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Trace element levels in the liver of rats with acute and chronic fascioliasis and after treatment with zinc-copper hydroxochloride mixed crystals

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Abstract

Quantities of trace elements including copper, zinc, cobalt, manganese and iron were investigated in the liver tissue of rats at the acute or chronic stages of fascioliasis following treatment with zinc-copper hydroxochloride mixed crystals. Oral dosing (with food) of zinc–copper mixed crystals to healthy rats increased zinc and copper levels in the liver and decreased the iron content compared with controls. Manganese and cobalt levels did not change significantly. Significant reductions in all trace elements except manganese occurred in the liver of rats with acute or chronic fascioliasis. Manganese levels were slightly increased in rats at the acute stage and slightly decreased in rats at the chronic stage of fascioliasis. The application of mixed zinc–copper crystals at the acute or chronic stages of fascioliasis lead to a restoration of zinc and copper levels and a slight reduction in the iron levels in liver tissue of rats. The beneficial effects of applied salts were more apparent in rats chronically infected with *Fasciola hepatica*.

Introduction

The structural, functional and metabolic changes in host tissues caused during penetration, migration and localization of helminths in the host body, are associated with specific changes in trace element levels in body tissues, including the liver (Davtyan, 1982; Mansour *et al.*, 1985; Pienaar *et al.*, 1999).

Salts of copper (Cu) and zinc (Zn), e.g. $ZnSO_4.H_2O$ and $CuSO_4.5H_2O$ are used during parasite infections to correct these mineral deficiencies. However, the prolonged use of these salts often leads to undesirable side effects, such as depressed growth, higher mortality and vomiting (Vazquez *et al.*, 1990; Gabrashanska *et al.*, 1993).

Recently, experiments for treating mineral imbalances with basic salts of certain transitional elements or their mixtures indicated that the application of basic copper or zinc salts is better tolerated by the body than was the application of normal salts. Benefits associated with the application of basic salts persisted, even after more prolonged treatment of human and non-human hosts (Vazquez *et al.*, 1991; Galvez-Morros *et al.*, 1992; Gabrashanska *et al.*, 1993; Galvez-Morros *et al.*, 1995). In general, treatment with basic salts led to improved effects on the growth, survival and the balance of trace elements in the host, relative to normal salts.

The application of basic salts of transitional elements is not well investigated for the treatment of host trace element imbalances associated with infections by endoparasites. Data exist for the successful use of basic salts of Cu, Zn or Mn to correct body weight loss, mortality and parasite burdens in chicks infected with *Ascaridia galli* (Nematoda) (Gabrashanska *et al.*, 1993, 1999b; Galvez-Morros *et al.*, 1995). Also, these salts have been used to replace neutral Cu and Zn salts as therapeutic agents in

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ascaridiasis (Gabrashanska *et al.*, 1999a). No data exist for the effects of basic salts or their mixtures on hosts infected with other endoparasites.

The present study aims to examine the trace element content in the liver of rats with acute or chronic fascioliasis after treatment with Zn–Cu hydroxochloride mixed crystals $(Cu_{0.78}Zn_{0.22})_2(OH)_3Cl$.

Materials and methods

One hundred 30-day-old male albino Wistar rats were divided into four groups (I–IV). Each group was further divided into two treatment groups: A, rats treated with salts and examined at the acute stage of fascioliasis; and B, rats treated and examined at the chronic stage of the disease. Group I (A and B) acted as controls ($n_A = 7$; $n_B = 10$); group II (A and B) included rats treated with zinc-copper (Zn–Cu) hydroxochloride mixed crystals ($n_A = 8$; $n_B = 15$); group III (A and B) included rats experimentally infected with *Fasciola hepatica* (Trematoda) ($n_A = 10$; $n_B = 20$); and group IV (A and B) rats were infected with *F. hepatica* and treated with mixed Zn–Cu hydroxochloride mixed crystals ($n_A = 10$; $n_B = 20$). Rats in groups III and IV were infected orally with 15 metacercariae of *F. hepatica* on day 1 of the experiment.

Treatment with Zn–Cu hydroxochloride mixed crystals was initiated on day 20 post-infection (p.i.) in acute stage groups and on week 28 p.i. in chronic stage groups. The salt was applied in the food, daily, for 10 consecutive days. Daily doses of 3 mg and 6 mg Zn–Cu mixed crystals were administered to each rat from groups A and B, respectively (Galvez-Morros *et al.*, 1992). Rats were fed twice a day, with those from group A receiving 5 g food containing the Zn–Cu crystals in the morning and 10 g food in the afternoon. Rats from group B received 10 g food with Zn–Cu mixed crystals in the morning and 20 g food in the afternoon. Food given in the morning was ground and mixed with salts, whereas in the afternoon the food was granulated. The basic diet comprised: ground wheat 50%, ground barley 25%, fish flour 7%, bone meal 6%, dried brewer's yeast 5%, salt mixed 4%, vitamin mixed 2%, fish oil 1%. There were no Zn or Cu-salt supplements in the diet.

Mixed crystals $(Cu_{0.78}Zn_{0.22})_2(OH)_3Cl$ were synthesized by the method of continuous co-precipitation under standard conditions with a $pH \approx 7$ (Markov, 1987). Diluted solutions of zinc and copper chloride and sodium hydroxide were used. Crystals were highly soluble in mineral acids but not in water and their composition was determined by chemical X-ray and thermal analyses.

Rats at the acute and chronic stages of fascioliasis were killed on day 30 p.i. and week 30 p.i., respectively. Liver samples were dried for 24 h at 100°C, weighed, ground and then burned slowly for 48 h in a muffle furnace up to 480°C. The ashes were treated with a mixture of concentrated H_2SO_4 and HNO_3 (1:5) in a sand bath and wet residues were dissolved in 1 M HCl. The concentration of the trace elements zinc, copper, manganese, iron and cobalt in the rat liver were determined by atomic absorption spectrophotometry using a PU-900 (Pye Unicam) spectrometer (Cambridge, UK).

Trace elements were expressed as μg per g of dry liver tissue and a Student's t-test was used for statistical analyses of results.

Results

Quantities of trace elements Cu, Zn, Mn, Fe and Co in the liver and data for all rat groups are presented in tables 1 and 2. For all treatment groups the percent of variation ($V_{\%}$) ranged from 2.2% to 23%. Trace element levels in normal liver tissue were used as controls (groups IA and IB) in tables 1 and 2.

Levels of Cu and Zn significantly increased in rat liver tissue following treatment with Zn–Cu mixed crystals (groups IIA and IIB) ($P < 0.001$, $P < 0.001$ and $P < 0.001$, $P < 0.05$ respectively). The concentrations of Mn and Co in these groups were equivalent to control levels ($P > 0.1$), and Fe content was reduced ($P < 0.001$ and $P < 0.1$) (tables 1 and 2).

Rats at the acute stage of fascioliasis (group IIIA) showed significantly reduced levels of Zn ($P < 0.001$), Cu ($P < 0.01$), Fe ($P < 0.001$) and Co ($P < 0.001$), and slightly increased levels of Mn ($P < 0.01$) compared with controls (table 1).

Trace elements in the liver of rats with chronic fascioliasis (group IIIB) showed reduced levels of all trace elements compared with controls: Zn ($P < 0.001$),

Table 1. Liver trace element content and statistical analysis of data for rats treated with Zn–Cu hydroxochloride mixed crystals during the acute stage of fascioliasis.

Groups	Liver trace element content (μg per g dry tissue) and statistical analysis				
	Cu	Zn	Mn	Fe	Co
IA	23.10 ± 2.070	41.96 ± 1.760	5.37 ± 0.408	81.65 ± 1.695	0.57 ± 0.109
IIA	30.72 ± 2.096	48.02 ± 1.427	5.74 ± 0.662	72.97 ± 1.639	0.48 ± 0.111
	$P < 0.001$	$P < 0.001$	$P > 0.1$	$P < 0.001$	$P > 0.1$
IIIA	17.90 ± 1.133	21.35 ± 1.118	6.34 ± 0.484	45.89 ± 3.204	0.30 ± 0.024
	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$
IVA	28.05 ± 3.000	35.81 ± 1.579	5.23 ± 0.857	41.50 ± 1.746	0.26 ± 0.044
	$P < 0.02$	$P < 0.001$	$P > 0.1$	$P < 0.001$	$P < 0.001$
IIA/IIIA	$P < 0.001$	$P < 0.001$	$P > 0.1$	$P < 0.001$	$P < 0.01$
IIA/IVA	$P > 0.1$	$P < 0.001$	$P > 0.1$	$P < 0.001$	$P < 0.01$
IIIA/IVA	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.05$	$P < 0.10$

Table 2. Liver trace element level and statistical analysis of data for rats treated with Zn–Cu hydroxochloride mixed crystals during the chronic stage of fascioliasis.

	Liver trace element content (µg per g dry tissue) and statistical analysis				
Groups	Cu	Zn	Mn	Fe	Co
IB	22.50 ± 3.841	48.60 ± 4.435	1.78 ± 0.376	81.43 ± 3.900	0.41 ± 0.126
IIB	36.08 ± 2.776	54.62 ± 2.990	1.85 ± 0.403	73.32 ± 5.417	0.45 ± 0.125
	$P < 0.001$	$P < 0.05$	$P > 0.1$	$P < 0.1$	$P > 0.1$
IIIB	16.25 ± 1.498	33.18 ± 2.994	1.10 ± 0.354	44.19 ± 2.722	0.35 ± 0.091
	$P < 0.01$	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P > 0.1$
IVB	24.73 ± 2.866	42.83 ± 5.573	0.93 ± 0.061	42.13 ± 1.555	0.35 ± 0.077
	$P > 0.1$	$P > 0.1$	$P < 0.01$	$P < 0.001$	$P > 0.1$
IIB/IIIB	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P > 0.1$
IIB/IVB	$P < 0.001$	$P < 0.01$	$P < 0.01$	$P < 0.001$	$P > 0.1$
IIIB/IVB	$P < 0.001$	$P < 0.02$	$P > 0.1$	$P > 0.1$	$P > 0.1$

Fe ($P < 0.001$), Cu ($P < 0.01$), Mn ($P < 0.05$) and Co ($P > 0.1$) (table 2).

The liver of rats infected with *F. hepatica* and treated with Zn–Cu mixed crystals at the acute stage of the disease (group IVA) showed slightly increased levels of Cu ($P < 0.02$), decreased levels of Zn ($P < 0.001$), Fe ($P < 0.001$) and Co ($P < 0.001$) and unchanged levels of Mn ($P > 0.1$) compared with controls (table 1).

Levels of Cu and Zn were significantly higher in group IVA compared with group IIIA ($P < 0.001$) and Cu levels were almost equivalent to the levels in group IIA ($P > 0.1$). Manganese levels in group IVA were slightly lower than in group IIIA ($P < 0.05$) but, nonetheless, were close to those of controls ($P > 0.1$). Levels of Fe and Co in group IVA were reduced and were the lowest compared with all A-groups ($P < 0.001$) (table 1).

Trace element levels in rats infected with *F. hepatica* and treated with Zn–Cu crystals at the chronic stage of fascioliasis (group IVB) showed Cu, Zn and Co levels equivalent to those of controls ($P > 0.1$) but Fe ($P < 0.001$) and Mn ($P < 0.01$) were significantly reduced (table 2).

Levels of Cu and Zn in group IVB were significantly increased compared with group IIIB ($P < 0.001$ and $P < 0.02$ respectively). The level of Fe in group IVB was the lowest of all B-groups. Levels of Mn in group IVB and group IIIB were similar ($P > 0.1$), but they were lower than those recorded for groups IB and IIB. Levels of Co were the same in all groups ($P > 0.1$) (table 2).

Discussion

Results of the present study indicate that the application of Zn–Cu mixed crystals increases Cu and Zn levels in the rat liver. Increased Zn or Cu in liver tissue following experimental treatment with pharmacological doses of Zn or Cu normal salts have been reported for a range of animals (Keen *et al.*, 1985; Yu & Beynen, 1994; Du *et al.*, 1996). The well-known antagonistic interaction between Zn and Cu was not observed after an application of low doses of Zn and Cu simple salts (Nozdryuhina, 1977) or Zn–Cu mixed crystals. Absorption of Fe in hepatocytes declines during an application of Zn, Co or Cu normal salts. This effect is also observed following excess feeding with Zn, which leads to a lowering of Fe levels in the liver of some experimental animals (Fedosova, 1978; Storey & Greger, 1987). In the present study, reduced levels of liver Fe were observed in association with the feeding of Zn–Cu mixed crystals. Fedosova (1978) demonstrated that Co levels in rat liver were reduced by treatment with Cu salt but the present data indicates that the application of mixed Zn–Cu basic salt does not evoke any significant changes in Mn or Co levels in the liver of rats.

The present results relating to the status of liver trace elements at the acute stage of fascioliasis are in agreement with data in the literature showing reduced levels of Fe, Zn, Co and Cu and slightly increased levels of Mn in comparison with controls (Davtyan, 1982; Damyanova & Gabrashanska, 1988; Tsocheva & Gabrashanska, 1992). The low levels of Fe, Zn and Co are likely to be due to the disturbance of oxy-reduction processes in the liver cells and the increased permeability of cell membranes (Nozdryuhina, 1977; Fedosova, 1978). These data are consistent with observations demonstrating altered structures and biochemical processes in hepatocytes during the acute and chronic stages of fascioliasis, caused by the direct or indirect action of parasites as they migrate through the liver tissue or during their localization (Tsocheva, 1986; Tsocheva *et al.*, 1992a,b).

The effect of Zn–Cu mixed crystals on host tissues is not well investigated. Until now data are available only for treatment with single normal Zn or Cu basic salts, which maintain the balance of liver trace elements more effectively than treatment with single neutral Zn or Cu salts in *Ascaridia galli*-infected chicks (Gabrashanska, 1993; Gabrashanska *et al.*, 1993; Galvez-Morros *et al.*, 1995).

The application of Zn–Cu mixed crystals to rats during the acute stage of fascioliasis causes an increase in Zn and Cu levels compared with levels in infected but untreated rats. Levels of Cu in this group were restored to control levels, demonstrating the beneficial effect of the salts in compensating for any mineral deficiency. Concurrently, significantly reduced levels of liver Fe and Co were established. The reduced levels of liver Fe and Co after treatment of *F. hepatica* infected rats with Zn–Cu mixed crystals may be due to disturbances in oxy-reduction processes in the hepatocytes. Alternatively, the addition of Cu salts to the diet of rats with a deficit of Cu may lead to reduced Co levels in the liver by other compensatory mechanisms (Keen *et al.*, 1985). The reduced Co interferes with resorption of Fe by the intestine leading to reduced Fe levels in the liver (Keen *et al.*, 1985).

Additional studies are required to established the optimum Cu:Zn ratio for mixed Zn–Cu crystals for prophylactic and therapeutic application in rats and other mammals which are at risk for infection by *F. hepatica*.

In summary, treatment with Zn–Cu hydroxochloride mixed crystals has a beneficial effect on liver trace element imbalances associated with acute and chronic fascioliasis. Deficiencies of Zn and Cu in the liver of rats infected with *F. hepatica* were restored almost to control levels.

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References

Davtyan, E.A. (1982) On trace elements-like factors enhancing host-parasite relationships during helminthoses and in the possibility for their application for raising at animal production. *Biological Journal of Armenia* **30**(4), 2–5 (in Russian).

Damyanova, A. & Gabrashanska, M. (1988) Mineral contents of helminths and their hosts. I. *Fasciola hepatica* and tissues of *Bos taurus*. *Khelmintologiya* **26**, 3–9 (in Bulgarian).

Du, Z., Hemken, R.M., Jackson, J.A. & Trammell, D.S. (1996) Utilization of copper in copper proteinate, copper lysine, and cupric sulfate using the rats as an experimental model. *Journal of Animal Sciences* **74**, 1657–1663.

Fedosova, E. (1978) Dynamics of iron and cobalt content in some organs and tissues of white rats of different ages. pp. 181–184 in *Trace elements in medicine*. Zdorovya, Kiev (in Russian).

Gabrashanska, M. (1993) On the effect of copper salts (basic and neutral) during ascaridiosis on chicks. *Comptes Rendus de l'Academie Bulgare des Sciences* **46**, 97–99.

Gabrashanska, M., Galvez-Morros, M. & Garcia-Martinez, O. (1993) Application of small doses copper salts (basic and neutral) to *Ascaridia galli*-infected chicks. *Journal of Helminthology* **67**, 287–290.

Gabrashanska, M., Teodorova, S., Galvez-Morros, M. & Garcia-Martinez, O. (1999a) A kinetic model for *Ascaridia galli* populations in chickens treated with mixed salts of copper and zinc. *Journal of Helminthology* **73**, 45–50.

Gabrashanska, M., Tepavitcharova, S., Balarew, C., Galvez-Morros, M.M. & Arambarri, P. (1999b) The effect of excess dietary manganese on uninfected and *Ascaridia galli*-infected chicks. *Journal of Helminthology* **73**, 313–316.

Galvez-Morros, M., Garcia-Martinez, O., Wright, A. & Southon, S. (1992) Bioavailability in the rat of zinc and iron from the basic salts $Zn_5(OH)_8Cl_2.H_2O$, $Fe(OH)SO_4$ and $Fe_4(OH)_{11}NO_3.2H_2O$. *Food Chemistry* **43**, 377–381.

Galvez-Morros, M., Gabrashanska, M. & Garcia-Martinez, O. (1995) Comparison of the effects of basic and neutral zinc salts on chickens infected with *Ascaridia galli*. *Parasitology Research* **56**, 199–205.

Keen, C., Reinstein, N., Goudey-Lefevre, J., Lefevre, M., Lonnerdal, B., Schneeman, B. & Hurley, L. (1985) Effect of dietary copper and zinc levels on tissue copper, zinc and iron in male rats. *Biological Trace Element Research* **8**, 123–136.

Mansour, M.M., Francis, W.M. & Farid, Z. (1985) Prevalence of latent iron deficiency in patients with chronic *S. mansoni* infection. *Tropical and Geographical Medicine* **37**, 124–128.

Markov, L. (1987) Synthesis and thermal decomposition of Me(II)-Co(II) hydroxonitrate mixed crystals with layer structure type (Me=Mg, Ni, Cu, Zn). PhD thesis, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Nozdryuhina, L.R (1977) Biological role of trace elements in the constitution of animals and humans. Moscow, Nauka, 86–119 (in Russian)

Pienaar, J.G., Basson, P.A., du Plessis, J.L., Collins, H.W., Naude, T.W., Boyazoglu, P.A., Boomker, L., Reyers, F. & Pienaar, W.L. (1999) Experimental studies with *Strongyloides papillosus* in goats. *Onderstepoort Journal of Veterinary Research* **66**, 191–235.

Storey, M.L. & Greger, J.L. (1987) Iron, zinc and copper interactions: chronic versus acute responses of rats. *Journal of Nutrition* **177**, 1434–1442.

Tsocheva, N. (1986) Combined action of *Fasciola hepatica* infection and diethylnitrosamine intoxication on the rat liver. PhD thesis, Bulgarian Academy of Sciences, Sofia (in Bulgarian).

Tsocheva, N. & Gabrashanska, M. (1992) Trace element content of the rat liver after *Fasciola hepatica* infection and diethylnitrosamine intoxication. *Comptes Rendus de l'Academie Bulgare des Sciences* **45**(9), 115–117.

Tsocheva, N., Kadiiska, M., Yanev, S., Poljakova-Krusteva, O., Krustev, L. & Stoychev, T. (1992a) Changes in some parameters of liver drug metabolism in *Fasciola hepatica* infected and diethylnitrosamine injected rats. *Helminthologia* **29**, 39–42.

Tsocheva, N., Kadiiska, M., Yanev, S., Poljakova-Krusteva, O., Krustev, L. & Stoychev, T. (1992b) Combined effect of fascioliasis and diethylnitrosamine carcinogenesis on the activity of the rat liver monooxygenase system. *Comparative Physiology and Biochemistry* **101C**, 475–479.

Vazquez, C., Galvez-Morros, M., Millan, P. & Garcia-Martinez, O. (1990) Hidroxicloruro de zinc versus sulfato en la sobre carga oral de zinc. *Nutricion Clinica* **10**, 33–37.

Vazquez, C., Millan, P., Galvez-Morros, M. & Garcia-Martinez, O. (1991) Zinc serico y talla en una poblacion infantil de 5 a 15 anos. *Nutricion Clinica*, 158–163 Edition especial.

Yu, S. & Beynen, A.C. (1994) The combined effect of high iron and zinc intake on copper status in rats. *Biological Trace Element Research* **42**(1), 71–79.

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