
Research Note

Effects of *Echinostoma caproni* infections on metallic ions in the intestinal mucosa of ICR mice

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Abstract

Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used to study metallic ions in the intestinal mucosa of ICR mice infected with *Echinostoma caproni* and the mucosa of uninfected control mice. Infected mucosa ($n=9$ with about 100 mg wet weight per sample) were examined at 2 weeks p.i. in mice that were infected with about 25 worms per host. Uninfected mucosa ($n=9$ with about 100 mg wet weight per sample) were examined in the same time frame as the infected mucosa. Five metals were measured in the mucosa by ICP-AES analysis, as follows: calcium, potassium, magnesium, sodium and zinc. There were no significant differences (Student's t-test, $P>0.05$) in the concentrations of calcium, potassium or zinc in infected versus uninfected mucosa. The concentration of sodium was significantly greater ($P<0.05$) in the mucosa of infected versus uninfected mucosa, but the situation was reversed in regard to magnesium.

Fried & Huffman (1996) suggested that the *Echinostoma caproni*-mouse model is useful for studying the pathological effects of an intestinal trematode on a laboratory host. Using this model, Horutz & Fried (1995) showed that the infected intestinal mucosa of ICR mice had elevated amounts of free fatty acids compared with that of the controls. Rivas *et al.* (1998) demonstrated a significant decrease in the free sterols of the intestinal mucosa of infected ICR mice. Albrecht *et al.* (1998) showed a significant decrease in the concentrations of both phosphatidylcholine and phosphatidylethanolamine in the intestinal mucosa of infected ICR mice compared with the controls; they also noted an apparent increase in the cerebroside content of the mucosa of infected mice.

Little information is available on the effects of intestinal helminth parasitism on the metallic ion content of the

vertebrate intestine. Von Brand (1973) noted that the sodium and potassium content of the jejunal mucosa of rodents infected by *Nippostrongylus brasilienses* (Nematoda) were elevated. Similar studies on the effects of gastrointestinal trematodes on the metallic ion content of the vertebrate intestinal mucosa are not available. The purpose of this study was to examine the effects of intestinal parasitism with *E. caproni* on the metallic ion content of the mucosa of the ICR mouse.

Intestinal mucosa from ICR mice infected with *E. caproni* were obtained at 2 weeks p.i. as described in Frazer *et al.* (1997). Each exposed mouse ($n=9$) received about 50 cysts, and worm recovery per mouse averaged about 25 (approximately 50% of the cyst inoculum as reported in Hosier & Fried, 1991). Worms were removed and discarded, and the intestinal mucosa was scraped with a wooden applicator (Horutz & Fried, 1995). Approximately 100 mg (wet weight) was obtained from each infected host. Controls for the intestinal mucosa studies consisted of nine ICR mice not infected with

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E. caproni. These mice were necropsied at the same time as the experimentals, and the mucosal samples (about 100 mg per mouse) were prepared as for the experimentals.

Prior to use in an analysis, each sample was rinsed several times with ultrapure water (Milli-Q, Millipore, Bedford, Massachusetts) and digested in boiling nitric acid. Each digested sample was diluted to 10.0 ml with 2% (v/v) nitric acid. Sample solutions were analysed for 28 elements by ICP-AES using a Thermo Jarrell Ash (Franklin, Massachusetts) simultaneous-reading spectrometer with autosampler. The instrument was calibrated following EPA Method 6010A, which uses a two-point calibration, a blank, and multielement standards. Inter-element correction factors were employed to minimize any interferences between elements in samples. Reagent blank samples were also analysed. Each sample, standard and blank was analysed using three 30-sec integrations. The results for each sample were averaged and the reagent blank subtracted to calculate the final analysis values listed in table 1. A number of quality control checks were made during the analyses to verify the calibration curve, blank, and interelement correction factors.

Table 1 presents the data obtained from ICP-AES analysis of intestinal mucosa samples from infected and uninfected ICR mice. Each value in the table represents the average and standard deviation of a group of nine samples. The following 23 elements were found to be present below the level of quantification for the method and were not considered further: Ag, Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sb, Sn, Ti, Tl and V. The five elements that could be quantitatively measured are listed in table 1. The means were compared for significant differences between infected and uninfected mice using Student's *t*-test. Two elements (Na and Mg) were found to be significantly different ($P < 0.05$). Sodium was significantly greater in the mucosa of infected hosts (980 ± 100) compared to that in uninfected hosts (620 ± 120), whereas magnesium was significantly

less in the mucosa of infected hosts (72 ± 7) compared to uninfected hosts (106 ± 19). While the amounts of the other three elements showed variation, the differences were not significant ($P > 0.05$).

Comparative studies on metallic ions in the intestine of vertebrate hosts infected with adult trematodes are not available. Layman *et al.* (1996), using flame and graphite furnace atomic absorption spectrometry and ICP-AES, showed changes in some metallic ions in the digestive gland-gonad complex (DGG) of *Helisoma trivolvis* infected with larval stages of *E. trivolvis*. There was a significantly higher amount of sodium and significantly lower amount of magnesium in the DGG of the infected snails. It is interesting that in the present study, sodium was elevated significantly and magnesium decreased significantly in the intestinal mucosa of mice infected with *E. caproni*. Therefore, both larval and adult echinostomes were found to cause similar changes in metallic ions in target host sites.

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Table 1. Mean \pm standard deviation in $\mu\text{g g}^{-1}$ wet weight of intestinal mucosa of ICR mice as determined by ICP-AES.

Element	Uninfected	Infected	Value of <i>t</i>
Ca	198 \pm 73	290 \pm 110	0.96
K	1200 \pm 220	1020 \pm 90	1.23
Mg	106 \pm 19	72 \pm 7	2.60*
Na	620 \pm 120	980 \pm 100	3.30*
Zn	4.8 \pm 3.7	10 \pm 3	1.52

*Differences significant at $P < 0.05$ if $t > 1.83$.

Mice infected with *Echinostoma caproni* ($n = 9$) and uninfected mice ($n = 9$).

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