitis macularis (strawberry cervix) can sometimes be seen with the aid of a colposcope as a "speckling" of hemorrhagic spots on the mucosa. Malodorous (fishy) discharge is frothy, yellow or green, mucopurulent, and copious (43, 127), as described in "classic" severe vaginitis.

The severity of symptoms in *T. vaginalis* infection is linked to a number of factors. Trichomoniasis is characterized by a rise in the vaginal pH from approximately 4 to as high as 7 (12), brought about by a decrease or elimination of endogenous *Lactobacillus* species. This increase in pH creates a better environment for the growth of the parasite (40). Additionally, certain factors contributing to pathogenicity, such as cell-detaching factor, are inactivated at a pH of less than 5. Cell-detaching factor activity had also been found to be inhibited by estrogen, although it is unknown whether hormones have any other effect on the parasite (37). The roles played by pH and hormones in trichomoniasis may explain the observation that symptoms of the disease are often worse during menstruation (43, 107). Menstrual blood creates a rich milieu for *T. vaginalis* reproduction at a higher pH than is normally found in the vagina. Additionally, the blood provides increased amounts of iron, which enhances the ability the of *T. vaginalis* to attach to the vaginal epithelium (66).

T. vaginalis adheres primarily to the squamous epithelium of the genital tract (41). Infection is multifocal but usually does not involve the invasion of the parasite into tissue. In men, the external genitalia, prostate, and epididymis can be infected.

The oxidative nature of the male genital tract is hypothesized to be inhibitory to certain pathogenic factors of the protozoan (10). Zinc, present in prostatic fluid, is also cytotoxic to the parasite (55). These factors may explain the usually transient nature of infection. Complications, although rare, can result in genitourinary inflammatory disease and sterility. In infected women, the parasite may be found in the vagina, cervix, and bladder, as well as Bartholin's, Skene's, and periurethral glands (41). The vagina is a reducing environment, which may contribute to the activation of some pathogenic mechanisms of *T. vaginalis* (10). Complications associated with trichomoniasis in women include various inflammatory conditions, cervical erosion, cervical cancer, and infertility. Premature rupture of the placental membranes, contributing to premature labor, and low-birth-weight babies are known perinatal complications (107, 108, 127). Increased risk of HIV infection due to trichomoniasis is seen in both sexes (115).

TREATMENT

Topical vaginal medications (creams and gels) and pessaries can be prescribed for the treatment of *T. vaginalis* in women. Modern preparations include clotrimazole, povidone-iodine, nonoxynol-9, and arsenical pessaries. These preparations provide local symptom relief, but documentation on their effectiveness as cures has been inconsistent. There are no topical treatments for trichomoniasis in men (77).

The only curative treatment currently available for *T. vaginalis* infection in the United States is metronidazole. Usually prescribed as a single or multiple oral doses, metronidazole can also be administered intravenously. Vaginal metronidazole creams and pessaries have also been available but are no longer favored due to their poor rate of cure compared to oral metronidazole (11, 23).

METRONIDAZOLE

General Structure

Metronidazole (α-hydroxyethyl-2-methyl-5-nitroimidazole) is a 5-nitroimidazole, a heterocyclic compound with a nitro group on the fifth position of an imidazole ring. It is derived from the *Streptomyces* antibiotic azomycin. Developed in 1959, metronidazole was approved for the treatment of trichomoniasis in the early 1960s and was the first drug to have a cure rate approaching 100% with systemic treatment (22). It is the 5-nitro group that, when reduced to a nitro radical within the *T. vaginalis* parasite, is responsible for the antiprotozoal activity.

Treatment Regimens

Current Centers for Disease Control and Prevention guidelines recommend that metronidazole be administered orally, with dose regimens of 250 mg three times a day for 7 days, 500 mg twice a day for 7 days, or a single 2-g dose. The 2-g dose is usually favored because patient compliance is better and less total drug is required for successful treatment. However, there may be a slightly increased risk of side effects with this larger dose. Additionally, patients treated over 7 days are protected for this period from immediate reinfection whereas this pro-

tection is not as reliable with a single dose unless the sexual partner(s) is treated simultaneously (76).

Metronidazole can also be administered intravenously, with a dose of 500 mg to 2 g of metronidazole administered over 20 min. Intravenous administration, although rarely used, is associated with less severe side effects than oral dosing. Cure rates for oral and intravenous regimens are similar, at 85 to 95%, and increase if the sexual partner(s) is treated simultaneously (76). Therefore, given the high incidence of asymptomatic trichomoniasis, concurrent treatment of sexual partner(s) is highly recommended to prevent recurrent infections.

Treatment of Pregnant Women and Children

Use of metronidazole during pregnancy has been debated because the drug is known to cross the placenta. In addition, metronidazole is mutagenic in bacteria and carcinogenic in mice, so that teratogenicity has been a concern (20, 69). This has led the Food and Drug Administration to classify metronidazole as a class B risk factor for pregnancy, a possible but unconfirmed risk to the fetus. Therefore, the drug has been contraindicated in the first trimester and is considered a second line of therapy in the latter stage of pregnancy.

More recent retrospective analyses, however, have failed to establish a link between metronidazole exposure during pregnancy and birth defects (16, 24, 109). This fact, coupled with the known risk of pregnancy complications associated with *T. vaginalis* infection, has led many clinicians and researchers to propose that the risk to the fetus from a maternal trichomoniasis is far greater than any risk related to metronidazole exposure. As such, a single 2-g dose of metronidazole is the treatment currently recommended by the Centers for Disease Control and Prevention for women at any stage of pregnancy. Alternatively, a daily intravaginal dose of 100 mg of clotrimazole for 6 days can provide temporary relief in the first trimester, followed by standard metronidazole therapy in the second trimester.

Metronidazole is secreted in breast milk in small quantities. It is recommended that lactating mothers be treated with a single 2-g dose of metronidazole, followed by a 24-h interruption in breast feeding to prevent neonatal exposure to the drug. This recommendation has become controversial, with some authors citing the lack of evidence of risk to the neonate and the small amount of drug exposure as a poor reason to interrupt breast feeding (76).

Neonatal trichomoniasis is the only nonsexually transmitted form of the disease. It is usually asymptomatic or mildly symptomatic and is dependent on maternal estrogen levels. Spontaneous resolution of the condition is common as the level of estrogen wanes in the third to sixth week of an infant's life. If infection persists after the sixth week or is symptomatic, the infant may be treated with metronidazole as a single 50-mg/kg dose or a 10- to 30-mg/kg dose daily for 5 to 8 days (76). Canadian guidelines for the treatment of prepubescent *T. vaginalis* infections with metronidazole recommend 15 to 20 mg/kg divided into three doses daily for 7 days, or a single dose of 40 mg/kg to a maximum of 2 g.

Side Effects

Metronidazole is generally well tolerated, with patients suffering few or no side effects due to standard regimens. Common adverse reactions include nausea, vomiting, headache, insomnia, dizziness, drowsiness, rash, dry mouth, and metallic taste (the last two generally being associated with oral metronidazole only). These side effects are usually mild, although some patients do have reactions severe enough to necessitate halting metronidazole therapy. More serious side effects are rare but include eosinophilia, leukopenia, palpitation, confusion, and peripheral neuropathy. Side effects have been found to be temporary and resolve on cessation of the therapy (75).

Adverse reactions to metronidazole are of greater concern in patients with refractory infections. Since metronidazole is the only approved treatment for trichomoniasis in the United States, recurrent or resistant *T. vaginalis* infection is treated with increasing doses of the drug for longer periods. Side effects from metronidazole treatment are much more common at these higher doses, leading to patient intolerance, incomplete treatment courses, and treatment failure (68, 121).

Nitroimidazole hypersensitivity is also an issue in the treatment of trichomoniasis with metronidazole. Incremental dosing has been successful in treating some women with reactions to metronidazole, but it must be carefully monitored. However, such regimens could potentially facilitate the emergence of metronidazole-resistant strains of *T. vaginalis*.

Pharmacokinetics

Metronidazole is metabolized primarily in the liver by side chain oxidation and glucuronidation. Oxidative metabolism yields two main products, 1-acetic acid-2-methyl-5-nitroimidazole (acid metabolite) and 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (hydroxy metabolite). The hydroxy metabolite can be further oxidized into an acid metabolite or 1-(2-hydroxyethyl)-2-carboxylic acid-5-nitroimidazole (117). Glucuronidation of both metronidazole and its hydroxy metabolite also occurs (116).

Hydroxymetronidazole is the main product yielded when metronidazole is metabolized. It maintains 35 to 60% of the antimicrobial efficacy of the parent drug (121). The half-life of metronidazole is about 8.7 h, compared to approximately 12 h for the hydroxy metabolite. Renal clearance is low, with only 15 to 20% of the administered dose being recovered in the urine after 3 to 7 days (104). Of the drug recovered in urine, 60 to 70% is hydroxymetronidazole and unchanged metronidazole, indicating that active drug or metabolite is present throughout the course of treatment (48). Metronidazole follows linear pharmacokinetics and is eliminated by first-order kinetics (105).

Metronidazole is a small molecule, does not bind to serum proteins, and is well distributed through bodily tissue and fluids. Therapeutic levels of the drug have been found in blood, cerebrospinal fluid, pulmonary exudates, bile, and seminal fluid, as well as bone, brain, and pelvic tissue. Unfortunately, metronidazole levels present in the vagina are less well defined. One group has reported that the vaginal drug concentration is approximately 50% that in serum (75), while another has found the level of metronidazole in the vagina to be com-

parable to that in serum and saliva after 24 h (25). The lack of information about vaginal metronidazole levels is problematic because low vaginal penetration could affect the efficacy of treatment.

The bioavailability of metronidazole is dependent on the route of administration. Metronidazole taken orally or given intravenously has a bioavailability of 93 to 100% (45). Rectal suppositories, which have been tested in clinical trials but are not used clinically, show peak concentrations in plasma that are about 50% of those of oral metronidazole (86). Vaginal absorption of the drug is lower still, with bioavailability being only about 20% of that of oral metronidazole (23). Vaginally administered metronidazole is also much slower to achieve peak concentration in the plasma, taking 9 to 16 times longer than an oral dose (11). Absorption of metronidazole in the vagina is dependent on a number of factors, including drug formulation (insert or cream), dose, and physicochemical characteristics of the vagina during treatment. These variables may explain why some women respond better to vaginal treatment with metronidazole than others.

Concentration of metronidazole in serum, specifically peak concentration in plasma and minimum lethal concentration (MLC), are dependent on dosage. All oral dose regimens (250 mg three times a day, 500 mg twice a day for 7 days, and a single 2-g dose) achieve peak concentrations in plasma in 1 to 2 h (11, 23). The height of the peak is proportional to the dose, and the peak is followed by slow, steady elimination. After a single 2-g dose of metronidazole, a mean maximum concentration of the drug in serum greater than the MLC is maintained for 24 to 48 h, providing efficient elimination of infection (97). However, gastrointestinal intolerance and other side effects are more common with single large doses. Studies have shown that metabolism of metronidazole is reduced at high dosages, making this regimen undesirable for elderly and ill patients and those with liver dysfunction that could result in reduced drug clearance (94, 121). Metronidazole regimens involving multiple doses over 7 days ensure the maintenance of concentrations of metronidazole in plasma exceeding the MLC for the duration of the treatment period. However, since body weight plays a role in drug excretion (121), some fixed-dose regimens may not be appropriate for all patients.

Mode of Action

Metronidazole is a small molecule that enters *T. vaginalis* via passive diffusion. The drug itself is inactive, but anaerobic reduction results in the formation of a cytotoxic nitro radical anion. This nitro radical is hypothesized to bind transiently to DNA, disrupting or breaking the strands and leading to cell death (33, 70). The action of a short-lived reactive intermediate (the nitro radical) rather than an irreversible binding of the drug to DNA is suspected, since no evidence of the formation of a stable metronidazole-DNA complex has been found (26). In addition, studies with *E. coli* have shown that damage due to the direct binding of drugs to DNA is repaired by the excision pathway and that this pathway is not activated in *T. vaginalis* exposed to metronidazole (60).

The nitro radical is thought to target sections of DNA rich in thymine and adenine residues for disruption, since studies have shown that maximal damage is observed in DNA rich in these

nucleotides (32, 98). It has also been found that the action of metronidazole is associated with the release of certain thymine and thymidine phosphates (54). Since the genome of *T. vaginalis* has a high A+T content (approximately 71%), this could explain the specificity of metronidazole for the parasite. In vitro, the response of *T. vaginalis* to metronidazole is rapid. DNA synthesis is inhibited within 30 min, and death occurs in 5 h (47).

Metronidazole is reduced in the hydrogenosomes of *T. vaginalis* by the enzyme pyruvate:ferredoxin oxidoreductase (PFOR) (17). Electron shuttling between PFOR and metronidazole occurs via ferredoxin (33). This system is very sensitive to the presence of molecular oxygen, since oxygen is a strong competitor for ferredoxin-bound electrons. To tolerate increased levels of cellular oxygen, *T. vaginalis* strains that have adapted to a microaerophilic or aerobic environment are thought to have reduced function of hydrogenosomal oxidases. This would diminish oxygen reduction and concomitant formation of toxic oxygen species. It also inhibits the conversion of metronidazole into its active, reduced forms, which may contribute to resistance. Additionally, as metronidazole enters *T. vaginalis* by passive diffusion, slower metabolism of the drug would result in a lower diffusion gradient and less uptake of metronidazole by the cell (68).

METRONIDAZOLE RESISTANCE

Resistance of *T. vaginalis* to metronidazole is classified as either aerobic or anaerobic. In aerobic resistance, oxygen-scavenging pathways (106, 123) and possibly ferredoxin (103, 124) are involved. These pathways are not implicated in anaerobic resistance, which is driven instead by a reduction or cessation of activity of PFOR and hydrogenase (29, 59).

Anaerobic Resistance

Although metronidazole-resistant clinical isolates of *T. vaginalis* have generally been found to utilize aerobic mechanisms of resistance, an anaerobically resistant strain has been isolated from a patient with trichomoniasis (125). This could be of clinical importance since it is unknown if this type of resistance can develop in response to routine patient treatment with metronidazole (29). In vitro, induction of anaerobic metronidazole resistance has been achieved by cultivating trichomonads with sublethal, increasing drug pressure (1 to 100 mg/ml) for a period of 12 to 21 months. *T. vaginalis* with this type of resistance showed decreased or absent PFOR activity. Trichomonads also failed to take up [^{14}C]metronidazole, indicating both that metabolism of the drug had ceased and that the pyruvate-oxidizing pathway is critical to this metabolism. Hydrogenase activity is also impaired or absent in anaerobic resistance, inhibiting the production of molecular hydrogen by the hydrogenosome (59). It had previously been hypothesized that metronidazole-induced cell death occurs by inhibiting hydrogenase and hydrogen production (30). However, since hydrogenase activity is also impaired in anaerobic resistance, it is clear that multiple factors contribute to the trichomonicidal effects of metronidazole and that all these factors must be considered in dealing with drug resistance.

Aerobic Resistance

Aerobic resistance is important clinically since it can develop in vivo in trichomonads treated with therapeutic levels of metronidazole and does not require the prolonged, incremental exposure used to create anaerobic resistance in vitro. One in vitro study has shown that transcription of the ferredoxin gene is reduced in aerobically resistant *T. vaginalis* strains. This finding indicates that reduced transcription may be the result of a point mutation in the 5′ region of the ferredoxin gene. Located at position −239 from the start site, this mutation was found to cause a reduced binding affinity for a 23-kDa transcriptional protein and subsequent reduction in the expression of the ferredoxin gene (103). It was theorized that reduced ferredoxin expression would result in lowered activation of metronidazole and less drug trafficking into the cell. The extent to which ferredoxins are involved in metronidazole resistance is unclear, since some studies have shown that ferredoxin is involved in nitroimidazole susceptibility (124) while others demonstrate that disruption of the ferredoxin gene does not lead to in vitro metronidazole resistance (63).

Decreased hydrogenase activity and its concomitant reduction in hydrogen production play a factor in impaired oxygen-scavenging mechanisms in the hydrogenosome. Since oxygen is a highly efficient electron receptor, increased levels of cellular (hydrogenosomal) oxygen result in impaired reduction and activation of metronidazole. Oxygen concentrations in resistant strains are higher than those in susceptible ones when extracellular oxygen levels are between 0 and 20 μM. The vaginal environment is microaerophilic, with an oxygen concentration in this range (or slightly higher) (34). Increased oxygen levels inhibit the accumulation of [^{14}C]metronidazole in resistant strains. This is due to oxygen competition with metronidazole for ferredoxin-bound electrons. If metronidazole is not reduced, the concentration of the drug is the same in the intra-and extracellular environments, and no additional drug enters the cell. Additionally, in the presence of oxygen, a reduced nitro free radical may be oxidized back to the original compound and then reduced to become a superoxide anion. This process is known as futile cycling and results in only limited cell damage via superoxide anions rather than cell death due to nitro free radicals (100).

The degree of aerobic resistance of wild-type *T. vaginalis* strains is variable and depends on the degree to which oxygen-scavenging mechanisms, and potentially ferredoxin activity, are impaired. In vitro, aerobic resistance can be induced by culturing trichomonads in TYM medium containing sublethal concentration of metronidazole. *T. vaginalis* cultured in this manner can be differentiated from strains with anaerobically induced resistance because they retain PFOR activity (120).

Role of Oxygen

T. vaginalis is not strictly anaerobic and consumes oxygen at low levels (70, 123). PFOR and hydrogenase may play roles in protecting the parasite from the toxic products of oxygen reduction. *T. vaginalis* also contains cytosolic and hydrogenosomal oxidases and an iron superoxide dismutase, all of which are involved in protecting the cell from reactive oxygen species (34). *T. vaginalis* iron superoxide dismutase is of particular interest since mammalian cells do not contain this enzyme, making it a possible target for drug therapy.

Clinically resistant strains of *T. vaginalis* have poor peroxide-reducing enzymes and impaired oxygen-scavenging pathways (34). This leads them to be more sensitive to increased levels of oxygen than their metronidazole-sensitive counterparts (106). When oxygen concentrations exceed 60 μM, clinically resistant strains are inactivated and killed more quickly than drug-sensitive trichomonads. Cell death is the result of an accumulation of toxic oxygen metabolites normally prevented by effective oxygen scavenging and peroxide reduction (34).

Iron is also important in regulating a number of the activities of *T. vaginalis* (66) and has been proposed to be involved in metronidazole resistance, particularly that involving the PFOR/ferredoxin pathway. Research in this area is mostly at the in vitro stage, however, and correlations with clinical findings have not yet been firmly established.

Treatment Failure

Treatment of trichomoniasis with oral or intravenous metronidazole is effective in 85 to 95% of cases, and when treatment fails to cure, a number of factors must be considered. Reinfection can be ruled out if the sexual partner(s) of a patient is treated concurrently, and noncompliance can be eliminated if a single-dose rather than multiple-dose regimen is used. Relapse in patients for whom reinfection and noncompliance have been ruled out has been reported, but it appears to be rare, and little is known about the mechanisms that lead to such a recurring trichomoniasis (51). Low zinc concentrations in serum (126), inactivation of metronidazole by vaginal bacteria, and ineffective delivery of the drug to the vaginal area have been proposed as explanations for treatment failure in patients for whom reinfection and noncompliance are not a factor (31, 87). Vaginal preparations of metronidazole (cream or insert) have much lower cure rates (20%), probably because low absorption into the serum leads to poor delivery to the genitourinary glands and organs other than the vagina that may have become infected by *T. vaginalis* (49). A protocol exists that takes the above factors into consideration when determining further treatment for refractory trichomoniasis (6).

Diagnosis of Resistance

Once other causes of treatment failure have been ruled out, the possibility of a resistant *T. vaginalis* infection must be considered. It is estimated that at least 5% of all clinical cases of trichomoniasis are caused by *T. vaginalis* strains with at least some resistance to metronidazole (96, 101). Most of these strains have low or moderate resistance to the drug, although highly resistant trichomonads have also been recovered from patient samples. Metronidazole susceptibility testing is important if a resistant *T. vaginalis* infection is suspected, since it can help determine the course of subsequent treatment.

Anaerobic assays have been used to measure *T. vaginalis* resistance to metronidazole, but the results were often controversial because there is poor discrimination of susceptibility in anaerobic culture (46). In vitro testing of aerobic culture offers the best results in determining susceptibility, as well as reflecting the fact that therapeutic use of metronidazole occurs under

microaerophilic conditions (92). MLCs determined in such assays do not directly reflect the curative concentration of metronidazole in serum (74), but the degree of drug resistance may be useful in estimating the dosage that is likely to be effective (77, 93).

REFRACTORY CASES

Refractory cases of trichomoniasis, defined as cases in which two standard courses of treatment fail to cure, can be extremely difficult to treat. Increasing drug dosages may reach levels that are toxic to the patient. To avoid such toxicity, standard therapy may be combined with anecdotal cures found in case studies, such as vaginal clotrimazole, arsenical pessaries, betadine douches, and the SolcoTrichovac lactobacillus vaccine. However, limited information about the effects of these drugs on *T. vaginalis* makes it questionable whether these regimens are curative or merely palliative.

Extended and Combined Therapies

When metronidazole is the only approved treatment of trichomoniasis, a treatment regimen for refractory infection involves increased doses of oral metronidazole (often double doses) for extended periods. Extended therapy is effective in only about 80% of these patients (in contrast to a 95% cure rate in compliant, nonrefractory patients) (96). In cases in which trichomonad drug resistance is very high and toxic levels of metronidazole are required, administration of the drug intravenously or in combination with oral and vaginal therapy may minimize side effects. One treatment protocol is available that recommends regimens for marginal, low, moderate, and high levels of metronidazole resistance (6). This highlights the need for metronidazole susceptibility testing in refractory *T. vaginalis* infection, as well as the need for alternate therapies to avoid increasing resistance in wild-type strains as a result of increased metronidazole pressure.

Tinidazole

Tinidazole is a 5-nitroimidazole currently in use for the treatment of trichomoniasis in countries outside the United States and under Food and Drug Administration review. It has a longer half-life than metronidazole and is eliminated at a significantly lower rate (86). It shows superior tissue distribution to that of metronidazole, and concentrations of the drug found in vaginal secretions are close to the levels found in serum, showing that it is delivered more effectively to this area than is metronidazole (73). MLCs of tinidazole for various *T. vaginalis* strains are consistently lower than MLCs for metronidazole, and this is reflected clinically: tinidazole is curative at lower doses then metronidazole. The therapeutic doses of tinidazole result in fewer and milder side effects (76, 81, 96).

Since tinidazole is a nitroimidazole, however, its mode of action is similar to that of metronidazole, and cross-resistance to *T. vaginalis* is a concern. Studies have shown that cross-resistance among nitroimidazoles does occur but is incomplete. Both in vitro and in vivo assays have shown that MLCs of tinidazole for metronidazole-resistant trichomonads are generally significantly lower than the MLCs of metronidazole (77,

96). Thus, metronidazole-resistant trichomoniasis may be treated with tinidazole, but rapid development of tinidazole resistance due to the similarity of metabolic pathways should be a concern.

Other Nitroimidazoles

A number of nitroimidazole derivatives other than metronidazole and tinidazole have been investigated for the treatment of *T. vaginalis* infection. The modes of action of these derivatives are similar, but the pharmacokinetics, tissue distribution, levels in serum, trichomonicidal activity, and toxicity are variable.

Ornidazole and secnidazole are similar to tinidazole in that they have longer half-lives and lower rates of elimination than metronidazole. In contrast, nimorazole is rapidly absorbed and metabolized. It retains significant antiprotozoal activity, however, since its two major metabolites are much more active than the metabolites of metronidazole (65). More recently, the nitroimidazole EU11100 was developed in an attempt to obtain a drug with the trichomonicidal activity of metronidazole but without the side effects. In vitro testing has shown that EU11100 has a very low MLC compared to other nitroimidazoles, but no clinical trails have been published (28).

Comparative studies of metronidazole and other nitroimidazoles have shown that most drugs in this family are effective at similar dosages (1.5 to 2 g one-time dose) and that the majority of patients suffer similar, usually mild, side effects. Severe adverse reactions (especially related to single high doses) and hypersensitivity are usually related to nitroimidazoles in general and not to one drug in particular. The one exception to this is misonidazole, which has consistently been shown to cause more serious side effects (peripheral neuropathy). Interest in misonidazole has therefore shifted away from a possible role in the treatment of trichomoniasis and is focusing on its radiosensitizing properties (65).

Other Chemotherapeutic Agents

A number of studies have been published demonstrating the in vitro trichomonicidal activities of nonimidazole drugs. These compounds include both agents currently in use in industry or for the treatment of other infectious diseases and drug derivatives synthesized specifically for the treatment of *T. vaginalis* infection.

Hamycin is an aromatic polyene related to amphotericin B. It can induce cell death in *T. vaginalis* and other eukaryotic cells by binding to ergosterols in the plasmalemma and causing the formation of pores, leading to cytoplasmic leakage and cell death. Studies have shown that hamycin at low concentrations effectively kills both metronidazole-sensitive and -resistant strains of *T. vaginalis*. The drug is currently in use in India as a topical treatment for trichomoniasis. Unfortunately, reported side effects in patients and laboratory animals, along with in vitro studies with mammalian tissue cultures, indicate that the toxicity of hamycin may limit future clinical applications (78).

Intravaginal application of paromomycin has been successfully used to treat recurrent trichomoniasis. However, severe

side effects, including pain and mucosal ulceration, make it an unlikely candidate for clinical therapy (102).

Sodium nitrite, sodium nitroprusside, and Roussin's black salt, traditionally used to prevent food contamination, exhibit trichomonicidal activity. The mode of action of these compounds is unknown, but they are active against both metronidazole-sensitive and -resistant strains of *T. vaginalis* (110).

The nitrothiazole derivative niridazole has a broad spectrum of antimicrobial activity and is active against *T. vaginalis*. Both metronidazole-sensitive and -resistant strains are inhibited by this drug. Chemical analyses have shown that niridazole may have multiple modes of action, accounting for its wide range of inhibitory effects, but specific mechanisms are unknown (129).

Nitazoxanide is a 5-nitrothiazolyl that has a broad spectrum of activity against protozoan parasites in vivo. In vitro studies have shown that the compound is active against both metronidazole-sensitive and -resistant strains of *T. vaginalis*, with some formulations being about five times more active against trichomonads than is metronidazole (4).

An in vitro comparison study of antimicrobial drugs established that of the 50 compounds tested, only metronidazole and tinidazole (no other nitroimidazoles were tested) and three others possessed significant trichomonicidal activity. Both metronidazole-sensitive and -resistant strains of *T. vaginalis* were inhibited by the heterocyclic antibiotic anisomycin; an antigiardial nitrofuran, furazolidone; and mebendazole, a microtubular inhibitor commonly used to treat helminth infections. Unfortunately, the level of toxicity exhibited by all three of these compounds is cause for concern and may limit their potential as intravaginal preparations; clearly, more research is required (111).

An in vitro comparison of two synthetic derivatives of benzoizothiazolinone showed that the drugs exhibited significantly higher trichomonicidal activities than metronidazole. The antimicrobial mechanisms of the derivatives remain largely undefined (130).

A partial list of other drugs investigated for antitrichomonal activities include sulfimidazole, a 5-nitroimidazole with a functional sulfonamide group (80); nifuratel, a nitrofuran derivative (75); berberine sulfate, a plant alkaloid (50); MDL 63,604, a derivative of the antibiotic purpuromycin (122); lipophilic tetracyclines (52); thiadiazine derivatives (13); some 4-nitrobenzimidazole derivatives (7); specific benzimidazole derivatives (53); acetylated derivatives of sugar hydrazones (79); spiroarsoranes, "spiranized" arsonic acids (71); and disulfiram, a drug often used to treat alcoholism (14). These compounds have all shown some promise in the treatment of trichomoniasis, but research either is in the preliminary stages or is not being systematically pursued at this time.

VACCINES

With an increasing incidence of refractory infection and the lack of a safe and effective alternative to nitroimidazoles, disease prevention with a vaccine would clearly be desirable. As with many sexually transmitted diseases, infection with *T. vaginalis* does not induce long-term immune protection (3, 44). A complicated host defense system restricts *T. vaginalis* infection to the genitourinary tract and may play a role in limiting the immune response and preventing long-term immunity.

The host defense network has three components: nonimmunologic factors such as zinc concentration and iron availability; nonspecific innate mechanisms including complement, natural antibodies, and phagocytes; and specific adaptive B- and T-cell responses (3, 27, 64, 112). The antibody response to *T. vaginalis* infection is well documented and includes circulating serum and cervicovaginal immunoglobulin A (IgA), IgG, and IgM (8, 19, 83, 113, 114, 118, 119). However, these antibodies appear to provide only limited protection from invading parasites, and antibody titers progressively dwindle after infection is eradicated by treatment (44). At 6 to 12 months after infection, neither *T. vaginalis*-specific antibodies nor memory B cells are present in the circulation, leaving the host with no defense against subsequent infection. A T-cell-mediated response has also been demonstrated in trichomoniasis, but its mechanisms remain largely undefined and it is likely to be too limited to provide sustained protective immunity (84, 128).

Research into the development of a vaccine for *T. vaginalis* has shown some promise, elucidating a number of mechanisms by which protection could potentially be achieved. One study has shown that many women develop an immune response to the 115-kDa α-actin protein of *T. vaginalis* (5). Elevating this response through the use of an adjuvant or other costimulation might contribute to a stronger immune reaction. It has also been shown that *T. vaginalis* strains possess both unique and common antigenic epitopes (36, 85, 128). The presence of shared epitopes suggests that it may be possible to provide protection to a cross section of *T. vaginalis* strains in a single vaccine. Potential antigen candidates could include a 100-kDa protein that was found to be immunogenic across a broad sampling of *T. vaginalis* isolates (36). Essential adherence molecules, including adhesins, mucinases, and cysteine proteinases (67), are also potential targets, given the importance of adherence for the pathogenicity of the organism.

Two distinct *T. vaginalis* vaccine candidates have progressed to the stage of human clinical trials. The first was studied in the 1960s and involved a trial of 100 women with refractory trichomoniasis receiving intravaginal inoculations with increasing numbers of heat-killed *T. vaginalis* cells. A 100% improvement in clinical symptoms was reported, but the procedure has not been repeated and this method of vaccination has been left unpursued (2).

In the late 1970s, another *T. vaginalis* vaccine, under the commercial name SolcoTrichovac or Gynatren, became available. The vaccine was derived from heat-inactivated "abnormal strains of lactobacilli" that had been isolated from the vaginal secretions of women with trichomoniasis (99). These lactobacilli were reported to have lost the ability to produce lactic acid, and their morphology was described as shortened or coccoidal rather than the traditional elongated bacillus (90).

A diverse spectrum of mechanisms were proposed to explain the activity of SolcoTrichovac. It was proposed that aberrant lactobacilli associated with *T. vaginalis*, leading to the formation of bacterial-trichomonad immune complexes which immobilized the parasites and induced cytolysis and/or stimulated macrophage activity. SolcoTrichovac-induced production of lymphokines, leading to the activation and attraction of T cells to the infected area, was another proposed mechanism. Since the antibody response is strong during *T. vaginalis* infection, enhanced trichomonicidal activity was hypothesized to be the

result of either antilactobacillus antibodies cross-reacting with *T. vaginalis* or trichomonad uptake of *Lactobacillus* membrane components, leading to antibody recognition and destruction of the parasites (91). It was also suggested that the abnormal lactobacilli in some way reestablished a "normal" vaginal flora, which would lead to a reduction of pH that would inhibit the growth of *T. vaginalis* (72, 99). None of these mechanisms of action were experimentally proven to be the mode of action of the vaccine.

Although SolcoTrichovac was initially purported to both cure existing infection and provide protection from reinfection, subsequent studies cast doubt on the actual efficacy of the vaccine against *T. vaginalis*. Clinical trial results were positive, but these studies often were not very thorough or lacked the proper control groups. The fundamental theory behind the vaccine was tenuous, since no particular antigenic similarity between lactobacilli and *T. vaginalis* was ever shown. Given these facts, it is unlikely that any trichomonidical activity of SolcoTrichovac was the result of a direct immune or antibody response to *T. vaginalis*. It is more likely that vaccination stimulated an increase in nonspecific immune factors capable of alleviating symptoms and eliminating infection (9, 39).

Given that the immune response to vaginal *T. vaginalis* infection is comparatively weak and does not elicit protection, introduction of a vaccine via a systemic route has been considered as a method of inducing a stronger and more lasting immune response. A murine model of *T. vaginalis* infection was established, in which mice were immunized with two subcutaneous injections of whole trichomonads, first in Freund's complete adjuvant and later in Freund's incomplete adjuvant (1). The mice were then estrogenized and vaginally preinoculated with *Lactobacillus acidophilus* to establish a vaginal milieu comparable to that found in humans (88). Infection with *T. vaginalis* was then performed, and vaginal washes were used to compare infection rates between immunized and control mice. Results showed that immunized mice either were protected or cleared the infection faster than sham-vaccinated or naive mice did. Nonvaccinated mice cured with metronidazole were not protected from vaginal infection and showed no immune response, whereas vaccinated mice had elevated levels of anti-*T. vaginalis* antibodies in both serum and vaginal secretions (42).

A similar murine model has been developed for *Tritrichomonas foetus*, a protozoan related to *T. vaginalis* that causes vaginal infection and spontaneous abortion in cattle. *T. foetus* is closely related to the human pathogen, and the model has the advantage of not requiring preestrogenization of mice (95). A bovine *T. foetus* model has also yielded promising results. Employing the natural host of the parasites enables a more accurate analysis of the natural process of infection and immunity. A whole-cell *T. foetus* vaccine in now commercially available for cattle and has been found both to provide protection and to accelerate eradication of infection. Antibody titers after vaccination were similar to those in *T. vaginalis* infection studies (given the immunologic differences between species), namely, IgG in serum and IgA and IgG in vaginal secretions. A more recent study compared the duration of infection and reproductive losses in heifers vaccinated with *T. foetus* whole cells or cell membrane alone and challenged with an infected bull. The results showed that infection was eradicated somewhat faster

and calf mortality was reduced in animals vaccinated with the membrane vaccine (18).

Comparison of *T. vaginalis* and *T. foetus* infections has shown that at least some disease mechanisms are shared (21). The existence of a successful *T. foetus* vaccine and the development of a subunit vaccine are encouraging in the search for a vaccine against *T. vaginalis*.

CONCLUSIONS

T. vaginalis infection has in the past been considered a "nuisance" disease of women and a problem of developing countries. However, increasing global infection rates, pregnancy complications, and increased susceptibility to HIV and other sexually transmitted diseases make it clear that safe, effective, and affordable treatment of trichomoniasis is essential.

Increases in both the prevalence of metronidazole-resistant *T. vaginalis* infection and the degree of drug resistance in the parasites indicate a need for non-nitroimidazole treatment for refractory trichomoniasis. A number of drug candidates have been investigated, but research must now continue past the initial in vitro investigations to the point of clinical trials. A successful vaccine could limit the human cost of trichomoniasis and its related complications. It could also control the real cost of repeated treatments, an important consideration given the incidence of *T. vaginalis* every year in developing countries. The mouse model of intravaginal infection provides an excellent launchpad for such investigation, and the success of the related model and vaccine for *T. foetus* indicates that such research can yield beneficial results.

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