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Fasciola hepatica: Effects on the antioxidative properties and lipid peroxidation of rat serum

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Abstract

Fasciola hepatica infection is accompanied by increased formation of reactive oxygen species. The aim of this study was to analyze antioxidative properties of rat serum in the course of fasciolosis. Wistar rats were infected per os with 30 metacercariae of *F. hepatica*. Activities of antioxidant enzymes and concentrations of non-enzymatic antioxidants in serum were determined at 4, 7, and 10 weeks post-infection (wpi). Activity of superoxide dismutase (Cu, Zn-SOD) significantly decreased (by 35% during the migratory phase, by 40 and 23% at 7 and 10 wpi, respectively), while glutathione reductase activity significantly increased (by 62, 65, and 41%, at 4, 7, and 10 wpi, respectively). No significant changes were found in the activity of glutathione peroxidase. Significant decreases in concentrations of reduced glutathione, vitamins C, E, and A were observed, particularly during the migratory phase of fasciolosis (at 4 wpi). These changes were accompanied by enhancement of lipid peroxidation processes as evidenced by increased levels of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). Concentrations of MDA and 4-HNE at 4 wpi increased by 38% and by 59%. MDA increased by 51% at 7 wpi and by 79% at 10 wpi, while 4-HNE increased by 87 and 118%, respectively. The results indicate that fasciolosis is associated with enhanced oxidative reactions and reduced antioxidant defense capability of rat serum.

Index Descriptors and Abbreviations: *Fasciola hepatica*; Trematode; Fasciolosis; Rat; Serum; Lipid peroxidation; Antioxidants; ROS, reactive oxygen species; SOD, superoxide dismutase; GSSG-R, glutathione reductase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal

1. Introduction

The liver fluke (*Fasciola hepatica*) is a cosmopolitan trematode found in the liver and bile ducts of many mammal species, especially ruminants and man. Fasciolosis causes considerable economic losses in animal farms through reduced production of milk, wool, and meat, particularly in cattle and sheep.

Hepatic lesions produced by *F. hepatica* in various host species are associated with the number of ingested metacercariae. Migration of juvenile flukes in the liver is associated with mechanical damage to the parenchyma in the form of haemorrhage, necrosis, fibrosis, and cirrhosis. Beside many histological similarities of fasciolosis in different hosts, i.e., hyperplasia of the bile duct epithelium in cattle, sheep, mice or rats (Foster, 1981; Kolodziejczyk, 1994; Lee et al., 1992; Ross et al., 1966), some specific differences are evident, e.g., calcification of the walls of intrahepatic bile ducts in cattle (Ross et al., 1966). Other changes accompanying fasciolosis include hypoalbuminemia, anemia, and weight loss (Abbas et al., 1990; Anderson et al., 1977; Dargie and Mulligan, 1971).

Recent investigations indicate that parasitic infections with high tolerance of the host are the result of defense mechanisms which include enhanced generation of reactive oxygen species (ROS) (Boczon et al., 1996; Maffei Facino et al., 1989; Sanchez-Campos et al., 1999; Smith and Bryant, 1989). Cells and biological fluids form an antioxidant defense

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system aimed at suppression of ROS generation and prevention of ROS reactions with cellular components. A balance between oxidants and antioxidants is known to exist under physiological conditions. However, even small changes in oxidant or/and antioxidant levels may disturb this balance. Many parasitic infections, whether single or combined, enhance ROS generation including superoxide radical, nitric oxide, and hydrogen peroxide, produced especially by leukocytes (Abo-Shousha et al., 1999; Sibille et al., 2004; Smith and Bryant, 1989; Smith et al., 1992; Wandurska-Nowak and Wisniewska, 2002). Increased levels of ROS generated by peritoneal leukocytes of rats infected with *Fasciola* were found by Smith et al. (1992). Fasciolosis leads to oxidative stress which is the causative agent in the initiation and progress of lipid peroxidation (Maffei Facino et al., 1989). This situation becomes dangerous when the antioxidant system is unable to prevent oxidative reactions triggered by ROS and directed at oxidative modification of lipids, proteins, and DNA. Although it is generally believed that ROS generation serves to combat infection (Sadeghi-Hashjin and Naem, 2001), research has shown that newly excysted juveniles of *F. hepatica* are relatively resistant to free radicals (Piedrafita et al., 2000).

Having these facts in mind, the present study was designed to compare the antioxidant capacity of rat serum during sequential phases of fasciolosis.

2. Materials and methods

2.1. Animal treatment

The experiment was carried out in male Wistar rats aged 5 weeks. The rats were housed in groups with free access to granulated standard chow and water. A normal light–dark cycle was maintained. All experiments were approved by the local Ethic Committee for Scientific Experiments on Animals in Szczecin (Poland) and were carried out in accordance with the Polish Act on Animal Protection of 1997. Rats were infected orally with 30 metacercariae of *F. hepatica* passed through a stomach tube. Metacercariae were obtained from *Lymnaea truncatula* snail culture according to Taylor and Mozley (1948) and were classified as viable only if excretory granules were seen under an optical microscope (Boray, 1969). Groups of 10 control and 10 infected rats were anaesthetized with ketamine after 4, 7, and 10 weeks from infection. Blood was collected by cardiac puncture and serum was obtained after incubation (30 min, 37 °C). Recovery of flukes from bile ducts was done at 10 wpi.

2.2. Biochemical assays

Cu, Zn-SOD (EC 1.15.1.1) activity was determined by the method of Misra and Fridovich (1972) as modified by Sykes et al. (1978). A standard curve for SOD activity was made using SOD from bovine erythrocytes (Sigma Biochemicals, St. Louis, MO, USA). One unit of SOD was defined as the amount of the enzyme inhibiting by 50% the oxidation of epinephrine to adrenochrome. Activity was expressed in units per milligram of protein. Reaction rates were determined at 25 °C using 10 mM hydrogen peroxide.

Glutathione peroxidase (GSH-Px; EC 1.11.1.9) activity was measured spectrophotometrically using a technique according to Paglia and Valentine (1967). GSSG formation was assayed by measuring the conversion of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP. One unit of enzyme activity was defined as the amount of enzyme catalyzing the conversion of 1 μmol of NADPH/min at 25 °C and pH 7.4. Activity was expressed in units per milligram of protein.

Glutathione reductase (GSSG-R; EC 1.6.4.2) activity was measured with the method of Mize and Langdon (1962) by monitoring the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. One unit of enzyme activity was defined as the amount of enzyme catalyzing the conversion of 1 μmol of NADPH/min at 25 °C and pH 7.4. Activity was expressed in units per milligram of protein.

Glutathione (GSH) concentration was measured using Bioxytech GSH-400 test. The method consists of two steps. The first step leads to the formation of substitution products between a patented reagent and mercaptans (RSH) present in the sample. The second step specifically transforms the substitution products obtained with GSH into a chromophoric thione exhibiting maximal absorbance at 400 nm.

HPLC methods were used to determine the levels of vitamins C (Ivanovic et al., 1999), A, and E (De Leenheer et al., 1979). The ascorbic acid assay started with protein precipitation by metaphosphoric acid (100 g/L). Next, samples were centrifuged (3500*g*, 4 min) and assayed immediately. Vitamins A and E were extracted from serum with hexane containing 0.025% butylated hydroxytoluene. The hexane phase was removed, dried with sodium sulfate, and 50 μl of the hexane extract was injected onto the column.

Lipid peroxidation was assayed using HPLC for the measurement of malondialdehyde (MDA) as malondialdehyde–thiobarbituric acid adducts (Londero and Lo Greco, 1996) and of 4-hydroxynonenal (4-HNE) as a fluorimetric derivative (Yoshino et al., 1986).

2.3. Statistics

Data were expressed as means ± SD. Standard statistical analyses were done, including one-way ANOVA with Scheffe's F test for multiple comparisons of groups and taking $p < 0.05$ as the level of significance.

3. Results

We found juvenile forms of the fluke in the liver parenchyma and mature forms in the bile ducts of rats at 7 wpi. The average of 10 mature flukes was recovered from the bile ducts at 10 wpi (mean ± SD: 10.0 ± 1.01).