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OXYGEN FREE RADICAL AND NITRIC OXIDE PRODUCTION IN SINGLE OR COMBINED HUMAN SCHISTOSOMIASIS AND FASCIOLIASIS

By

SEHAM ABO SHOUSHA[1], SAFIA S. KHALIL[2]

AND EMAN A. RASHWAN[1]

Departments of Immunology[1] and Parasitology[2], Medical Research Institute, Alexandria Unviersity, Alexanria, Egypt.

ABSTRACT

The levels of superoxide (O^-_2) and nitric oxide (NO) production by monocytes have been measured in 15 patients with *S. mansoni*, 15 patients with *Fasciola* and six patients with combined infection as well as in control group (15). The levels of both radicals were significantly higher in all patient groups than in the control group, indicating that these radicals may have a role in the immunity against such infections. Patients with chronic fascioliasis showed lower level of O^-_2 and NO than those with schistosomiasis. This may be due to the lodging of the mature *Fasciola* spp. away from the immune system and subsequently decreased amount of antigens reaching the circulation. In combined infection, the levels of these products were at the highest value, due to increased antigenic stimulation and cross reactivity between the two parasites which may have lead to augmented immune response.

INTRODUCTION

Concurrent infection with more than one parasite sometimes occurs. Human fascioliasis is nowadays reported more frequently among Egyptians, and may be in conjunction with schistosomiasis (Abou Basha, 1992). This combined infection may lead to antagonistic or synergistic heterologous interactions that may be mediated via the host's specific and nonspecific

responses to infection (Abdel Rahman et al., 1995). The generation of reactive oxygen radicals and nitrogen radicals may be an important mechanism by which mononuclear phagocytes deal with parasites (James, 1991 and Brophy et al., 1992). Exposure of these cells to appropriate stimuli results in increased oxygen consumption leading to release of toxic oxygen radicals principally O^{-}_{2} and H_2O_2 during the respiratory burst. These two radicals may interact to produce hydroxyl radical, which is even more reactive. Available evidence indicates that these products are toxic and thus may contribute significantly to the destruction of extracellular and intracellular pathogens (Docampo and Moreno, 1984, and Hughes, 1988). These reactive oxygen metabolites may also initiate lipid peroxidation, a process that leads to membrane damage and generation of further toxic products (Clark et al., 1985). Nitric oxide is a soluble free radical that is formed by the deamination of the amino acid arginine by nitric oxide synthase (NOs). It is involved in a variety of biological functions such as vasodilatation and neuronal signaling. A number of cells including mononuclear phagocytes when activated by immunological stimuli produce large amounts of NO as a part of effector mechanisms for the destruction of the pathogens and tumours (Liew, 1993).

Free radicals involvement in direct killing of worms by macrophages has been demonstrated in vitro in some helminths as *S. masnoni* (Nare et al., 1990), *Trichinella spiralis* (Kazura and Meshnick, 1984), and *Hymenolepis nana* (Niwa and Migazato, 1996). However, free radicals are not only essential for host defence against parasites but may also contribute to inflammatory injury and immunopathology of parasitic diseases (Liew, 1993).

The aim of the present work was to study the role of free radicals (oxygen derived free radicals and nitric oxide) in immunity against human *S. mansoni* and *Fasciola* spp. infection as well as in combined infection with both parasites. This has been achieved by assaying the monocyte production of oxygen free radicals and nitric oxide.

PATIENTS AND METHODS

Fifty one patients, 12-30 years of age, enrolled in the present study

were divided into three groups: 15 with *S. mansoni* infection, 15 with *Fasciola* spp. infection, and six with combined *Fasciola* and *S. mansoni* infection. Also, 15 healthy individuals were taken as a control group. All patients were excreting ova as established by repeated stool examiantions using saline sedimentation and Kato Katz technique. Clinically, all schistosomiasis patients were in the early stage of infection.

Isolation and stimulation of mononuclear cells: Peripheral blood mononuclear cells were freshly prepared by centrifugation of heparinized blood over Ficoll-Hypaque gradients and cultured in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum, 50 µg/ml penicillin, 50 µg/ml streptomycine and 2 mML-glutamine. Cells were cultured for an hour in glass petri-dishes for separation of monocyte cells. Cells at 2 x 10^6 cells/ml were stimulated by concavalin A (con A) at 10µg/ml (Boyum, 1977).

Measurement of superoxide generation was determined by reduction of cytochrome c test (Johnston et al., 1978). Briefly, 500 µl of monocytes ($2x10^6$ cells/ml) were mixed with 250 µl of serum treated Zymosan and 100 µl of cytochrome c. The mixture was incubated at 37°C for 60 minutes. Absorbance at 550 nm was determined after incubation.

Nitric oxide production was evaluated by measuring nitrite accumulation in the culture supernatant by the Griess colorimetric reaction (Ding et al., 1988). Briefly, 100 µl of supernatant was reacted in a mixture of 50 µl of 0.1% sulfanilamide and 50 µl of 0.1% N-(1-naphthyl) ethylene- diamine dihydrochloride in 3% phosphoric acid at room temperature. Absorbance was measured at 540 nm in reference to a standard nitrite quantitative curve.

RESULTS

For comparison (table, 1) one way analysis of variance (ANOVA) and the least significant difference (LSD) were employed. It was observed that the levels of O^-_2 were significantly higher in all patient groups compared to control group. The levels were higher in patients with schistosomiasis than those with fascioliasis. The highest levels were observed in patients with combined infection. Similar findings were observed with nitric oxide (table, 2).

Table 1: O^{-}_{2} levels (nmol/min) in patients with single or combined schistosomiasis and fascioliasis.

O^{-}_{2} level	Control group (n = 15)	Schistosomiasis (n = 15)	Fascioliasis (n = 15)	Combined (n = 6)
Range	0.6 - 1.3	1.7 - 2.8	1.3 - 2.0	2.5 - 3.2
Mean	0.95	2.15	1.63	2.8
±S.D.	0.25	0.31	0.23	0.26

One way (ANOVA), Between groups F = 91.40 (P<0.001).

Table 2: NO levels (nmol/10^{6} cells) in patients with single or combined schistosomiasis and fascioliasis.

NO level	Control group (n = 15)	Schistosomiasis (n = 15)	Fascioliasis (n = 15)	Cobined (n = 6)
Range	6.2 - 9.6	22.5 - 25.2	15.5 - 19.8	25.5 - 27
Mean	7.73	23.77	17.69	26.14
±S.D.	0.98	0.96	1.3	0.65

One way (ANOVA), Between groups F = 712.2 (P<0.001).

DISCUSSION

The level of oxygen free radical (O^{-}_{2}) and nitric oxide (NO) produced by monocytes isolated from all patient groups were found significantly higher than those of normal control. Patients with single *S. mansoni* infection showed higher level of oxygen free radical (O^{-}_{2}) and NO than those with *Fasciola* spp. infection. This could be attributed to the fact that in established fascioliais, the localization of mature worms in the biliary tract is accompanied by low release of antigens in the circulation. In schistosomiasis, the high level of worm antigens released in the circulation together with

antigens of eggs lodged in the portal veins induce higher stimulation of the immune system with subsequent more production of oxygen free radical and NO than in fascioliasis. El-Nassery et al. (1992) reported a highly significant increased amount of oxygen metabolites produed by murine peritoneal phagocytic cells stimulated by *S. mansoni* infection. Osman et al. (1995) found that oxygen free radical produced by neutrophils was significantly increased in patients with acute fascioliasis with a relative decrease in chronic infection. In vitro study, Baeza et al. (1993) demonstrated that in the presence of specific antibodies, excretory-secretory products of *F. hepatica* stimulate free radicals production by bovine PMN cells. The harmful effect of O^{-}_{2} has been studied by Docampo and Moreno (1984); Clark et al. (1985); Hughes, (1988). They can damage cell membrane, inactivate proteins, degrade nucleic acid, kill cells and eventually may kill the parasite itself. However, the parasites show variation in susceptibility to oxidant killing that appears to be dependant on the species or even cyclic stage. In this regad, Mkoji et al. (1988) in vitro, found that adult *S. mansoni* showed a high resistance to oxidative killing while the majority of schistosomules were killed under the same condition. Smith et al. (1992) demonstrated that O^{-}_{2} correlates with the immune status of the host. They added that free radicals generation by leucocytes of rats (host resistant to *Fasciola* infection) was higher than that produced by mouse (non resistant host). These findings clearly indicate that oxygen free radicals are very important contributors to the parasite's killing mechanism though, the participation of other oxygen independent factors could not be excluded.

It has been appreciated that NO is a major effector molecule of macrophage cytotoxicity against variety of microbial targets including some helminth parasites (Ahmed et al., 1997). NO is cytotoxic through, its ability to bind and deplete intracellular iron and inactivate cellular enzymes. Hence, the high level of NO production by monocytes reported in the present study may be an important contributor in the host immunity against *S. mansoni* and *Fasciola* spp. James and Glaven (1989) found that macrophage cytotoxicity against schistosomule of *S. mansoni* involves production of NO and is inhibited by NO synthase inhibitors. Also, Cervi et al. (1998) reported enhanced NO synthesis production by spleen monocyte cells from *F. hepatica* infected rats.

In the present study, O^{-}_{2} and No were at the highest values in combined *S. mansoni* and *Fasciola* spp. infections. The prominent level of these free radicals may be due to the increased antigenic stimulation by the two parasites. In addition, it is known that there are cross- reactive antigens between *S. mansoni* and *Fasciola* spp. (Hillyer and Serrano, 1982). So, the primary immune response to antigens of one parasite will be augmented by secondary stimulation of the second parasite. Wynn et al. (1994) demonstrated elevated expression of NO synthase in the lung of vaccinated mice after challenge infection with *S. mansoni.*

In conclusion, oxygen free radical and nitric oxide were lower in chronic fascioliasis as compared to active schistosomiasis. This is due to low level of antigens reaching the circulation and subsequently low stimulation of immune system. In combined infection, increased antigenic stimulation and the presence of cross reactive antigens between the two parasites could explain the highest level of the free radicals.

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