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REVIEW

Strategies for Prevention and Treatment of *Trichomonas vaginalis* Infections

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SUMMARY The last estimated annual incidence of *Trichomonas vaginalis* worldwide exceeds that of chlamydia and gonorrhea combined. This critical review updates the state of the art on advances in *T. vaginalis* diagnostics and strategies for treatment and prevention of trichomoniasis. In particular, new data on treatment outcomes for topical administration of formulations are reviewed and discussed.

KEYWORDS *Trichomonas vaginalis*

INTRODUCTION

Trichomonas vaginalis is a human protistan parasite responsible for the most common nonviral sexually transmitted disease in the world. *T. vaginalis* is recognized as a cause of vaginitis. It can also infect the urethra and prostate in men. Although men are often asymptomatic carriers of *T. vaginalis*, dysuria and discharge have also been reported (1). In women, the disease may range from asymptomatic (up to 50% of women) to severe with serious sequelae. Classical symptoms include a malodorous and purulent discharge which results in local pain and irritation. *T. vaginalis* is implicated in reproductive tract postsurgical infections that usually remain localized to the lower part of the urogenital area. This parasite can also result in serious consequences, such as infertility, premature rupture of placental membranes, premature delivery, low-birth-weight infants, and neonatal death (2). Moreover, an increased predisposition to HIV infection has been reported for both men and women (3).

Oral metronidazole remains the recommended regimen for the treatment of trichomoniasis. However, treatment failure does occur, mainly due to significant gastrointestinal adverse effects, which have been found to be temporary and disappear after the cessation of treatment. Systemic delivery of metronidazole may also result in

TABLE 1 Incidences and prevalences of *T. vaginalis* infection in women and men between the ages of 15 and 49 years in different world regions in 2008[a]

Region	Incidence				Prevalence			
	Women		Men		Women		Men	
	Per 1,000	Millions of individuals	Per 1,000	Millions of individuals	%	Millions of individuals	%	Millions of individuals
Africa	146	28.1	164.8	31.6	20.2	38.9	2	3.9
The Americas	177.7	42.5	180.6	43	22.0	52.7	2.2	5.2
Southeast Asia	40.3	18.5	50.1	24.3	5.6	25.7	0.6	3.0
Europe	51.7	11.6	48.4	10.9	5.8	13.0	0.6	1.3
Eastern Mediterranean	64.0	9.7	66.1	10.6	8.0	12.0	0.8	1.3
Western Pacific	45.6	21.9	47	23.8	5.7	27.2	0.6	2.9

[a]The data used are from the WHO (137).

allergy and drug resistance (4). In this context, intravaginal drug delivery allows better-tolerated prevention and treatment options for trichomoniasis that avoid systemic adverse effects.

This article offers an update on the progress in *T. vaginalis* diagnostics and chemotherapy for the treatment of trichomoniasis. A vaccine strategy for the prevention of *T. vaginalis* vaginal infections and formulations for the treatment of vaginal trichomoniasis are discussed. Although reviews of the literature do exist (5–14), documentation on the vaginal administration of drugs and strategies for the prevention of *T. vaginalis* infection has, until now, been inconsistent.

EPIDEMIOLOGY AND TRANSMISSION

According to the WHO estimate, among the total number of new cases of curable sexually transmitted infections (STIs) in 2008 (498.9 million adults aged 15 to 49 years), 276.4 million resulted from *T. vaginalis*. The prevalence and incidence of vaginal *T. vaginalis* infection are higher in African regions and in the Americas than in other parts of the world (Table 1). Different studies conducted in Denmark, Great Britain, and France showed that the prevalence is declining in industrialized nations (15, 16). Iranian studies determined the prevalence to be 2 to 8% (17). However, based on cultural and social factors, this rate is underestimated and may be higher than 30% (17).

No association was found in the United States between trichomoniasis and HIV infection (9), while a study conducted in Africa demonstrated that HIV infection increased the prevalence of vaginal trichomoniasis (18). The exact mechanism by which *T. vaginalis* facilitates HIV transmission is poorly understood, but it is probably related to a higher susceptibility to bacterial vaginosis (19). *T. vaginalis* infections also involve inflammatory processes, which may facilitate vaginal infection with HIV (20, 21).

Trichomoniasis is a venereal disease that affects members of both sexes. Successful treatment of *T. vaginalis* infection reduced HIV transmission by mucosal routes (20, 22). A Rwandan study showed a difference in the prevalences of trichomoniasis in pregnant women who were infected with HIV or not (20.2% and 10.9%, respectively) (23). Another study, conducted in the United States, demonstrated that *T. vaginalis* contributes to HIV acquisition and exceeds the relative contributions of other STIs (22). Metronidazole used for the treatment of *T. vaginalis* infection significantly decreased the number of HIV (RNA)-free cells (24). Moreover, in HIV-infected women, complications of reproductive tract infection increased significantly with *T. vaginalis* and may decrease if *T. vaginalis* infection is controlled (3, 25).

A study conducted on 43,016 Norwegian women showed that trichomoniasis increased the risk for cervical neoplasia (CN) caused by human papillomavirus (HPV) (26). The same correlation between trichomoniasis and cervical cancer induced by HPV was found during studies conducted in Finland (27) and India (28). Another study showed that *T. vaginalis* infection promoted HPV infection by a factor of 6.5, increasing the risk for CN (29).

Some strains of *T. vaginalis* carry their own viruses that amplify inflammatory

responses (30). Trichomonasvirus released from infected *Trichomonas vaginalis* induced inflammation upon metronidazole treatment (30).

It is well known that infected pregnant women can transmit *T. vaginalis* to their fetuses (2). Unlike other nonviral STIs, trichomoniasis does not primarily reach young women (15 to 25 years of age). It affects women during the reproductive years, and high rates of infection are found in women between the ages of 35 and 40 (31, 32). Predisposing factors comprise older age, use of oral contraceptives, trading sex, smoking, single marital status, and low socioeconomic class (9, 33, 34). The prevalence and average duration of infection depend on the health care-seeking behaviors of populations and their access to health care (34).

CLINICAL PRESENTATION

While it is usually isolated from the vagina, *T. vaginalis* can also infect the urethra and Skene's gland. The infection, once established, may persist for long periods in women. Asymptomatic *T. vaginalis* infections are well documented; up to 25 to 50% of infected women do not show clinical signs. However, women can also develop symptoms that may be cyclic and often become worse during menstruation. Among women with culture-proven *T. vaginalis* infection, only 11 to 17% present abnormal discharge, odor, pruritus, dysuria, or vaginal burning (35). A "strawberry cervix" is observed in only 2% of women (36).

In healthy adult women, the vaginal pH is around 4. During trichomoniasis, the vaginal pH increases to >7 (37), which is favorable to parasite growth. The fact that trichomoniasis symptoms are worse during menstruation can be explained by changes in pH and hormones. It was proved that the activity of cell detaching factor is inhibited by estrogen (38). Furthermore, menstrual blood creates a rich medium with a high concentration of iron at a higher pH. Consequently, *T. vaginalis* reproduction and attachment to the vaginal epithelium are promoted, resulting in the worsening of symptoms (10).

Even if trichomoniasis usually remains localized in the lower part of the urogenital area, it can occasionally provoke adnexitis or pyosalpinx and may potentially have serious sequelae in women, especially during pregnancy.

BIOLOGY AND PATHOGENESIS OF *TRICHOMONAS VAGINALIS*

T. vaginalis is a parasitic protozoan that is typically pyriform, but its appearance is modified under physicochemical conditions (39) (Fig. 1). *T. vaginalis* has five flagella. Four flagella of about 7 to 18 μm are located at its anterior end and give its characteristic twisting and wriggling movement. The fifth flagellum is incorporated within the undulating membrane, whose length is equivalent to half that of the cell, and is supported by a slender, noncontractile costa. An axial axostyle starting at the centrosome is extended by a small posterior tip end (Fig. 1). *T. vaginalis* has a large nucleus characteristic of eukaryotic cells as well as a highly developed Golgi apparatus. As it lacks mitochondria, *T. vaginalis* contains hydrogenosomes as alternative providers of energy (40).

After cytoadherence, the parasite changes its structure to an amoeboid form, which allows an increased contact surface with vaginal epithelial cells followed by adhesion to target cells (for a review, see references 11 and 41). Five *T. vaginalis* attachment proteins (AP), named adhesins, are able to mediate parasite attachment; these are AP23, AP33, AP51, AP65, and AP120. Other surface proteins are also implicated in the attachment of *T. vaginalis*, including fibronectin binding protein and glycolipids, such as lipophosphoglycan (10). Lipophosphoglycan is a pure carbolipid (no peptide component) that, similarly to prokaryotic glycoconjugates, is anchored to the *T. vaginalis* surface via inositol-phosphoceramide.

The immunoinflammatory response to infection has been investigated *in vitro* and in different animals, including murine, bovine, and nonhuman primate models (42–44). *In vitro* experiments have been performed using cervical and vaginal cells and various immune cell types.

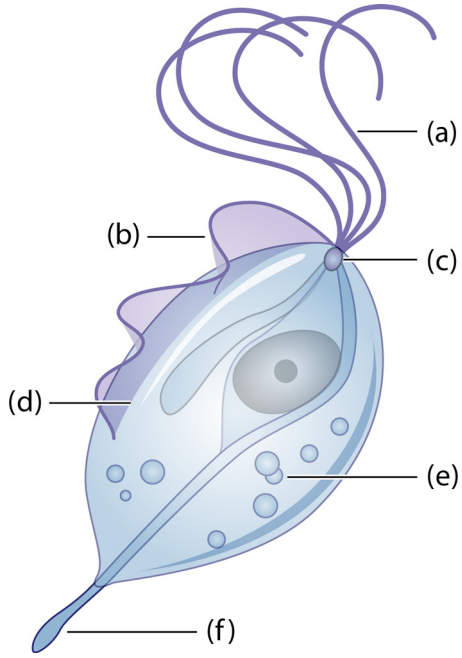

FIG 1 Schematic drawing of *Trichomonas vaginalis*. (a) Anterior flagellum; (b) undulating membrane; (c) pelto; (d) costa; (e) hydrogenosomes; (f) axostyle. The parasite has an average length and width of 9 to 23 and 7 μm, respectively.

The vaginal discharge of infected women contains polymorphonuclear leukocytes. Interaction between *T. vaginalis* and cells triggers an active involvement of signaling pathways. This leads to the production of interleukin-8 (IL-8), IL-6, macrophage chemoattractant protein 1 (MCP-1), and tumor necrosis factor alpha (TNF-α). Several mitogen-activated protein kinase (MAPK) signaling pathways can be activated, such as c-Jun N-terminal kinase (pJNK), p38, and extracellular signal-regulated kinase 1/2 (ERK1/2) pathways. During *T. vaginalis* infection, the signaling pathways involved are ERK1/2, p38, and NF-κB pathways (45). These signaling pathways also lead to increased mRNA expression of Toll-like receptors (TLRs) (30, 46).

Finally, it appears that MAPKs are also involved in the establishment of cell death in the form of apoptosis by activating Bcl-XL (a Bcl-2-like protein), but not via the Bcl-2 pathway (47), as well as NF-κB in macrophages (46). Implementation of this apoptosis and autophagy pathway may be demonstrated in epithelial cells to decipher more mechanisms related to the pathogenesis of the parasite. An understanding of the host-parasite interaction mechanism is still under investigation by researchers and may lead to the identification of molecular targets on *T. vaginalis* for the design of new trichomonacidal drugs.

DIAGNOSIS

T. vaginalis was identified more than 150 years ago (in 1836), by Donné, when he visualized motile microorganisms in vaginal fluid from women with symptoms of infection. However, the sensitivity of this microscopic visualization technique, also called the wet mount test, is variable, with sensitivities of 38% to 82% among symptomatic women (12). Despite these statistics, the wet mount test is still used in clinical trials for evaluation of drug activity (48). This test must be performed within a few minutes after sample collection to observe viable parasites.

Broth culture of vaginal fluid requires the use of a specialized medium, such as Diamond's or Trichosel medium. Unfortunately, this diagnostic test has different drawbacks: it is limited to laboratories with access to the culture medium and an incubator and has a delay of up to 7 days for *T. vaginalis* identification (49). The culture method was improved by the development of the InPouch device, which is commercially

available. This device consists of a plastic bag with two chambers connected by a narrow passage. The collected specimen is placed in the upper chamber, while the lower chamber is for culture and further observation when necessary. Comparison of the effects of culture medium on sensitivity showed that the InPouch system is at least as sensitive as culture with Diamond's modified medium (50).

PCR-based tests can detect very few trichomonads in a sample, as well as nonviable organisms. The Affirm VPIII test (Becton Dickinson, Sparks, MD) is an amplification test for RNA that allows *T. vaginalis* detection within 30 to 60 min. The specificity and sensitivity of such tests are 99% and 90%, respectively (10).

The OSOM *Trich* rapid antigen test is an immunochromatographic capillary-flow enzyme immunoassay dipstick test that detects *T. vaginalis* membrane protein. Compared to culture, the OSOM *Trich* test has the advantage of being rapid, as the result is available in 10 min. It can be conducted on frozen samples without altering the test characteristics (51).

Nowadays, newer diagnostic options are available (49). Commercially available nucleic acid amplification tests (NAATs) have been validated for use for asymptomatic and symptomatic women and are highly sensitive tests that can be used on multiple specimen types, including urinary, urethral, vaginal, and endocervical specimens (52–54). In a study conducted during 2012 and 2013 in Jefferson County, AL, endocervical, urethral, or urinary specimens from 3,821 women and 2,514 men were collected for *T. vaginalis* detection by use of wet mount detection and a *T. vaginalis* NAAT. The results showed that the *T. vaginalis* NAAT detected infections in women at a rate that was 1.3 times higher than that for wet mount detection (53). In summary, detection of *T. vaginalis* by NAATs, even for asymptomatic patients, should result in better control and treatment of *T. vaginalis* infection (53).

Other NAATs are available, such as the AmpliVue and Solana (Quidel) tests (55). The GenXpert (Gx) assay (Cepheid) (56, 57) is the only NAAT cleared for use for men.

SYSTEMIC CHEMOTHERAPY OF UROGENITAL TRICHOMONIASIS

Currently, 5-nitroimidazole drugs are commonly used for treatment of trichomoniasis by oral and parenteral routes. Among these drugs, only metronidazole and tinidazole are available in the United States and are authorized by the Food and Drug Administration (FDA) for the treatment of trichomoniasis.

Metronidazole (α,β-hydroxyethyl-2-methyl-5-nitroimidazole; Flagyl) (Fig. 2a) was developed in 1959 and approved in the 1960s for the treatment of trichomoniasis, and it was the only drug with a high cure rate after systemic treatment. Despite the fact that the partners of patients with *T. vaginalis* infection are often infected as well, high rates of asymptomatic infection mean that they do not always seek treatment and may reinfect the partner who was treated. As a consequence, sexual partners of patients should routinely be treated (58, 59).

Metronidazole is relatively cheap, effective, and generally well tolerated. Common side effects, such as gastrointestinal disturbances, are usually mild. Occasional hematologic and neurotoxic side effects have also been reported. Concerning patients with refractory trichomoniasis infection, side effects due to metronidazole become a real problem. In fact, recurrent or resistant trichomoniasis infections are treated for longer periods with increasing doses of metronidazole. However, with higher doses, side effects are much more frequent, leading to patient discomfort and treatment failure (60). In the absence of an alternative to nitroimidazole treatment, cure can be achieved only by increasing dosages of metronidazole. However, side effects may limit the dose of metronidazole and sometimes necessitate stoppage of treatment (61). Cases that cannot be treated with higher doses of metronidazole are a real challenge for both patient and practitioner. Different treatment options with higher doses of oral metronidazole or tinidazole for longer periods have been used together with intravaginal drug delivery (62).

Tinidazole (Fig. 2b) is a nitroimidazole that was introduced in 1969 for the treatment of infections caused by *T. vaginalis*. Tinidazole is curative at lower therapeutic doses

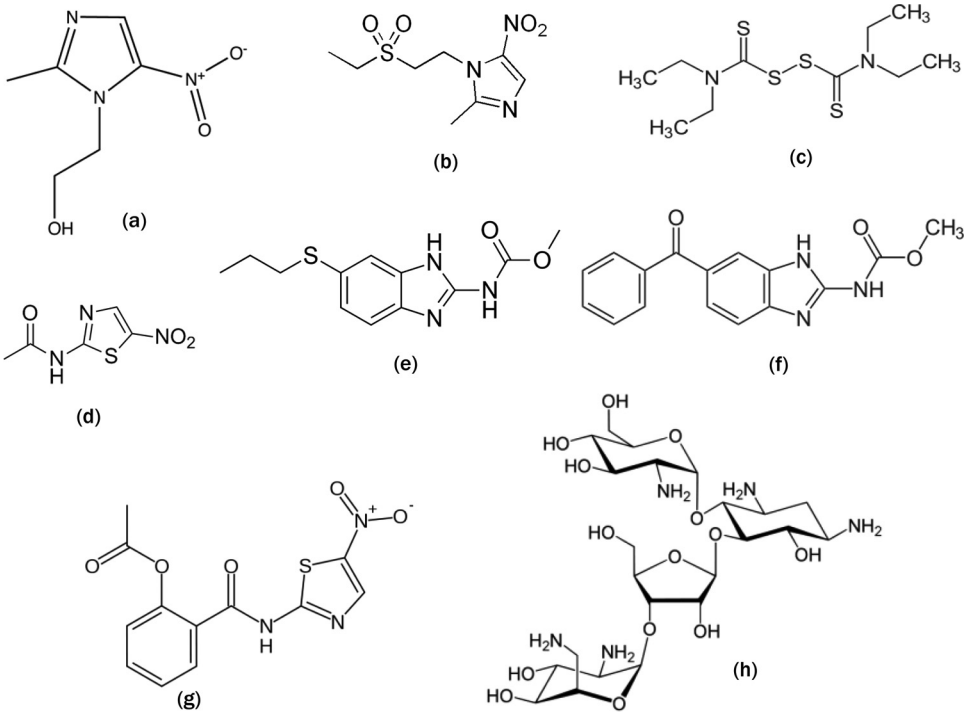

FIG 2 Chemical structures of anti-*T. vaginalis* drugs. (a) Metronidazole; (b) tinidazole; (c) disulfiram; (d) nithiamide; (e) albendazole; (f) mebendazole; (g) nitazoxanide; (h) paromomycin.

than those for metronidazole and results in fewer and milder side effects (63). Minimum lethal concentrations (MLCs) of tinidazole are lower than those of metronidazole for *T. vaginalis* isolates, and tinidazole resistance is not detected (64).

The mechanism of metronidazole resistance is not totally elucidated, but it is assumed that it may be due to several mutations (4). In anaerobic resistance, the activity of the key enzyme pyruvate:ferredoxin oxidoreductase (PFOR) decreases (65) or disappears in *T. vaginalis*, and the metabolism of the drug is disrupted. In aerobic resistance, transcription of the ferredoxin gene is reduced in resistant *T. vaginalis* strains (66). Aerobic and anaerobic metronidazole resistances have in common a decrease or loss of flavin reductase activity (67).

Although the 5-nitroimidazole compounds are the most effective drugs for treating *T. vaginalis* infection, disulfiram and nithiamide (Fig. 2c and d) might represent alternatives for treating patients with hypersensitivity to 5-nitroimidazole drugs. In another study, the 50% inhibitory concentrations (IC_{50}s) for albendazole (Fig. 2e) against *T. vaginalis* were 13.2 μg/ml (50 μM) and 0.899 μg/ml (3.39 μM) after 4 and 48 h of exposure, respectively (68, 69). Albendazole and mebendazole (Fig. 2f) have so far been found to be effective *in vitro* against *T. vaginalis* (70).

Nitazoxanide (Fig. 2g), a 5-nitrothiazolyl derivative, has shown *in vitro* activity against *T. vaginalis*, with IC_{50} and IC_{90} values of 0.034 and 2.046 μg/ml, respectively (71). However, in 2007, there was a failure to cure trichomoniasis with nitazoxanide in three women who presented with nitroimidazole resistance (72).

Clearly, there are only a few data on alternatives to nitroimidazole derivatives, and new antitrichomonal agents or new pharmaceutical formulations are needed to treat infections with resistant *T. vaginalis* organisms.

PREVENTION OF *T. VAGINALIS* INFECTIONS

Condom use remains the best and most reliable protection against STIs. However, due to religious or cultural reasons, condom use may be limited, particularly in some developing countries. Concurrent treatment of sexual partners is recommended to prevent reinfection. However, systemic administration of chemotherapeutics to prevent

infection results in increased incidences of nitroimidazole-refractory strains. Prevention methods using local intravaginal formulations or vaccines are thus necessary.

Vaccination against *T. vaginalis* is particularly interesting for high-risk individuals to protect themselves and their partners. This strategy would solve many of the issues that currently undermine control efforts. Vaccination against trichomonads is already commercially available against *Tritrichomonas foetus* (TrichGuard; Boehringer Ingelheim Vetmedica). This parasite is a flagellate protozoan similar to *T. vaginalis* that infects cattle. Many recent research works and reviews on vaccination against *T. foetus* have been published (73–75). These studies have shown benefits of vaccinating cattle against trichomoniasis by prevention or clearing of genital infections due to *T. foetus*. Given the similarity between *T. foetus* and *T. vaginalis*, development of a vaccine against human trichomoniasis seems achievable and has the potential for significant social and health impacts. However, the severe economic implications of *T. foetus*, which are estimated to be $800 to $6,030 per bull per year in the United States, stimulated research into development of a *T. foetus* vaccine. The calf crop can be reduced by up to 50% in beef enterprises, which partly explains why research has been funded for this bovine vaginal infection and not for the equivalent human infection.

Long-term immune protection is not induced by infection with *T. vaginalis* (76), and the design of an efficacious vaccine still remains a challenge. Due to the limited success of intravaginal vaccination reported in the 1960s with heat-killed *T. vaginalis* and in 1970 with Solco Trichovac, derived from heat-inactivated abnormal strains of lactobacilli, systemic administration of vaccines against *T. vaginalis* was considered. More recently, subcutaneous vaccination of mice reduced the incidence and increased the clearance of *T. vaginalis* infection (77). In the vaccination schedule, mice were vaccinated 56 and 28 days prior to vaginal infection. The vaccine consisted of whole-cell *T. vaginalis*, with aluminum hydroxide used as an adjuvant.

Male circumcision represents another means for the prevention of *T. vaginalis* transmission, since different relevant randomized trials have proven beyond a doubt that partners of circumcised men are less at risk for viral and bacterial infections than those of uncircumcised men (78–80). A clinical study demonstrated that male circumcision reduced *T. vaginalis* infection transmission from male to female partners (81). This study, conducted in South Africa, also demonstrated that woman-to-man transmission of *T. vaginalis* was reduced among circumcised men. In another clinical study, conducted in Rakai, Uganda, Gray and coworkers also observed that trichomonad infection was reduced in women with circumcised partners (82). One possible explanation for this protective effect is that the subpreputial space in uncircumcised men is moist (83), thus enhancing the survival of trichomonads.

However, different conclusions were drawn from a randomized clinical study conducted in Kenya that found no reduction of the risk of acquiring *T. vaginalis* infection (84). No protective effect of circumcision was observed for nonulcerative bacterial STIs (*Neisseria gonorrhoeae* and *Chlamydia trachomatis*) (84). Another study, conducted in Uganda, Zimbabwe, and Thailand, showed that women with circumcised partners had risks of chlamydial, gonococcal, and trichomonal infections similar to those of women with uncircumcised partners (85).

Vaginal administration of microbicide constitutes an alternative to prevent the acquisition of *T. vaginalis* infections. Microbicides are self-administered by women before intercourse, allowing more control over the acquisition of microbial infections. Microbicide administration before intercourse may limit *T. vaginalis* interaction with host cells. In a study conducted by Lushbaugh and coworkers (86), hydrogels composed of hydroxyethylcellulose (3.25% [wt/wt]) containing an antimicrobial peptide (D2A21) (0.5 or 2% [wt/vol]) or metronidazole (500 μg/ml) were administered to *Lactobacillus*-pretreated estrogenized young mice before inoculation of a *T. vaginalis* suspension. The results showed that the intravaginal metronidazole (500 μg/ml) gel completely prevented infection in all mice. Three groups of 10 mice were used for each formulation. The peptide D2A21 gel with a peptide concentration of 2% (wt/vol) was significantly more effective than the drug-free hydroxyethylcellulose gel at preventing

T. vaginalis infection. Indeed, 90% of mice treated with the peptide at 2% (wt/vol) did not develop infection. Interestingly, even without drug, the hydroxyethylcellulose hydrogel showed a reduction of infection, by a factor of 2. Only 53% of mice treated with hydroxyethylcellulose hydrogel developed the infection (86).

VAGINALLY ADMINISTERED FORMULATIONS FOR TREATMENT OF *T. VAGINALIS* INFECTIONS

The major limitation related to vaginal administration of drugs for the treatment of trichomoniasis is the nonaccessibility of other infected organs (cervix, bladder, and Bartholin's, Skene's, and periurethral glands). Despite this limitation, vaginal administration of drugs represents an interesting alternative to systemic administration (i) in cases of nitroimidazole allergy, (ii) in cases of pregnancy, (iii) when desensitization is not possible, (iv) when other systemic treatment options are limited, or (v) when severe side effects due to systemic administration are observed.

The Vagina as a Site for Drug Administration

The vagina is composed of 26 to 29 layers of epithelial cells, depending on age and the stage of the menstrual cycle (87, 88). The state of the epithelium is highly dependent on hormonal activity. In the first part of the cycle, the epithelium has a proliferative activity accompanied by significant glycogen synthesis (89). After ovulation, glycogen synthesis slows down, the cells desquamate, and the epithelial thickness decreases.

The vaginal mucosa is lined with mucus, which is a viscoelastic gel composed of organic and inorganic salts, mucin proteins (including immunoglobulins), carbohydrates, urea, and fatty acids. Mucus protects the vaginal mucosa from the external environment and ensures lubrication. Its composition and physical characteristics vary according to the menstrual period. It is capable of capturing foreign particles and removing them, but in contrast, some particles can be directed to the uterus. This is the case, for example, for sperm during ovulation.

Vaginal fluid is composed of secretions and transudations from blood vessels, cells derived from desquamation of the vaginal epithelium, leukocytes, and secretions of the endometrium and fallopian tubes. The amount and composition of vaginal fluid change during the menstrual cycle. The amount of vaginal fluid is estimated to be between approximately 0.5 and 0.75 g (90, 91).

The normal vaginal pH is acidic (approximately 4 to 4.5) and usually varies between 3.5 and 5. This value is maintained by the resident microbiota and by lactobacilli (in particular *Lactobacillus* spp. in the vagina, also called Döderlein flora) that convert glycogen from exfoliated epithelial cells into lactic acid and produce other fatty acids. Lactobacilli also produce bactericidal substances, such as hydrogen peroxide and surfactants, that play a role in preventing infections (92). Menstrual secretions, cervical mucus, and sperm can raise the vaginal pH (93).

The vaginal route has many advantages for drug delivery. It allows drugs to avoid the hepatic primary channel, as demonstrated with propranolol (a sympatholytic nonselective beta blocker), which has a better bioavailability after administration by the vaginal route than after oral administration (94). There is also a decrease in observed side effects by vaginal administration of bromocriptine, a dopamine agonist, compared to those with oral administration (95). Hepatic side effects induced by hormone replacement therapy or birth control are also greatly reduced (96).

Pharmaceutical formulations for vaginal administration are various and include liquid solutions, emulsions, suspensions, and solids, such as pessaries, vaginal tablets, vaginal capsules, and vaginal films. There are also other pharmaceutical formulations that are semisolid, including creams, ointments, and gels. Vaginal rings, unlike semisolid formulations used to line the vaginal mucosa, are positioned at a precise place in the upper third of the vagina (which is particularly susceptible to pathogen infection), near the cervicovaginal junction. They may liberate drug in a controlled way and during a long period (up to several months) in the lumen of the vagina. Vaginal rings are

generally constituted of a polymeric matrix, such as silicone, and thermoplastic materials containing drugs.

Vaginally Applied Formulations for Treatment of *T. vaginalis* Infections

In 1956, povidone-iodine (Betadine) was introduced as an antiseptic agent. It was shown to be an active agent against *T. vaginalis* (97–101). However, other studies observed treatment failure (102). The antiprotozoal action of povidone-iodine is dependent on the release of iodine. Thus, a 2-min douche with povidone-iodine is less effective than a 10-min douche (103). The use of povidone-iodine is counterindicated for pregnant women because of neonatal hypothyroidism reported after maternal use of povidone-iodine in pregnancy.

The use of intravaginal nonoxynol-9 administration was considered at the beginning of the 1990s for the treatment of trichomoniasis (104), but it was abandoned because of toxicity revealed during clinical trials of anti-HIV-1 microbicides (105–107). Studies have shown that nonoxynol-9 results in rupture of the vaginal epithelial barrier and accelerates HIV replication (108).

Nitroimidazoles for *T. vaginalis* treatment have been formulated for vaginal application. Metronidazole (500 mg) vaginal tablets (Tergynan) or ovules (Flagyl) are commonly used for the treatment of vaginal trichomoniasis. The classical treatment schedule consists of one application per day for 10 days (109–111).

The anti-*T. vaginalis* activity of vaginal tablets containing a lower dosage of metronidazole (100 mg) was compared to those of vaginal tablets containing placebo and 7-day oral metronidazole (500 mg twice a day). Significant differences in cure rates were observed between the placebo group and the two metronidazole groups. Oral and bioadhesive treatments did not lead to significant differences in clinical efficacy (112). Furthermore, a cure rate of 64% was obtained after administration of vaginal tablets containing 100 mg of metronidazole (113).

A pilot study compared the efficacies of 7-day treatment with oral metronidazole tablets (250 mg; three times daily) and vaginal gel treatment (0.75%; twice daily) (114). The 5-g dose of hydrogel contained only 37.5 mg of active drug. At the end of the treatments, significant reductions of genitourinary symptoms were observed with both vaginal hydrogel and tablets. However, the wet mount test showed that vaginal metronidazole administration failed to treat trichomoniasis compared to treatment with oral metronidazole. It is noteworthy that the composition of the hydrogel used in this study is unknown. Hydrogel properties can affect the distribution of the drug on the vaginal mucosa and, in turn, the anti-*T. vaginalis* activity. The cure rate was 44% with metronidazole gel, which is comparable to rates reported in previous studies of intravaginal metronidazole administration for the treatment of *T. vaginalis* infections, which ranged from 30 to 60% (109, 111). Although it failed to completely cure the infection, metronidazole gel was suitable for reducing side effects due to systemic passage. In order to facilitate treatment of resistant trichomoniasis, one strategy consisted of concomitant vaginal and oral administration of metronidazole. The success of combined oral and vaginal therapy has been known since the 1960s. A study conducted on 2,002 incarcerated women in California over 36 months compared the efficiencies of oral metronidazole (250 mg; three times daily for 3, 5, 7, or 10 days), vaginal metronidazole (500-mg vaginal inserts; once daily for 7 days), and a combination of oral and vaginal therapies (250-mg oral tablets given 3 times a day and concurrent 250-mg vaginal tablets for 5 days) (109). The results showed that the combined oral and vaginal 5-day treatment had the highest activity against *T. vaginalis* infections (109).

Combination therapy of metronidazole with other drugs is a good alternative strategy to administration of metronidazole alone. Vaginal ovules or cream containing the same metronidazole dosage (500 mg) combined with nystatin (100,000 IU) (Flagystatin) was used to treat mixed vaginal infections due to *T. vaginalis* and *Candida albicans*. Metronidazole has also been combined with other antimicrobial drugs, such as miconazole (115).

Alternative treatments include intravaginal preparations of paromomycin cream (116–118) (Fig. 2h). In 1964, paromomycin was used to treat trichomoniasis, with cure achieved in 85% of patients who received the drug topically as a vaginal pessary (119).

A case of trichomoniasis that was particularly resistant to metronidazole was successfully treated with intravaginal application of paromomycin (116). That study showed that the patient failed to respond to high-dose oral and topical metronidazole. The highest dose of metronidazole used was 7 days of oral tablets (800 mg) given three times daily, with 1 g intravaginal metronidazole given nightly. Over the next few months, the patient was treated without success with oral tinidazole, nimorazole, mebendazole, intravaginal clotrimazole, povidone-iodine, Aci-Jel, nonoxynol-9 pessaries, and hydrogen peroxide douches. This was followed by a full course of inactivated lactobacillus vaccine (Solco Trichovac) and a booster 1 year later. The patient continued to be symptomatic, and *T. vaginalis* was isolated repeatedly throughout this period. The initial MIC showed that the organism was resistant to metronidazole (116). Complete cure was achieved with 250 mg of paromomycin administered vaginally for 5 days.

In a study conducted on nine patients infected with *T. vaginalis*, among whom four patients had strains that were metronidazole resistant and five patients were allergic to metronidazole, treatment with paromomycin cream (250 mg per 4-g applicator nightly) was achieved for 2 weeks (117), and six of nine women were cured.

However, in a case study reported by Muzny and colleagues (102), intravaginal paromomycin failed to treat a patient with symptomatic *T. vaginalis* infection. This patient developed hypersensitivity to nitroimidazole drugs. Complete symptomatic cure was observed after vaginal pH acidification by intravaginal administration of boric acid for 2 months (102). Two case studies previously showed that vaginal acidification with intravaginal boric acid, for 1 and 5 months in two patients who tolerated this therapy, resulted in the treatment of recalcitrant *T. vaginalis* (120).

In a more recent study, a case report demonstrated that drug-resistant trichomoniasis can be treated successfully by concomitant administration of tinidazole (orally) and paromomycin cream nightly (121). However, frequent local vulvovaginal adverse reactions were reported for patients treated with paromomycin (62, 116, 118, 121). The side effects, such as ulcers of the vulvar and vaginal mucosal surfaces and vulvar pain, could be so severe as to require interruption of treatment (116).

During pregnancy, a daily dose of 100 mg of clotrimazole can be delivered intravaginally at bedtime for 14 days and can provide temporary relief to patients in the first trimester. With this treatment schedule, a cure rate of 50% was obtained (122).

To sum up, topically applied treatments are generally limited to adjunctive therapy or particular cases of allergy or resistance. Nevertheless, some data have shown that treatment of trichomoniasis by the vaginal route can be a real alternative to systemic treatment. All the described strategies for local delivery of anti-*T. vaginalis* formulations are based on the inclusion of a drug in a vehicle. Generally, little attention is paid to improving the formulation vehicle residence time on the mucosa infected with *T. vaginalis*.

General Considerations for Designing Vaginally Applied Anti-*T. vaginalis* Formulations

Efficacious vaginally applied formulations must have different properties, such as stability in acidic medium, adhesion, nonliquefaction at body temperature, slow dissolution, lubricant properties, and nongreasiness (123). Moreover, when formulations are intended for the vagina, other elements should also be taken into account. In fact, the formulations should (i) be stable at the acidic vaginal pH, (ii) be easily applied to obtain a homogeneous distribution of the drug, (iii) be retained in the vagina as long as possible, (iv) be compatible with other coadministered substances, and (v) be nontoxic to the vaginal mucosa (124). Vaginally applied formulations can also be used as controlled-release devices for vaginally applied drugs.

pH plays a role in the amount of drug absorbed from the vaginal mucosa and must be considered in the formulation of drug delivery systems. An increase of the absorption of leuprolide (a gonadotropin-releasing hormone analog) was observed after adding organic acids which partially dissolved the cellular cement. Furthermore, the efficacy of a gel containing dinoprostone (prostaglandin E2) was significantly related to the pH of the vagina.

The apparent aqueous solubility of anti-*T. vaginalis* drugs (i.e., albendazole or clotrimazole) represents another parameter to be considered for their formulation, as it determines the choice of formulation (solid, semisolid, liquid, solution, suspension, etc.). The apparent aqueous solubility of albendazole was increased 7,600 times by using randomly methylated β-cyclodextrin (Rameb Me-β-CD) at a concentration of 40% (wt/wt) (125), while the solubility of clotrimazole was improved 9,980 times by using the same cyclodextrin (126). In another study, chitosan nanoparticles exhibited strong anti-*T. vaginalis* activity *in vitro* even without addition of drugs (127).

Semisolid drug delivery forms, such as gels, are widely used in the development of topical formulations against vaginal microbial infections (128). Gels are semisolid substances generally constituted of a polymeric matrix. Pharmaceutical gels consist of natural polymers, in particular some proteins (collagen and gelatin) and polysaccharides (alginate, carrageenan, and guar gum), semisynthetic polymers (carboxymethyl cellulose and hydroxypropyl methylcellulose), and other synthetic (carbomer and Pluronic) or inorganic (aluminum hydroxide, bentonite, and laponite) substances (123). Hydrogels have many advantages: they are generally colorless, odorless, and tasteless and can be used in combination with vaginal devices or condoms (129).

In terms of their effectiveness, the most important problems presented by these forms are their low remanence at the epithelial surface and their rapid detachment from the application site (129, 130). In order to overcome these problems, mucoadhesive polymers are often added to formulations to immobilize the gel as long as possible on the vaginal mucosa (131).

For all these reasons, the use of thermosensitive and mucoadhesive gels was recently presented as an alternative strategy for treatment of *T. vaginalis* infection (127, 132, 133). These gels are liquid at low temperature, thus facilitating the spreading of the formulation on the whole vaginal area, even in difficult-to-access places, and they become semisolid at body temperature, increasing the residence time of the formulation on the mucosa (134–136).

CONCLUSIONS

The progressive abandoning of condom use relative to discomfort and linked to the forgetting of HIV risk and the increase of poverty worldwide may partially explain the increase in the annual number of urogenital trichomoniasis cases. In addition, drug resistance emergence and intolerance to nitroimidazoles contribute to making trichomoniasis treatment a societal challenge to be addressed. Because of this, chemotherapy and vaccines are the best ways to control the expansion of this cosmopolitan disease. Besides *in vitro* screening of new compounds, all strategies that attempt to improve the biodistribution of anti-*Trichomonas* compounds provide real added value to the fight against this disease. In particular, approaches consisting of the prevention of side effects linked to parenteral treatments need to be prioritized. Thus, any investigation with the aim of developing local treatments is promising. In this regard, advances in mucoadhesive polymer formulation should particularly be supported in the future.

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