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UP TO DATE

MODE OF ACTION OF THE MAIN ANTI-PARASITIC DRUGS

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ABSTRACT

Neglected tropical diseases affect more than 1 billion people in tropical and subtropical regions most of them are caused by parasites. Several of these diseases already have effective drugs capable of eliminating the causative parasite, however are not capable of interrupting the transmission cycle. This fact induces the continuous of repetitive treatments, which may result in the parasite's resistance. This review aims to show the mechanism of action of the main drugs used to treat parasitic neglected tropical diseases in order to determine the drug's target and help the understanding of how the parasites are killed within the host.

KEY WORDS: Parasitic neglected tropical diseases; mode of action; drugs.

INTRODUCTION

Neglected tropical diseases (NTD) are transmissible diseases that prevail in tropical and subtropical regions affecting more than 1 billion people. Most of the infected individuals are living in poverty conditions without adequate sanitation, treated water and in close contact with vectors and domestic animals (WHO, 2017).

In spite of having effective anti-parasitic drugs against most of the NTD, their incidence is increasing worldwide especially due to its mode of transmission and its close relation to poor hygienic habits and degrading general condition of life (Andrews et al., 2014; Colley et al., 2014).

The mechanism of action of anti-parasitic drugs have been investigated because it can point to the drug's target whether biochemical or structural within the parasite. As the target is determined also resistance mechanisms are found which help to understand why certain parasites present an increasing incidence and how the host-parasite relationship is established (Bergquist et al., 2017; Genetu et al., 2017).

The drugs indicated by WHO to treat the most prevalent parasitic NTD's are described in Table 1.

This review aimed to describe the mode of action of the main drugs used to treat the most common parasitic NTD's found in the Americas.

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Table 1. Parasitic neglected tropical diseases, etiological agent, mode of transmission and main anti-parasitic drug used in its treatment.

Parasitic neglected tropical disease	Etiological agent	Transmission	Anti-parasitic drug
Chagas disease	*Trypanosoma cruzi*	Vector borne – insects of the Triatominae family; oral, blood transfusion, vertical transmission	Benznidazole and Nifurtimox
Leishmaniasis	*Leishmania* spp	Vector borne – flies of the Phlebotomus or Lutzomyia genus	Pentavalent antimonials (sodium stibogluconate), miltefosine. Others
Taeniasis	*Taenia solium* *Taenia saginata* *Taenia asiática*	Food borne – ingestion of raw or undercooked meat containing cysticerci	Praziquantel, Niclosamide, Nitazoxanide
Cysticercosis	*Taenia solium*	Food borne – ingestion of water or raw food contaminated with eggs of the parasite	Praziquantel and/ or Albendazole
Echinococcosis	*Echinococcus granulosus* *Echinococcus multilocularis*	Food/water borne - Ingestion of parasite's eggs, dirty hands disease	Albendazole
Foodborne trematodiasis	*Clonorchis sinensis* *Opistorchis viverrinin* *Fasciola hepatica* *Fasciola gigantica* *Gnathostoma spinigerum*	Food borne – ingestion of fish, vegetables, crustaceans contaminated with larval parasites	Clonorchiasis and Opisthorchiasis – Praziquantel; Fascioliases – Triclabendazole; Gnathostomiasis – Albendazole
Schistosomiasis	*Schistosoma mansoni* *Schistosoma haematobium* *Schistosoma japonicum, others*	Active skin penetration of cercariae released from freshwater snails	Praziquantel
Soil-transmitted helminthiases	*Ascaris lumbricoides* *Ancylostoma duodenale* *Necator americanus* *Trichuris trichiura*	Soil contaminated with human faeces, dirty hands diseases	Albendazole or mebendazole

Adapted from WHO, 2016; WHO, 2017; Aronson et al., 2016

Benznidazole (BZN) and Nifurtimox (NFX)

BZN (N-benzyl-2-(2-nitro-1H-imidazol-1-yl)acetamide) and NFX ((E)-N-(3-methyl-1,1-dioxo-1,4-thiazinan-4-yl)-1-(5-nitrofuran-2-yl) methanimine) belong to the nitroimidazole drug family therefore containing a nitro group linked to an imidazole ring (Trochine et al., 2014).

BZN is a pro-drug that requires activation within the parasite as to perform its activity. An unusual prokaryotic type I nitroreductase was identified in trypanosomatid parasites which is responsible for the reductive activation of BZN (Trochine et al., 2014), while the bioactivation of NFX is dependent on a type II nitroreductase (Patterson & Wyllie, 2014).

Both BZN and NFX activities are related to their reduced nitrointermediates, which covalently modify and inactivate macromolecules such as lipids, DNA and proteins (Trochine et al., 2014). Also reactive oxygen species (ROS), generated by the interaction of reduced nitrointermediates with oxygen, lead to an intense intracellular oxidative stress and are particularly effective against *T. cruzi* because the parasite lacks catalases and is partially deficient in peroxidases (Docampo & Moreno 1984). Additionally, non-enzymatic reactions with BZN intermediates generate glyoxal which is highly toxic and may contribute for the parasite's cell death in spite of the low velocity of this metabolite formation (Patterson & Wyllie, 2014; Trochine et al., 2014).

Since BZN and NFX are mostly effective in the treatment of the acute phase of the infection, congenital transmission and children with chronic infection the search for new active compounds is paramount for the control of this disease (Paucar et al., 2016). Several studies have been performed as to determine new eligible targets within the parasite such as key metabolic enzymes: farnesyl pyrophosphate synthase, trans-sialidase, cruzipain (a cysteine protease), trypanothione reductase, glucose 6-phosphate-dehydrogenase, glyceraldehyde 3-phosphate-dehydrogenase and alpha-hydroxy acid dehydrogenase (Rivera et al., 2009; Urbina, 2010). Inhibitors of *de novo* sterol biosynthesis are one of the most advanced strategies for the development on novel anti-*T. cruzi* agents as they block *de novo* production of alkyl-sterols which is an essential biochemical pathway for the parasite's survival and is not replaced by the host's cholesterol synthesis (Urbina, 2009). Some of these compounds are experimentally active against acute and chronic murine Chagas disease and against NFX and BZN-resistant strains. One of the advantages described in this approach is the selectivity and potency of the treatment, less side effects and better tolerability. On the other hand, the limitations are the cost and complexity to manufacture these compounds (Urbina, 2009). Antifungal drugs such as azoles in clinical use or undergoing clinical trials have been considered promising on *in vitro* and *in vivo* assays against *T. cruzi*. Some of these, such as posaconazole and a pro-drug of

ravuconazole, are being evaluated in studies aiming Chagas disease treatment (Buckner & Urbina, 2012).

The evaluation of other nitroheterocyclic drugs on acute and chronic phases (Francisco et al., 2016), natural compounds on *in vitro* analysis (Ebiloma et al., 2017) are approaches that may result in active compounds against the parasite.

Also the improvement of chemical characteristics of the drugs such as the enhancement of BZN and NFX dissolution rate (Fonseca-Berzal et al., 2015; Figueredo et al., 2017) may result in a better reach of the drug metabolites to the therapeutic targets within the parasite when it is in the amastigote form within the parasitophorous vacuole (Campo et al., 2016).

Pentavalent antimonials (PA) – sodium stibogluconate

Sodium stibogluconate (trisodium (3R,4S,5R)-1-{[(3R,4S,5R)-3-carboxylato-5-[(1R)-1,2-dihydroxyethyl]-1-oxido-2,6,7-trioxa-1-stibabicyclo[2.2.1]heptan-1-yl]oxy}-5-[(1R)-1,2-dihydroxyethyl]-1-hydroxy-2,6,7-trioxa-1-stibabicyclo[2.2.1]heptane-3-carboxylate nonahydrate) is indicated in the treatment of leishmaniasis as an alternative to trivalent antimonials such as tartar emetic. It has been proposed that the drugs enter the parasitic cell via a phosphate transporter. Once inside the cell they induce the oxidation of thiols (glutathione, cysteine and cysteine-glycine) and inhibit trypanothione reductase (Wyllie et al., 2004). It is believed that PA needs to be reduced to the trivalent form as to be active, however it is not clear how this occurs inside the parasite as well as inside macrophages (Singh et al., 2012). Other studies suggest that after the activation of PA's there is a depletion of purine nucleosides (Frezard et al., 2009). On the other hand, after reduction of PA's to form trivalent antimonials there is a complexation with glutathione and other thiols which lead to a series of reactions that result in the apoptosis of the amastigote (Frezard et al., 2009). Other studies show that there is a 50% decrease in the parasite DNA, RNA protein and purine nucleoside triphosphate levels added to the reduction in ATP and GTP synthesis leading to a decrease in macromolecular synthesis within the parasite which contributes to its death (Mukherjee et al., 2016). Specifically, sodium stibogluconate inhibits DNA topoisomerase I leading to the inhibition of DNA replication and transcription (Walker & Saravia, 2004).

As PA are mostly effective against amastigotes which are the parasitic form inside macrophages it is highly recommended that drug trials even the ones selected from *in silico* platforms are performed in amastigote cultures and in animal experimental models (Andrews et al., 2014).

The development of liposomal and cyclodexitrin-based formulations may enhance the therapeutic activity of PA's as these formulations increased

the reach of the active compound within the parasite's biochemical/molecular target (Frezard et al. 2009).

Miltefosine

Miltefosine (1-O-hexadecylphosphocholine) is used in the treatment of visceral leishmaniasis. Its mode of action is not entirely understood and the most reports and investigations regarding it have been made in promastigote assays (Aronson et al., 2016).

Its mode of action has been related to the impairment of the alkyl-phospholipid metabolism and the biosynthesis of alkyl-anchored glycolipids and glycoproteins. It has been reported that in *L. donovani* promastigotes miltefosine is capable of inducing an apoptosis-like death (Verma & Dey, 2004; Paris et al., 2004). Several reports have described that miltefosine affects the lipid metabolism of promastigotes with emphasis on sterols and fatty acids oxidation (Rakotomanga et al., 2005; Rakotomanga et al., 2007). Studies have shown that miltefosine also impairs the calcium homeostasis leading to the cell death by apoptotic mechanisms. The calcium regulation is especially compromised in the mitochondrion membrane, endoplasmic reticulum and acidocalcisomes. This process results in a large increase in intracellular calcium concentrations inducing the parasite death (Serrano-Martin et al., 2009; Benaim & Garcia, 2011). In combination with amiodarone, miltefosine is capable of inducing the cure of an experimental model of cutaneous leishmaniasis by *L. mexicana* through the disruption of the calcium homeostasis, inhibiting the proliferation of intracellular amastigotes (Serrano-Martin et al., 2009).

Metabolomics analyses have determined that miltefosine is capable of altering around 10% of the metabolome of sensitive *L. donovani* promastigotes, mainly linked to the lipids metabolism (Vincent et al., 2014). On amastigotes it has been described the impairment of the polyamine metabolism from arginine to trypahothione added to an increase in the production of reactive oxygen species (Canuto et al., 2014).

Miltefosine inhibits cytochrome c in *Leishmania donovani* promastigotes leading to an impairment of the respiratory chain, reduction in the oxygen consumption rate and mitochondrial depolarization (Luque-Ortega & Rivas, 2007).

Amphotericin B in its liposomal formulation (AmBisome) is the most effective and frequently used drug for the treatment of visceral leishmaniasis worldwide, as monotherapy or in combinations with pentavalent antimonials or miltefosine (Rama et al., 2015). Clinical trials have demonstrated that patients present better tolerability to liposomal amphotericin B with greater effectiveness of the treatment (Freire et al., 1997). It has demonstrated excellent efficacy against visceral leishmaniasis and has been adopted as first-line regimen in its treatment (Rahman et al., 2017).

There is a need for new compounds against *Leishmania* species that are not only potent but also less toxic and more cost effective in humans (Rama et al., 2015).

Praziquantel (PZQ)

Praziquantel (2-cyclohexylcarbonyl(1,2,3,6,7,11 b)hexahydro-4H-pyrazin(2,1-a)isoquinolin-4-0ne) is a pyrazinoisoquinoline and is the drug of choice to treat several helminthiasis such as schistosomiasis, teniasis, cysticercosis and others. In spite of the extensive studies of its mechanism of action it is still not totally elucidated (Thomas & Gonnert, 1977; Chai, 2013). It is interesting to highlight that PZQ is active against several flatworms and not nematodes and this occurs because the main target of the drug is a unique gene product which is found only in flatworms (Greenberg, 2005) or this target might be encoded in genes that are transcripted in different structural signatures that do not enable the interaction with PZQ (Greenberg, 2005).

Since the first studies of the PZQ's mechanism of action the tetanic contraction of the musculature and structural damage of the syncytial tegument were described (Andrews, 1985, Greenberg, 2005). These effects lead to exposure of parasite antigens on the worm surface enabling the immunological attack (Greenberg 2005).

Tegumental damage is observed by vacuolization and blebbing in *in vitro Schistosoma mansoni.* This effect is dose dependent and may be so severe that parts of the parasite are lost (Andrews, 1985; Doenhoff et al., 2008). Both muscle contraction and tegumental damage are Ca^{2+} - dependent processes as the removal of calcium from the medium blocks these responses (Greenberg, 2005). There are several targets related to calcium homeostasis within the parasite which are voltage-, ligand- and second messenger-gated calcium channels, intracellular calcium release channels and intracellular calcium buffers which alter the intracellular calcium concentrations resulting both in impaired membrane fluidity and in the spastic contracture of the muscle (Greenberg, 2005, Jeziorski & Greenberg, 2006; Aragon et al., 2009).

Transcriptomic assays that analyzed both sensitive and resistant miracidia and developing adult *Schistosoma mansoni* determined that susceptibility to PZQ is linked to genes involved in aerobic metabolism and cytosolic calcium regulation (Aragon et al., 2009).

One problem of the PZQ efficacy is that it shows parasite stage and sex dependent differences in susceptibility when used in the schistosomiasis treatment (Greenberg, 2005). The importance of ATP-binding cassette (ABC) multidrug transporters in the praziquantel resistant *Schistosoma mansoni* strains have been reported. These studies have enabled the determination of the role of these transporters both in drug resistance and in several physiological functions such as excretion and permeability barriers (Greenberg 2014). Also

transient receptor potential (TRP) channel which is an ion channel related to the pharmacological properties of PZQ in *S. mansoni*. These channels are essential to transducing sensory signals and in the regulation of the intracellular calcium and therefore are related to sensitivity to PZQ (Bais &Greenberg 2016).

Nanoformulations of PZQ have been developed as to increase the efficacy of the drug as described in *in vivo* and *in vitro* essays in *S. mansoni* (Kolenyak-Santos et al., 2014) and in the experimental model of cysticercosis (Silva et al., 2016)

Niclosamide

Niclosamide (5 - chloro - N - (2 - chloro - 4 - nitrophenyl) - 2 - hydroxy - benzamide), is a benzenoid and is indicated to treat intestinal tapeworms. It has a poor absorption and does not reach active concentrations in plasma as neither the drug nor its metabolites have been recovered from the blood or urine, therefore it does not have efficacy against tissue parasites (Pearson & Hewlett, 1985; Swan, 1999).

Adult worms, not larval stages nor eggs, are killed through an impairment of the oxidative phosphorylation or stimulation of ATPase activity. Parts of the parasites are eliminated with the feces while others are destroyed within the intestine (Poole et al., 1971; Pearson & Hewlett, 1985).

The most reported use of niclosamide is as moluscicide in order to eliminate the intermediary hosts of *Schistosoma* sp. In *Oncomelania hupensis*, the intermediate host of *S. japonicum*, niclosamide induced a significant decrease in the number of mitochondria which presented morphological alterations of their cristae, associated to polarized heterochromatin, decreased number of ribosomes in the rough endoplasmic reticulums, damaged cell structures and organelles, leading to the death of the snails (Xiong et al., 2016). Also molecular analyses performed in *Biomphalaria glabrata* showed that niclosamide interfered on the transcriptional responses of genes involved in the biotransformation of xenobiotics such as cytochrome P450, glutathione S-transferase, drug transporters, multi-drug resistance protein as efflux transporters and solute linked carrier as influx carriers (Zhang et al., 2015).

Nitazoxanide (NTZ)

NTZ (2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl acetate) is a synthetic nitrothiazol-salicylamide derivative. Initially indicated as anti-protozoal agent used against *Giardia intestinalis* and *Cryptosporidium* sp is now indicated in the treatment of both intestinal and tissue parasites, whether protozoans, flatworms or nematodes (White, 2004).

After oral administration it is rapidly hydrolyzed into tizoxanide. However both NTZ and tizoxanide have efficacy against parasites evaluated in *in vitro* and *in vivo* essays (Palomares-Alonso et al., 2007).

The biochemical target of NTZ and tizoxanide is the pyruvate ferredoxin oxidoreductase, the enzyme responsible for the decarboxylation of pyruvate into acetyl-CoA (White, 2004; Hoffman et al., 2007). It impairs the tricarboxilic acid cycle as it decreases the acetyl-CoA supply for the cycle to continue, forcing the parasite to use alternative sources of acetyl-CoA such as fatty acids oxidation and proteins catabolism (Isac et al. 2016). Also the excess of pyruvate induces acidosis due to an increase in lactate concentrations which also induces gluconeogenesis (Isac et al., 2016).

Benzimidazoles – albendazole, mebendazole and triclabendazole

Albendazole (methyl N-[6-(propylsulfanyl)-1H-1,3-benzodiazol-2-yl]carbamate), mebendazole (methyl N-(6-benzoyl-1H-1,3-benzodiazol-2-yl)carbamate) and triclabendazole (6-chloro-5-(2,3-dichlorophenoxy)-2-methylthiobenzimidazole) are indicated in the treatment of both intestinal and tissue flatworms and nematodes (Gottschall, 1990; Lacey, 1990).

The anti-parasitic main mode of action of benzimidazole drugs is to impair the tubulin polymerization into microtubules and therefore disrupting microtubule-based processes (Lacey, 1990; Fairweather & Boray, 1999). Tissue parasites, such as vascular and interstitial ones, are less sensitive than intestinal ones. Also activity against developing stages is superior to that against adult ones (Lacey, 1988). After the drug activation within the host, usually a sulfoxidation reaction in the liver, the active compound is capable of inducing impairment of β-tubulin polymerization, perturbation in parasite motility, nutrient uptake, enzyme secretion and glycolytic enzyme activities (Lacey, 1988, Martin et al., 1997).

Biochemical effects are also described in the activity of fumarate reductase and on traditional and alternative energetic pathways both in *in vitro* and *in vivo* assays (Lacey, 1988; Vinaud et al., 2007; 2008; 2009; Fraga et al., 2012). Glucose uptake impairment, uncoupling of oxidative phosphorylation, depression of ATP levels, inhibition of transmembrane proton discharge and increase in Na^+ uptake were also described (Lacey, 1988).

The resistance against benzimidazoles is increasing due to mutations in the tubulin molecule and to increased active cellular efflux of the drug (Gottschall et al., 1990). Therefore the importance of the development of benzimidazole derivatives that present similar efficacy as albendazole but with different targets within the parasite. This is the case of benzimidazole derivatives which target α-tubulin subunit of microtubules and not the β-one. Studies have shown that these derivatives present similar efficacy both in biochemical and in mortality parameters (Hernández-Luiz et al., 2010; Márquez-Navarro et al.,

2013; Fraga et al., 2016; Fraga et al., 2017). Mebendazole, on the other hand, elicits changes in adenine nucleotides, glucose uptake, glycogen depletion and in the respiratory end product (Behm & Bryant, 1979).

Therefore we conclude that the understanding of the mechanism of action of the different anti-parasitic drugs help to determine how the parasite is killed within the host, how the parasite develops resistance and what can be done to prevent this. Especially when there are so many cases of drug resistance in veterinary helminthes showing the capability of the parasites to remain viable and contaminating the environment (Wolstenholme et al., 2004). It is of paramount importance that these drugs be used correctly and judiciously as to ensure the adequate response.

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