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Review

Chemotherapeutic options for the treatment of human trichomoniasis

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ABSTRACT

Trichomonas vaginalis is the causative agent of the most common non-viral sexually transmitted disease worldwide. The infection may be associated with severe complications, including infertility, preterm labour, cancer and an increased risk of human immunodeficiency virus (HIV) transmission. Treatment remains almost exclusively based on 5-nitroimidazoles, but resistance is on the rise. This article provides an overview of clinically evaluated systemic and topical treatment options for human trichomoniasis and summarises the current state of knowledge on various herbal, semisynthetic and synthetic compounds evaluated for their anti-*Trichomonas* efficacy in vitro.

1. Introduction

Trichomonas vaginalis is the causative agent of human trichomoniasis, with an estimated worldwide prevalence of 168 million cases per year. This rate has significantly increased in the past 10 years [1]. Overall, trichomoniasis was estimated to have caused 194 000 years of life with disability in 2015 [1]. Reported percentages of asymptomatic cases vary from 10–85% in women [2–14] and 56.25–76.8% in men [3,15], and the estimated number of unreported cases of individuals exposed to infection can be assumed to be much higher. Trichomoniasis is associated with a variety of pregnancy complications, including an increased risk of preterm labour [16–18] as well as preterm, premature membrane rupturing [16,19–21] and infants small for gestational age [16,17]. Moreover, cervical neoplasia [22–25], infertility both in men [26] and women [27,28], and increased risk of human immunodeficiency virus (HIV) acquisition [29] have been associated with the presence of *T. vaginalis*.

The standard treatment for human trichomoniasis has been unchanged since 1959 [30,31] and is based on 5-nitroimidazoles, mainly metronidazole. The US Centers for Disease Control and Prevention (CDC) recommend a 2 g single-dose treatment in pregnant and non-pregnant women and in men [32]. In HIV-infected women and patients with treatment failure due to resistance, treatment with 500 mg twice daily for 7 days is recommended [32]. If this treatment fails, infections are treated with 2 g of metronidazole or tinidazole for 7 days [32]. Most cases of metronidazole-resistant trichomoniasis can be cured by the use of tinidazole. The CDC recommends an increased dosage of 2–3 g for 14 days in combination with intravaginal treatment [32]. *Trichomonas vaginalis* strains resistant to metronidazole are prevalent in 4.3–13.3% of cases [33–35], and resistant cases appear to be rising [36]. Resistance is rather relative than absolute. Resistance in vivo can, but need not be, detectable in vitro [33]. The combination of high infection rates, high percentages of asymptomatic cases, possible severe complications, treatments based on only one class of antimicrobials, and increasing resistance drives the search for alternative treatment options.

Whilst several reviews providing detailed information on particular compounds, structures and modes of action have been published in the recent past [37–39], the current study gives an overview of all compounds tested in the past decades, including dosage regimens used in different case studies and their corresponding efficacies, as well as smaller dose–response studies over the years. This study is intended to complement the partly detailed information available for several particular compounds and classes in previous reviews.

Generally, the efficacy of topical treatments in comparison with systemic (mainly oral) treatments in sexually transmitted diseases was long questioned, particularly with respect to low compliance by patients. However, in 2013 a randomised controlled, dose-ranging pilot study found no significant differences in efficacy between oral metronidazole and topical treatment with miconazole and metronidazole [40]. In recent years, a wide variety of compounds have been tested for their trichomonacidal activity. Most compounds are intended for topical application, thereby avoiding complications associated with systemic application.

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2. Alternative compounds clinically tested or in use

Several compounds have been tested for their suitability as alternative drugs to metronidazole in clinical trials and case series in women. However, the only compound evaluated with a level of evidence comparable with 5-nitroimidazoles and concurrent with evidence-based medicine is nifuratel. In a rather limited number of randomised controlled trials (RCTs), additional compounds, including herbal medications and established antimicrobials, revealed high efficacy. Unfortunately, several promising candidates failed to achieve satisfactory cure rates. Table 1 gives an overview of trials completed in the past years. The following compounds are ranked according to their level of evidence, defined by the Centre for Evidence-Based Medicine (CEBM Levels of Evidence) [41]. The CEBM Levels of Evidence is a tool provided by the Oxford Centre for Evidence-Based Medicine that allows for accurate descriptions and thereby compares the degree of evidence for a therapy, diagnosis or intervention. The levels are ranked by numbers ranging from 1 (a systematic review of several RCTs) to 4 (a case report) [41]. All studies except two used wet mounts for diagnostics. Wet mounts have a high specificity of 99.3–100% [42–45] but a low sensitivity of 38–68.7 % [42–44,46–50]; thus, they are often insufficient for proof of cure.

2.1. CEBM Level I: Nifuratel alone or in combination with nystatin

Nifuratel is a nitrofuran derivative with known activity against *T. vaginalis* in vitro [51–53] (Table 2). Its mode of action is based on interactions with gene expression and DNA damage, while simultaneously inhibiting repair [54]. In 2002, a meta-analysis of multiple clinical trials comparing nifuratel with metronidazole in the treatment of trichomoniasis and mixed infections revealed equal efficacy of nifuratel (88.5%) and metronidazole (90.0%) both in oral and topical applications [55]. The evaluated studies consisted of slightly different treatment regimens of nifuratel and metronidazole, as presented in Table 1. The highest cure rate (96%; 462/482) was achieved with a dosage of 600 mg nifuratel orally combined with 250 mg topically, both for 10 days [55]. All control groups in these studies used metronidazole at lower concentrations than recommended by the CDC; nevertheless, the cure rates in the control groups were comparable with the reported cure rates in a meta-analysis evaluating the efficacy of metronidazole against *T. vaginalis* [56]. The results are limited by the use of wet mounts and culture for diagnostics in the evaluated studies [55].

Nifuratel was also evaluated in combination with nystatin, a polyene antibiotic [57] that interacts with ergosterol and cholesterol [58]. A combination of 500 mg of nifuratel and 200 000 IU of nystatin per vaginam (p.v.) for 6 days was able to cure 14/14 patients in two different case series [59,60], whilst lower concentrations achieved lower cure rates [60]. The most common side effect was itching during the first days of treatment [60].

2.2. CEBM Level II: Mentha crispa

The dry extract from leaves and stems of *M. crispa* has known in vitro activity against *Entamoeba histolytica* and *Giardia duodenalis* [61]; however, it has a low clinical cure rate (47.83%) against giardiasis [62]. In the treatment of trichomoniasis, a double-blind RCT in 60 women with a single-dose treatment of 24 mg of *M. crispa* (Giamebil®; commercially available) presented equal efficacy with significantly ($P = 0.0006$) lower side effects compared with 2 g of secnidazole [63]. Secnidazole and tinidazole are known to have equivalent efficacy compared with metronidazole [64,65]. This double-blind RCT evaluating *M. crispa* was limited by the number of patients and the use of wet mounts for diagnostics.

2.3. CEBM Level II: Zataria multiflora

The mode of action of *Z. multiflora* has not been entirely elucidated but is probably based on an interaction with the plasma membrane [66]. In a double-blind RCT in 140 women with trichomoniasis, a vaginal cream (Leucorex®; commercially available) applied once daily in combination with an overnight applicator containing 5 g of cream, both for 7 days, presented lower cure rates compared with 250 mg of metronidazole administered orally twice daily for 7 days (71.43% and 92.86%, respectively) but provided equal relief of symptoms [67]. Although comparable numbers of patients were included, this study was limited by the use of wet mounts for diagnostics and the dosage of metronidazole in the control group, which was not in line with the recommended dosage [32].

2.4. CEBM Level II: Fenticonazole

Fenticonazole is an antifungal azole that inhibits ergosterol synthesis [68,69]. In a double-blind RCT with 61 women, intravaginal ovules containing 1000 mg or 600 mg of fenticonazole used on two consecutive days revealed cure rates of 58.8% (10/17) for 1000 mg and 65.0% (13/20) for 600 mg compared with 15.0% (3/20) in the placebo group [70]. In a small observational study, the same dosage of 1000 mg on Day 1 and Day 3 revealed a cure rate of 70.0% (7/10) [71]. Overall, the cure rates were lower than the reported cure rates with 5-nitroimidazoles in single-dose and long-term treatment [56]. Furthermore, these studies were limited by the number of patients and the use of wet mounts for diagnostics.

2.5. CEBM Level IV: Paromomycin

Paromomycin is an aminoglycoside antimicrobial that inhibits protein synthesis with known efficacy against Gram-negative and Gram-positive bacteria as well as protozoa and cestodes [72]. In 1964, vaginal pessaries containing 25 mg of paromomycin applied daily for 20 days were reported to cure 85% of patients with trichomoniasis ([73], cited in [74]). However, vaginal pessaries containing 30 mg of paromomycin three times daily achieved cure rates of only 37% (57 patients) in a study by Spitzbart and Wilk ([75], cited in [76] and [74]). In one case study, 500 mg of paromomycin applied intravaginally for 2 days failed to cure one patient [77]. In four different case studies, 250 mg of paromomycin applied intravaginally twice daily for 2–10 days cured 75% (3/4) of patients (1 of them post-delivery) [74,78–80]. All treatment courses were stopped due to small ulcers or vaginal soreness. The woman post-delivery had to be treated for 4 weeks until cultures were negative [79]. In four different case studies, treatments using 250 mg of paromomycin cream nightly for 5–21 days were able to cure 57.1% (8/14) of cases [80–83]. Paromomycin was effective in several cases when other therapies failed but caused ulcerations and irritation within a short time.

2.6. CEBM Level IV: Acetarsol

Acetarsol is a pentavalent arsenical compound with known antiprotozoal and antihelminthic properties [84]. In a case study, 500 mg p.v. treatment with acetarsol pessaries twice daily for 6 days cured 0/1 patients with recalcitrant trichomoniasis [85]. In three other case studies, 500 mg p.v. once daily for 7–14 days cured 75% (3/4) of patients [86–88]. Interestingly, all patients treated with nightly applications were cured. In three more case studies, 250 mg acetarsol pessaries twice daily for 7–14 days cured 100% of patients (5/5) [85,89,90], whilst 250 mg pessaries of acetarsol nightly for 10–12 days cured 2/2 patients [86,90].

Table 1
Compounds clinically tested against trichomoniasis in women and the level of evidence according to the CEBM Levels of Evidence [41] (L) of the respective number (*n*) of trials (T) with the pooled number (*N*) of participants or studies included in the trial or meta-analysis, the diagnostics (D) used including microscopy (m), culture (c) or PCR (pcr), and the efficacy (E) observed after certain treatment regimen (Dosage).

Compound	*n* × T (*N*)	L	D	Dosage	E	Control	*P*-value	Reference(s)
Systemic treatment								
Any 5-NTZ	SR (54)	1	m/c	1 × >1.5 g	>90%			[56]
		1	m/c	1–3 × 200–600 mg, 5–10 d	>90%			[56]
Mentha crispa	RCT (60)	2	m	1 × 24 mg	90%	1 × 2 g SCZ	0.61	[63]
Aminitrozole	OS (125)	3	m/c	4 × 150 mg p.o., 6 d	37%	–	–	[113]
	CS (13)	4	m	300–400 mg p.o., 6–14 d	0/13	–	–	[114]
Nitazoxanide	2 × CS (4)	4	m/c	2 × 1–2 g, 7–14 d	0/4	–	–	[82,122]
Mebendazole	CS (3)	4	m/c	2–3 × 400 mg, 7 d	0/3	–	–	[123]
+ doxycycline	CS (1)	4	m	+ 2 × 100 mg, 7 d	0/1	–	–	[86]
Combined systemic (p.o./i.m.) and topical (p.v.) treatment								
Nifuratel	SR (7)	1	m/c	600–1200 mg p.o., 7–10 days ± 250–500 mg p.v. nightly, 10 days	88.5%	MTZ	0.582	[55]
Aminitrozole	OS (104)	3	c	3 × 100 mg p.o., 10 days + 2 × acidic jelly p.v.	45.8%	–	–	[111]
	CT (46)	3	m/c	Same dosage	20%	Placebo	N/A	[112]
L-Vacc	CS (1)	4	m/c	3 × 0.5 mL i.m. + 500 mg CTZ p.v.	0/1	–	–	[90]
	CS (6)	4	m	N/A	3/6	–	–	[85]
Mebendazole	CS (1)	4	m/c	2 × 100 mg p.o. + p.v., 3 d	0/1	–	–	[123]
Topical (p.v.) treatment								
Zataria multiflora	RCT (140)	2	m	0.1%, 7 d	71.43%	2 × 250 mg MTZ, 7 days	0.001	[67]
Fenticonazole	RCT (37)	2	m	1000 mg, 2 d	58.8%	Placebo	<0.005	[70]
	RCT (40)	2	m	600 mg, 2 d	65%	Placebo	<0.005	[70]
	OS (10)	3	m	1 g, on days 1 and 3	70%	–	–	[71]
Nonoxynol-9	RCT (33)	2	m	150 mg, 3 d	17.6%	2 g MTZ	<0.001	[103]
	CS (1)	4	m/c	100 mg + condom during sex	1/1	–	–	[104]
Furazolidone	OS (48)	2	m	Powder every 36 h + nightly pessaries + daily vinegar douches	93.8%	–	–	[108]
	OS (26)	2	m	Same dosage	92.3%	–	–	[105]
	OS (26)	2	m	Same dosage	65.4%	–	–	[105]
	3 × CS (4)	4	m/c	2 × 100 mg, 12–14 d	1/4	–	–	[82,109,110]
	CS (2)	4	m	N/A	0/2	–	–	[124]
Clotrimazole	CT (90)	3	m	2 × 100 mg, 7 d	11.1%	2 g MTZ	<0.001	[115]
	CS (1)	4	m/c	2 × 100 mg, 14 d	0/1	–	–	[74]
	CS (3)	4	m	N/A	1/3	–	–	[124]
Nifuratel + nystatin	2 × CS (14)	4	m	500 mg, 6–10 d + 200 000 IU, 6–10 d	14/14	–	–	[59,60]
	CS (8)	4	m	250 mg, 10 d + 100 000 IU, 10 d	6/8	–	–	[60]
	CS (7)	4	m	125 mg, 10 d + 50 000 IU, 10 d	4/7	–	–	[60]
Paromomycin	1 × CS (1)	4	pcr	500 mg, 2 d	0/1	–	–	[77]
	4 × CS (4)	4	m/c	2 × 250 mg, 2–10 d	3/4	–	–	[74,78–80]
	4 × CS (14)	4	m/c	250 mg, 5–21 d	8/14	–	–	[80–83]
	CS (4)	4	m	N/A	1/4	–	–	[124]
Povidone-iodine	CS (1)	4	m	250 mg, 14 d	1/1	–	–	[89]
	CS (1)	4	m	2 × 20 mL of 10%, 2 d/w, 2 w, pause, 1 w	1/1	–	–	[118]
	CS (1)	4	m/c	2 × 20 mL of 10%, 2 d/w, 4 m	0/1	–	–	[82]
	4 × CS (17)	4	m/c	N/A	4/17	–	–	[74,85,110,124]
Acetarsol	CS (1)	4	N/A	2 × 500 mg, 6 d	0/1	–	–	[85]
	3 × CS (4)	4	m	500 mg nightly, 7–14 d	3/4	–	–	[86–88]
	3 × CS (5)	4	m/c	2 × 250 mg, 7–14 d	5/5	–	–	[85,89,90]
	2 × CS (2)	4	m	250 nightly, 10–12 d	2/2	–	–	[86,90]
	CS (1)	4	N/A	0/1	–	–	–	[124]
Boric acid	CS (1)	4	c	600 mg alt. CTZ, 150 d	1/1	–	–	[93]
	2 × CS (2)	4	m/c/pcr	2 × 600 mg, 30–61 d	3/3	–	–	[82,93,94]
	2 × CS (2)	4	pcr	600 mg, 45–92 d	0/2	–	–	[77,94]
Punica granatum	CS (20)	4	m	–	18/20	–	–	[95]
Pentamycin	CS (4)	4	m	3 mg, 10 d/6 mg, 5 d	4/4	–	–	[97]
Zinc sulfate	CS (2)	4	m	2 × 1%, 14–28 d	2/2	–	–	[100]
Nitazoxanide	CS (1)	4	m/c	2 × 500 mg, 14 d	0/1	–	–	[110]

CEBM, Centre for Evidence-Based Medicine; 5-NTZ, 5-nitroimidazoles; p.o., oral; i.m., intramuscular; p.v., per vaginam; L-Vacc, *Lactobacillus acidophilus* vaccination; SR, systemic review of multiple randomised controlled trials; RCT, randomised controlled trial; OS, observational study; CS, case series; CT, non-randomised controlled trial; SCZ, secnidazole; MTZ, metronidazole; N/A, not available; CTZ, clotrimazole; alt., alternating.

Table 2
Compounds tested against *Trichomonas vaginalis* in vitro after different incubation periods (Time) and their respective effective concentrations for 50% growth inhibition (IC_{50}), minimum inhibitory concentrations (MICs) and minimum lethal concentrations (MLCs).

Compound	Time (h)	IC_{50} (μg/mL)	MIC (μg/mL)	MLC (μg/mL)	Reference(s)
Metronidazole	48	1.03	0.4–1.6	1.6–3.2	[207–209]
	72		0.8–0.9	1.6–3.83	[210–212]
Artemisia absinthium	24		500		[168]
Arbutus unedo	24 or 48		500		[167]
Albendazole	48	0.9–1			[145]
Anisomycin	72		<0.1–0.4	<0.2–1.6	[107]
Auranofin	24			1.36–4.07	[148]
ASCA	48		1.56–31.2		[185]
Amomum tsao-ko	48	22.49–44.97		44.97–89.93	[157]
BPQ-OH	24	12.85			[184]
Bee venom	0.5		75		[203]
Boric acid	48			3000–6000	[92]
Cussonia holstii	72	1.9–4.2			[160]
Curcumin	24			400	[161]
Deer musk	24		300 000		[204]
Disulfiram	48		0.03–100		[144]
Doxycycline	48	13–15			[129]
	72		>100		[107]
Eucalyptus camaldulensis	24		12 500–25 000		[171]
Epinecidin-1	4		12.5–25		[186]
Esomeprazole	72		400	800	[125]
Furazolidone	48			0.8–3.1	[106]
	72		1.6–3.1	3.1–6.2	[107]
Ginger	24	93.8			[170]
	48			800	[170]
Gonocaryoside A	24		3.25		[154]
Hypericum polyanthemum	24	206.98	325		[163]
Hypocrea lixii	24		2500		[164]
Kingiside	24		2.53		[154]
Maytenus imbricata	48	1.09–1.57			[153]
Lavandula angustifolia	0.33		5,000		[169]
Micana cordifolia				1000	[156]
Mebendazole	48	0.029–0.080			[145]
	72		0.8–6.2	<0.1–0.8	[107]
Miltefosine	24	5.9			[142]
Minocycline	48	18			[129]
	72		>100		[107]
MPC	48		13.86–250		[187]
Nigella sativa	48			1000–10 000	[162]
Chitosan-coated nanoparticles	24			100	[201]
Nifuratel	24		50		[213] (in [53])
	48		0.09–1.5	0.52–3.05	[51–53] (in [53])
Nitazoxanide	48			6–12	[119]
Polygala decumbens	24		1560		[166]
Ocimum basilicum oil	24		30		[155]
Passiflora alata	N/A		250		[172]
Penicillium citrinum	24		2500		[164]
Pistacia lentiscus mastic	24		15 000		[155]
Pantoprazole	72		200	400	[125]
PCG	24		3.85		[154]
Purpuromycin derivatives	48		0.5–16		[178]
Quillaja saponaria	N/A		250		[172]
Rheum ribes	24		500		[165]
Scutellaria havanensis	48	12.9			[158]
Sulfimidazole	24–48			0.4–11	[191]
Tetracycline	8		300–500	500–700	[130]
	48	62–95			[129]
Tomatine	24	8.17	103		[152]
Tomex®	24		100	100	[159]
Torvoside A	24		5.66		[154]
Verbascum thapsus	24	39.17	400	800	[132]
Ursolic acid	24		22.8		[180]
Wogonin	48	16.1			[158]

ASCA, ammonium salts of carbamodithioic acid; BPQ-OH, 3-(biphenyl-4-yl)-3-hydroxyquinuclidine; MPC, morpholin/piperidin-1-yl-carbamodithioate; N/A, not available; PCG, plumieride coumarate glucoside.

2.7. CEBM Level IV: Inactivated *Lactobacillus acidophilus* vaccination

Heat-inactivated *L. acidophilus* vaccination (Gynatren®; commercially available) is believed to cause a non-specific immunostimulatory effect rather than an antibody-mediated reaction, as previously suggested [91]. It was evaluated with a vaccine approach but its role in acute infection is unclear. Inactivated *L. acidophilus* was able to cure 3/6 cases of recalcitrant trichomoniasis [85]. However, this vaccination in combination with a 500 mg clotrimazole pessary failed to cure another patient [90].

2.8. CEBM Level IV: Boric acid

Boric acid is an inorganic acid with an unknown mode of action. It is used against bacterial vaginosis and vulvovaginal candidiasis and has a proven trichomonacidal effect in vitro [92] (Table 2). In one case study, topical application of 600 mg of boric acid alternated with clotrimazole for 150 days cured 1/1 patient [93]. In two other case studies, 600 mg vaginal capsules of boric acid applied twice daily for 30–61 days cured 100% of patients (3/3) after multiple treatment failures with several other compounds [82,93,94]. In two more case reports, 600 mg nightly or daily for 45–92 days cured 0/2 patients [77,94]. In another case report, only the combination of 500 mg intravenous metronidazole every 8 h with 2 g of liquid oral tinidazole daily and 600 mg of intravaginal boric acid for 14 days was effective [77]. Based on these reports presenting effective treatment in patients after multiple treatment failures, boric acid might be used in patients when other approaches fail.

2.9. CEBM Level IV: Punica granatum

Natural plant extract of *P. granatum* has been shown to have in vitro and in vivo efficacy against *T. vaginalis* with an unknown mode of action [95]. In one case series, natural extract used in an unspecified dosage cured 18/20 women with metronidazole-resistant trichomoniasis [95].

2.10. CEBM Level IV: Pentamycin

Pentamycin is a polyene macrolide antibiotic altering the barrier function of cell membranes of micro-organisms [96,97]. Pentamycin was reported to have a strong effect against *T. vaginalis* in vitro [effective concentration for 50% killing (EC_{50}), 1.74–2.62 µg/mL after 1 h] [98]. It is well tolerated in vivo and has been registered for the topical treatment of bacterial and fungal vaginitis [99]. In a case series, 6 mg daily for 5 days or 3 mg for 10 days cured 4/4 patients [97]. Pentamycin was safe and well tolerated in a randomised, double-blind, dose-ranging (3–100 mg) study in 19 women [99].

2.11. CEBM Level IV: Zinc sulfate

Zinc is an essential trace element for humans with known antimicrobial properties [100]. Men with <1.6 mmol zinc in their prostatic secretions have an increased risk of chronic prostatitis [101]. In one case series, a 1% zinc sulfate douche daily for 14–28 days cured 2/2 patients [100].

3. Tested compounds with limited efficacy

A number of additional compounds have been evaluated for their anti-*Trichomonas* efficacy in clinical trials and case series in women but presented comparably low cure rates or are impractical to use. Table 1 gives an overview of trials completed in the recent past. The following compounds are ranked according to their CEBM Level of Evidence [41].

3.1. CEBM Level II: Nonoxynol-9

Nonoxynol-9 is a topical contraceptive with known antimicrobial activity [102]. In a RCT in 33 women, intravaginal treatment with 150 mg of nonoxynol-9 for three consecutive days presented significantly lower cure rates compared with a 2 g single dose of metronidazole (17.6% and 70%, respectively) [103]. A combination of topical application with 100 mg and contraception using latex condoms led to the cure of metronidazole-resistant trichomoniasis in one woman with multiple treatment failures [104]. In this case, the infection might have been caused by re-infection from the untreated partner.

3.2. CEBM Level II: Furazolidone

Like nifuratel, furazolidone is a nitrofuran antibiotic presenting considerable efficacy against *T. vaginalis* in vitro [105–107]. In 1956, a cohort study of 48 women with trichomoniasis evaluating furazolidone intravaginal powder insufflation every 36 h, nightly pessaries of furazolidone and daily vinegar douches [108] achieved a cure rate of 93.8% (45/48) [108]. In the same year, studies on the same treatment regimens found cure rates of 92.3% (26 patients) and 65.4% (26 patients), whilst topical treatment without powder insufflation presented lower cure rates [105]. In three other case studies, 100 mg of furazolidone per 5 g applicator of 3% nonoxynol-9 twice daily for 12–14 days cured 25% (1/4) of the respective patients from trichomoniasis [82,109,110]. Based on the low efficacy of furazolidone alone, the high cure rates observed in the cohort studies might be connected to the complex treatment regimen, thereby making it an impractical approach for standard treatment of trichomoniasis.

3.3. CEBM Level III: Aminitrozole

Aminitrozole is a historically used antiprotozoal drug. In 1956, an observational trial evaluated the efficacy of 100 mg of aminitrozole taken orally three times daily for 10 days in combination with intravaginal acidic jelly twice daily for 14 days for trichomoniasis [111]. One course of treatment cured 48.1% (50/104) and two courses cured 45.8% of cases [111]. The lower cure rate after two courses of treatment was caused by selection of recalcitrant infections and a lower number of patients. In a controlled trial including 46 women, the same dosage cured 20% (5/25) in contrast to 19% in the placebo group [112]. In an observational trial, 150 mg of aminitrozole taken orally four times daily for 6 days presented cure rates of 37% (46/125) with side effects in 35% (44/125) of patients [113]. In a case series of 13 women, treatment with 300–400 mg taken orally daily for 6–14 days was unsuccessful [114]. In conclusion, aminitrozole as an oral or combined (oral and topical) treatment presented cure rates similar to placebos and is therefore not sufficient for the treatment of metronidazole-resistant trichomoniasis.

3.4. CEBM Level III: Clotrimazole

Clotrimazole is an antifungal azole that inhibits ergosterol synthesis. A multicentre, open-label trial with 168 women comparing 100 mg of topical clotrimazole applied twice daily for 7 days with vaginal suppositories containing sulfanilamide, aminacrine and allantoin, and oral metronidazole achieved a cure rate of 11.1% [115]. The same dosage failed in one patient with resistant trichomoniasis [74]. Therefore, clotrimazole might not be a sufficient treatment for trichomoniasis.

3.5. CEBM Level IV: Povidone-iodine douches

Povidone-iodine, also known as betadine, is an iodinated polyvinyl polymer delivering diatomic free iodine to intracellular structures [116]. It has been broadly used in antiseptics since 1956 [117] and is known to have trichomonacidal activity [116]. In a case study, treatment with daily pessaries containing 250 mg of povidone-iodine for 14 days cured 1/1 patient [89]. In two other case studies, treatment with twice-daily 20 mL vaginal douches of a 10% solution on 2 days of the week cured 1/2 patients [82,118]. Although efficient in antiseptics, povidone-iodine achieved low cure rates in the treatment of trichomoniasis.

3.6. CEBM Level IV: Nitazoxanide

Nitazoxanide is a nitrothiazolide antiparasitic agent with known trichomonacidal activity [119–121] that blocks anaerobic metabolism. However, in two case studies 1–2 g orally two times daily for 7–14 days cured 0% (0/4) of patients [82,122], and topical application of 500 mg twice daily for 14 days cured 0/1 patient [110]. In the reported cases, treatment with nitazoxanide was unsuccessful for trichomoniasis.

3.7. CEBM Level IV: Mebendazole

Mebendazole is a benzimidazole with established antihelminthic activity. Its mode of action is based on selectively and irreversibly blocking glucose uptake and inhibiting the polymerisation of microtubules. It has been shown to have strong efficacy against metronidazole-susceptible and -resistant strains of *T. vaginalis* in vitro [107]. However, all in vivo studies of mebendazole taken orally, intravaginally or combined with doxycycline failed to cure any patient. In one case study, 400 mg taken orally two or three times daily for 7 days cured 0/3 patients [123]. A combination of 400 mg of mebendazole with 100 mg of doxycycline taken orally two times daily for 7 days cured 0/1 patient [86]. Application of 100 mg of mebendazole taken orally twice daily combined with 100 mg applied intravaginally two times nightly for 3 days also cured 0/1 patient [123]. In conclusion, trichomoniasis could not be cured with either oral or combined systemic and topical treatment with mebendazole.

4. In vitro-tested compounds

In the search for alternatives in the treatment of trichomoniasis, numerous additional compounds have been tested in vitro. The following paragraphs list the different compounds, their calculated EC_{50} value, IC_{50} value (concentration that inhibits 50% growth), and minimum inhibitory concentration (MIC) or minimum lethal concentration (MLC). Table 2 gives an overview of tested compounds and their calculated parameters.

4.1. Compounds already in use or with other applications

Some compounds with antitrichomonal activities are already in use for the treatment of other diseases or used in traditional medicine.

4.1.1. Proton pump inhibitors

In vitro antiprotozoal effects of the proton pump inhibitors esomeprazole [125], pantoprazole [125,126], omeprazole, lansoprazole and rabeprazole [126] have been identified. The mode of action is believed to be inhibition of uridine ribohydrolase, required by the parasite for scavenging uracil [127]. These results are consistent with the reported lower likelihood of trichomoniasis in women actively treated with proton pump inhibitors (odds ratio = 0.75, 95% confidence interval 0.65–0.86) [128].

4.1.2. Tetracycline

Tetracyclines are a class of broad-spectrum antibiotics with known activity against several protozoan parasites. However, studies on their efficacy against *T. vaginalis* revealed contradictory results [107], particularly with lipophilic compounds (doxycycline and minocycline) [129]. A recent analysis revealed a strong effect based on enhanced production of reactive oxygen species (ROS), leading to cell death [130]. Interestingly, the efficacy of tetracycline was higher in a metronidazole-susceptible strain compared with a metronidazole-resistant strain. The maximum plasma levels of minocycline and doxycycline, the most effective tetracyclines, were reported to be 2.18–2.35 μg/mL after 2 h [131], thus suggesting a topical approach.

4.1.3. Anisomycin

Anisomycin is an antimicrobial agent mainly used in diagnostics that inhibits eukaryotic protein synthesis. It was shown to have considerable efficacy against metronidazole-susceptible and -resistant strains of *T. vaginalis* [107].

4.1.4. Verbascum thapsus

Verbascum thapsus (common mullein) ethanol extract has been reported to be used against genitourinary infections in Iran [132]. It shows considerable efficacy against *T. vaginalis*; however, its efficacy was lower compared with metronidazole.

4.1.5. Extracts from Amaryllidaceae

Extracts from plants of the family Amaryllidaceae are reported to be used for sexually transmitted diseases in South Africa [133]. Eight extracts of these plants presented trichomonacidal activity, but no MICs, MLCs or EC_{50} values were calculated [134].

4.1.6. Octenidine dihydrochloride

The common antiseptic octenidine dihydrochloride, in combination with phenoxyethanol, is a registered drug for intravaginal use [135] and has been shown to have high antitrichomonal activity in vitro (EC_{50}, 0.68–2.11 μg/mL after 30 min of incubation) [136]. In clinical trials, octenidine hydrochloride/phenoxyethanol as a local treatment has shown similar efficacy to metronidazole against bacterial vaginosis [137] and to clotrimazole against vaginal candidiasis in non-pregnant and pregnant women [138,139], and it was very well tolerated [138–140].

4.1.7. Miltefosine

Miltefosine (hexadecylphosphocholine) has been shown to be highly active against *T. vaginalis* [141,142]. It is not registered for intravaginal use but may be used off-label.

4.1.8. N-Chlorotaurine

N-Chlorotaurine showed trichomonacidal activity (EC_{50}, 9–28.7 mmol after 1 h of incubation) in an in vitro study [143] but is not registered for intravaginal use.

4.1.9. Disulfiram

Disulfiram is used in the treatment of alcoholism. It presented MICs of 0.11–1.3 μM against *T. vaginalis* in aerobic conditions and 0.3–337 μM in anaerobic conditions [144].

4.1.10. Benzimidazoles

The antihelminthic drug albendazole was shown to have trichomonacidal activity in vitro [145]. In a recent study, newly synthesised bis-benzimidazoles presented considerable efficacy against *T. vaginalis* in vitro and were also effective in mouse models [146]. 2-(Methylthio)-1*H*-benzimidazole-5-carboxamide derivatives were shown to have comparable efficacy to metronidazole against *T. vaginalis* in vitro (IC_{50} values of 0.012–0.515 μM and 0.236 μM, respectively) [147].

4.1.11. Auranofin

Auranofin, an organic gold compound used since 1985 for the treatment of rheumatoid arthritis, exhibited high trichomonacidal activity [148] by targeting thioredoxin reductase [148–150]. In a phase 1 trial, auranofin was generally well tolerated and achieved high plasma concentrations [150].

4.2. Novel and experimental approaches

The following compounds are known to have an effect on *T. vaginalis* but are either in experimental status, have high or unknown side effects, or the active compound has not yet been determined. A summary is given in Table 2.

4.2.1. Herbal products

The efficacy of many herbal and natural products against *T. vaginalis* might be based on molecular docking of antiprotozoal phytochemical agents to methionine γ-lyase and purine nucleoside phosphorylase. The best docking ligands are polyphenolic compounds, including aurones, chalcones, flavonoids and lignans [151]. Several phytotherapeutic compounds show similar in vitro efficacy to metronidazole (IC_{50} or MIC between 0.1–10 μg/mL after 48 h), including commercially available tetrasaccharide tomato glycoalkaloid tomatine extracted from the leaves and stems of wild tomato [152]. In addition, extracts obtained from the roots of *Maytenus imbricata* [153], β-glycosides isolated from Thai plants (torvoside A, plumieride coumarate glucoside, gonocaryoside A, kingiside [154]), essential oil from *Ocimum basilicum* [155] and a nanoemulsion of *Micana cordifolia* [156] showed promising effects against *T. vaginalis* in vitro.

Some plants or fungal-derived compounds showed moderate efficacy (IC_{50} or MIC between 10–100 μg/mL after 48 h), including *Amomum tsao-ko* essential oil, used in traditional Chinese medicine [157], the compound wogonin, *Scutellaria havanensis* chloroform extract [158], Tomex® (a garlic-based product) [159] and *Cussonia holstii* [160]. Curcumin was highly effective against metronidazole-resistant strains of *T. vaginalis* in vitro (EC_{50}, 73–105.8 μg/mL after 24 h) [161] and has no known side effects.

Compounds based on plants or fungi that showed low efficacy (IC_{50} or MIC >100 μg/mL after 48 h) include oil of *Nigella sativa* [162], extracts of *Hypericum polyanthemum* [163], filtrate samples of two marine-associated fungi (*Hypocrea lixii* and *Penicillium citrinum*) [164], preparations from *Rheum ribes* [165], *Polygala decumbens* [166], ethyl acetate extract of *Arbutus unedo* [167], essential oils from *Artemisia absinthium* [168], *Pistacia lentiscus* [155], essential oils from *Lavandula angustifolia* [169], ginger [170] and extract of *Eucalyptus camaldulensis* [171]. In addition, saponins from *Passiflora alata* and *Quillaja saponaria* exhibited rather low trichomonacidal efficacy with unknown treatment times [172]. *Phaseolus vulgaris* [173], methyl jasmonate [174] and lycorine [175] have been described as effective against *T. vaginalis*, but neither EC_{50} values, MICs nor MLCs have been calculated. Amaurocine produced by the mushroom *Amauroderma camerarium* presented MICs between 2.6–5.2 μM after 24 h and increased human neutrophil nitric oxide release [176]. Isoaustrobrasilol showed an anti-*T. vaginalis* IC_{50} of 38 μM following incubation for 24 h [177].

4.2.2. Semisynthetic compounds

Semisynthetic derivatives of purpuromycin, an antibiotic produced by *Actinoplanes ianthinogenes*, presented trichomonacidal activity [178]. Piperazine, derived from betulinic acid, presented an MIC of 91.2 μM after 2 h of incubation [179]. Ursolic acid of *Manilkara rufula* revealed an IC_{50} of 35 μM and ultrastructural alterations of *T. vaginalis* following incubation for 24 h as well as high cytotoxicity against HMVII and HeLa cell lines [180]. Prophenin-2 presented an EC_{50} of 47.66 μM after 24 h of incubation [181]. Two derivates of betulinic acid presented MICs of 25–50 μM and 50–100 μM following 24 h of incubation and reduced ROS production by neutrophils; furthermore, an additional anti-inflammatory effect was induced [182].

4.2.3. Synthetic compounds

The rather new class of ceragenins, or cationic steroid antimicrobials, are synthetically derived compounds with a sterol backbone and amino acids and have shown trichomonacidal activity similar to cationic surface-active substances, but they require further analysis for clinical application [183]. 3-(Biphenyl-4-yl)-3-hydroxyquinuclidine, a derivative of hydroxyquinuclidine, presented lower efficacy in vitro towards *T. vaginalis* than metronidazole and presented lower toxicity towards human cell lines [184]. The mode of action is based on inhibition of β-haemolysis of red blood cells caused by *T. vaginalis* [185]. In addition, synthetic peptides derived from epinecidin-1 showed trichomonacidal activity [186]. Morpholin/piperidin-1-yl-carbamodithioates presented trichomonacidal, fungicidal and spermicidal activity in vitro with efficacy depending on the metronidazole resistance of the tested strain [187]. Ammonium salts of carbamodithioic acid are suspected to inhibit three thiols, subsequently causing chemical attenuation of the ROS. The MICs varied for metronidazole-susceptible and -resistant strains [185]. Synthesised tetrazolylmethyls exhibited rather high IC_{50} values [188]. The bimetallic iridium-ferrocene isonicotinyl complex showed high antitrichomonal activity in vitro [189]. In addition, nitrothiazole–NSAID chimaeras presented high efficacy (IC_{50}, 30–45 nM [190]).

The hybrid drug sulfimidazole, combining a sulfonamide and the standard therapeutic 5-nitroimidazole, presented equal efficacy to metronidazole alone in metronidazole-susceptible *T. vaginalis* strains and stronger efficacy in resistant strains [191]. Bis-uracil isatin conjugates showed IC_{50} values of 9.86–9.79 μM after 24 h [192], and nitroquinoxalins had IC_{50} values of 18.26–255.68 μM after 24 h [193]. Dioxolanes exhibited MICs of 90 μM and an IC_{50} of 60 μM after 24 h [194], synthetic endoperoxides showed IC_{50} values of 41–82 μM against metronidazole-susceptible strains and 60–300 μM against metronidazole-resistant strains after 24 h [195]. Nitroimidazole-carboxamides presented similar efficacy to metronidazole (EC_{50}, 0.6–1.4 μM) against *T. vaginalis* after 24–48 h [196]. Finally, chlorinated metronidazole presented significantly stronger efficacy against metronidazole-susceptible (0.006 μM vs. 0.068 μM) and metronidazole-resistant strains (0.24 μM vs. 0.49 μM) compared with standard metronidazole [197].

Polyamine quinoline rhodium complexes presented IC_{50} values between 4.8–12 μM following 24 h of incubation [198], and the most promising nitrothiazole presented an IC_{50} of 2.24 μM [199]. Synthesised chalconyl blended triazole allied silatranes showed stronger trichomonacidal activity (IC_{50}, 18.24–101.26 μM) after 48 h than metronidazole, but until now have only been screened in silico for their physiochemical properties [200]. Drug-free poly(isobutylcyanoacrylate) nanoparticles coated with chitosan were effective against *T. vaginalis* in vitro and non-toxic towards vaginal mucosa in pigs [201]. Evaluation of the antiretroviral lectins on adherence of *T. vaginalis* in vitro suggested a modest potential for preventing or treating human infections by causing self-aggregation and precipitation by the same pathway as galectin-1 [202].

4.3. Other approaches

Bee venom presented a growth-inhibiting effect on *T. vaginalis* [203] but the active compound and the effects of inducing a histamine response in the vaginal flora must be determined. In addition, deer musk presented a growth-inhibiting effect [204].

Photodynamic therapy using the combination of methylene blue with exposure presented high trichomonacidal efficacy in vitro and offers a completely different approach [205].

4.4. Screened in silico for possible efficacy

The purine-specific adenosine/guanosine-preferring ribohydrolase of *T. vaginalis* was screened in silico as a druggable target and eight compounds had very low IC_{50} values, the lowest being isoquercitrin with a value of 0.3 μmol [206]. In silico evaluation of natural products revealed new specific drug targets, namely *T. vaginalis* methionine γ-lyase and *T. vaginalis* purine nucleoside phosphorylase, both with promising ligands [151].

5. Conclusion

In the past years, several compounds have been tested for their efficacy against *T. vaginalis* in vivo and in vitro. However, only a few compounds have been evaluated in controlled clinical studies, and of these only nifuratel achieved a similar efficacy to metronidazole. The highest cure rates were achieved with 600 mg of oral nifuratel combined with 250 mg topical for 10 days. Two commercially available compounds containing extracts of the plants *M. crispa* or *Z. multiflora* achieved similar cure rates to metronidazole in double-blind RCTs. Paromomycin was shown to be effective in several cases where other therapies failed but occasionally caused ulcerations and irritations within a short time. Other effective drugs in complicated cases were pentamycin, zinc sulfate, acetarsol and, particularly, boric acid.

Of the compounds only tested in vitro, the most promising candidates are octenidine dihydrochloride and miltefosine, both for topical application and commercially available. Octenidine dihydrochloride is already in use against bacterial vaginosis and vulvovaginal candidiasis. The most promising candidate for systemic application from in vitro studies is auranofin.

In conclusion, in cases where treatment with metronidazole or tinidazole is not advised or unsuccessful, nifuratel, *M. crispa* and *Z. multiflora* may be alternatives. In cases of recalcitrant trichomoniasis, treatment with nifuratel in combination with nystatin, boric acid or (nightly) acetarsol, and, as a last resort, paromomycin has been successful.

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