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Schistosoma mansoni: *N*-acetylcysteine downregulates oxidative stress and enhances the antischistosomal activity of artemether in mice

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ABSTRACT

Artemether (Art), a derivative of the antimalarial artemisinin, also exhibit antischistosomal properties. *N*-acetylcysteine (NAC) has a diversity of applications, largely because of the chemical properties of the thiol moiety present in its structure. The ability of this moiety to sweep reactive oxygen species is well-established with NAC. This study investigates the ability of NAC to enhance the therapeutic potential of Art against adult *Schistosoma mansoni* infection and evaluates the protective role of this antioxidant on *S. mansoni*-induced oxidative stress. Mice were divided into five groups; normal (i), infected control (ii), infected treated with NAC, 300 mg/kg 5 days a week/4weeks (iii), infected treated with Art (300 mg/kg) 7 weeks post infection (iv) and infected treated with both NAC and Art (v). Results showed that Art produced a significant reduction in total number of worms when used alone. Also, it decreased hepatic ova count significantly accompanied with an increase in the percentage of dead ova. Treatment with NAC alone increased the percentage of dead ova; meanwhile, it enhanced the decrease in total number of worms and hepatic ova count when used with Art. Infection with *S. mansoni* significantly increased tissue GSH, GR, SOD and serum ALT and GGT, while decreased the activities of GST, GPx and the levels of proteins and albumin compared to normal control. Treatment with NAC alone approximately recovered the contents of GSH, activities of GPx and levels of serum albumin, ALT and GGT relative to normal control. A tendency for normalization in activities of the antioxidant enzymes mentioned above and serum levels of liver function tests was observed in the groups treated with Art alone or Art + NAC. *Conclusion:* NAC downregulates oxidative stress induced by *S. mansoni* infection and enhances the therapeutic potential of artemether against adult schistosomes.

1. Introduction

Schistosomiasis continues to rank, following malaria, at the second position of the world's parasitic diseases in terms of the extent of endemic areas and the number of infected people (Utzinger et al., 2001; Pyrrho et al., 2004). There is yet no vaccine available and the current mainstay of control is chemotherapy with praziquantel used as the drug of choice (WHO, 2002). In view of concern about the development of tolerance and/or resistance to praziquantel, there is a pressing need for research and development of novel drugs for the prevention and cure of schistosomiasis (Cioli, 2000; Utzinger and Keiser, 2004). Artemisinin (qinghaosu) is the main active agent extracted from the leaves of *Artemisia annua*, a plant that is widely disseminated in China and grows naturally in Central Europe, the United States and Argentina. Its antimalarial activity was confirmed in 1971 and more than two million patients with malaria have been treated with this drug and its derivatives (artemether, artesunate and arteether) over that last 20 years (Utzinger et al., 2001). Interestingly artemether and artesunate also exhibit antischistosomal properties (Xiao, 2005). In contrast to praziquantel, artemether exhibits the highest level of activity against 1–4 weeks old *Schistosoma japonicum*, *Schistosoma mansoni* and *Schistosoma haematobium* schistosomula, while the invasive stages and adult worms are less susceptible (Xiao et al., 2000a; Utzinger et al., 2002). These laboratory findings have been confirmed in seven and one randomized controlled clinical trials for *S. japonicum* and *S. mansoni*, respectively, with more than 5000 study participants (Utzinger et al., 2001).

Schistosome eggs induced liver fibrosis which is a common pathological process leading to the development of irreversible cirrhosis (Xiong et al., 2003) and inability of the liver to perform its biochemical functions (Rehermann and Nascimbeni, 2005). Glutathione (GSH) plays an important role in the detoxification of reactive oxygen intermediates generated at sites of inflammation and during metabolism of xenobiotics and as a source of cysteine for various organs (Liang et al., 1991). A decreased concentration of GSH is of pathogenic significance in these disease processes, and

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increasing the concentration of GSH in target cells and plasma might be beneficial. Prevention of oxidative stress and lowering the level of intracellular peroxides depends mainly on elevating the levels of reduced glutathione (GSH) and promoting its metabolizing enzymes to confront the liberated free radicals (Pocernich et al., 2000). Under such conditions, and taking into consideration that some studies have reported that schistosomiasis is associated with free radical liberation and disturbance in the cellular antioxidant system (Abdallahi et al., 1999; Gharib et al., 1999), much interest has been focused on compounds that are capable of stimulating glutathione synthesis and acting as antioxidants.

One of the compounds which possesses antioxidative and cytoprotective properties is *N*-acetylcysteine (NAC), whose molecule contains free sulfhydryl groups, so it may directly react with electrophilic compounds such as free radicals (Cuzzocrea et al., 2000). It is expected that glutathione and its precursors will soon be important pharmacological tools and will contribute to the treatment of several pathophysiological conditions (Wernerman and Hammarqvist, 1999). In this regard, Chyka et al. (2000) reported that acetylcysteine, a common precursor of glutathione, showed promising results for treatment of several toxic and adverse effects of drugs. In the light of the above-mentioned information, this work aimed at: (i) assessing the ability of NAC to enhance the therapeutic potential of Art against adult *S. mansoni* infection; (ii) evaluating the protective role of this antioxidant on *S. mansoni*-induced oxidative stress; and (iii) determining the induced alterations in serum markers enzymes of liver function.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), Tween-80, ethanol, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithiobis (2-nitrobenzoic acid) [DTNB, (Ellman's reagent)], ethylene diamine tetraacetic acid (EDTA), hydrogen peroxide (H_2O_2), nicotinamide adenine dinucleotide reduced (NADH), nitro blue tetrazolium (NBT), phenazine methosulphate (PMT), potassium dihydrogen phosphate (KH_2PO_4), reduced glutathione (GSH), glutathione oxidized (GSSG), glutathione reductase, sodium pyrophosphate and trichloro acetic acid (TCA) were purchased from Sigma–Aldrich chemical Co. (St. Louis, MO, USA).

2.2. Animals and infection

Male Swiss albino mice (CD-1 strain) obtained from Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt, and weighing 18–20 g were used in this study. The animals were maintained on a standard commercial pellet diet and were kept in air-conditioned animal house at 20–22 °C. All animal studies presented here were approved by the local government based on guidelines of ethical research committee of TBRI. Mice were infected with 70 ± 10 *S. mansoni* cercariae/mouse (provided by SBSC of TBRI) according to the method described by Liang et al. (1987).

2.3. Drugs and dosages

Artemether (Art) was the product of Kunming Pharmaceutical Corp. (Kunming, China; Batch No. 010377) and it was freshly suspended in 7% Tween-80 and 3% ethanol and given as a single oral dose of 300 mg/kg seven weeks post infection. *N*-acetylcysteine; NAC (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) was dissolved in distilled water and given orally in a dose of 300 mg/kg 5 days a week from the 3rd to the 7th week after infection.

2.4. Animal groups

In the present study, mice were randomly allocated into five groups each of 8–10 mice at the beginning of the experiment:

Group 1:	Normal control.
Group 2:	Infected control and treated with vehicle only.
Group 3:	Infected treated with NAC.
Group 4:	Infected treated with Art.
Group 5:	Infected treated with both NAC and Art as previously described.

The number of animals in each group slightly varied, due to mortality, and is denoted between parentheses in the presented tables. All animals were killed by rapid decapitation 10 weeks post infection (i.e., 3 weeks post treatment). Immediately after death of mice, blood samples were collected, and sera were separated by centrifugation at 1850*g* and stored at −70 °C pending assay.

2.5. Study of parasitological criteria

After killing, hepatic and portomesenteric vessels of mice were perfused according to Duvall and De Witt (1967) for worms' recovery and subsequent counting. Immediately after perfusion, the liver was isolated, chilled on ice and divided into two unequal pieces. The small piece of liver was used for determining the number of ova per gram liver (Kamel et al., 1977), while the other piece of liver was used for hepatic enzyme assessment. The percentage of dead ova was also determined according to the method of Pellegrino et al. (1962).

2.6. Preparation of liver homogenates

Liver tissue was homogenized in four volumes (w/v) of ice cold 100 mM KH_2PO_4 buffer containing 1 mM EDTA, pH 7.4 and centrifuged at 10,000*g* for one hour at 4 °C. The supernatant was collected and kept at −80 °C for subsequent analysis for determination of liver levels of glutathione-related antioxidant enzymes. Protein concentration of the supernatant was measured (Lowry et al., 1951) using crystalline BSA as standard.

2.7. Assay of antioxidant enzymes in liver homogenates

GSH content was measured by the method of Ellman (1959). GST activity was measured according to the method of Habig et al. (1974) using chlorodinitrobenzene (CDNB) as a substrate. Glutathione reductase (GR) activity was assayed by using oxidized glutathione as a substrate according to the method described by Zanetti (1979). Glutathione peroxidase (GPx) catalyzes the oxidation of glutathione and its activity was measured based on the method described by Paglia and Valentine (1967). Superoxide dismutase (SOD) activity was assayed spectrophotometrically by the procedure of Winterbourn et al. (1975).

2.8. Assessment of liver function tests

Serum alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), total proteins and albumin levels were estimated by the methods of Reitman and Frankel (1957); Persijn and van der Slik (1976), Weichselbaum (1946) and Doumas et al. (1971), respectively using available commercial kits.

2.9. Statistical analysis

All data are expressed as mean ± SD. Mean values were assessed for significance using one-way analysis of variance (ANOVA)