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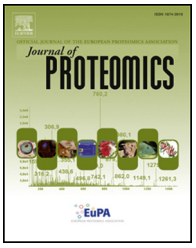

Inhibition of thioredoxin reductase by CDNB induces apoptosis in filarial parasite *Setaria cervi*: A proteomic and biochemical approach

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ABSTRACT

Thioredoxin reductase plays a crucial role in the maintenance of cellular redox homeostasis. In this study, we have targeted TrxR in *Setaria cervi*, a bovine filarial parasite using its inhibitor CDNB. It caused significant decrease in the motility and viability of these parasites leading to their death. Inhibition of TrxR leads to the downregulation of the antioxidant system followed by generation of oxidative stress in these parasites. The increased ROS level induced lipid peroxidation and protein carbonyl formation which might alter the mitochondrial membrane permeability leading to release of cytochrome c. CDNB significantly downregulated the level of ced-9 and activity of tyrosine phosphatases, cytochrome c oxidase. It also upregulated ced-3, homolog of mammalian caspase 3 suggesting initiation of intrinsic pathway of apoptosis. The proteomic profile of CDNB treated parasites showed marked alteration in abundance of different protein spots with 20% downregulated and 13% unregulated spots in comparison to control parasites. We observed a downregulation in the glycolytic enzymes such as enolase, PGK, and GAPDH thereby blocking the ATP formation in the parasite. This study suggests that TrxR inhibition disrupts the cellular homeostasis thereby generating oxidative stress followed by mitochondrial mediated apoptosis in filarial parasites leading to the death of the parasites.

Biological significance

Lymphatic filariasis is one of the most prevalent tropical diseases caused by tissue dwelling parasitic nematodes viz., *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Currently available antifilarial drugs effectively eliminate larval stages of the parasite but are ineffective against the adult worms. Therefore, there is an urgent need for finding proteins/enzymes which play a crucial role in the persistence of these parasites. Our study for the first time reports the important role played by *S. cervi* TrxR in its survival. Thus, suggesting filarial TrxR as a potent chemotherapeutic target against lymphatic filariasis. This would help in screening of new compounds having macrofilaricidal activity.

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1. Introduction

Lymphatic filariasis (LF), commonly known as elephantiasis is one of the most prevalent tropical diseases. It is primarily caused by tissue dwelling parasitic nematodes viz., *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. This disease is considered as a major obstacle to socioeconomic development in endemic countries [1]. In India, an estimated 374 million people are living in endemic area of which 45 million are infected with the causative organism [2]. The current treatment strategies are based on a limited number of drugs such as DEC, albendazole and ivermectin either given alone or in combination. These drugs effectively eliminate larval stages of the parasite but are ineffective against the adult worms [3]. Therefore, there is an urgent need for development of potent macrofilaricidal drugs to eradicate the lymphatic filariasis.

The filarial parasites are long lived and are capable of surviving in host's environment by employing a variety of evasion strategies and defence mechanisms. They possess a strong anti-oxidant system to protect themselves from hostile environment of the host. These parasites possess different antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSHPx), glutathione-S-transferase (GST), glutathione reductase (GR) and thioredoxin reductase (TrxR). One of these enzymes, TrxR plays an important role in the survival of the parasites [4]. The TrxR is a homodimeric flavoenzyme which belongs to the pyridine nucleotide-disulphide oxidoreductase family and catalyses NADPH dependent reduction of thioredoxins (Trx). It comprises of a NADPH binding domain, FAD prosthetic group and a redox active catalytic site at the C-terminal [5]. It is known to be conserved throughout the evolution, from bacterium to human. In higher organisms, two major ubiquitous TrxRs have been identified viz TrxR1 (cytosolic form) and TrxR2 (mitochondrial form). However, TrxR exists as a single enzyme in the bacteria and lower eukaryotes [6]. It plays a crucial role in the maintenance of cellular redox homeostasis. It also plays an important role in the redox regulation of multiple intracellular processes including DNA synthesis, transcriptional regulation, and protecting the cell from oxidative stress [7].

The presence and role of TrxR have been well studied in different organisms such as *Caenorhabditis elegans*, *Fasciola hepatica*, *Plasmodium falciparum* and *Haemonchus contortus* [8–11]. It has been reported that inhibition of TrxR leads to alteration in the redox state, elevating oxidative stress followed by mitochondrial mediated apoptosis in human carcinoma cell [12]. However, its role in the filarial parasites is still unknown. The presence of TrxR in the adult and microfilarial stages of the bovine filarial parasite, *Setaria cervi* has already been reported earlier in our laboratory [13]. The *S. cervi* resembles *W. bancrofti*, a human filarial parasite in its nocturnal periodicity and antigenic cross reactivity. Several *S. cervi* proteins and enzymes show more than 98% homology with *W. bancrofti* [14,15].

Therefore, we have designed this study to elucidate the importance of TrxR in the survival of filarial parasites. The ex vivo effect of CDNB (1-chloro 2,4-dinitrobenzene), a TrxR inhibitor on parasite survival was observed. The CDNB irreversibly inhibits selenocysteine and cysteine residues present in the active site of the enzyme TrxR [16]. As filarial parasites possess a strong antioxidant system [4], we have evaluated the levels of various other enzymatic and non-enzymatic antioxidants in *S. cervi*. Different oxidative stress parameters like reactive oxygen species generation, protein carbonyls, lipid peroxidation, and NADPH oxidase were further examined. Several reports have demonstrated that TrxR inhibition causes apoptosis due to mitochondrial dependent intrinsic pathway triggered by oxidative stress [17,18]. Therefore, we have assessed different apoptotic markers viz: cytochrome C-oxidase, protein tyrosine phosphatases (PTPs), ced-9/Bcl_2 and ced-3/caspase 3. Furthermore, effect of TrxR inhibition on the proteome expression profile of the filarial parasite was analysed through 2D gel electrophoresis and MALDI mass spectrometry. To the best of our knowledge, this is the first report which shows the importance of TrxR in the survival of any filarial parasite.

2. Material and methods

2.1. Collection of parasites and preparation of extract

The adult female *S. cervi* parasites were procured from the peritoneal folds of freshly slaughtered Indian water buffaloes. Worms were washed with phosphate buffer saline (PBS) and maintained in the Krebs Ringer Buffer (KRB) supplemented with streptomycin, glutamine and 1% glucose (maintenance medium). Microfilariae (mf) were obtained by dissecting the distal portion of the uterus of adult female parasite. A 10% w/v homogenate of adult parasite was prepared using standard laboratory protocol [19]. The parasites were homogenized in 50 mM Tris–HCl, pH 8.8 containing 0.15 M NaCl, 1 mM EDTA, 1 mM DTT and 50 mM PMSF using motor driven REMI homogenizer (RQ127A) at 4 °C. The homogenate was then centrifuged at 5000 *g* for 10 min at 4 °C. The supernatant was collected which was again centrifuged at 15,000 *g* for 30 min at 4 °C, the clear supernatant thus obtained was used for various assays.

2.2. Exposure of worms to inhibitors

An equal number (n = 10) of adult worms were incubated in the 20 ml maintenance medium containing 10, 25 and 50 μM concentration of CDNB for 6 h at 37 °C, 5% CO_2 and 95% humidity. Worms incubated in the maintenance medium only, served as control. After 6 h worms were washed with PBS buffer, homogenized and kept at −20 °C for further use. The concentration of CDNB used in our study is safe and non-toxic.

2.3. Effect on parasite motility

The motility of parasites was recorded by visual observation up to 6 h and scored either positive or negative (+/−) depending on their motility. After 6 h, the recovery of motility was checked by transferring the worms to fresh KRB medium for 1 h.

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