Evaluation of hepatic antioxidant changes in ovine dicrocoeliosis

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Received: 31 August 2013 / Accepted: 9 December 2013 / Published online: 21 December 2013
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Abstract Dicrocoeliosis, caused by *Dicrocoelium dendriticum* is a hepatic parasitic disease of clinical and financial significance in ruminant breeding, which causes direct losses due to condemnation of parasitized livers. The purpose of our study was to assess the effects of natural dicrocoeliosis on the antioxidant defence capability of the liver in sheep. For this purpose, livers of 40 infected sheep with *D. dendriticum* along with livers of 20 healthy (control) sheep were collected from animals slaughtered in Khuzestan province, Iran. An increase in malondialdehyde concentrations accompanied by decreased activities of SOD and GPX of infected liver was noticed when compared with control values. Our data indicate that through dicrocoeliosis insufficient scavenging of reactive oxygen species takes place and caused oxidative liver damage.

Keywords *Dicrocoelium dendriticum* ·
Lipid peroxidation · Antioxidant enzyme · Liver

Introduction

The small liver fluke, *Dicrocoelium dendriticum* Dujardin, 1845 (Trematoda, Digenea) is a parasite of the bile ducts of a wide variety of wild and domestic mammals (sheep, goats, cattle, buffaloes, roe deer, and camels) and occasionally affect rabbits, pigs, dogs, horses, and humans (Otranto and Traversa 2003). *D. dendriticum* is found in America, Asia, North Africa, and Europe. It has a very complex life cycle because it involves numerous species of

land mollusks and ants as first and second intermediate hosts, respectively. The economic and health significance of dicrocoeliosis is due to the direct losses occasioned by the confiscation of altered livers and also to the indirect ones caused by the digestive disorders derived from the hepatobiliary alterations, such as decreased animal weight, growth delay, and reduced milk production and feed efficiency (Manga-González et al. 2001).

Many investigations indicate that parasitic infections with high tolerance of the host are the result of defence mechanisms which include enhanced generation of reactive oxygen species (ROS) (Sanchez-Campos et al. 1999). These molecules can exist in a free form and interact with various vital tissue and cell components which results in organism dysfunction. The generation of oxygen-derived free radicals may be an initial, non-specific defence reaction of the host toward parasitic infection. Lipid peroxidation is one of the best parameters used for indicating the level of ROS-induced systemic biological damage (Kühn and Borchert 2002). Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant malondialdehyde (MDA) (Romero et al. 1998). MDA is isolated in urine, blood and tissues and is used as a biomarker for radical-induced damage (Day 1996). On the other hand sheep possess both enzymatic and non-enzymatic antioxidant mechanisms of defence that prevent ROS formation or limit their toxic effects. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) are antioxidant enzymes involved in endogenous antioxidant defences against ROS (Fridovich 1978). From the available literature, altered activities of some enzymatic and non-enzymatic antioxidants in parasitic diseases have been reported (Dede et al. 2002; Pabon et al. 2003), but information on

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liver levels of MDA and activities of SOD and GPX in sheep infected with *D. dendriticum* are lacking. Since, it has been reported that the increased plasma lipid peroxides in hepatic diseases originates essentially from the affected liver (Suematsu and Abe 1982) this study was therefore carried out to investigate the relation of oxidative stress by estimating the level of MDA, SOD and GPX enzymes in the liver of infected sheep.

Materials and methods

Study area

The study was done in the southwest region of Iran (Khuzestan province) where dicrocoeliosis is prevalent. Khuzestan province has a border of about 64,236 km², between 47° and 41 min to 50° and 39 min of eastern longitude from prime meridian and 29° and 58 min to 33° and 4 min of northern latitude from equator (Statistical book of Khuzestan province 2006). The province has hot and wet summers, mild spring and cold winters.

Sampling

40 sheep with liver dicrocoeliosis along with 20 healthy sheep were selected from animals admitted for slaughtering at slaughterhouse located in Ahvaz, Khuzestan province. All the ewes were of native breed, and their age ranged from 3 to 4 years. The selection of infected animals was restricted to those affected with dicrocoeliosis only. Also, the negative control animals did not show any pathology in the carcass.

Preparation of tissue extract

Liver were placed in ice-cold 0.15 M NaCl solution, perfused with the same solution to remove blood cells, blotted on filter paper and weighed. 200 mg of liver were homogenized in 2 ml potassium phosphate buffer to obtain 10 % homogenate. The liver homogenate were centrifuged at 4,000×g for 10 min. The supernatant was used for the experiment.

Determination of lipid peroxidation levels

MDA levels in liver samples were measured using the thiobarbituric acid reaction method of Placer et al. (1966). Quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. Values of MDA were expressed as nmol/ml.

Antioxidant enzyme assay

The determination of SOD activity was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazon dye. The absorbance was measured at 505 nm and the SOD activity was then calculated according to the manufacturer's instruction (Ransod[®]-Randox Lab, Antrim, UK). GPX activity was determined based on the fact that GPX catalyzed the oxidation of glutathione by cumen hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione was immediately converted to the reduced form with concomitant oxidation of NADPH to NADP⁺. The absorbance was measured at 340 nm and the GPX activity was then calculated according to the manufacturer's instruction (Ransel[®]- Randox Lab, Antrim, UK). The enzymes activities were expressed as U/ml.

Statistical analysis

Data were analyzed statistically by independent-samples *t* test using SPSS 16. Differences of *P* < 0.05 were considered significant.

Results

The activities of SOD and GPX in infected livers were 1.68 ± 0.23 and 0.76 ± 0.14 U/ml, respectively. The control value for the activity of SOD was 4.02 ± 0.87 U/ml and for GPX was 1.62 ± 0.31 U/ml in uninfected groups. Concentration of MDA in the liver of infected and control groups were 9.3 ± 1.1 nmol/ml and 3.7 ± 0.13 U/ml, respectively. The values of the liver MDA, SOD and GPX activities in dicrocoeliosis and non-infected sheep are shown in Table 1. In livers with dicrocoeliosis, levels of MDA were higher and SOD and GPX activities were lower than in controls, and these differences were significant.

Discussion

Data described in this study provide a reliable biochemical evidence for the generation of oxidative stress as detected by enhancement of lipid peroxidation and reduction of the enzymatic antioxidant ability in grazing sheep naturally infected with *D. dendriticum* in comparison with the healthy group. In the current work, the higher MDA concentration of infected sheep suggests increased production of lipid peroxidation in the liver, and indirectly pointed to

Table 1 Liver MDA and antioxidant enzyme activities in ovine dicrocoeliosis
Data represent the mean $\pm$ SD of triplicate experiment

	MDA (nmol/ml)	SOD (U/ml)	GPX (U/ml)
Control ($n = 20$)	3.7 ± 0.13	4.02 ± 0.87	1.62 ± 0.31
Infected ($n = 40$)	9.3 ± 1.1	1.68 ± 0.23	0.76 ± 0.14

enhanced free radical generation. Free radicals, such as hydroxyl ions and superoxide, occur on the activation of phagocytes. They are used by host phagocytes to kill internal pathogens, or they can be released by activated phagocytes onto a parasite surface. Although they act in a non-specific manner as effectors of resistance, their generation usually follows the activation of macrophages by cytokines from specifically stimulated lymphocytes. Free radicals are also believed to be responsible for the parasite-induced pathology in the host (Morel et al. 1991).

Lipid peroxidation represents oxidative tissue damage caused by hydrogen peroxide, superoxide anion and hydroxyl radicals, resulting in structural alteration of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products. MDA is a major end product of free radical reaction on membrane fatty acids. The sharp increase of MDA concentration showed that *D. dendriticum* infection evokes significant changes in the oxidative status of infected host. An increase of MDA, due to enhanced production of ROS, was also disclosed in trichinellosis (Derda et al. 2004), experimental hamster dicrocoeliosis (Sanchez-Campos et al. 1999) and fascioliosis (Kolodziejczyk et al. 2006).

The presence of lipid peroxidation could contribute to hepatic injury and to the reduced capacity for handling of drugs and xenobiotics previously reported for this parasitosis (Sanchez-Campos et al. 1996). The biological damage caused by free-radical-mediated mechanisms can be prevented by specific chemical scavengers which trap the specific radicals, as well as by protective antioxidant enzymes including SOD and GPX, which remove hydrogen peroxide or superoxide radicals. SOD and GPX are part of the defence mechanisms against free radicals (Sies 1993). SOD neutralizes the superoxide anion, while selenium-dependent GPX detoxifies hydrogen and lipid peroxides formed as a result of membrane peroxidation. SOD and GPX belong to the main defence antioxidants that prevent the formation of new free radical species by converting the existing free radicals into less harmful molecules before they have a chance to react, or by preventing the formation of free radicals from other molecules. In the latter case, SOD converts superoxide into hydrogen peroxide, and GPX converts hydrogen peroxide into harmless molecules before they form free radicals. These antioxidant enzymes prevent superoxide and hydrogen peroxide from interacting

to produce hydroxyl and singlet oxygen through a Fe-dependent Fenton reaction. In this study, while the level of MDA showed an increase, the significant decrease of antioxidant enzymes were detected. These changes indicated that *D. dendriticum* led to lipid peroxidation. The decrease in antioxidant enzymes activities proved that the formation of reactive compounds was much higher than the level which could be compensated by the cellular defence system, thus, these compounds may not be converted to less harmful or ineffective metabolites at the sufficient level. Another reason might be the formation of reactive compounds in the body, which inhibited the enzyme.

The present findings suggest that enhanced lipid peroxidation observed in the liver in the course of *D. dendriticum* infection is a general process reflected by increased peroxidation and deregulation of antioxidative enzymes in sheep liver. Dicrocoeliosis is associated with a noticeable reduction in the availability of important endogenous antioxidants and the presence of an ongoing oxidative process.

Acknowledgments This study was supported by the research grant provided by Shahid Chamran University of Ahvaz.

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