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Oxidative Stress in Human Infectious Diseases – Present and Current Knowledge About Its Druggability

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1. Introduction

Infectious diseases caused by parasites are a major threat for entire mankind, especially in the tropics. These infections are not only restricted to humans, they are also predominant in animal health. Just a few years ago infectious diseases caused by parasites were classified as an issue of the past. Due to the elevating level of drug resistance of these pathogens against the current chemotherapeutics, the need for new drugs became even more important. In particular parasitic diseases such as malaria, leishmaniasis, trypanosomiasis, amoebiasis, trichomoniasis, soil-transmitted helminthiasis, filariasis and schistosomiasis are major health problems, especially in "developing" areas (Renslo and McKerrow, 2006; Pal and Bandyopadhyay, 2012). A variety of these parasitic diseases, which comprises the so called neglected diseases Chagas disease, leishmaniasis, sleeping sickness, schistosomiasis, lymphatic filariasis, onchocerciasis and of course malaria (Chatelain and Ioset, 2011), are transmitted by vectors and therefore attempts to combat transmission became prominent. In contrast to the treatment of bacterial infections with antibiotics there are no "general" antiparasitic drugs. The use of a specific drug is dependent on the parasitic organism and therefore has to be individually chosen (Khaw et al., 1995).

Reactive oxygen species (ROS) and oxidative stress are the inevitable consequences of aerobic metabolism, with partially reduced and highly reactive metabolites of O_2 being formed in the mitochondria (Andreyev et al., 2005) or as by-products of other cellular sources such as the cytoplasm, the endoplasmatic reticulum, the plasma membrane and peroxisomes. Furthermore, environmental agents such as ionizing and UV radiation or xenobiotic exposure can generate intracellular ROS. O_2 metabolites include superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2), formed by one- and two electron reductions of O_2 or the highly reactive hydroxyl radical ($\cdot OH$) which is formed in the presence of metal ions via Fenton

and/or Haber-Weiss reactions (Massimine et al., 2006). At physiologically low levels, ROS can function as second messenger in redox signaling, with H_2O_2 best providing the specificity in its interaction with effectors in signaling processes (Forman et al., 2010). Balancing the generation and elimination of ROS maintains the proper function of redox-sensitive signalling proteins. However, severe increases of ROS induce oxidative modifications in the cellular macromolecules DNA, proteins and lipids, this leading to a disruption of redox homeostasis and irreversible oxidative damage (Trachootham et al., 2008). Depending on the cellular context, the levels of ROS and the redox state of the cells, alterations of the delicate redox balance can promote cell proliferation and survival or induce cell death.

To maintain redox homeostasis and eliminate ROS, aerobes are equipped with enzymatic/ nonenzymatic antioxidants and metal sequestering proteins to either prevent or intercept the formation of pro-oxidants. Furthermore, protective mechanisms are put in place to repair and replace damaged macromolecules. Two central thiol/disulfide couples are involved in controlling the redox state of the cell: glutathione/glutathione disulfide (GSH/GSSG) is the major redox couple that determines the antioxidative capacity of cells, other redox couples include the active site dithiol/disulfide of thioredoxins (Trx_{red}/Trx_{ox}) interacting with a different subset of proteins and thus forming a distinct but complementary redox system (Jones and Go, 2010).

Enzymatic antioxidants can be categorized into primary or secondary antioxidants, the first reacting directly with pro-oxidants (e.g. catalase, superoxide dismutase), the latter are involved in the regeneration of low molecular weight antioxidant species (Halliwell, 1999). Here, the reduced state of GSSG and Trx-enzymes is restored by the glutathione reductase (GSR) and the Trx reductase using electrons obtained from NADPH. Additionally glutaredoxins (Glrx) utilize GSH for the reduction of intracellular disulfides (Fernandes and Holmgren, 2004). While Trx, Trx reductase and Trx peroxidase (peroxiredoxin, Prx) constitute the Trx-system, the versatile GSH-system includes enzymes required for GSH synthesis and recycling, for its use in metabolism, in defense against ROS-induced damage and in a multitude of detoxification processes. Furthermore, for normal GSH turnover and disposition of GSH-conjugated metabolites and xenobiotics, export from the cell is required that is carried out by GSH efflux transporters and pumps (Sies, 1999) (Fig. 1).

In spite of the diversity of parasites, all are faced with similar biological problems that are related to their lifestyle. Besides coping with ROS levels generated from intrinsic sources, all have to deal with the oxidative stress imposed by the host's immune response. Furthermore, parasites are faced with ROS that are produced during the epithelial innate immune response of their vector, by vector-resident gut bacteria (Cirimotich et al., 2011) or during melanotic encapsulation processes (Kumar et al., 2003).

Since the redox system plays such a fundamental and indispensable role for parasite survival within their host (Massimine et al., 2006), drugs that either promote ROS generation or inhibit cellular antioxidant systems will lead to redox imbalance by pushing ROS levels above a certain threshold level that will ultimately lead to parasite death (Müller et al., 2003). In general, drugs that target vital redox reactions or promote oxidative stress are

named redox-active antiparasitic drugs (Seeber et al., 2005) on which we will mainly focus within this chapter.

2. The role of the antioxidant system in *Leishmania*

Leishmaniasis is caused by the protozoan flagellate *Leishmania* which is transmitted by sand-flies of the genus *Phlebotomus* (Sharma and Singh, 2008). There are several species of the genus *Leishmania* which are known to cause this infectious disease. Leishmaniasis shows a broad spectrum of clinical manifestations and includes visceral, cutaneous and mucocutaneous leishmaniasis. Whereas the two latter ones are not considered to be lethal (Herwaldt, 1999), infection with *Leishmania donovani/infantum* – resulting in kala azar or visceral leishmaniasis - can be lethal without treatment. Although treatment of leishmaniasis with chemotherapeutics is the only current option, drug resistance to first-line drugs is increasing which is accompanied by frequently occurring toxic side effects and by the high cost of treatment (Van Assche et al., 2011). Additionally, the small number of novel drugs combined with the low number of identified and subsequently validated number of *Leishmania* drug targets in clinical use, reveals an alarming situation for the current status in chemotherapy. The predominant target for the application of chemotherapy is the amastigote stage which proliferates intracellularly in tissue macrophages (Dedet et al., 2009), thereby hindering the accessibility of antileishmanial drugs to the pathogen.

3. Antimonials

Despite the fact that antimonials were already identified in 1921, they still remain the first-line treatment, although the precise mode of action is not known. But it is generally accepted that pentavalent antimonials (Sb^V) represent a pro-drug which is converted to trivalent antimonials (Sb^{III}) for antileishmanial activity. Recently it has been indicated that thiols act as reducing agents in this conversion. Furthermore, the participation of a unique parasite-specific trimeric glutathione transferase TDR1 in the activation of antimonial prodrugs has been suggested (Fyfe et al., 2012).

Treatment with antimonials requires parenteral administration and is accompanied by toxic side effects such as cardiac arrhythmia and acute pancreatitis (Sundar and Rai, 2002). Some studies have been carried out to investigate the activity mechanism of antimonials which correlates with an interference with the antioxidant defence system of the parasite: Trivalent antimonials decrease the thiol-reducing capacity of *Leishmania* by inducing an efflux of trypanothione. In contrast to *Leishmania*, mammalian cells depend on GSH to control their intracellular thiol-redox status. Here, ROS and oxidized cell components are efficiently reduced by GSH, thereby generating GSSG. The glutathione disulfide form can then be regenerated by the GSR (Monostori et al., 2009). In contrast, the redox metabolism of *Leishmania* relies on a modified GSH-system, N1,N8-bis(l-γ-glutamyl-l-hemicystinylglycyl)

spermidine, also known as trypanothione (Fairlamb et al., 1985). The oxidised form, trypanothione disulfide, is generated when trypanothione reduces ROS and its reconversion is catalysed by the trypanothione reductase. Antimonials inhibit this enzyme, leading to an accumulation of trypanothione disulfide, which subsequently is not accessible for the reduction of ROS (Krauth-Siegel and Comini, 2008). The influence of antimonials on the parasite's redox biology has been verified on cellular level by the fact that trivalent antimonials-resistant parasites display an increased IC_{50}-value for nitric oxide donors such as $NaNO_2$, SNAP, and DETA NONOate compared to antimonial-sensitive strains (Souza et al., 2010; Holzmüller et al., 2005; Vanaerschot et al., 2010). Whether nitric oxide resistance is due to elevated trypanothione levels or due to another antioxidant mode of action is not yet clear.

4. Amphotericin B

Amphotericin B (Fig. 2), a polyene macrolide, has been employed in the treatment of *Leishmania* since 1960, but just as a second-line drug. This drug exhibits an excellent antileishmania activity with more than 90% cure rates. Because the pure compound creates severe side effects and requires long-term treatment and extensive monitoring, liposomal application of amphotericin B is used at the moment which results in cure rates of 3–5 days (up to 100%), is convenient for the patient and is less expensive (Gradoni et al., 2008; Manandhar et al., 2008; Sundar et al., 2002; Thakur et al., 1996). The mode of action can be explained based on its chemical structure, polyene macrolide has been shown to bind to ergosterol, one of the main sterols within *Leishmania* membranes. Interference with this molecule results in an increasing permeability of the cell membrane which leads to the parasite's death (Balana-Fouce et al., 1998; Amato et al., 2008). Additionally there is some evidence that amphotericin B has an effect on the oxidative response of macrophages (Mukherjee et al., 2010), however further experiments are required to verify this effect.

5. Miltefosine

Miltefosine (hexadecylphosphocholine) is the first and currently the only, orally administered antileishmanial drug (Fig. 2). However, despite cure rates of up to 98% (Roberts, 2006), the drug reveals serious side effects such as vomiting, diarrhea and can cause abnormal physiological development of the foetus. Furthermore, the drug has a relatively long half-life of about 150 hours (Seifert et al., 2007; Maltezou, 2010) which could lead to the development of rapid resistance. Related to its structure, the drug possibly interferes with membranes and membrane-linked enzymes. Currently no verified implications of the drug within the redox biology of the parasite have been found (Rakotomanga et al., 2004; Saint-Pierre-Chazalet et al., 2009).

6. Oxidative chemotherapeutic intervention of Trypanosoma infections

Trypanosoma infections, caused by the flagellate protozoan *Trypanosoma* are responsible for a high degree of health problems in endemic countries. They can be divided into two types of pathogens: *Trypanosoma cruzi*, the causative agent of Chagas disease, also known as American trypanosomiasis, since it occurs in Latin America and *Trypanosoma brucei ssp.*, the causative agent of sleeping sickness, or human African trypanosomiasis, since it is endemic to sub-Saharan Africa. The current medication is known for its toxicity, poor activity in immune-suppressed patients and long-term treatment combined with high costs. Moreover, vaccines are not foreseeable in the near future. The *T. cruzi* life cycle includes three fundamental forms characterized by the relative positions of the flagellum, kinetoplast and nucleus: Trypomastigotes, epimastigotes and amastigotes, the latter one characterized by their proliferation in any nucleated cell (Prata, 2001). On the one hand Chagas' disease is controlled through elimination of its vectors by using insecticides and on the other side by chemotherapy. Currently, the drugs used are nifurtimox (4[(5-nitrofurfurylidene)amino]-3-methylthiomorpholine-1,1-dioxide), derived from nitrofuran, and benznidazole (*N*-benzyl-2-nitroimidazole-1-acetamide), a nitroimidazole derivative. Nifurtimox and benznidazole (Fig. 2) are trypanocidal to all forms of the parasite (Rodriques Coura and de Castro, 2002). However, severe side effects and toxicity have been observed (Kirchhoff, 2000). In addition, there are also reports of mutagenesis resulting in DNA damage (Zahoor et al., 1987). An additional aspect that complicates treatment is the different susceptibility of different parasite strains to the applied chemotherapeutics (Filardi and Brener, 1987). The mode of action of nifurtimox and benznidazole (Fig. 2) is via the formation of free radicals and/or charged metabolites. The nitro group of both drugs is reduced to an amino group by the catalysis of nitro-reductases, leading to the formation of various free radical intermediates. Cytochrome P450-related nitro-reductases initiate this process by producing a nitro anion radical (Moreno et al., 1982). Subsequently, the radical reacts with oxygen, which regenerates the drug (Mason and Holtzman, 1975). For example, nifurtimox-derived free radicals may undergo redox cycling with O_2, thereby producing H_2O_2 in a reaction catalysed by the SOD (Temperton et al., 1998). Furthermore, in the presence of Fe^{3+} the highly reactive ·OH is also being formed according to the Haber-Weiss reaction. These free radicals can subsequently bind to cellular macromolecules such as lipids, proteins and DNA, resulting in severe damage of parasitic cells (Díaz de Toranzo et al., 1988). In contrast, the trypanocidal effect of benznidazole does not depend on ROS but it is likely that reduced metabolites of benznidazole are covalently binding to cellular macromolecules, thereby revealing their trypanocidal effect (Díaz de Toranzo et al., 1988; Maya et al., 2004). Additionally, it has been demonstrated that benznidazole inhibits the *T. cruzi* NADH-fumarate reductase (Turrens et al., 1996).

7. Approaches to increase oxidative stress within the malaria parasite

Malaria is a devastating and quite often a deadly parasitic disease, which causes important public health problems in the tropics. The population in more than 90 countries, with more than 2000 million citizens, is exposed to the infection. Malaria infection is responsible for an estimated 500 million clinical cases per annum, causing more than one million deaths; most of these are children in Africa. The malaria parasite *Plasmodium falciparum*, the causative agent of Malaria tropica, is proliferating within human red blood cells, thereby exploiting host's nutrient sources and hiding from the human immune response. A vaccine is not available and the control of the disease depends solely on the administration of a small number of drugs. Due to mutational modification of the genome of the malaria parasite, an ongoing rapid adaptation to environmental changes and drug resistance is occurring (Greenwood et al., 2008). At the moment – which is just a question of time - solely artemesinin is still effective against the malaria parasite. However, first reports already demonstrated drug resistance against artemisinin (Wangroongsarb et al., 2011). Therefore, continuous discovery and development of new drugs are urgently needed. A variety of the current anti-malaria drugs are targeting the redox balance of the parasite. As outlined above, redox systems are essential for the intracellular proliferation of the plasmodial pathogen.

In general, *P. falciparum* uses the two interacting systems, GSH- and TRX-system, to protect against reactive ROS (Kanzok et al., 2002; Kanzok et at., 2000; Kawazu et al., 2001; Kehr et al., 2011; Krnajski et al., 2001; Krnajski et al., 2002; Kumar et al., 2008; Liebau et al., 2002). Both systems can be link by the redox protein plasmoredoxin (Becker et al., 2003). Active interference by employing redox-active antiparasitic drugs, however, harms the parasite and results in its death. Compounds which disturb the redox balance can be categorised into three different groups: (i) molecules that are responsible for the *de novo* synthesis of ROS and thus lead to parasite death, (ii) molecules which inhibit the activities of redox balancing enzymes and (iii) molecules that interfere in the scavenging of pro-oxidant metabolic products like hemozoin.

8. Molecules which inhibit the activities of redox balancing enzymes

The GSH-system plays an important role in the maintenance of the redox status in *Plasmodium* (Kehr et al., 2011). It is involved in detoxifying free heme (ferriprotoporphyrin IX) (Atamna et al., 1995; Müller, 2003) and in the termination of radical-based chain reactions (Frey, 1997). Therefore, enzymatic reactions within this system are highly druggable. The GSR is one of the key enzymes of the GSH-system and consequently several compounds have been synthesized to successfully interfere with its catalysis *in vitro* and *in vivo* (Biot et al., 2004; Gallo et al., 2009; Grellier et al., 2010; Muller et al., 2011). Inhibitory compounds comprise for example isoalloxazines, quinacrines, tertiary amides that reveal antimalarial activity at low doses against the chloroquine sensitive *P. falciparum* strain 3D7 (Sarma et al., 2003; Friebolin et al., 2008; Chibale et al., 2001). Methylene blue (Fig. 2), a noncompetitive

inhibitor of the *P. falciparum* GR, shows antiplasmodial activity against all blood stage forms, whereas only a marginal cytotoxic effect against mammalian cells has been reported (Biot et al., 2004; Buchholz et al., 2008; Atamna et al., 1996; Akoachere et al., 2005; Badyopadhyay et al., 2004; Krauth-Siegel et al., 2005; Garavito et al., 2007).

The GST is one of the most abundant proteins expressed in *P. falciparum*. Additionally to its detoxifying role, it efficiently binds parasitotoxic heme not only in the presence of GSH, but also when GSSG is present, thereby protecting the parasite from hemin even under severe oxidative stress conditions. Here, a peculiar loop region, that is both crucial for the gluta‐thione-dependent tetramerization/inactivation process and for hemin-binding, represents an ideal drug target (Liebau et al., 2005; 2009). Recently chemical synthesis to design effective compounds to target GST has been performed which show some promising antiplasmodial activity (Ahmad et al., 2007; Sturm et al., 2009). Furthermore, the development of drugs that overcome resistance to available antimalarial drugs also are of great interest. The action of multidrug resistance protein (MRP)-like transporters is associated with the efflux of xenobi‐otics in both unaltered and GSH-conjugated form and it is conceivable that they are in‐volved in the development of drug resistance in malarial parasites (Koenderink et al., 2010). Since coordinated expression and synergistic interactions between GST and efflux pumps have been observed (Sau et al., 2010), a promising new intervening strategy might be the in‐hibition of GST and/or the development of GST-activated pro-drugs that overcome drug re‐sistance by blocking the drug binding sites of the transporters.

Another promising antimalarial drug target is the *P. falciparum* TrxR (Banerjee et al., 2009). Chalcone derivatives and Eosin B exhibit antiplasmodial activity by inhibiting the plasmo‐dial TrxR (Li et al., 1995; Massimine et al., 2006).

For many years it was thought that the malaria parasite had no need for an endogenous SOD and simply adopted the host's enzyme for its purpose. However, in 2002, an iron-de‐pendent SOD was described in *P. falciparum* (Boucher et al., 2006). Being quite distinct from the human tetrameric Mn and Cu/Zn SOD, it is exploited as anti-malaria drug target (Sou‐lere et al., 2003).

9. Drugs inhibiting hemozoin formation and thereby inducing oxidative stress

Besides the attacks of the immune systems of the respective host, where ROS are deployed to kill invading pathogens, the parasite faces another even bigger challenge: *Plasmodium* relies al‐so on the digestion of human haemoglobin to obtain amino acids for its metabolism (Sherman, 1977). Haemoglobin is the major protein inside the erythrocyte and the parasite has evolved a unique pathway to utilise this molecule (Muller et al., 2011). Heme is the degradation product of haemoglobin, which has to be detoxified and stored as hemozoin within the food vacuole of the parasite – the place where the haemoglobin degradation occurs. Non-detoxified heme is extremely toxic (Papalexis et al., 2001) and leads not only to the generation of H_2O_2, $^{.}OH$ and $O^{2-.}$ (Francis et al., 1997), but also to the highly reactive, non-radical molecule, singlet oxygen ($^1O_2$)

(Freinbicherler et al., 2011). Moreover, one $^1O_2$ molecule can be either synthesised by the reaction of $\cdot$OH and $O^{2-\cdot}$ or two $O^{2-\cdot}$ with two hydrogen ions (Khan and Kasha, 1994). In order to detoxify these ROS, *Plasmodium* has developed – as outlined above - multiple antioxidant defence systems. However - excluding the membrane located lipophilic tocopherol (vitamin E) (Wang and Quinn, 1999) - none of the above mentioned defense systems are capable to detoxify $^1O_2$. The fact that vitamin B6 is linked to the defense against $^1O_2$ in plants and fungi (Tambasco-Studart et al., 2005; Ehrenshaft et al., 1999), suggests that the vitamin B6 biosynthesis might also play a yet unrecognized role in combating $^1O_2$ in the malaria parasite *P. falciparum*. Very recently this role of $^1O_2$ detoxification has been verified in the malaria parasite (Wrenger et al., 2005; Knöckel et al., 2012; Butzloff et al., 2012).

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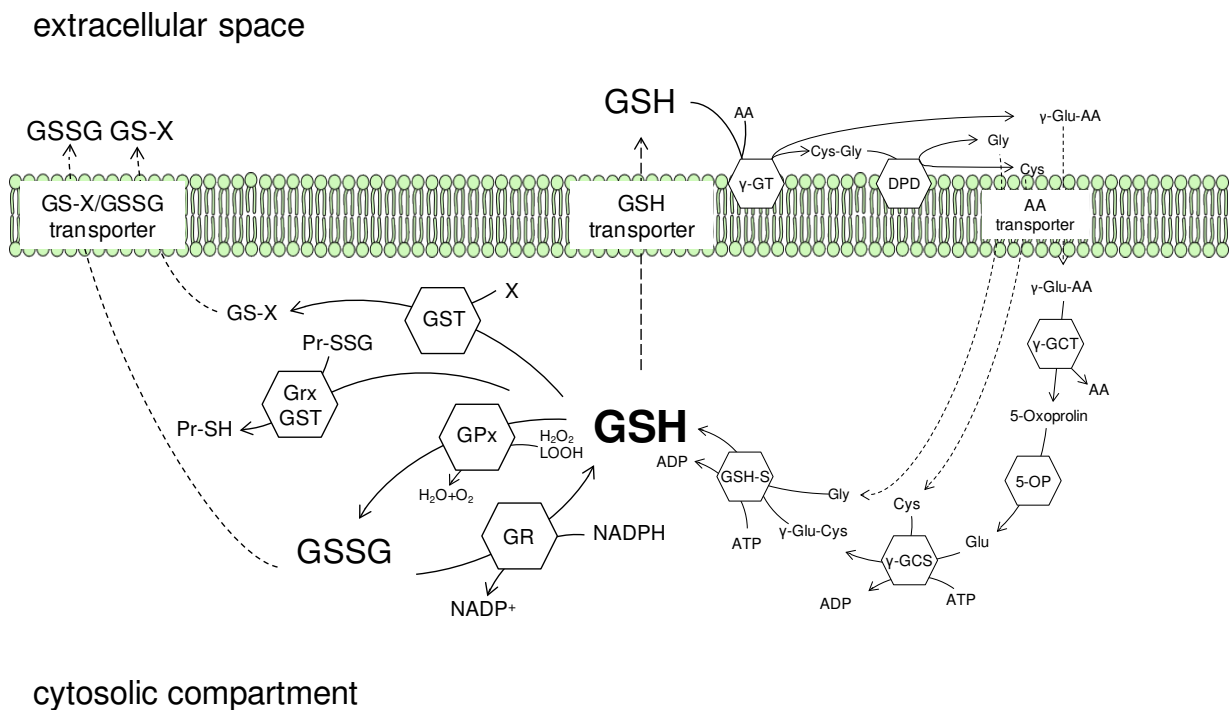
Figure 1. Schematic illustration of the glutathione (GSH) system. GSH homeostasis involves intra- and extracellular mechanisms. GSH is synthesized from amino acids (AA) by the action of γ-glutamylcysteine synthetase (γ-GCS) and glutathione synthase (GSH-S), both requiring ATP. As antioxidant, GSH participates in the reduction of peroxides, catalysed by glutathione peroxidase (GPx), in the reduction of protein-disulfides, catalysed by glutaredoxins (Grx) and in conjugation reactions with electrophils (eg. xenobiotics, X), catalysed by glutathione transferases (GSTs). The glutathione conjugates (GS-X) and GSSG are transported out of the cell via GS-X/GSSG pumps. The NADPH-dependent GSH reductase (GR) is responsible for the intracellular recycling of GSH, while extracellular GSH gets sequentially hydrolysed by γ-glutamyl transpeptidase (γ-GT) and dipeptidase (DPD), with glutamate, cysteine and glycine being recycled for GSH synthesis (γ-glutamyl cycle).

A number of drugs have been identified that act as inhibitors of the hemozoin formation by binding to heme. This leads to an accumulation of free heme, causes high levels of oxidative stress and ends in the death of the parasite (Meunier et al., 2010). Quinoline-containing derivatives such as amopyroquine, amodiaquine, tebuquine, halofantrine, pyronaridine, quinine, mepacrine, epiquinine, quinidine, bisquinoline chloroquine (see

figure 2) are highly potent antimalarials that inhibit hemozoin formation at EC_{50}-values in the low nano-molar range (Egan et al., 2000; Kotecka et al., 1997; O'Neill et al., 2003; Vennerstrom et al., 1992). Azole derivatives are also inhibitors of the hemozoin formation and reveal efficacy against chloroquine sensitive as well as resistant plasmodial strains (Banerjee et al., 2009; Rodrigues et al., 2011). Another novel class, which has been identified to interact with heme and thereby prevent the hemozoin formation, are xanthones (Docampo et al., 1990; Ignatushchenko et al., 1997; Xu Kelly et al., 2001). Moreover, a variety of isonitrile derivatives gain their antimalarial activity from inhibition of the hemozoin synthesis (Kumar et al., 2007; Wright et al., 2001) resulting in EC_{50}-values in the low nano-molar range (Badyopadhyay et al., 2001; Singh et al., 2002; Kumar et al., 2007). Benzylmenadione derivatives do not show any cytotoxicity against two human cell lines while they are effective against the chloroquine resistant *P. falciparum* strain Dd2 (Muller et al., 2011). The precise mode of action of benzylmenadione remains for elucidation, but it has been suggested that the molecule is initially oxidized to a naphthoquinone derivative within the food vacuole of the parasite which leads subsequently to the inhibition of the hemozoin formation (Davioud-Charvet et al., 2003).

10. Druggability of oxidative stress systems in helminths

Helminths are parasitic worms that encompass nematodes (roundworms), cestodes and trematodes (flatworms) and affect humans in all areas of the world, with more than one-third of humans harbouring these parasites that cause chronic, debilitating morbidity. Furthermore, co-endemicity and polyparasitism increase the burden of millions (Hotez et al., 2008). In the absence of vaccines, control relies on pharmacotherapy and pharmacoprophylaxis to easy symptoms and reduce transmission. Helminthosis are treated with a limited number of anthelmintics by chemotherapy of symptomatic individuals or, more general, by control programmes that rely on mass drug administration (MDA) and require annual or bi-annual treatment of at-risk populations over prolonged period of time (Prichard et al., 2012). A major problem, however, is the development of resistance or tolerance by the parasites to these common antiparasitic drugs (Vercruysse et al., 2011). It is therefore essential to understand the underlying mechanisms of drug resistance and find new drugs to circumvent it.

Praziquantel has been used for over 20 years to treat a variety of human trematode infections. Its precise mechanism of action has not been fully elucidated, however, there is experimental evidence that praziquantel acts by increasing the permeability of cell membranes towards calcium ions and/or by interfering with adenosine uptake (Jeziorski and Greenberg, 2006; Angelucci et al., 2007). Furthermore, it has been suggested that praziquantel reduces GSH concentrations, making the parasite more susceptible to the host immune response (Ribeiro et al., 1998). Interestingly, exposure to sub-lethal concentrations of praziquantel shows that schistosomes undergo a transcriptomic response similar to that observed during oxidative stress (Aragon et al., 2009).
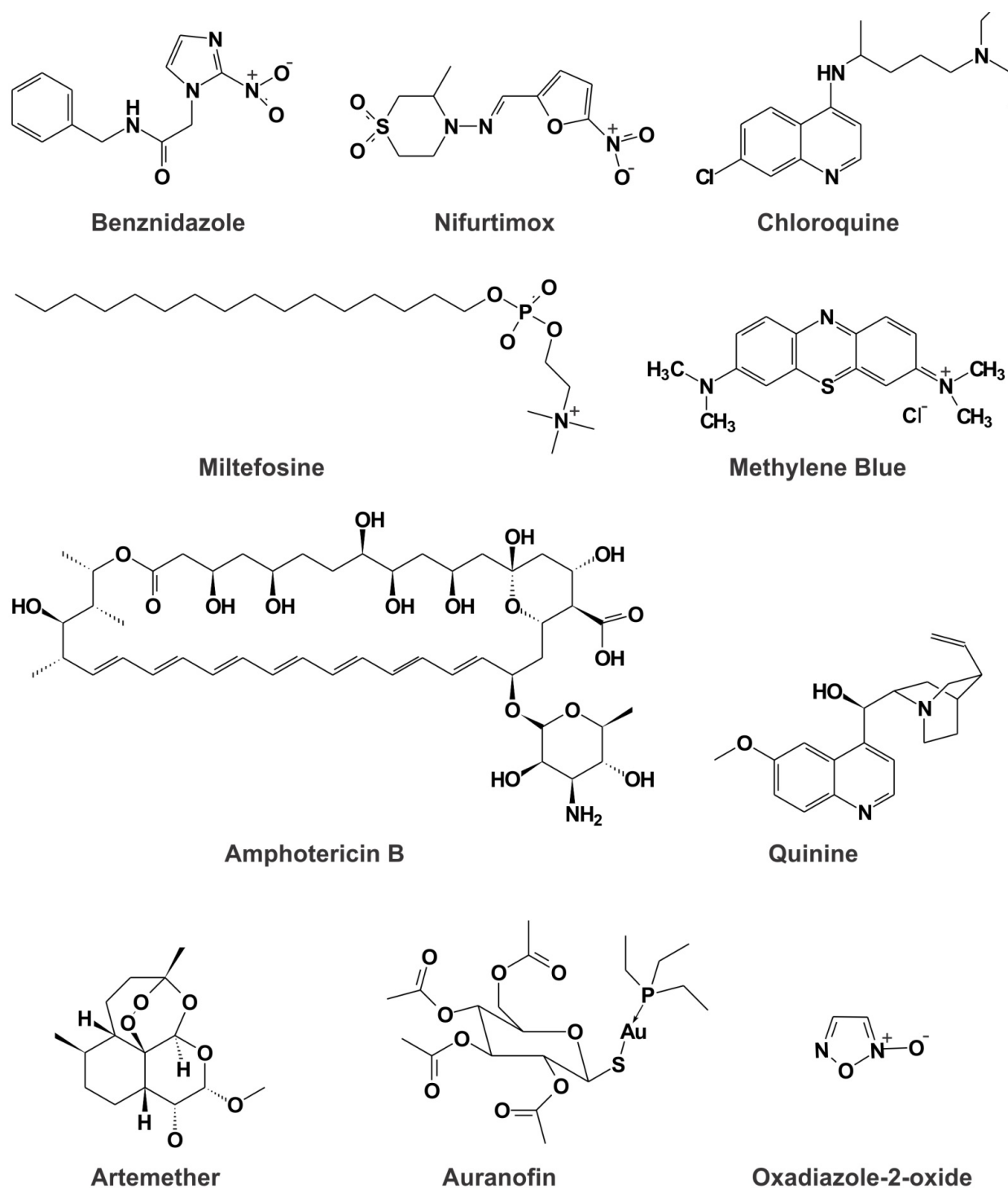
Figure 2. Molecular structures of chemotherapeutics which are used to treat infectious disease by generating directly or indirectly high levels reactive oxygen species.

Reliance on a single drug for mass treatment is risky. Therefore, anti-schistosomiasis drug development is on the way to identify new compounds with different modes of action. Recently it was demonstrated that artemisinin-based compounds (e.g. artemether, figure 2) are

active against immature stages of schistosomes. Although a number of potential drug targets have been proposed, the mode of action remains ambiguous (O'Neill et al., 2010). It is thought that the primary activator of the drug is an iron source. Therefore, interaction with heme in the worm gut has been suggested, leading to the formation of an unstable species that generates ROS and thus kills the worm (Utzinger et al., 2001). Since artemisinins are critically important for malaria chemotherapy, they are not available for MDA.

Schistosomes seem to be poorly adapted to cope with oxidative stress. This is surprising, since they have to deal with host-immune and self-generated ROS and, furthermore, with ROS generated during the consumption of host haemoglobin (Huang et al., 2012).The highly restricted antioxidant network has been widely accepted as an excellent drug target for schistosomes and other platyhelminths, since it is unique and differs significantly from the human host. Interestingly, the parasites have merged the Trx- and GSH-system using a hybrid enzyme, the thioredoxin-glutathione reductase (TGR) (Salinas et al., 2004, Huang et al., 2012). Using RNA interference, the TGR was found to be essential for parasite survival (Kuntz et al., 2007). TGR was indicated to be the main target of schistosomicidal drugs used in the past (antimonyl potassium tartrate and oltipraz) and of the anti-arthritic drug auranofin (Fig. 2), with a significant worm reduction observed in infected mice (Kuntz et al., 2007; Angelucci et al., 2009). A quantitative high-throughput screen identified highly potent lead compounds against the Schistosoma TGR (Simeonov et al., 2008), with low inhibitory constants being found with derivatives of phosphinic amides, isoxazolones and the oxadiazole-2-oxide chemotype (Furoxan) (Fig. 2) (Huang et al., 2012).

Preventive chemotherapy is the mainstay in the control of human soil-transmitted helminthiasis (STH). STH is primarily caused by the nematodes *Ancylostoma duodenale* and *Necator americanus* (hookworms), *Ascaris lumbricoides* (roundworm) and *Trichuris trichiura* (whipworm) that parasitize the human gastrointestinal tract. Four anthelminthics that exhibit a broad spectrum of activity are currently recommended by the World Health Organization: The benzimidazoles albendazole and mebendazole, the synthetic phenylimidazolthiazole levamisole and the pyrimidine derivative pyrantel pamoate. While benzimidazoles bind to free β-tubulin, leading to tubule capping and degradation (Beech et al., 2011), the cholinergic agonist levamisole activates ligand-gated acetylcholine receptors (Lewis et al., 1980) and the pyrimidine derivative pyrantel pamoate induces persistent activation of nicotinic acetylcholine receptors (Utzinger and Keiser, 2004). The GABA agonist piperazine, the nicotinic acetylcholine receptor agonist tribendimidine are further drugs used in STH. Currently neither drug class used to control or treat STH, has been implicated as influencing the redox biology of parasites. Instead, most of the currently used or proposed drugs (Olliaro et al., 2011) of gastro-intestinal nematodes affect ion channel function of the neuromuscular synapses. These neuroactive drugs cause paralysis of the worm and result in its rapid expulsion or killing.

Filarial parasites are classified according to the habitat of the adult worms in the vertebral host, with the cutaneous (*Loa loa* and *Onchocerca volvulus*) and lymphatic (*Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*) groups being the most clinically significant. Chemotherapeutic approaches to control parasite transmission and to treat onchocerciasis rely on the

macrocyclic lactone ivermectin, an effective and safe microfilaricide (Basáñez et al., 2008). Ivermectin is an agonist of ligand-gated Cl^- channels, with particular activity against glutamate-gated Cl^- channels of invertebrates (Martin et al., 1997). While ivermectin is less effective against adult worms, it causes reproductive quiescence and disappearance of microfilaria from skin or blood. Interestingly, cultured microfilariae are unaffected by ivermectin at concentrations found in treated patients (Bennett et al., 1993), making interference of the drug with protective mechanisms employed against the human immune response feasible (Geary et al., 2010). The development of ivermectin-resistant strains of *Caenorhabditis elegans* has shown that resistance to low levels of ivermectin is associated with an increased expression of drug efflux pumps and an increase in GSH-synthesis and -conjugation is observed. Since the overall levels of glutathione decrease, increased drug conjugation and removal from the cells is suggested (James and Davey, 2009). In a recent study, ivermectin has been identified as a cytotoxic agent to leukemia cells and a previously unknown indirect influence of ivermectin on the intracellular redox balance was demonstrated. Mechanistically, ivermectin induced chloride influx, membrane hyperpolarization, and generated ROS, the latter being functionally important for ivermectin-induced cell death (Shrameen et al., 2010).

Diethylcarbamazine (DEC) is still the mainstay for the treatment of lymphatic filariasis and first choice of therapy of loiasis. Surprisingly, its molecular mechanism of action is still not completely understood. Since pharmacologically relevant concentrations of DEC do not have an effect on microfilariae in culture, its mode of action must involve both the worm and its host. A possible involvement of host arachidonate- and NO-dependent pathways was observed (McGarry et al., 2005). Currently no verification of an influence on the redox biology of helminths is available.

It has been postulated that antioxidant enzymes, that defend against host-generated ROS, are of particular importance for long-lived tissue-dwelling parasites that are involved in chronic infections. Here, surface or secreted antioxidant enzymes are of great importance since they can directly neutralize ROS that pose real danger, thereby protecting surface membranes against peroxides. Secreted filarial antioxidant enzymes include SOD, GPx and Prx (Henkle-Dührsen and Kampkötter, 2001). Additionally to their antioxidant role, the Prx have recently been shown to contribute to the development of Th2-responses by altering the function of macrophages (Donnelly et al., 2008). Interestingly, GSH-dependent proteins have been observed that are capable of modifying the local environment via modulation of the immune response. Here the secretory GSTs from *O. volvulus* combine several features that make them excellent drug target: they are accessible since they are located directly at the parasite–host interface, they detoxify and/or transport various electrophilic compounds and secondary products of lipid peroxidation and they are involved in the synthesis of potential immunmodulators. Significant structural differences to the host homologues are observed in the xenobiotic binding site; this may support the structure-based design of specific inhibitors (Sommer et al., 2003; Perbandt et al., 2008; Liebau et al., 2008).

As outlined above, GSH-dependent detoxification pathways defend against current drugs and also play a role in mediating resistance to anthelmintics. The antioxidant pathways also provide the parasite with a means to protect against ROS-attack by its host and/or vector. In

the model nematode *C. elegans*, GSH-synthesis and a large variety of primary and secondary antioxidant enzymes and GSH-dependent detoxification enzymes are tightly regulated by the sole NF-E2-related (Nrf) transcription factor SKN-1 (An and Blackwell, 2003). Inhibiton of SKN-1 would thus target the expression of a multitude of enzymatic antioxidants and detoxification enzymes rather than affecting only one single protein or protein class, resulting in the downregulation of xenobiotic detoxification and in an enormous increase of oxidative stress. Since SKN-1 is also essential for embryonic development, this would be an additional bonus. Nematode-specific structural differences are observed that make SKN-1 an excellent candidate for the development of specific nematocidal drugs (Choe et al., 2012).

11. Conclusion

The current bottle-neck for the treatment of parasitic diseases with chemotherapeutics is the increasing drug resistance which forces the continuous discovery and development of new antiparasitic drugs. There is an urgent need for novel chemotherapeutic targets. New drugs should be generated to specifically target the parasite with minimal (or no) toxicity to the human host. Therefore, good drug targets should be distinctly different from processes in the host, or ideally be absent in the latter. Targeting the peculiarities - which are absent in the host - is proposed as such a strategy. In this sense, the parasite-specific biosyntheses represent ideal drug targets; similar to the already exploited antifolate interference with the parasite's dihydrofolate (vitamin B9) biosynthesis. There are a variety of reports about reactive compounds that have antiparasitic activity; however, not all of these are therapeutically viable drug-like molecules due to various limitations such as toxicity, low bioavailability, rapid inactivation under *in vivo* conditions and development of resistance. Recently studies on drug synergism raised special attention, which can open new avenues to improve the efficacy of antiparasitic drugs in combination with others. Since parasites such as *Plasmodium*, *Trypanosoma* or helminths are highly susceptible to oxidative stress - as outlined within this chapter - the identification of new lead compounds that target the parasite's redox systems by inducing oxidative stress, will be an efficient approach to discover novel drugs.

In this chapter we have tried to give an outline of the present situation of redox-active antiparasitic molecules that target human infectious diseases. In future the mechanisms, evolutionarily developed by the parasite to circumvent the crucial presence of ROS, will open new avenues for the development of novel antiparasitic drugs that combat resistant human pathogens effectively.

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