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Profile of Manganese Accumulation in the Host-Parasite (Rattus norvegicus-Fasciola hepatica) System after Manganese Salt Treatment

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The aim of our study was to evaluate the combined effect of manganese salt ($MnCl_2.H_2O$) and experimental *Fasciola hepatica* infection on the Mn-concentration in the system rat/ *F. hepatica*. Mn concentration was determined in the liver, kidney and the musculature of the host and as well as in F. *hepatica*, using ICP method. Results: the treated non-infected rats showed a significantly increased Mn level in the kidney and musculature, and slightly higher in the liver. In the infected rats, Mn-concentration in the liver was reduced, increased – in the musculature and non-changed in the kidney in comparison to those observed in the non-infected host. The *F. hepatica* isolated from the Mn treated rats showed double increased Mn concentration than that in the *F. hepatica* found in non-treated hosts. Conclusion: Mn homeostasis may play an important role at the rat/ *F. hepatica* interaction.

Key words: host-parasite system, Mn, bioaccumulation

Introduction

Manganese (Mn) is one of several first-order transition elements involved in many metabolic processes in animal organisms. This metal is involved in different biological activities dependent on multiple Mn-dependent enzymes [1]. Mn-enzymes have an important role in protecting cells from damage caused by free radicals and determined the oxidative status of animals. Infection with endoparasites affects the trace element balance due to redistribution among host tissues [3, 4].

Data from targeted studies on the effects of parasitic infection on host Mn homeostasis are scarce. This comparative study was conducted to determinate the Mn levels in the host (*Rattus norvegicus*) tissues and in the parasite (*F. hepatica*).

Material and Methods

Rat and parasite. The Wistar rats (Rattus norvegicus) used were aged 30 days, male and weighed 100 ± 10 g each. *Fasciola hepatica* used where obtained from laboratory maintained life cycle of the parasite, using *Galba truncatula* snails as intermediate host, and male Wistar rats as definitive host [2].

All animal experiments were carried out in accordance with the U.K. Animals (Scientific procedures) Act, 1986 and the associated guidelines EU Directive 2010/ 63/ EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No, 8023, revised 1978).

Experimental design. At the beginning of the experiment (day 0) rats (n = 40) were divided into 4 groups with 10 animals each (n = 10): 1st – control, uninfected, untreated; 2nd – uninfected, treated with $MnCl_2.4H_2O$, (Sigma-Aldrich US); 3rd – untreated, experimentally infected with *Fasciola hepatica*; 4th – infected with *F. hepatica* and treated with Mn salt. On day 0, each rat in groups 3 and 4 was experimentally infected orally with 15 viable *F. hepatica* metacercariae. All rats in groups 2 and 4 were treated with Mn salt solution through drinking water at a concentration of 0.323 mg Mn/ animal/day for 2 weeks before the end of experiment (day 60).

Sampling and analytical procedure. After exposure, rats (400 ± 20 g each) were anesthetized, slaughtered and dissected. Samples of liver, kidney and muscle (pectoralis major) as well as parasites, removed from the rats, were used to determine the concentration of Mn using Inductively coupled plasma-optical emission spectrometry (ICP – OES, VISTA MPX CCD Simultaneous VARIAN). The statistical analysis was carried out on the Prism 6 program. Variation analysis was used for determining the mean values, the standard deviation (SD) and the significance criterion (P). The comparison of the mean values of parameters was carried out using the one-way analysis of variance, Dunnett's Multiple Comparison Test. The results from these comparisons were also statistically significant: * ($p \leq 0.05$), ** ($p \leq 0.01$).

Results

The Mn content of host (Wistar rat) and parasite (*F. hepatica*) tissues after experimental exposure to Mn salt is presented in **Table 1.**

The content of Mn in the liver of all rats was the highest, compared to that of the other organs. The lowest concentration was found in the muscles. Treatment of control, non-infested rats with Mn salt increased the level of Mn significantly in the kidney and muscle, and insignificantly in the liver. The concentration of Mn in the liver of the invaded rats was decreased, in the kidney – unchanged, and increased in the muscle significantly compared to the control levels. The Mn content in the tissues of the infected rats was close to that of the controls, except for a twofold increase in Mn in the muscles. The concentration of Mn in the parasites isolated from the untreated rats was slightly increased compared to that in the parasitized liver. Parasites from treated rats showed a two-fold increase in Mn concentration compared to untreated rats. The concentration of Mn does not exceed the permissible concentrations in animals.

Table 1. Mn concentration values (µg/g d.m.) in host (Wistar rat) and parasite (*Fasciola hepatica*) tissues after treatment with $MnCl_2$. $4H_2O$).

Tissue / Groups	Liver	Kidney	Muscles	*F. hepatica*
1st gr. – control, untreated, non-infected	2.6 (±0.8)	1.3 (±0.4)	0.25 (±0.01)	
2nd gr. – treated, non-infected	3.0 (±0.11)	2.5* (±1.2)	0.56*(±0.04)	
3rd gr. – infected	1.15* (±0.06)	1.33 (±0.15)	0.42*(±0.07)	1.49 (±0.18)
4th gr. – infected and treated	2.71 (±1.1)	1.62 (±0.28)	0.5*(±0.06)	2.9** (±0.94)

Data were presented as means ± standard deviation. * Significant differences between tissues of rats in control and other groups ($p < 0.05$). ** Significant differences between *F. hepatica* tissues in 3rd and 4rd group ($p < 0.01$).

Discussion

This study investigates the content of Mn in the rat tissues (liver, kidney and muscle) affected by *F. hepatica* and supplied with Mn salts. Different concentrations of Mn were detected in the test samples. Comparing its content in the tissues of healthy rats, it is observed the higher concentration of Mn in the rat livers than that of rat kidneys and muscles. The highest level of Mn was measured in the parasite. Our results clearly show that liver fluke infection affects the host's metabolism by redistribution of Mn content among its tissues and accumulating Mn in the helminth tissues. In the present study, the experimental exposure to Mn salt to uninfected rats shows identified kidney and muscle as organs for Mn accumulation. Our results show a slight increase in the level of Mn in rat liver tissue, although the liver is considered to be the site that accumulates the highest metal content in organisms [1]. Comparing the Mn content in the tissues of healthy and *F. hepatica* – infected rats, an imbalance was found in the presence of parasite. The liver fluke infection induces the significant reduction of Mn in liver and enhancement in muscle tissues of its host. *F. hepatica* and the liver accumulate Mn^{2+} in similar extend. It shows that Mn^{2+} is involved in the metabolic processes in the parasite.

Conclusion

Rats chronically treated with Mn salt and infected with *F. hepatica* showed bio-redistribution of Mn among internal organs accompanied with Mn accumulation in the infected livers. The livers accumulated Mn regardless in the presence of the infection. Mn homeostasis may play an important role at the rat/ *F. hepatica* interaction.

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