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Short communication

The antioxidant defence mechanisms of parasite and host after chronic *Hymenolepis diminuta* infestation of the rat

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Abstract

The aim of the present study was to determine antioxidant defence mechanisms in the rat and *Hymenolepis diminuta* after long-term infestation. We determined levels of oxidative stress markers, and activity of antioxidant enzymes in the rat small intestine and in particular parts of *H. diminuta*. Observed changes in antioxidant enzymes activity in *H. diminuta* and the rat intestine indicate the defence against parasitic infestation and probably allowed parasite to adapt and live in oxidative stress.

Key words: *Hymenolepis diminuta*, rats, chronic infection, antioxidant defence mechanisms, enzymatic antioxidant system

Introduction

Inflammatory reaction induced by parasites e.g. *Hymenolepis diminuta* invasion leads to oxidative stress in small intestine of definitive host (Shin et al. 2009). Reactive oxygen species (ROS) produced by host's organism during parasitic infestation, mediate interactions between host and parasite (Dzik 2006, Barrett 2009). Many parasitic helminths may survive in host body for a very long period of time due to their high degree of antioxidant enzymes activity (Dzik 2006, Chiumiento and Bruschi 2009).

The aim of the present study was to determine the antioxidant defence mechanisms in definitive host and *H. diminuta* after chronic infestation in rats.

Materials and Methods

The study was performed on 6 mature male Lew/Han rats (aged 8-10 weeks). The animals were divided into 2 groups (n = 3 rats). One group was subjected to infestation with *Hymenolepis diminuta* larvae WSJ strain, lasting 1.5 years. The control group consisted of rats not infested with *H. diminuta* tapeworm larvae. Tapeworm specimens were divided into 5 fragments; immature proglottids; genital primordia; hermaphroditic proglottids, early and gravid uterus. The part of the small intestine, where scolex and proximal part of the tapeworm was located, was harvested for the study.

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Table 1. GSH and TBARS concentrations and activity of anti-oxidative enzymes in the rat small intestine (A) and in particular parts of *H. diminuta* (B) after long – term (1.5 years) infestation with *H. diminuta*.

(A)

Tested parametrs	GSH	TBARS	SOD1	SOD2	CAT	nonSe GSHPx	SeGSHPx	GST	GSHR
Rats non infested with *H. diminuta*	14.74 ± 1.28	0.15 ± 0.09	0.47 ± 0.1	0.32 ± 0.06	0.021 ± 0.001	0.09 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.06 ± 0.02
Rats infested with *H diminuta*	5.56 ± 2.2*	0.29 ± 0.10*	0.25 ± 0.05*	0.05 ± 0.01*	0.029 ± 0.006*	0.14 ± 0.02*	0.09 ± 0.02*	0.06 ± 0.02	0.16 ± 0.05*

* – statistically significant versus the small intestine of rats non infested with *H. diminuta* ($p \leq 0.05$)

(B)

Tested parametrs	GSH	TBARS	SOD1	SOD2	CAT	nonSe GSHPx	SeGSHPx	GST	GSHR
Immature proglottids	5.40 ± 1.00	3.20 ± 0.5	0.16 ± 0.06	0.12 ± 0.02	0.015 ± 0.002	0.09 ± 0.02	0.06 ± 0.02	0.32 ± 0.03	0.03 ± 0.01
Genital promordia	7.00 ± 2.50[a]	4.75 ± 0.6[a]	0.18 ± 0.08	0.05 ± 0.015[a]	0.014 ± 0.001	0.07 ± 0.01	0.04 ± 0.01	0.21 ± 0.09[a]	0.04 ± 0.02
Hermaphroditic proglottids	9.32 ± 3.30[a/b]	0.82 ± 0.43[a/b]	0.13 ± 0.02	0.04 ± 0.01[a]	0.009 ± 0.003	0.06 ± 0.02	0.04 ± 0.01	0.20 ± 0.05[a]	0.04 ± 0.02
Early uterus	13.20 ± 2.21[a/b]	1.42 ± 0.60[a/b]	0.17 ± 0.07	0.02 ± 0.01[a]	0.014 ± 0.003	0.05 ± 0.02	0.04 ± 0.01	0.22 ± 0.09[a]	0.04 ± 0.01
Gravid uterus	15.64 ± 4.89[a/b]	1.6 ± 0.35[a/b]	0.13 ± 0.03	0.06 ± 0.01[a]	0.007 ± 0.005	0.05 ± 0.01	0.03 ± 0.01	0.18 ± 0.09[a]	0.03 ± 0.01

[a] – statistically significant versus immature proglottids *H. diminuta* ($p \leq 0.05$)

[b] – statistically significant versus genital promordia *H. diminuta* ($p \leq 0.05$)

The results are expressed as arithmetical means of three independent experiments each carried out in triplicate ± S.D.

Abbreviations:

Animals (n-3) were infested *per os* with *H. diminuta* cysticercoids from *Tribolium destructor* (one cysticercoid of *H. diminuta* per animal). Strobilae and fragments of the small intestine were dissected from each rat (control and infested with *H diminuta*).

Concentration of GSH in μmol/mg protein and TBARS in nmol/mg protein; activity of enzymes in U/mg protein.

NonSe-GSHPx activity was determined using cumene hydroperoxide (CHP) as a substrate.

SeGSHPx activity was measured using H_2O_2 as a substrate

Determination of oxidative stress markers and antioxidant enzymes activity

Fragments of the rat small intestine and tapeworms were cut into smaller pieces, homogenized, centrifuged, and stored as previously described (Skrzycki et al. 2011). In supernatants obtained we determined the concentration of thiobarbiturate reactive substances (TBARS) and reduced glutathione (GSH), and the activity of antioxidant enzymes – superoxide dismutase: SOD1 and SOD2, catalase (CAT), selenium-dependent (SeGSHPx) and selenium-non-dependent (nonSeGSHPx) peroxidases, glutathione transferase (GST), and glutathione reductase (GSHR) according to previously described methods (Skrzycki et al. 2011).

Statistical analysis

Significance of differences was calculated by Student-t, Mann-Whitney U and ANOVA tests (significant if $p \leq 0.05$). Data were analysed by STATISTICA 9.0 program (StatSoft 9.0).

Results and Discussion

In the small intestine of infested rat lipid peroxidation increased, GSH concentration decreased, and activity of SODs decreased. Activity of GSHPxs and GSHR increased. The lowest activity we observed for CAT ($p \leq 0.05$) (Table 1A). In *H. diminuta* TBARS level was the highest in genital primordia, and the

lowest in hermaphroditic proglottids. The lowest level of GSH was in immature proglottids and the highest in the gravid uterus. Activity of SOD2 and GST was the highest in immature proglottids ($p \leq 0.05$), and activity of nonSeGSHPx decreased in subsequent parts of *H. diminuta.* Very low activity of CAT was determined particularly in hermaphroditic proglottids and gravid uterus (Table. 1B).

In our previous papers we reported differences between antioxidant systems in young and old forms of *H. diminuta* (Skrzycki et al. 2011). Similar results were also reported by other authors (Kosik-Bogacka et al. 2011). Now we obtained especially important and valuable results in parasite-host setting concerning the influence of long-term *H. diminuta* parasitosis on the antioxidant system in definitive host intestine, and simultaneously showing a defense strategy of parasite against oxidative stress. Chronic exposure of the rat intestine to direct or indirect contact with a tapeworm leads to weakening of antioxidant defence mechanisms. Profile of antioxidant enzymes activity in the intestine of infested rat and in particular parts of *H. diminuta* indicates the adaptation of definitive host to oxidative stress and defence against parasitic invasion. We found that *H. diminuta* is very well equipped in antioxidant mechanisms, indicating unique homeostasis of host-parasite setting, between two defensive reactions: host against parasite and parasite against host.

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