Evaluation of *Giardia lamblia* thiorexin reductase as drug activating enzyme and as drug target

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**ABSTRACT**

The antioxidative enzyme thioredoxin reductase (TrxR) has been suggested to be a drug target in several pathogens, including the protist parasite *Giardia lamblia*. TrxR is also believed to catalyse the reduction of nitro drugs, e.g. metronidazole and furazolidone, a reaction required to render these compounds toxic to *G. lamblia* and other microaerophiles/anaerobes. It was the objective of this study to assess the potential of TrxR as a drug target in *G. lamblia* and to find direct evidence for the role of this enzyme in the activation of metronidazole and other nitro drugs.

TrxR was overexpressed approximately 10-fold in *G. lamblia* WB C6 cells by placing the trxR gene behind the arginine deiminase (ADI) promoter on a plasmid. Likewise, a mutant TrxR with a defective disulphide reductase catalytic site was strongly expressed in another *G. lamblia* WB C6 cell line. Susceptibilities to five antigiardial drugs, i.e. metronidazole, furazolidone, nitazoxanide, albendazole and auranofin were determined in both transfectant cell lines and compared to wildtype. Further, the impact of all five drugs on TrxR activity in vivo was measured.

Overexpression of TrxR rendered *G. lamblia* WB C6 more susceptible to metronidazole and furazolidone but not to nitazoxanide, albendazole, and auranofin. Of all five drugs tested, only auranofin had an appreciably negative effect on TrxR activity in vivo, albeit to a much smaller extent than expected. Overexpression of TrxR and mutant TrxR had hardly any impact on growth of *G. lamblia* WB C6, although the enzyme also exerts a strong NADPH oxidase activity which is a source of oxidative stress.

Our results constitute first direct evidence for the notion that TrxR is an activator of metronidazole and furazolidone but rather question that it is a relevant drug target of presently used antigiardial drugs.

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1. Introduction

*Giardia lamblia* (syn duodenalis, intestinalis) is a microaerophilic protist parasite that occurs in all parts of the world and infects hundreds of millions of people every year (Centers for Disease Control and Prevention, CDC). It colonizes the small intestine and causes gastrointestinal symptoms like nausea, diarrhea, bloating and malabsorption of nutrients. Although not life-threatening in most cases, *Giardia* infections can be persistent and cause growth retardation in children (Buret, 2008). Treatment mainly relies on 5-nitroimidazoles, such as metronidazole and tinidazole, or albendazole, a benzimidazole drug (Leitsch, 2015). 5-nitroimidazoles have been in use against practically all microaerophilic or anaerobic pathogens for more than 50 years due to the comparably low rate of resistance (Leitsch, 2015). However, metronidazole-resistant microaerophiles and anaerobes, including isolates of *G. lamblia*, do occur. Due to the importance of 5-nitroimidazoles, especially metronidazole which is listed among the “essential medicines” by the WHO (World Health Organisation, 2015), a large number of studies on 5-nitroimidazole action and resistance have been conducted throughout the last 30 years.

5-Nitroimidazoles are essentially prodrugs and not very reactive. Reduction at the nitro group, however, activates nitroimidazoles which react with numerous cell constituents - in *G. lamblia* e.g. DNA (Uzlikova and Nohynkova, 2014), proteins (Leitsch et al., 2012), and thiols (Leitsch et al., 2012). Due to the extremely low reduction potential of 5-nitroimidazoles reduction at the nitro group occurs quantitatively only in microaerophilic and anaerobic...
organisms (Müller, 1983). In the protist parasites *G. lamblia*, *Entamoeba histolytica* and *Trichomonas vaginalis*, several enzymatic pathways were identified that are likely to play a role in 5-nitroimidazole reduction, including the central metabolic enzyme pyruvate/ferredoxin oxidoreductase (PFOR) together with ferredoxin (Townson et al., 1996; Rasoloson et al., 2002; Leitsch et al., 2011) and thioredoxin reductase (TrxR) (Leitsch et al., 2007, 2009, 2011), a central redox regulator. Further, another 5-nitroimidazole reducing enzyme, nitroreductase 1 (NR1), was identified in *G. lamblia* (Müller et al., 2007). A correlation between expression levels of nitroreductase 1 and PFOR/ferredoxin and metronidazole sensitivity in *G. lamblia* is well documented, as PFOR and nitroreductase 1 are less strongly expressed in many metronidazole-resistant cell lines (Müller et al., 2008; Leitsch et al., 2011). Moreover, overexpression of NR1 from a plasmid renders *G. lamblia* more sensitive to metronidazole (Nillius et al., 2011). Direct evidence for a role of TrxR in 5-nitroimidazole reduction in vivo, however, has been missing so far.

Importantly, TrxR was not only found to reduce 5-nitroimidazoles but also to be targeted by reduced nitroimidazole intermediates (Leitsch et al., 2007, 2009, 2011), resulting, at least in *E. histolytica* (Leitsch et al., 2007) and *T. vaginalis* (Leitsch et al., 2009), in a diminished thioredoxin reducing activity of the enzyme (Leitsch et al., 2007, 2009). Thus, TrxR has an intriguing double role as an activator and target of 5-nitroimidazoles. It was hypothesised that inhibition of TrxR could be one of the major toxic effects brought about by 5-nitroimidazoles (Leitsch et al., 2007, 2009). The TrxR/thioredoxin (Trx) redox system has multiple effects brought about by 5-nitroimidazoles (Leitsch et al., 2007, 2009; 2011), resulting, at least in *T. vaginalis* and *G. lamblia*, in a diminished thioredoxin reducing activity of the enzyme (Leitsch et al., 2007, 2009). Overexpression of NR1 from a plasmid renders *G. lamblia* more sensitive to metronidazole (Nillius et al., 2011). Direct evidence for a role of TrxR in 5-nitroimidazole reduction in vivo, however, has been missing so far.

2.2. Cell culture

*G. lamblia* WB C6 (ATCC 50803) trophozoites were axenically cultivated in Keister’s modified Diamond’s medium. Media were sterile-filtered. Subcultures were performed every third day.

2.3. Construction of a TrxR overexpressing transfectant

The TrxR gene (GL50803_9827; XM_001707116) was amplified from genomic DNA isolated from WB C6 (ATCC 50803) with primers bearing 50 bp of the upstream region and 50 bp of the downstream region, respectively, of the arginine deiminase gene (GL50803_112103; XM_001705703), and Pacl and XbaI restriction sites for cloning into the pPac-Vinteg vector (Stefaníč et al., 2009). The primer sequences were as follows: (forward) CTCTA-GAAACCTCTACACGTAGGT TGAATACCTCCGGAGAACAAAATCTCTA GTACATGTCTGCTCAAGCATTCGA, (reverse) CATTAATTAAC TGGAAGACGTCTACACGTGAGGTGTGTAAACTTCCGGAGAAAAAAATCCTAGT ATGAAAGTGGATATTTGGATCCATTCTACATGGTTTGTAGATGTGATGA. Transfections of the new plasmid pTrxR into WB C6 trophozoites were performed in a BTX Electro Cell manipulator 600 (Harvard Apparatus) with the settings 500 V, 800 μF, and 720 Ω. Transfectant WB C6 cells were selected via the plasmid-encoded puromycin N-acetyl-transferase (pac) gene by adding puromycin to the growth medium (100 μg/l). The plasmid constructs are schematically depicted in Supplementary Fig. 1.

2.4. Construction of an episomal mutagenized TrxR gene

The second cysteine of the active site of TrxR on pTrxR was mutated to serine using the QuickChange II XL Site-Directed Mutagenesis kit (Agilent) according to the manufacturer’s instructions. The mutagenesis primers introduced one single nucleotide exchange in order to alter a cysteine codon (TGG) to a serine codon (AGC). The sequences of the primers were as follows: (forward) GTGCCCGTCGCTCTGACGGATTCTGC, (reverse) GCAACTATGCTGACGGCGAC. The resulting plasmid pTrxR-mut was transfected into WB C6 as described above.

2.5. Two-dimensional gel electrophoresis of *G. lamblia* protein extracts (2DE)

Two-dimensional gel electrophoresis (2DE) with *G. lamblia* cell extracts was performed as described previously (Leitsch et al., 2011, 2012). Gels were stained with Coomassie Blue R-250 and evaluated using Melanie™ 4 software (Genebio).

2.6. mRNA quantification of expression by real-time RT–PCR

For quantification of TrxR mRNA expression, cells were harvested as described above and RNA was extracted using a Qiagen RNeasy Kit (Qiagen, Hilden, Germany). Synthesis of first-strand cDNA was performed using a Qiagen OmniscriptRT Kit (Qiagen). The primers used for the amplification of a 189 bp TrxR gene fragment: (forward) CTTGGCCTGGCTGTACGAGTCC, (reverse) GGCGATCGTGGCCCAAGC. TrxR mRNA levels were calculated using actin mRNA as internal standard (primers: ACTquantF, ACATAGCGCTGCAAGATG; ACTquantR TGCGGAGAGCATGCAAC). Quantitative RT–PCR was performed on a LightCyclerTM Instrument (Roche Diagnostics, Rotkreuz, Switzerland) as described previously (Nillius et al., 2011). Expression levels of TrxR mRNA were calculated as arbitrary units in relation to the quantity of actin mRNA. PCRs were performed in triplicate in three independent experiments.
2.7. Drug susceptibility and growth assays

Culture medium (10 ml) was inoculated with 10,000 trophozoites/ml and drugs were added in appropriate amounts. After incubation (37 °C, 48 h), cell numbers were determined in a Bürker-Türk chamber and IC50 values were calculated using Grafit 7 software (Erithacus software). The generation times of the cell lines containing 1 mM EDTA, 0.5 mM NADPH and 50 μM furazolidone or nitazoxanide were added to Tris pH 7.5 buffer.

2.8. Nitroreductase assay

5 μg/mL recombinant G. lamblia TrxR and 100 μM of either furazolidone or nitazoxanide were added to Tris pH 7.5 buffer, containing 1 mM EDTA, 0.5 mM NADPH and 50 μM cytochrome c (Leitsch et al., 2011). Reduction of cytochrome c (Sigma, St. Louis, Mo, USA) was measured in a Lambda 25 UV/Vis spectrometer (Perkin Elmer) at λ = 550 (Δε550 = 20 mM−1 cm−1). Recombinant G. lamblia TrxR was expressed and purified as described (Leitsch et al., 2011).

2.9. Disulphide reductase and NADPH oxidase assays with G. lamblia cell extracts

Disulphide reductase and NADPH oxidase activities of TrxR were measured photometrically using cell extracts of the respective G. lamblia cell lines. Cells were harvested and lysed by resuspension of the pellet in 10 times the volume of ultrapure water. Cell debris was removed (20,000 × g for 10 min) and protein concentrations of the supernatants determined by Bradford assay. 50 μg protein/mL were used in all measurements. Disulphide reductase activity in G. lamblia extracts was measured at 37 °C by quantifying reduction of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) to TNB (2-nitro-5-thiobenzoate) at λ = 412 (Δε412 = 13.6 mM−1 cm−1) in 100 mM Tris pH 7.5 buffer containing 0.5 mM NADPH, 1 mM EDTA and 1 mM DTNB (Merck). Net TrxR activity without background reduction of DTNB by thiols and proteins was calculated by adding 10 μM of the non-competitive flavin inhibitor diphenyleneiodonium (DPI). NADPH oxidase activity was measured in 100 mM Tris pH 7.5 buffer containing 0.2 mM NADPH and 1 mM EDTA by determining oxidation of NADPH at λ = 340 (Δε340 = 6.2 mM−1 cm−1). Again, 10 μM DPI were added in order to determine the net NADPH oxidase activity of TrxR.

2.10. Western blotting

Polyclonal antibodies were raised in rabbit against G. lamblia TrxR (GenicBio Biotech, Shanghai, China). Sera were diluted 1:1000 for the detection of TrxR on western blots of G. lamblia cell extracts using anti-rabbit IgG (Sigma-Aldrich; 1:5000). Western blotting was performed according to standard protocols.

3. Results

3.1. Generation of TrxR overexpressing transfectant WB C6 lines

In order to assess the role of TrxR in the reduction of metronidazole and other drugs containing a nitro group, an expression construct based on pPAV-VInteg (Stefanic et al., 2009) was devised for the manipulation of TrxR expression levels in G. lamblia. Preliminary experiments harnessing 2DE and RT-qPCR had shown that episomal expression of the TrxR gene under its own promotor did not result in recognisable higher copy numbers of TrxR protein and mRNA, respectively (data not shown). The position of TrxR in 2DE gels had been identified earlier (Leitsch et al., 2011) and was hence known to us at the start of the study. Several promoters of very highly expressed proteins were tested as replacement of the trxR promotor on the plasmid, including 50 bp of the upstream and downstream regions, respectively, of the arginine deiminase (ADI) gene, one of the most strongly expressed proteins in G. lamblia as recognisable on 2DE gels (Supplementary Fig. 2). The resulting vector was named pTrxR and transfected into G. lamblia WB C6 trophozoites. Transfectants bearing pTrxR (WB TrxR) had an approximately 10-fold higher expression level of TrxR (Fig. 2) than wildtype WB C6, rendering TrxR one of the most prominent proteins in 2DE gels. The elevated expression level of TrxR in WB pTrxR was further confirmed in a western blot assay using an anti-TrxR antibody (Supplementary Fig. 3). The increase in protein levels was mirrored by strong increases of TrxR mRNA levels (Table 1). In order to check whether TrxR was functional in WB TrxR, disulphide

![Fig. 1. The five antigiardial drugs tested in this study. 1, metronidazole; 2, furazolidone; 3, nitazoxanide; 4, albendazole, and 5, auranofin.](image-url)
TrxR did hardly, if at all, affect growth of the transfected cell lines in reductase and NADPH oxidase activities were completely inhibited, when 10 \( \mu \text{M} \) of flavin inhibitor diphénylione (DPI) were added to reactions, both disulphide activity in wildtype cells was low and definitely lower than that found in WB TrxR-mut. Surprisingly, the effect of TrxR overexpression was not due to much higher concentrations of the target molecule, alcohol dehydrogenase, the benzimidazole albendazole, and aurano. The first three drugs have nitro groups which need to be reduced for activity (Müller et al., 2007; Leitsch, 2015) and are thus potential substrates for TrxR. Albendazole, however, lacks a nitro group and does not require reduction for toxicity. Also Aurano, a novel antigiardial drug known to semicompetitively inhibit TrxR in vitro (Tejman-Yarden et al., 2013), lacks a nitro group. Thus, prior to the susceptibility assays we hypothesised that WB TrxR would be more susceptible to the three nitro drugs than wildtype or WB TrxR-mut. Further, no effect of altered TrxR levels on motility or shape could be observed under the light microscope. WB TrxR-mut was used as a control in the ensuing drug susceptibility assays in order to demonstrate that increased nitroreductase activity of the enzyme and not overexpression of the enzyme by itself is responsible for altered drug susceptibilities.

### Table 1

<table>
<thead>
<tr>
<th>TrxR expression</th>
<th>Relative expression level (protein)</th>
<th>Relative expression level (mRNA)</th>
<th>Disulphide reductase activity in cell extract (nmol min(^{-1}) mg(^{-1}))</th>
<th>NADPH oxidase activity in cell extract (nmol min(^{-1}) mg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB C6 (wildtype)</td>
<td>(\times 1)</td>
<td>(\times 1)</td>
<td>10.2 ± 1.5</td>
<td>27 ± 7</td>
</tr>
<tr>
<td>WB TrxR</td>
<td>(\times 10)</td>
<td>(&gt;25)</td>
<td>109 ± 14</td>
<td>213 ± 17</td>
</tr>
<tr>
<td>WB TrxR-mut</td>
<td>(\times 6.5)</td>
<td>(&gt;11)</td>
<td>12.1 ± 2.3</td>
<td>279 ± 27</td>
</tr>
</tbody>
</table>

reductase and NADPH oxidase activities of TrxR were measured in cell extracts. Indeed, WB TrxR displayed approximately tenfold disulphide reductase and NADPH oxidase activities as compared to wildtype (Table 1). It is important to note that disulphide reductase activity in wildtype cells was low and difficult to measure because background reduction of DTNB cell constituents of the added extract was several times higher. Another expression construct was designed that placed a mutated TrxR gene behind the ADI promoter. To this end the second cysteine of the enzyme’s active site was mutated to serine which nullifies disulphide reductase activity because thioredoxin reductases require two cysteines in their active site to reduce disulfide bonds in the substrate (Schlosser et al., 2013). Mutant TrxR could be overexpressed from pTrxR-mut in WB C6 trophozoites (WB TrxR-mut) to levels comparable to those in WB TrxR but disulphide reductase activity was practically identical to wildtype (Fig. 2, Table 1). NADPH oxidase activity, however, was even slightly higher (\(P < 0.05\)) in WB TrxR-mut than in WB TrxR (Table 1). When 10 \(\mu\)M of the flavin inhibitor diphénylione (DPI) were added to reactions, both disulphide reductase and NADPH oxidase activities were completely inhibited, showing that NADPH oxidase activity is independent of the catalytic cysteines but requires the cofactors NADPH and FAD. Importantly, the very high expression levels of native TrxR and mutant TrxR did hardly, if at all, affect growth of the transfected cell lines in *G. lamblia* growth medium although the at least tenfold NADPH activity would be expected to cause considerable oxidative stress through generation of superoxide radicals. Still, wildtype WB C6 displayed practically the same generation time (410 ± 10 min) as WB C6 pTrxR (435 ± 15 min) and WB C6 pTrxR-mut (430 ± 10 min). Further, no effect of altered TrxR levels on motility or shape could be observed under the light microscope. WB TrxR-mut was used as a control in the ensuing drug susceptibility assays in order to demonstrate that increased nitroreductase activity of the enzyme and not overexpression of the enzyme by itself is responsible for altered drug susceptibilities.

3.2. Evaluation of *G. lamblia* TrxR as an activator of and target of antigiardial drugs

Five antigiardial drugs (Fig. 1) were selected for susceptibility testing in the transfected cell lines: the 5-nitroimidazole metronidazole, the 5-nitrofuram furazolidone, the nitrothiazolide nitazoxanide, the benzimidazole albendazole, and aurano. The first three drugs have nitro groups which need to be reduced for activity (Müller et al., 2007; Leitsch, 2015) and are thus potential substrates for TrxR. Albendazole, however, lacks a nitro group and does not require reduction for toxicity. Also Aurano, a novel antigiardial drug known to semicompetitively inhibit TrxR in vitro (Tejman-Yarden et al., 2013), lacks a nitro group. Thus, prior to the susceptibility assays we hypothesised that WB TrxR would be more susceptible to the three nitro drugs than wildtype or WB TrxR-mut. Indeed, overexpression of TrxR had no influence on the susceptibility to albendazole (Table 2). In contrast to our prediction, however, overexpression of TrxR did not confer more tolerance to aurano but had no effect at all (Table 2). In accordance with TrxR’s capacity to function as a nitroreductase, however, overexpression of functional TrxR rendered WB TrxR more sensitive to metronidazole and furazolidone (Table 2) than wildtype or WB TrxR-mut. Surprisingly, the effect of TrxR overexpression was not

![Fig. 2. Sections of 2D-gels from cell extracts of *G. lamblia* WB C6 without plasmid (wildtype), with a plasmid harbouring the trxR gene behind the ADI promoter (WB TrxR), and with a plasmid harbouring a mutated trxR gene behind the ADI promoter (WB TrxR-mut). TrxR is encircled. The degree of overexpression of TrxR (functional or mutated) in relation to wildtype is indicated beneath the gel sections. The gel shown is representative for three biological replicates in total.](image)
significant in case of nitazoxanide although this drug has a nitro group which was shown to be essential for toxicity (Müller et al., 2007). In order to check whether nitazoxanide is a potential substrate for G. lamblia TrxR, we used purified recombinant G. lamblia TrxR for measuring reduction of nitazoxanide along the lines described for metronidazole and other 5-nitroimidazoles in an earlier study (Leitsch et al., 2011). Indeed, no nitroreductase activity of recombinant TrxR activity could be observed with nitazoxanide as substrate (Table 3). In contrast, furazolidone, was strongly reduced by recombinant G. lamblia TrxR (Table 3). This suggests that the elevated expression levels of TrxR in WB TrxR are responsible for a higher susceptibility to metronidazole and furazolidone, whereas the susceptibility to nitazoxanide remains unchanged because it is not a substrate of TrxR.

As a second step we delineated the role of TrxR as a drug target and assessed the effect of the five drugs on disulphide reductase activity of TrxR in WB TrxR (Fig. 3). Inhibition of TrxR by metronidazole and furazolidone was not significant, whereas nitazoxanide and albendazole did not inhibit TrxR at all (Fig. 3). Auranofin was the only drug that substantially reduced disulphide reductase activity of TrxR in WB TrxR (Fig. 3). When applied at 20 μM auranofin reduced TrxR activity to 60% as compared to control but even at a concentration of 50 μM, which is about the 300-fold dose of the IC50 as determined with recombinant G. lamblia TrxR in vitro (Tejman-Yarden et al., 2013), residual activity of TrxR in WB TrxR did not drop below 40% as compared to untreated WB TrxR. This indicates that the dose-response curve is rather flat and that even much higher concentrations of auranofin would not reduce TrxR activity in WB TrxR below the rate as observed in wildtype (Fig. 3). Indeed, TrxR activity in WB TrxR treated with 50 μM auranofin, was still 4–5-fold higher than in untreated wildtype (Fig. 3), at least after 2 h of incubation. Our results demonstrate that TrxR is a target of auranofin in the living parasite. It is not likely, however, that TrxR is a critical target of auranofin in G. lamblia since the susceptibility of WB TrxR to auranofin is equal to that of wildtype (Table 2). In fact, the results of this experiment suggest that G. lamblia TrxR is not a relevant target of any drug presently used for the treatment of giardiasis.

4. Discussion

We devised a plasmid-based expression construct that coupled the trxR gene to the ADI promoter, resulting in approximately tenfold higher intracellular concentrations of the enzyme as compared to wildtype. Overexpression of TrxR rendered G. lamblia more susceptible to metronidazole and furazolidone (Table 2), two drugs which are reduced by recombinant TrxR in vitro (Leitsch et al., 2011) (Table 3). Although indirect evidence for the reduction of metronidazole in vivo by TrxR had already been presented for E. histolytica (Leitsch et al., 2007), T. vaginalis (Leitsch et al., 2009), and G. lamblia (Leitsch et al., 2007), this study constitutes first direct evidence that TrxR is an activator of nitro drugs in the living parasite. It can be ruled out that the higher susceptibility of WB TrxR to metronidazole and furazolidone was due to a decreased fitness caused by overexpression of TrxR as such because the susceptibilities to the other drugs tested were unaltered. Further, TrxR had to be functional in order to render WB C6 more susceptible to metronidazole and furazolidone (Table 2), as overexpression of mutated TrxR in WB TrxR-mut did not result in increased sensitivity to metronidazole and furazolidone (Table 2). Finally, since NADPH oxidase activity in WB TrxR-mut was even higher than in WB TrxR, it can be also ruled out that TrxR indirectly renders G. lamblia more susceptible to metronidazole and furazolidone by causing oxidative stress through generation of superoxide radicals. It is evident, however, that the effect of TrxR overexpression on metronidazole and furazolidone susceptibility was only moderate. This finding is in line with the notion that several factors contribute to nitro drug reduction in G. lamblia and other microaerophilic parasites. One well documented example is nitroreductase 1 from G. lamblia which, when overexpressed, increases susceptibility to metronidazole quite to the same extent as shown here for TrxR (Nillius et al., 2011; reviewed in Leitsch, 2015).

Surprisingly, the direct effect of four of the five drugs on TrxR activity was minute, if at all measurable. Only auranofin, a compound known to inhibit G. lamblia TrxR effectively in vitro also

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**Table 3**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reduction by G. lamblia TrxR</th>
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<tr>
<td>Metronidazole (1 mM)</td>
<td>81 ± 30 nmol min⁻¹ mg⁻¹⁻¹</td>
</tr>
<tr>
<td>Furazolidone (100 μM)</td>
<td>1094 ± 25 nmol min⁻¹ mg⁻¹⁻¹</td>
</tr>
<tr>
<td>Nitazoxanide</td>
<td>0</td>
</tr>
</tbody>
</table>

*already published in Leitsch et al., 2011.*
inhibited TrxR in vivo to a relevant extent (Fig. 3). Still, even if auranofin was applied in very high concentrations (50 μM) the residual TrxR activity was still 4 to 5-times higher than in untreated wildtype (Fig. 3). Since wildtype and WB TrxR were equally susceptible to auranofin (Table 2), we conclude that TrxR, quite in contrast to the commonly held belief (Tejman-Yarden et al., 2013; Watkins and Eckmann, 2016), is not the primary target of this drug in C. lamblia.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijpddr.2016.07.003.

References


