Abstract

*Giardia lamblia* (also known as *G. duodenalis* or *G. intestinalis*), a flagellated protozoan, is the most common causative agent of persistent diarrhea worldwide. The life cycle includes motile, flagellated trophozoites parasitizing the upper intestine and thick-walled cysts forming in the lower intestine, which are subsequently shed with the feces. Chemotherapy is directed against the trophozoite stage. The current anti-giardial therapy of choice is metronidazole. In the case of resistance, alternatives are other nitroimidazoles, quinacrine, albendazole, and nitazoxanide. Here, we present a review of current anti-giardial drugs and of their modes of action.

Introduction

For the treatment of giardiasis, chemotherapy is the method of choice. After the introduction of quinacrine as the first anti-giardial drug in the 1940s, metronidazole (commercially known as *Flagyl*®) and other nitroimidazoles such as tinidazole have been used as a therapy of choice for more than five decades. In the case of resistance formation, substitution therapies include benzimidazole albendazole, the acridine derivative quinacrine – historically the first anti-giardial drug –, or the aminoglycoside paromomycin alone or in combination with metronidazole (see for review Upcroft and Upcroft, 2001 and for a recent clinical study Morch et al., 2008). In the mid-1990s, a new antiparasitic agent, the thiazolidine nitazoxanide (commercially known as *Alinia*®), has been introduced in the market. Nitazoxanide has been approved in the USA for the treatment of persistent diarrhea due to cryptosporidiosis and giardiasis in people older than 12 years (reviewed by Hemphill et al., 2006). Moreover, *in vitro* effects of natural compounds such as isoflavones, polyenes or saturated fatty acids against trophozoites have been described, but clinical data are lacking. An overview of anti-giardial compounds with references is given in Table 1.

In this chapter, we will focus on the mode of action of these drug families (so far as known) and put some new light on resistance mechanisms.

Mode of Action and Drug Targets

General Remarks

The ideal situation for the mode of action of antiparasite drugs consists of binding to a target protein (enzyme or receptor) resulting in the inhibition of essential cellular functions. Such targets are identified by analyzing structural and metabolic alterations of the parasite due to the drug followed by the isolation of drug binding proteins or the analysis of resistant vs. sensitive parasites. The isolated targets are then validated by inhibition studies *in vitro*, structural analysis of protein-ligand complexes, and – if possible – knock-out and overexpression studies of the corresponding gene in the target organism (see e.g. Wang, 1997).

In an ideal situation, the drug-target interaction depends on distinct structural features of the target. In this case, point mutations causing the presence or absence of a single amino acid or nucleotide (in the case of rRNA as a target) discriminate between resistance or susceptibility. In reality, the situation is more complex. In resistant organisms, the “ideal” target may still be present, but the drug cannot access it due
In order to determine the efficacy of antigiardial drugs (e.g. in terms of IC$_{50}$ values), a couple of methods are currently in use. The most classical way is counting trophozoites grown axenically in the presence of drugs (see e.g. Müller et al., 2006). The incorporation of $^3$H-thymidine is less subjective (see e.g. Adagu et al., 2002). Both methods are time-consuming or need high-level equipment. An elegant alternative is the use of the vital staining resazurin (Alamar Blue; Bénéré et al., 2007). Viable cells reduce resazurin forming a pink compound absorbing at 550 nm and fluorescing at >590 nm. Although it is possible to use spectrophotometry for quantifying the product, the best sensitivity is obtained with fluorimetry. Fluorimeters are, however, expensive and therefore not present in many labs. Moreover, all these methods cannot be applied to uptake limitation or detoxification mechanisms. A search for drug targets (e.g. by protein binding studies) would thus yield detoxification enzymes, transporters or even irrelevant drug binding proteins besides the “true” target. Conversely, susceptibility to a drug may be triggered by the ability to convert a (ineffective) prodrug to the (effective) drug. In this case, search for targets have to be performed with the effective molecule, otherways, the transforming enzyme would be identified rather than the target itself.

In the case of antiparasite drugs (as well as other antibiotics), the situation is rendered more complicated by the fact that the drug acts in the vicinity or even inside the host cell (in the case of intracellular pathogens). Therefore, the mode of action of the drug may be modulated if not depend on the susceptibility of the host cell and not the parasite itself. In the following paragraphs, we will discuss some examples within the framework of antigiardial drugs.

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
<th>Efficacy</th>
<th>Reference</th>
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<tr>
<td>Acridines</td>
<td>Quinacrine</td>
<td>In vitro (10$^{-7}$ M)</td>
<td>Bell et al., 1991; Bénéré et al., 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In vivo (100 mg tid + MET 750 mg tid for 2–3 weeks)</td>
<td>Nash et al., 2001; Mørch et al., 2008</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Paromomycin</td>
<td>In vitro (10$^{-7}$–10$^{-5}$ M)</td>
<td>Edlind, 1989</td>
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<tr>
<td></td>
<td></td>
<td>In vivo: 500 mg tid for 1 week</td>
<td>Mørch et al., 2008</td>
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<tr>
<td>Benzimidazoles</td>
<td>Albendazole</td>
<td>In vitro (10$^{-7}$ M)</td>
<td>Katiyar et al., 1994, Upcroft and Upcroft, 2001; Cruz et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In vivo (400 mg bid + MET 250 mg bid or tid for 1 week)</td>
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<tr>
<td>Fatty acids</td>
<td>Dodecanolic acid</td>
<td>In vitro (10$^{-7}$ M)</td>
<td>Rayan et al., 2005</td>
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<td>Fluoroquinolones</td>
<td>Ciprofloxacine</td>
<td>In vitro (10$^{-8}$ M)</td>
<td>Sousa and Poiares-da-Silva, 2001</td>
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<td>Isoflavones</td>
<td>Genistein, Formononetin</td>
<td>In vitro (10$^{-8}$ M)</td>
<td>Khan et al., 2000; Sterck et al., 2008</td>
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<tr>
<td>Nitrofuranes</td>
<td>Furazolidone</td>
<td>In vitro (10$^{-7}$–10$^{-6}$ M)</td>
<td>Cedillo-Rivera and Munoz, 1992</td>
</tr>
<tr>
<td>Nitroimidazoles</td>
<td>Metronidazole, Tinidazole</td>
<td>In vitro (10$^{-6}$ M); in vivo</td>
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<td>Diaminidines</td>
<td>Pentamidine and derivatives</td>
<td>In vitro (10$^{-7}$–10$^{-5}$ M)</td>
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<td>Polyenes</td>
<td>Allicin and derivatives</td>
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<td>Thiazolides</td>
<td>Nitazoxanide</td>
<td>In vitro (10$^{-6}$ M)</td>
<td>Fox and Saravolatz, 2005; Hemphill et al., 2006</td>
</tr>
</tbody>
</table>

Methods for the Determination of Drug Efficacy

In order to determine the efficacy of antigiardial drugs (e.g. in terms of IC$_{50}$ values), a couple of methods are currently in use. The most classical way is counting trophozoites grown axenically in the presence of drugs (see e.g. Müller et al., 2006). The incorporation of $^3$H-thymidine is less subjective (see e.g. Adagu et al., 2002). Both methods are time-consuming or need high-level equipment. An elegant alternative is the use of the vital staining resazurin (Alamar Blue; Bénéré et al., 2007). Viable cells reduce resazurin forming a pink compound absorbing at 550 nm and fluorescing at >590 nm. Although it is possible to use spectrophotometry for quantifying the product, the best sensitivity is obtained with fluorimetry. Fluorimeters are, however, expensive and therefore not present in many labs. Moreover, all these methods cannot be
used when trophozoites are not grown axenically, but
in a coculture system with intestinal cells (e.g. Caco
2). In this system, trophozoites may be quantified using
real-time PCR with a Giardia-specific target (Müller et al., 2006).

A suitable alternative would be the use of a reporter strain as shown for instance for Toxoplasma gondii expressing β-galactosidase (McFadden et al., 1997; see for a recent application Müller et al., 2009a). In the case of Giardia, a reporter strain expressing glucuronidase A from E. coli has been created (Müller et al., 2009b). This strain is well suited for the determination of IC_{50} values and for high-throughput drug screening using simple colorimetric assays. Moreover, it can be used in a coculture system with host cells thus allowing the determination of the “therapeutic window”, i.e. the gap between drug efficacy and host cell toxicity in one screen.

**Nucleic Acids as Targets**

Early studies have shown that the acridine derivative quinacrine, the first anti-Giardia drug, interacts with DNA inhibiting replication and RNA and protein biosynthesis in prokaryonts (Ciak and Hahn, 1967). Like other acridine derivatives, quinacrine is an intercalating agent binding to DNA with a preference for (A+T)-rich regions and thereby blocking DNA replication and RNA biosynthesis (Wilson et al., 1994). In studies concerning pentamidine derivatives (Bell et al., 1991) and bis-benzimidazoles (Bell et al., 1993), very nice correlations between the binding affinities of these compounds to calf thymus DNA and their efficacies against Giardia lamblia have been pointed out. Since DNA binding is by far not species specific and therefore prone to side effects on host cells, the relevance of a DNA binding agent as an antimicrobial drug resides in other – more species specific – properties like a differential uptake or metabolism or the presence of other targets besides DNA. For instance, pentamidines and a variety of other DNA binding compounds such as etoposide induce the cleavage of Trypanosoma DNA minicircles in a pattern that resembles the action of topoisomerase II inhibitors (Shapiro and Englund, 1990). DNA modifying enzymes may thus constitute more selective targets for antimicrobial agents.

Fluoroquinolones such as ciprofloxacin inhibit DNA-gyrases and topoisomerases from prokaryonts (Kidwai et al., 1998) and are highly effective against G. lamblia as shown in a descriptive study (Sousa and Poiares-da-Silva, 2001). It is, however, unclear, which type of enzyme is inhibited there since more pronounced studies are lacking. A phylogeny analysis of type II topoisomerases shows that the gene of G. lamblia (GlTop2) is eukaryont-like and not closely related to prokaryont topoisomerases (He et al., 2005).

Aminoglycosides bind to the small subunit of ribosomes, more exactly to distinct features of the 16S-rRNA secondary structure and thereby inhibit protein biosynthesis (see e.g. Brodersen et al., 2000 for a detailed structural analysis). Therefore, specific mutations in this region confer resistance to various aminoglycosides in various prokaryonts. G. lamblia has a 16S-rRNA structure similar to prokaryonts with a primary sequence suggesting that only paromomycin and hygromycin are effective, other well known aminoglycosides such as kanamycin are not. This pattern correlates well with observed susceptibility or resistance to a panel of aminoglycosides (Edlind, 1989).

**Cytoskeleton Proteins as Targets**

Early studies with benzimidazoles such as albendazole showed effects on the cytoskeleton, especially in the region of the ventral disk (Fig. 1; see e.g. Chavez et al., 1992; Hemphill et al., 1996). Molecular genetics has revealed that sensitivity to albendazole (or other benzimidazoles) in evolutionary distant organisms such as fungi, nematodes, platyhelminthes, and various protozoa including G. lamblia is correlated to the presence of specific alleles of β-tubulin-genes (Kirk-Mason et al., 1988; Driscoll et al., 1989; Katiyar et al., 1994; Kwa et al., 1995; Henriquez et al., 2008), the substitution of Phe to Tyr in position 200 being sufficient for switching from benomyl sensitivity to resistance as shown in nematodes (Kwa et al., 1995). Molecular modeling of the putative albendazole binding site in albendazole resistant Acanthamoeba β-tubulin indicates that 4 of 13 residues are different from tubulins of sensitive organisms. Resistance is conferred when, besides Phe200, Phe167 is replaced (Henriquez et al., 2008).
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A mode of action similar to nitroimidazoles has been postulated for nitrothiazolides. Upon oral uptake, nitazoxanide, the best studied nitrothiazolide, is rapidly deacetylated to tizoxanide and further metabolized to tizoxanide-glucuronide (Fox and Saravolatz, 2005). Tizoxanide has been reported to display antimicrobial activity similar to nitazoxanide, while tizoxanide-glucuronide is largely inactive against a number of pathogens including *Giardia*.

Thiazolides without the thiazole-associated nitro group have been shown to exhibit decreased or no efficacy against *Giardia* (Adagu, et al., 2001; Müller et al., 2006). Therefore, an involvement of the nitro group in the mechanism of action, with the participation of PFOR similar to metronidazole, is likely (Wright et al., 2003). The nitro group is also required for *in vitro* activity against anaerobic bacteria (Pankuch and Apelbaum, 2006). Moreover, nitazoxanide inhibits activities of recombinant PFOR from various bacteria and parasites including *G. lamblia* (Sisson et al., 2002). A reduction of nitazoxanide, however, has never been observed in vivo. Instead, there is some evidence that unreduced nitazoxanide blocks an intermediate step of the reaction thus inhibiting PFOR in a non-competitive manner (Hoffman et al., 2007). When *G. lamblia* trophozoites are treated with nitrothiazolides, ultrastructural analyses

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**Drugs Interfering with Intermediary Metabolism**

The nitroimidazole metronidazole is clearly the best studied compound affecting intermediary metabolism. When trophozoites are treated with metronidazole the cell looses motility within a few hours followed by appearance of large zones of lesions on the dorsal shield and swelling of the cell body (Fig. 2; see e.g. Müller et al., 2006). According to a widely referred theory, metronidazole (as a prodrug) is reduced to a nitro radical by electrons coming from the enzyme pyruvate:flavodoxin/ferredoxin oxidoreductase (PFOR), a protein lacking in higher eukaryotic cells (Horner et al., 1999). The radical causes irreversible damages. Results obtained with the microaerophilic parasite *Trichomonas vaginalis* suggest, however, another mode of action. By comparing 2-D-protein electrophoresis patterns from treated and untreated cells, the authors suggest that metronidazole binds to the sulphhydryl group in the active center of various enzymes including thioredoxin reductase thereby impairing essential cellular functions (Leitsch et al., 2009). Also in this model, an activated form of metronidazole would act on multiple targets, the number being limited to enzymes with sulphhydryl groups in their active center.

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**Fig. 1** Examples of ultrastructural changes induced by albendazole and visualized by transmission electron microscopy, A, albendazole; B, control; N, nucleus
Recent studies have shown that not only nitazoxanide, but also derivatives lacking the nitro group (reviewed in Hemphill et al., 2007) exhibit *in vitro* activity against the intracellular apicomplexan parasites *N. caninum*, *Cryptosporidium parvum* and *Besnoitia besnoiti* (Hemphill et al., 2006; Cortes et al., 2007) and as well as proliferating host cells (Müller et al., 2008c). Therefore, it cannot be excluded that the efficacy of nitazoxanide against giardiasis and cryptosporidiosis in patients is partly due to effects on intestinal cells. *In vivo* studies on these diseases with non-nitro-thiazolides are lacking. In proliferating host cells (Caco2), inhibition of glutathione-S-Transferase P1 (GSTP1) by nitazoxanide reveal severe damages of the ventral disk instead of damages of the dorsal surface membrane as observed with metronidazole (Fig. 2; see e.g. Müller et al., 2006). In *G. lamblia*, a nitroreductase as a nitazoxanide-binding protein was identified and characterized. Nitroreductase is inhibited by nitazoxanide, and, as for PFOR, there is no evidence for a reduction by nitroreductase (Müller et al., 2007b). Overexpression of this nitroreductase is, however, correlated with increased sensitivity to metronidazole and nitazoxanide (Nillius et al., 2011). Besides nitroreductase, protein disulfide isomerases from *G. lamblia* (Müller et al., 2007a) and *Neospora caninum* (Müller et al., 2008b) are potential targets for nitazoxanide.

Fig. 2 Examples of ultrastructural changes induced by nitazoxanide and metronidazole and visualized by scanning electron microscopy. A, control; B, nitazoxanide (NTZ); C, D, metronidazole (MTZ)
and some non-nitro-thiazolides is well correlated with their efficacy to induce apoptosis. Overexpression and downregulation of GSTP1 is correlated with an increase, or decrease, of sensitivity, respectively (Müller et al., 2008c).

**Compounds of Plant Origin with Miscellaneous Targets**

In order to improve the situation in chemotherapy of metronidazole resistant giardiasis, new potential pharmaceuticals from natural sources have been evaluated regarding their antigiardial activity *in vitro*. In general, crude extracts from traditional medicinal herbs are tested with respect to antigiardial activities and then fractionated in order to identify the active principles (see for review Anthony et al., 2005). Essential oils are a well explored source for antimicrobial compounds. A good example for a study on antigiardial properties in oregano extracts is given by Ponce-Macotela et al. (2006). Often, essential oils have a broad spectrum of efficacy and therefore a potential toxicity to host cells. A good example is a study where active compounds have been identified from *Allium* extracts (Harris et al., 2000). In this study, the compound with the lowest IC₅₀ is allyl alcohol, a compound with an unspecified toxicity and a potential cancerogene. Allicin, another molecule effective against *Giardia* and other parasites such as *Entamoeba*, bind to SH-groups from cysteine proteases and other SH-proteins including thioredoxin reductase (see Ankri et al., 1997; Ankri and Mirelman, 1999), a mode of action recently postulated for metronidazole (Leitsch et al., 2009).

Another well studied group are isoflavones. Isoflavones are mainly found in Leguminosae where they have anti-oxidant, anti-microbial and signalling functions (e. g. reviewed in Dakora and Phillips, 1996; Dixon and Steele, 1999). Within this group of compounds, Khan et al. (2000) identified formononetin, a major isoflavone of *Ononis* sp, as the most potent antigiardial agent. *In vitro*, formononetin has an IC₅₀ value in the range of 10⁻⁷ M, thus one order of magnitude lower than the non-methylated daidzein and genistein, two isoflavones of soybean (Sterk et al. 2008). By daidzein-affinity chromatography, a nucleoside hydrolase was identified as daidzein-binding protein. Nucleoside hydrolase activity is inhibited by the three isoflavones mentioned above in a range of concentrations where daidzein and genistein inhibit trophozoite growth *in vivo*. In *Crithidia* (Parkin et al., 1991), *Leishmania* (Shi et al., 1999) or *Trypanosoma* (Parkin, 1996), the function of nucleoside hydrolase is central in salvaging purine nucleosides from the host and essential since the parasites lack purine *de novo* synthesis. Inhibition would thus lead ultimately to a block of DNA synthesis and thus cell division. This effect would be rather slow. Formononetin acts faster by stopping motility and inducing detachment within less than 2 h (Lauwaet et al., 2010, and own, unpublished data) and is inhibitory at ten times lower concentrations suggesting other mechanisms to be involved in addition such as depolarization of the membrane due to uncoupling effects or direct inhibition of membrane ATPases. However, experimental evidence for any of these possibilities is lacking.

**Resistance Formation**

Besides the search for drug binding proteins, another way to elucidate the action of drugs is the comparison of resistant vs. non-resistant trophozoites. Resistant trophozoites are created by selecting growing cells in the presence of increasing drug concentrations followed by cloning and characterization of the resistant clones vs. wildtype clones. Moreover, in some cases, drug (mainly metronidazole) resistant strains have been isolated from patients and compared to non-resistant strains. Table 2 gives an overview of selected studies.

Resistance to metronidazole and other nitroimidazoles has been induced *in vitro*; and various genotypically distinct isolates with reduced drug susceptibility have also been found in human patients (Gardner and Hill, 2001; Upcroft and Upcroft, 1993). Formation of resistance to metronidazole is associated with an altered uptake of the drug (Boreham et al., 1988; see also Upcroft et al., 1996 for quinacrine), downregulation of the PFOR activity (Upcroft and Upcroft 2001). This is consistent with the model claiming an involvement of PFOR in metronidazole activation (Townson et al., 1996; Sisson et al., 2002). Recent findings in *T. vaginalis* suggest, however, that metronidazole and other nitroimidazoles covalently bind and thereby
Table 2 Overview of studies on resistance formation in *G. lamblia*

<table>
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<tr>
<th>Drug</th>
<th>Source of resistance</th>
<th>Method</th>
<th>Important findings</th>
<th>Reference</th>
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<td>Albendazole</td>
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<td>Comparison to wildtype with respect to growth</td>
<td>Resistance at 4.5 µM for several weeks, normal cyst formation</td>
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<td></td>
<td>Induction of resistance <em>in vitro</em> in WB 1B</td>
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<td>Induced resistance not related to β-tubulin modifications</td>
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<tr>
<td>Patient isolates</td>
<td>Comparison of isolates with respect to growth</td>
<td></td>
<td>Isolates exhibit variations in their sensitivities prior to drug exposition</td>
<td>Argüello-García et al., 2004</td>
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<tr>
<td>Induction of resistance <em>in vitro</em> in WB 1</td>
<td>Representational difference analysis of gene expression</td>
<td></td>
<td>Induced resistance not related to β-tubulin modifications; antigenic variation</td>
<td>Argüello-García et al., 2009</td>
</tr>
<tr>
<td>Quinacrine</td>
<td>Induction of resistance <em>in vitro</em> in 7 different lines</td>
<td>Comparison to sensitive lines with respect to growth, investigation of quinacrine uptake by fluorescence</td>
<td>Quinacrine excluded from resistant lines, multidrug resistance in some lines</td>
<td>Upcroft et al., 1996</td>
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<td>Metronidazole</td>
<td>Patient isolates with different susceptibilities</td>
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<td>Patient isolates, induction of resistance formation <em>in vitro</em></td>
<td>PFOR assay</td>
<td>PFOR activity is lower in resistant isolates</td>
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</tr>
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<td>Argüello-García et al., 2009</td>
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<td>Induction <em>in vitro</em> T. vaginalis</td>
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<td>Nitazoxanide</td>
<td>Induction of resistance formation <em>in vitro</em> in WB C6</td>
<td>Comparison to wildtype with respect to expression of selected genes and by microarray</td>
<td>Cross-resistance to metronidazole. Complex changes of gene expression including antigenic variation and overexpression of chaperones in resistant clone</td>
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</tr>
<tr>
<td>Isoflavones</td>
<td>Induction of resistance formation <em>in vitro</em> in WB C6</td>
<td>Comparison to wildtype with respect to expression of selected genes and by microarray</td>
<td>Most pronounced changes of gene expression concern antigenic variation</td>
<td>Sterk et al., 2007 Unpublished data</td>
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</tbody>
</table>
inactivate proteins related to the thioredoxin reductase pathway. Resistant cells overcome this blocking by reregulating other enzymes involved in oxidoreductive processes, such as PFORs. In this model, downregulation of PFOR would be a consequence rather than a prerequisite of resistance formation (Leitsch et al., 2009). In another study, even a positive correlation between metronidazole-resistance formation and PFOR gene transcription activity has been observed (Argüello-Garcia et al., 2009). Furthermore, possible giardial metronidazole-resistance mechanisms involving reduced uptake of the drug (Boreham et al., 1988) or complex changes in the gene expression pattern including alterations of those genes (encoding variant surface proteins, VSPs) that mediate antigenic variation of the parasite (Müller et al., 2007; 2008; see also below) have been described.

In order to elucidate the biochemical nature of resistance formation against nitazoxanide metronidazole, and formononetin, G. lamblia WB C6 clones resistant to these drugs were generated (Müller et al., 2007a, 2008a; Sterck et al., 2007) and compared to the wildtype WB C6 with respect to their growth behavior and to the expression pattern of genes that are potentially involved in resistance formation. In all cases, resistance formation occurred after a small number of selection cycles. The pattern of up- and downregulated mRNAs was completely different in a nitazoxanide/metronidazole double resistant line, a line resistant for metronidazole only and a formononetin resistant line. The only annotated ORFs downregulated in all screens encoded the major surface-labeled trophozoite antigen 417 encoding the major surface antigen C6 (TSA417; Müller et al. 2007a, 2008a; Sterk et al., 2007 and unpublished data). In the nitazoxanide/metronidazole double resistant line, genes potentially involved in protein phosphorylation and network formations, especially ankyrins, and chaperonins (especially the cytosolic heat shock protein 70) are upregulated. The roles of these chaperonins in Giardia are not well understood. In human cells, molecular chaperones such as HSP72 and HSP27 prevent cells from apoptosis induced by heat shock (Gabai and Sherman, 2002). If similar mechanisms occur in Giardia, a general response to stress via induction of chaperonins could be responsible for thermotolerance as well as for drug resistance. Interestingly, a similar change in gene expression patterns has been found upon expression of neomycin phosphotransferase as a resistance marker and a subsequent selection for puromycin resistant trophozoites (Su et al., 2007).

As mentioned above, nitazoxanide inhibits protein disulfide isomerases (PDIs) from G. lamblia (Müller et al., 2007a) and N. caninum (Müller et al., 2008b). Inhibition of PDIs in vivo could lead to the formation of badly folded proteins and thus to an impairment of central metabolic and regulatory processes. Cells that overexpress chaperonins and proteins involved in the stabilisation of subcellular structures such as ankyrins could overcome the deleterious effects due to PDI inhibition.

Such indirect, stabilizing effects due to chaperonins or related proteins could also explain why artificially generated albendazole resistance (Upcroft et al., 1996; Argüello-Garcia et al., 2009) is not correlated to specific mutations of the β-tubulin gene as found in other albendazole resistant organisms. In this context, it is noteworthy that antigenic variation was also found to be a striking phenomenon associated with in vitro resistance formation against albendazole (Argüello-Garcia et al., 2009).

Giardia populations seem to have very flexible mechanisms in order to respond to changes in the environment such as drug pressure, presence of antibodies or even changes in medium composition and to changes of the internal milieu by introduction of transgenes (Su et al., 2007) in a way that complex gene expression patterns seem to be fine-tuned in order to find an optimal compromise between speed of growth and survival. How can these changes be explained? Individual trophozoites in cultures of G. lamblia (clone WB C6) exhibit substantial variations in their degree of susceptibility/resistance to different drugs even in absence of any previous drug pressure (Argüello-Garcia et al., 2004). This suggests that resistance formation within a G. lamblia in vitro culture seems to have its origin in a drug-independent process of continuous growth competition between relatively drug-susceptible and drug-resistant trophozoite subpopulations. The differences in these subpopulations may be explained by genetic effects, i.e. random point mutations all over the genome and selection of resistant phenotypes upon drug application or by epigenetic effects (transcriptional or posttranscriptional) resulting in subpopulations with different
gene expression patterns. In the latter case, exposure of the trophozoites to a drug in sublethal concentrations might even enhance conversion from a sensitive to a resistant phenotype.

The only common finding in all resistant lines is the change in variable surface protein gene expression (where investigated) resulting in antigenic variation (Müller and Gottstein, 1998; Svärd et al., 1998; Prucca and Lujan, 2009). Antigenic variation in *G. lamblia* allows the parasite to escape the host immune response (Müller and Gottstein, 1998; Müller, and von Allmen, 2005; Roxström-Lindquist et al., 2006). Epigenetic mechanisms at the transcriptional level involving histone acetylation (Kulakova et al., 2006) and at the post-transcriptional level involving RNA interference (Prucca et al., 2008) are considered as controlling antigenic variation (Prucca and Lujan, 2009). Thus, common epigenetic mechanisms may control both antigenic variation and drug resistance in *Giardia*.

The role of epigenetics in antigenic variation and drug resistance in *Giardia* and other parasites is – of course – far from being understood, but may be of a broader interest since evolution of antibiotic resistance initiated by increasing sublethal doses of various antibiotics has been attributed to epigenetic inheritance also in bacteria (Adam et al., 2008). The overview in Table 2 shows that most studies have been performed with the strain WB C6 showing a high degree of antigenic variation (Nash et al., 1990b). Drug resistance studies from other strains with little variation such as GS-M-83-H7 or WB A6 (Nash et al., 1990a) are lacking but would be most useful in putting the light on the relationship between antigenic variation and drug resistance formation.

## Conclusions

After more than 7 decades of anti-giardial chemotherapy, metronidazole is still the treatment of choice. Novel drugs such as nitazoxanide or plant-derived compounds do not offer obvious advantages with respect to efficacy or resistance formation. A couple of studies concerning resistance formation suggest a more complex mechanism than the mere mutation of a target protein. Where investigated, resistance formation is correlated with antigenic variation suggesting a common, underlying mechanism far from being understood. Future approaches aimed at transgenic up- or down-regulation of putative drug target proteins in *G. lamblia* are expected to provide conclusive insights into both the mode of action of anti-giardial drugs and the mechanisms that mediate resistance against these drugs.

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