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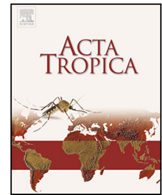

Inhibition of *Setaria cervi* protein tyrosine phosphatases by Phenylarsine oxide: A proteomic and biochemical study

Neetu Singh, Mohit Wadhawan, Savitri Tiwari, Ranjeet Kumar, Sushma Rathaur*

Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi 221005, India

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ABSTRACT

Phenylarsine oxide (PAO), a specific protein tyrosine phosphatase (PTP) inhibitor significantly decreased the motility and viability of *Setaria cervi* ultimately leading to its death. The PTP activity present in the cytosolic and detergent soluble fractions as well as on surface of these parasites was significantly inhibited by PAO. A marked alteration in protein spots abundance after proteomic analysis showed 14 down-regulated and 9 upregulated spots in the treated parasites as compared to the control. The PTP inhibition led to increase in the cytosolic and mitochondrial calpain activity in these parasites. PAO also blocked the ATP generation in the parasite depicted by reduced activity of phosphoglycerate kinase and expression of enolase. An increased ROS level, induced lipid peroxidation/protein carbonyl formation and decreased activity of different antioxidant enzymes like thioredoxin reductase, glutathione reductase and glutathione transferases was also observed in the PAO treated parasites. PAO, thus disturbs the overall homeostasis of the filarial parasite by inhibiting PTPs. Thereby suggesting that these molecules could be used as a good chemotherapeutic target for lymphatic filariasis.

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1. Introduction

The prolonged survival of filarial parasites inside the host suggests their successful evolutionary adaptation. They intervene with the immunological and physiological environment of the host, thus playing a critical role in the host-parasite relationship. The parasites need to maintain a homeostatic state for their survival. They exhibit strong antioxidant system, which shields them from the harmful reactive oxygen species (ROS) released during the cellular metabolism or by the immune system of the host (Rathaur et al., 2008). The existing antifilarial drugs, Diethylcarbamazine (DEC) and Albendazole though effective on the larval stages, are unsuccessful in killing the adult worms. Their extensive use also accelerates the probability of drug resistance (Nathan et al., 2005). It is therefore imperative to understand the underlying mechanisms, a parasite employs for its survival within the host, so that effective drugs/macrofilaricidal agents may be proposed and designed against them.

Protein tyrosine phosphatases (PTPs) are important signal transduction enzymes and considered as promising drug targets for the development of anti-parasitic chemotherapy in many parasitic protozoans like *Trypanosoma* spp. (Meyer-Fernandes et al., 1999), *Leishmania donovani* (Remaley et al., 1985), *Entamoeba histolytica* (Ruiz et al., 2003), *Crithidea* spp. (Lemos et al., 2002) etc. These PTPs accomplish diverse functions like actin filament disruption of host for tissue invasion, nutrition uptake, escaping the host immune system, virulence, cell adhesion, cell differentiation, growth and ROS sensing to rescue them from their host (Gomes et al., 2011). Numerous studies have demonstrated the antiparasitic effect of PTP inhibitors like Sodium orthovanadate and Bis-orthovanadate under *in vitro* and *in vivo* conditions against various protozoans such as *Plasmodium*, *Trypanosoma*, or *Leishmania* species (Heneberg, 2012). Many antifilarial compounds like Aspirin and SK7, a methyl chalcone have also been reported to significantly inhibit the PTP activity in the filarial parasites (Rathaur et al., 2011; Singh and Rathaur, 2010) and affect their survival. A specific inhibitor of PTP, Phenylarsine Oxide (PAO) (Sahara et al., 2004) has also been known to significantly inhibit the activity of important signalling molecules like FERM domain containing PTP, a cytosolic protein (Singh et al., 2015a,b); Ecto-PTP, a surface protein (Singh et al., 2014) and PRL PTP, a detergent soluble membrane bound protein (Singh et al., 2015a,b) isolated and purified from a bovine filarial parasite *Setaria cervi*. These proteins play important role in maintaining the structural and functional stability of the parasite.

PAO is a membrane permeable (Heneberg, 2012) and an organic trivalent arsenic compound which has been reported to be more

* Corresponding author.
E-mail address: surathaur@rediffmail.com (S. Rathaur).

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effective than inorganic arsenic in inhibiting proliferation of human acute myelocytic leukemic (AML) (Sahara et al., 2004) and erythroleukemia cell lines (Estrov et al., 1999). The trivalent arsenic and antimony compounds ranging from nM to μM concentration have also been reported to show noticeable lethal effects on *Trypanosoma brucei* and *Leishmania* parasites. These compounds form the chemical basis for development of anti-leishmanial/trypanasomal drugs till date (Chakraborty et al., 2014). The PAO LD_{50} value reported (RTECS Number, CH8100000) in case of mouse (intraperitoneal) and Rabbit (intravenous) are 1930 μg/ kg and 790 μg/ kg body weight respectively. The LD_{50} value of PAO reported in the case of the normal human PBMC is 40 nM (Deiss et al., 2013).

In the present study, we have exposed the *S. cervi* filarial parasites to different concentrations of PAO (0.25, 0.50, 0.75 and 1.0 nM). This concentration used in our study is safe and 40 times lower than the toxic dose of PAO in normal PBMC cells as stated above. We have also evaluated different biochemical and proteomic parameters to unravel the mechanism of action of PAO in these parasites. This study would aid to explore the role of PTP in survival of parasites and therefore help in designing of effective macrofilaricidal compounds.

2. Material and methods

2.1. Chemicals and reagents

Orthophospho-L-Tyrosine, Phenylarsine oxide, Trysin (proteomic grade), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), N-Succinyl-Leu-Leu-Val-Tyr 7-Amido-4-Methylcoumarin, Nitro blue tetrazolium (NBT) Sephadex G-25 beads were purchased from Sigma-Aldrich chemical company, USA. Prestained molecular weight markers for SDSPAGE were purchased from Biotech, India. EDTA, Ammonium molybdate, ascorbic acid, DTNB, GSSG, CDNB, sodium acetate, NaN3, Sodium deoxycholate, DTT, 2-ME, DNPH, TCA meta- phosphoric acid, n-Butanol, EtOH, ammonium bicarbonate, Malondialdehyde (MDA), Thiobarbituric acid, cytochrome c, Triflouroacetic acid, DMF, DMSO, KOH, Acrylamide, bis-Acrylamide, SDS, APS, etc. were purchased from Hi-Media Laboratories, Mumbai, India.

2.2. Collection of parasites

The *S. cervi* adult female parasites were acquired from the peritoneal folds of freshly slaughtered Indian water buffaloes and kept in the maintenance medium consisting of Krebs Ringer Buffer (KRB) supplemented with streptomycin, glutamine and 1% glucose. They were thoroughly washed with phosphate buffered saline (PBS) before the preparation of different extracts.

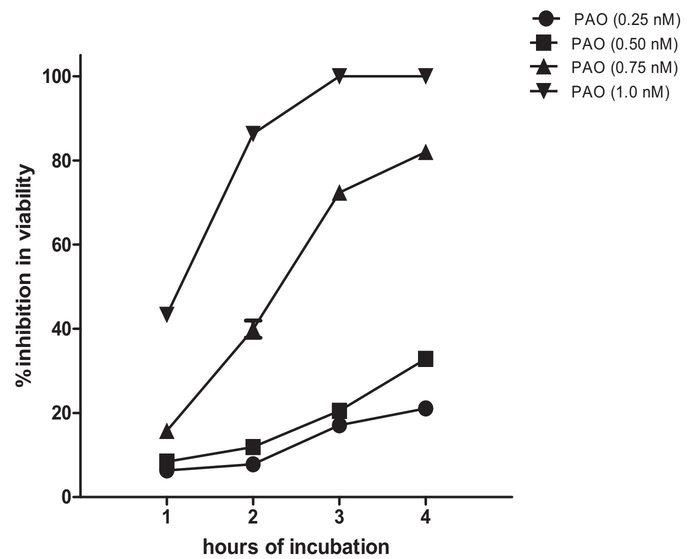

Fig. 1. Adult parasites (n = 15) of equal size were taken from the control and PAO treated sets after 3 h of incubation. The viability of the parasite was assessed using MTT assay as described in material and methods. The percentage inhibition in viability of the parasites after exposure to different concentration of PAO was calculated in comparison to the control. Values are Mean ± standard deviations of three experiments performed in duplicate.

2.3. Exposure of parasites to inhibitors

Equal number (n = 15) of adult parasites per 30 ml maintenance medium containing 0.25, 0.5, 0.75 and 1.0 nM concentration of PAO were incubated for 4 h at 37 °C, 5% CO_2 and 95% humidity. The parasites incubated in the maintenance medium alone were treated as control. After 3 h parasites were washed with PBS and kept at −20 °C for preparation of extracts.

2.4. Parasite motility and viability

The visual surveillance was employed to record the motility of adult parasites upto 4 h. The motility was assessed visually at 1 h time interval till 4 h and scored from + (least active) to ++++ (highly active) depending on parasite body movement. "–"shows no movement. As soon as the parasites were paralysed, they were transferred to the fresh KRB maintenance medium and checked

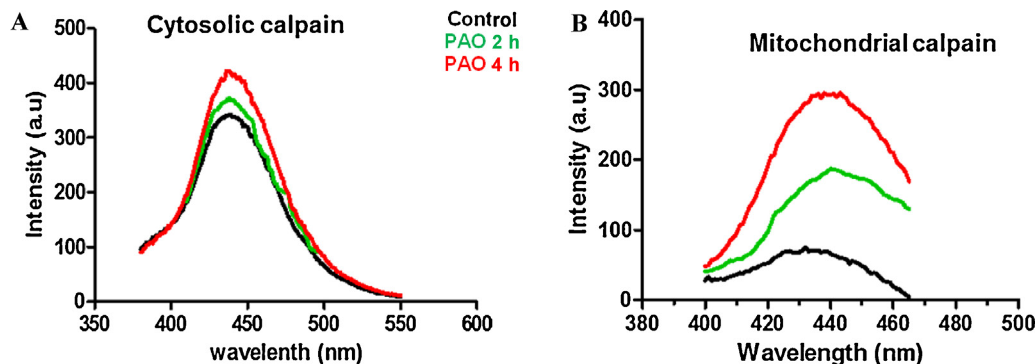

Fig. 2. The calpain activity was determined as the peak intensity (a.u.) by quantifying the fluorogenic substrate Suc- LLVY-AMC at 380 nm excitation/440 nm emission using a Nis –Elements F 3.0 fluorescence spectrophotometer. (A) Emission spectra in the cytosol and (B) Emission spectra in the mitochondria of the control extract (black line), PAO exposed extract after 2 h (green line) & PAO exposed extract after 4 h (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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