Drug resistance of Trichomoniasis: A Review

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***Abstract*: Trichomoniasis is the most common, sexually transmitted infection. It is caused by the flagellated protozoan parasite *Trichomonas vaginalis.* Symptoms include vaginitis and infections have been associated with preterm delivery, low birth weight and increased infant mortality, as well as predisposing to HIV/AIDS and cervical cancer. Trichomoniasis has the highest prevalence and incidence of any sexually transmitted infection. The 5-nitroimidazole drugs, of which metronidazole is the most prescribed, are the only approved, effective drugs to treat trichomoniasis. Resistance against metronidazole is frequently reported and cross-resistance among the family of 5-nitroimidazole drugs is common, leaving no alternative for treatment, with some cases remaining unresolved. The mechanism of metronidazole resistance in *T. vaginalis* from treatment failures is not well understood, unlike resistance which is developed in the laboratory under increasing metronidazole pressure. In the latter situation, hydrogenosomal function which is involved in activation of the prodrug, metronidazole, is down-regulated. Reversion to sensitivity is incomplete after removal of drug pressure in the highly resistant parasites while clinically resistant strains, so far analysed, maintain their resistance levels in the absence of drug pressure. Although anaerobic resistance has been regarded as a laboratory induced phenomenon, it clearly has been demonstrated in clinical isolates. Pursuit of both approaches will allow dissection of the underlying mechanisms. Many alternative drugs and treatments have been tested *in vivo* in cases of refractory trichomoniasis, as well as in vitro with some successes including the broad spectrum anti-parasitic drug nitazoxanide. Drug resistance incidence in *T. vaginalis* appears to be on the increase and improved surveillance of treatment failures is urged.**

***Keywords*: Metronidazole, Trichomoniasis, Ferredoxin, Hydrogenase, Drug Resistance.**

Introduction:

- Drug resistance amongst the protozoa is an increasing problem with relatively few available drug alternatives. Poorly supervised treatment regimes and chemoprophylactic use of antimicrobials has resulted in the inefficacy of valuable drugs such as chloroquine in the treatment of falciparum malaria[1]. Cross-resistance to chloroquine and its analogues now occurs in almost all endemic areas. Resistance in the anaerobic protozoan parasite, *Trichomonas vaginalis* which was reported as early as 1962 is similarly at risk of becoming resistant to all recommended therapeutics [2]. It is the most common sexually transmitted infection worldwide with an estimated billion people infected at any one time[3, 4]. The 5-nitroimidazole family of drugs, specifically metronidazole and tinidazole, is the only class of drugs approved for the treatment of trichomoniasis with estimates of up to 10% of infections not responding to treatment in the United States[4]. Resistant organisms are cosmopolitan in distribution and are of considerable concern as *Trichomonas* infections are linked to vaginal HIV transmission[5-7]. T. vaginalis discovered by Alfred Donn in 1836, is an amitochondrial, microaerotolerant flagellate of the human urogenital tract. Each trophozoite possesses 4 anterior flagella and a single recurrent flagellum incorporated into an undulating membrane which is supported by a non-contractile costa. The cell body is longitudinally pierced by a thin, hyaline structure called the axostyle, which protrudes at the posterior. The nucleus is contained within a porous envelope typical of higher eukaryotes. Both free, motile and adhered, amoeboid forms have been described[8]. Multiple granular organelles, the hydrogenosomes, are characteristic of *T. vaginalis* and are involved in the parasite's metabolic pathways. The origin of the hydrogenosome remains under debate and is believed to be either analogous with the mitochondria having shared a common ancestor, the a-Gram negative proteobacteria [9], or alternatively, a direct derivative of the mitochondrion itself[10]. T. vaginalis trophozoites divide via longitudinal binary fission. It has no known cyst stage. However, pseudocyst formation has been described in cells undergoing environmental stress[11]. *T. vaginalis* is found on every continent and within every culture with highest occurrences in South and South East Asia, sub-Saharan Africa and amongst the under-privileged[3]. Approximately 50% of infections present with non-typical symptoms ranging from vaginitis, cervicitis and vulvitis in women, to urethritis, prostatitis and pruritis in men[12, 13]. Neonatal ocular and respiratory infections have also been described in neonates of infected mothers[14, 15]. Chronic infections have been associated with pre-term birth and low birth-weight neonates, cervical cancer and increased HIV transmission amongst the most alarming [16-18]. Infection rates have been reported as high as 67% in Mongolia in 1998[19], 40-60 % in Africa, 40% in Indigenous Australians over 40 years of age[20], and 46% in highland women of Papua New Guinea[21, 22]. Trichomoniasis has the highest prevalence and incidence of any STI, and its eradication may well be the single most cost-effective step in HIV incidence reduction[23, 24].
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Materials and Methods:

A total of 346 articles were retrieved through the search engines namely PubMed, Science Direct, Springer and Google Scholar. Using combination of keywords “Trichomoniasis”,“ metronidazole”, “ferredoxin”, “hydrogenase” and “drug resistance”. The search was limited to English language. Resulting abstracts were searched and reviewed and articles were excluded if the focus was not relevant to the topic. Articles representing a developing body of literature were limited to the most recent publication date.

Result and Discussion:

Result:

We have scanned 346 titles and abstracts of scientific articles that contain the main word or sentences in the search engines we found 128 articles that math the aspect of our study. After reading them one by one the abstract from the title, we picked 56 articles that were worthy of review.

Discussion:

Treatment of trichomoniasis: Metronidazole was originally synthesized and approved for the treatment of trichomoniasis in 1959 [25]. Recommended treatment regimes range from a single 1.5 or 2 g oral dose to split doses of 500 mg twice daily over a period of 7 d[26, 27]. Although metronidazole and tinidazole are the recommended drugs to treat trichomoniasis, their broad antimicrobial spectrum extends across the other anaerobic protozoa including, Entamoeba histolytica, Giardia duodenalis and Blastocystis hominis and anaerobic bacteria, Helicobacter pylori, Bacteroides spp and Clostridium spp[28]. Metronidazole is also valued for its prophylactic qualities in gynecological and other surgery, ease of administration via oral, topical, intravaginal or intravenous routes, few side effects and it is relatively inexpensive[28].

Mechanisms of drug action: As well as being the metabolic powerhouse of the cell, the hydrogenosome plays a critical role in drug activation[29, 33]. Metronidazole enters both the cell and organelle via passive diffusion as a prodrug and competes with the terminal enzyme hydrogenase as an electron acceptor from Fd. Activation of the drug occurs when an electron is transferred to the all important nitro-group forming a toxic nitro-radical[4, 29, 34]. In bacteria, the radicals cause chromosome breakage and cell death[35]. However, the precise target in a nucleated organism is unknown with proteins and protein trafficking likely major targets [29]. In addition to the toxic radicals, the production of molecular hydrogen is impaired in the presence of metronidazole, resulting in an increase of intracellular hydrogen peroxide[29, 36]. Under aerobic conditions in vitro, oxygen converts radicals back to the prodrug. Thus, in susceptibility assays carried out aerobically, drug inhibitory concentrations are higher than those carried out anaerobically[4, 34, 37].

Clinical drug resistance: Clinical isolates derived from treatment failures typically show resistance to metronidazole under aerobic conditions while some also exhibit anaerobic resistance. In the assay system recently reported by us, a clinically resistant isolate BRIS/92/STDL/B7268 (B7268) has an anaerobic minimal inhibitory concentration (MIC) of 25 M metronidazole, while the MIC of susceptible isolates is 3-6 M[34]. aerobic conditions, B7268 has an MIC > 200 M (34 g/ml) metronidazole. Depending on the assay system used, aerobic MICs of metronidazole on clinically resistant isolates have been reported to range from 25 g/ml to > 200 g/ml. The assortment of assays reported in the literature prevent direct comparisons but the values incorporated give some idea of the levels of resistance reached by several metronidazole-resistant clinical isolates. Resistance typically arises following multiple courses of metronidazole treatment for either trichomoniasis or another condition and in most cases, symptoms can be finally resolved with high doses of tinidazole and/ or metronidazole over extended periods but in some instances these high dose, poorly tolerated treatments are also unsuccessful.

Mechanisms of resistance: Initially people induced high levels of resistance in the clinically susceptible strain BRIS/92/STDL/F1623 (F1623) by continuous culturing in sub-lethal, increasing concentrations (finally reaching 1 M) of metronidazole,[43]. This confirmed similar data by Kulda et al[42], where 2 strains, one susceptible and one aerobically resistant, were cultivated in increasing concentrations of metronidazole for almost 2 years until the parasites were able to survive very high concentrations of metronidazole. Since we had shown that the PFOR activity and Fd levels were decreased in highly metronidazole-resistant Giardia parasites[30, 45], we compared transcription levels of these two proteins in our syngeneic lines of metronidazole-resistant and -susceptible *T. vaginalis* parasites. Radioactively labelled probes derived from 764 bp of the 5' region of the PFOR gene and a 500 bp product of the Fd gene were hybridised with mRNA from the syngeneic lines. Northern blot analyses revealed that the 4. 4 kb band of PFOR mRNA and Fd mRNA were represented only in the susceptible strain[43]. Our data
[43], that of Kulda et al[42] and Quon et al[46], as well as the data of Land et al[36] working with the cattle parasite, *Tritrichomonas foetus[*36], revealed that mRNA levels of the hydrogenosomal proteins PFOR, HYD, Fd and malate enzyme (ME) were reduced by up to 100%, and hydrogenosomal function was generally depleted in the resistant lines. However, Land et al[36] and Jaroslav Kulda (personal communication) have recently reported that the depleted hydrogenosomes are present in the resistant cells in similar numbers to sensitive cells but they were significantly smaller in the resistant line. In our experience the typical brown colour of T. vaginalis trophozoites (particularly apparent in pelleted cells) due to Fe-S cluster containing proteins found in the hydrogenosomes is not evident in highly metronidazole- resistant cells, consistent with the down-regulation of hydrogenosome function.

Although reports of metronidazole-resistant Trichomonas have appeared regularly since the initial case in 1962[47] the incidence of resistance prior to 1996 remained low. However, Sobel et al[48] reported a 17 fold increase in incidence of resistance in 1997-1998 and urge closer surveillance of this resistance. No alternative drugs are approved for treatment of refractory cases which is highly problematic as described in this review with some cases remaining unresolved. Whether continued drug selection has now allowed increased transmission, more resistant organisms, or the emergence of a more serious sexually transmitted disease problem, remains to be monitored.

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