Biol Trace Elem Res (2007) 118:38–42
DOI 10.1007/s12011-007-0023-y

Serum Concentration of Copper, Zinc, Iron, and Cobalt and the Copper/Zinc Ratio in Horses with Equine Herpesvirus-1

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Received: 4 December 2006 / Revised: 6 February 2007 / Accepted: 24 February 2007 /
Published online: 16 May 2007

Abstract The serum concentrations of copper, zinc, iron, and cobalt and copper/zinc ratio were investigated in horses infected with equine herpesvirus-1 (EHV-1). Nine horses were naturally infected with the virus and nine healthy horses served as controls. The concentrations of copper, zinc, iron, and cobalt were determined spectrophotometrically in the blood serum of all horses. The results were (expressed in micrograms per deciliters) copper 2.80±0.34 vs 1.12±0.44, zinc 3.05±0.18 vs 0.83±0.06, iron 2.76±0.17 vs 3.71±0.69, cobalt 0.19±0.37 vs 0.22±0.45, and copper/zinc ratio 0.72±0.38 vs 1.41±0.36 for control vs infected group, respectively. In conclusion, copper and zinc concentrations of the infected group were lower than the control group ($p<0.001$), whereas iron concentration and the copper/zinc ratio of the infected group were higher than the control group ($p<0.05$ and $p<0.001$). The cobalt concentration was not found to be statistically different between two groups. It might be emphasized that copper/zinc ratio was significantly affected by the EHV-1 infection, so it could be taken into consideration during the course of infection.

Keywords Copper/zinc ratio · Equine herpesvirus · Horse · Serum · Trace elements

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Introduction

The extent of the impairment in the immune system because of trace element deficiency is sufficient to increase the risk of morbidity and mortality because of viral, microbial, and parasitic infections [1].

Several trace elements are essential micronutrients and are required for various body functions and well-being of the immune system. The deficiencies of trace elements and infectious diseases often coexist and exhibit complex interactions [2].

Trace elements such as selenium, zinc, copper, and manganese have immunomodulatory functions and thus influence the susceptibility to the course and the outcome of a variety of viral infections. Some trace elements inhibit virus replication in the host cells, thus showing antiviral activity. Many trace elements act as antioxidants or help such functions that not only regulate immune responses of the host, but also may alter the genome of the viruses [2, 3].

The fact that serum trace mineral levels change in response to infections is well known. Suttle and Jones [4] have reported that deficiencies in minerals such as copper and zinc can decrease an animal's immune response, thereby increasing susceptibility to disease. Shankarand and Prasad [5] stated that zinc deficiency depresses immune function and increases the risk of viral infection.

The aim of this study was to determine whether there was any difference between the serum copper, zinc, iron, and cobalt concentrations and copper/zinc ratio in the horses infected with EHV-1 and healthy horses.

Materials and Methods

Anatolian type local horses (n=18) that were used for the experiment had a body mass of 300–400 kg and age up to 6 years old. The animals were maintained in a controlled environment (paddock) that mimicked their natural habitat. All horses under study were examined serologically by using serum neutralization test [6] and clinically. According to the neutralization test and clinical examination, nine horses were found to be infected with equine herpesvirus-1 (EHV-1), other nine horses were virus-free.

Blood samples were taken into plain vacutainer tubes from the jugular vein of the infected and control groups. The serum was separated and diluted with double-distilled water. The samples were then analyzed following established procedures for copper, zinc, iron, and cobalt and by means of a Unicam 929 Atomic Absorption Spectrophotometer [7, 8].

All analyses were made using the SPSS statistical software package. Data are expressed as means±SD. Statistical analyses were made using the independent sample t test.

Results and Discussion

The blood serum copper, zinc, iron, and cobalt concentrations and copper/zinc ratio in control and infected groups are given in Table 1.

The mean serum zinc and copper concentrations in the infected group were significantly lower than the control group (p<0.001). Meanwhile, the mean serum iron concentration and the mean copper/zinc ratio in the infected group were significantly higher than that in

Table 1 Serum Copper, Zinc, Iron, and Cobalt Concentrations and Copper/Zinc Ratio in the Control and Infected Groups

Trace elements	Control group (X±Sx)	Infected group (X±Sx)
Copper (μg/dl)	2.80±0.34	1.12±0.44**
Zinc (μg/dl)	3.05±0.18	0.83±0.06**
Iron (μg/dl)	2.76±0.17	3.71±0.69*
Cobalt (μg/dl)	0.19±0.37	0.22±0.45
Copper/Zinc	0.72±0.38	1.41±0.36**
n	9	9

*$p<0.05$

**$p<0.001$

the control group ($p<0.05$ and $p<0.001$). However, there was no statistically significant differences for the mean serum cobalt concentration between the two groups.

Equine herpesvirus-1 is an important pathogen in horses, which is characterized with epidemic and sporadic respiratory disease, abortion, and encephalitis. The virus causes economic loss to the horse industry [9, 10].

Clinical illness after a virus infection depends upon factors both in the virus and the host. The most important factor in a virus is genomic alterations. The factors in the host mostly depend upon the nutritional status and the optimum functioning of the immune system [11].

The roles of zinc and copper on growth, immune system function, cellular respiration, redox processes, and protein synthesis are well known, as is their ability to prevent cancer and other noncancer diseases [12–14]. Serum trace mineral levels change in response to infections is well known. Infections are known to affect the extracellular concentrations of zinc and copper.

Serum copper concentration of the patients with hepatic viral fibrosis, chronic hepatitis B virus infection, and viral hepatitis were reported to be higher than the control [15, 16]. In our study, the serum copper concentration of the infected group was lower than the control group. This result is contradictory with the result in literature.

Studies indicate that copper has no significant role during some of the infections. It has been suggested that hypocupremia and hypocuprosis are not consistent features of Border disease and thus have no etiological significance [17]. However, Arthington et al. [18] have demonstrated that copper deficiency alters the acute-phase protein response to viral infection and may affect lymphocyte responsiveness to mitogen stimulation in young cow inoculated with live bovine HSV-1.

The relevance of zinc in resistance to infections by viruses, fungi, and bacteria is recognized because of its pivotal role in the efficiency of the entire immune system, in particular, in conferring biological activity to a thymic hormone called thymulin, which has differentiation properties on T cell lines [19, 20].

It is known that zinc deficiency, resulting from exposure of culture cells to membrane-permeable Zn^{2+} chelators, can induce apoptosis in virally transformed cells, whereas normal cells remain unaffected under these conditions [2]. Zinc metalloproteins may be useful to prevent transmission of viral disease [21].

The serum zinc concentration has been studied by Fota-Markowska et al. [22] and Nagamine et al. [23] in patients with viral hepatitis, by Koch et al. [24] in patient with AIDS, and by Wellinghausen et al. [25] in patient with HIV infection. They reported decreased serum zinc concentrations in these viral infections.

The serum zinc and copper concentrations of the patients with viral hepatitis have been investigated by Kalkan et al. [26]. They stated the decreased serum zinc concentrations and the increased serum copper concentrations. Similar results in the calves were stressed by bovine respiratory disease and infectious bovine rhinotracheitis [27]. In the present study, zinc concentration in the infected group was significantly lower than in the control group.

The increased serum cobalt concentrations have been reported in mice infected with Coxsackie B virus infection [28]. On the other hand, Greig et al. [29] determined cobalt deficiency in young calves suffering from tick-borne fever virus infection. In the present study, no statistically significant differences were found in the serum cobalt concentrations between the groups.

The micronutrient iron plays a central role in many biochemical and physiological processes. However, iron is known to play a role in the susceptibility to and outcome of several infections. High intracellular concentrations of iron are generally associated with the potential to catalyze oxidative damage through the generation of hydroxyl radicals [30]. Lin et al. [31] observed that decrease in the iron level in the serum of patients having hepatocellular carcinoma was induced by the hepatitis B virus. We did find significantly higher iron concentration in the infected group.

As a rule, infections also cause an increase of the copper/zinc ratio. Some researches found an increase in the serum copper levels, but a decrease in the zinc levels in different diseases when compared with those of the control group. They also found markedly elevated copper/zinc ratios [31, 32]. However, Ciftci et al. [33] stated that even if the copper level was not affected during the treatment of tuberculosis, a significant increase in the levels of zinc and a decrease in the copper/zinc ratio were observed.

Although we found significantly lower serum copper and zinc concentrations, the copper/zinc ratio was higher in horses infected with EHV-1 when the compared to control group. In the infected group, serum zinc concentration was decreased more than copper, so the copper/zinc ratio was found higher than the control group. Actually, calculated values for decrease of copper and zinc were 2.5–3.65 times lower than control values, respectively.

The results indicated significant differences of trace elements concentration between infected and control groups. It might be emphasized that the significantly affected copper/zinc ratio could be taken into consideration during the course of EHV-1 infection.

References

1. Failla ML (2003) Trace elements and host defense: recent advances and continuing challenges. J Nutr 133:1443–1447
2. Chaturvedi UC, Shrivastava R, Upreti RK (2004) Viral infections and trace elements: a complex interaction. Curr Sci 87:1536–1554
3. Erickson KL, Medina EA, Hubbard NE (2000) Micronutrients and innate immunity. J Infect Dis 182:5–10
4. Suttle NF, Jones DG (1989) Recent developments in trace element metabolism and function: trace elements, disease resistance and immune responsiveness in ruminants. J Nutr 119:1055–1061
5. Shankarand AH, Prasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68:447–463
6. Stokes A, Corteyn AH, Muray K (1991) Clinical signs and humoral immune response in horses following equine herpes virus type-1 infection and their susceptibility to EHV-4 challenge. Vet Sci 51:141–148
7. Bancroff JD, Cook HJ (1984) Mammal of histological techniques. Livingstone, New York
8. Greenberg WE, Trusell RR, Clescer LS (1996) Standard methods for the examination of water and wastewater, 60th edn. American Public Health Association, Washington DC
9. Kydd JH, Townsend HG, Hannant D (2006) The equine immune response to equine herpesvirus-1: the virus and its vaccines. Vet Immunol Immunopathol 111:15–30

10. Taouji S, Collobert C, Gicquel B, Sailleau C, Brisseau N, Moussu C, Breuil FM, Pronost S, Borchers K, Zientara S (2002) Detection and isolation of equine herpesvirus 1 and 4 from horses in Normandy: an autopsy study of tissue distribution in relation to vaccination status. J Vet Med B 49:394–399
11. Knipe DM (1990) Virus–host–cell interactions. In: Virology. Raven Press, New York
12. Underwood EJ (1977) Trace elements in human and animal nutrition. Academic Press, New York
13. Schwartz LH, Urban T, Herzberg S (1994) Antioxidant minerals and vitamins. Role in cancer prevention. Press Med 23:1826–1830
14. Dede S, Değer Y, Mert N, Kahraman T, Alkan M, Keleş I (2003) Studies on the effects of x-ray on erythrocyte zinc and copper concentrations in rabbits after treatment with antioxidants. Biol Trace Elem Res 92:55–60
15. Hatano R, Ebara M, Fukuda H, Yoshikawa M, Sugiura N, Kondo F, Yukawa M, Saisho H (2000) Accumulation of copper in liver and hepatic injury in chronic hepatitis C. J Gastroenterol Hepatol 15:786–791
16. Pramoolsinsap C, Promvanit N, Kurathongs S (1996) Serum trace metal levels in patients with acute hepatitis B. Southeast Asian J Trop Med Public Health 27:476–480
17. Barlow RM, Suttle NF, Derbyshire MB, Gardiner AC, Rennie JC (1976) Copper metabolism in experiment Border disease. Vet Rec 98:86–88
18. Arthington JD, Corah LR, Blecha F (1996) The effect of molybdenum-induced copper deficiency on acute phase protein concentrations, SOD activity, leucocyte numbers, and lymphocyte proliferation in beef heifers inoculated with bovine herpes-virus. J Anim Sci 74:211–217
19. Mocchegiani E, Muzzioli M (2000) Therapeutic application of zinc in human immunodeficiency virus against opportunistic infections. J Nutr 130:1424–1431
20. Baum MK, Shor-Posner G, Campa A (2000) Zinc status in human immunodeficiency virus infection. J Nutr 130:1421–1423
21. Fernandez-Pol JA, Hamilton PD, Klos DJ (2001) Essential viral and cellular and iron containing metalloproteins as targets for novel antiviral and anticancer agents: implications for prevention and therapy of viral diseases and cancer. Anticancer Res 21:931–957
22. Fota-Markowska H, Przybyla A, Borowicz I, Modrzewska R (2002) Serum zinc (Zn) level dynamics in blood serum of patients with acute viral hepatitis B and early recovery period. Ann Univ Mariae Curie-Sklodowska Sect D Med 57:201–209
23. Nagamine T, Takagi H, Hashimoto Y, Takayama H, Shimoda R, Nomura N, Suzuki K, Mori M, Nakajima K (1997) The possible role of zinc and metallothionein in the liver in the therapeutic effect of IFN-alpha to hepatitis C patients. Biol Trace Elem Res 58:65–76
24. Koch J, Neal EA, Schlott MJ, Garcier-Shelton YL, Chan MF, Weaver KE, Cello JP (1996) Zinc levels and infections in hospitalized patients with AIDS. J Nutr 12:515–518
25. Wellinghausen N, Kean WV, Jochle W, Kern P (2000) Zinc serum level in human immunodeficiency virus-infected patients in relation to immunological status. Biol Trace Elem Res 73:139–149
26. Kalkan A, Bulut V, Avci S, Celik I, Bingol NK (2002) Trace elements in viral hepatitis. J Trace Elem Med Biol 16:227–230
27. Orr CL, Hutcheson DP, Grainger RB, Cummins JM, Mock RE (1990) Serum copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. J Anim Sci 68(9):2893–2900
28. Funseth E, Lindh U, Friman G, Ilback NG (2000) Relation between trace element levels in plasma and myocardium during coxsackievirus B3 myocarditis in the mouse. BioMetals 13:361–367
29. Greig A, Macleod NS, Allison CJ (1977) Tick borne fever in association with nurusol disease and cobalt deficiency in calves. Vet Rec 100:562–564
30. Weinberg ED (1990) Cellular iron metabolism in health and disease. Drug Metab Rev 22:531–579
31. Lin CC, Huang JF, Tsai LY, Huang YL (2006) Selenium, iron, copper, and zinc levels and copper-to-zinc ratios in serum of patients at different stages of viral hepatic diseases. Biol Trace Elem Res 109:15–24
32. Fota-Markowska H, Rolla-Szczepanska R, Modrzewska R, Lis-Tonder J, Borowicz I (2003) Copper/zinc ratio in measles patients. Ann Univ Mariae Curie-Sklodowska 58(1):291–296
33. Ciftci TU, Ciftci B, Yis O, Guney Y, Bilgihan A, Ogretensoy M (2003) Changes in serum selenium, copper, zinc levels and Cu/Zn ratio in patients with pulmonary tuberculosis during therapy. Biol Trace Elem Res 95:65–71