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The Interaction of Cadmium and Selenium in Horse Kidney Cortex in Relation to Histopathological Changes

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Junnila, M., H. Korkeala, T. Rahko and A. Salmi: The interaction of cadmium and selenium in horse kidney cortex in relation to histopathological changes. Acta vet. scand. 1987, 28, 201–208. – The kidney cortex of 32 Finnish horses was analysed chemically for cadmium (Cd) and selenium (Se) content and by light microscopy for histopathological changes of the tissues. Cd concentrations in kidney cortex ranged from 6.9 to 91.6 mg/kg wet weight with an average of 31.9 mg/kg. Se concentrations ranged from 0.5 to 1.5 mg/kg with an average of 1.0 mg/kg. The age of the horses varied from 1.5 to 32 years; mean age was 16 years.

Cd levels in kidney cortex seemed to increase linearly up to an age of about 16 years. In old (over 16 years) horses no such correlation could be found. Se concentrations were found to decrease in relation to age. The horses with higher than 1 mg/kg Se in kidney cortex had less PAS-positive casts than horses with low Se content. Among the total population of the horses no correlation could be demonstrated between Cd and Se concentrations in kidney cortex. However, the horses with more than 40 mg/kg Cd in their kidney cortex had a slightly positive correlation between Cd and Se concentrations.

proteinuria; heavy metal; slaughter material; age.

Introduction

Cadmium (Cd) is regarded as one of the most toxic elements that accumulates in the mammalian body. Although it is quite a rare element in nature, there is increasing data showing that the amount of Cd in the environment has been rising in recent years. The toxicity of Cd to animals and man is well known. The main changes in man are renal tubular lesions followed by proteinuria and skeletal disturbances (*Friberg et al.* 1974).

In kidneys cadmium is known to accumulate in the cortex (*Gunn & Gould* 1957). It is bound by a protein, metallothionein (*Kägi & Vallee* 1960). *Elinder et al.* (1981) found that histopathological renal changes in horse kidneys were related to Cd concentrations.

The interactions of Cd with different cations, complexes, and biological compounds have been intensively studied (eg. *Chen et al.* 1975, *Piotrowski et al.* 1977, *Gasiewicz & Smith* 1978, *Flegal et al.* 1980, *Chavez* 1981, *Mahaffey et al.* 1981, *Bozkurt & Smith* 1981, *Flora et al.* 1982, *Teilmann & Hansen* 1984, *Nielsen & Winge* 1985). However, the information of the possible interaction of Cd and Se in kidneys is scanty.

Chen et al. (1975) reported that Se decreased the Cd content of kidneys in rats. *Chavez*

(1981) demonstrated that dietary Se increased the Se levels in the kidneys of pigs, and when given simultaneously with Cd, Se increased also the Cd concentration of the kidney cortex. On the other hand *Flegal et al.* (1980) found no effect of dietary Se on the Cd concentration of kidney cortex of pigs. *Teilmann & Hansen* (1984) found no relationship between Cd and Se in the horse kidney cortex either.

The purpose of this work was to study the Cd and Se levels in the kidney cortex of Finnish horses in relation to age and histopathological changes.

Materials and methods

Sampling

The kidneys of 32 horses were collected from 3 different slaughterhouses: Kaupunginteurastamo, Helsinki; Ok-liha, Helsinki; and Maan Liha Oy, Kauhajoki. One half of the samples were collected from the Helsinki area (Kaupunginteurastamo, Ok-liha), One half from the Ostrobothnia area (Maan Liha Oy). The horses were regarded as healthy animals slaughtered for meat purpose. The data concerning the ages of the animals and the geographical areas where the horses originated from was obtained from the owners. The kidneys for the chemical analyses and histological samples were collected within 2 h after the animals had been slaughtered.

Chemical analyses

The samples were frozen and kept at –18°C until preparation for the chemical analyses took place. The Cd levels of the kidney cortex were analyzed by atomic absorption spectrophotometer (Perkin Elmer 5000, Ueberlingen, FRG) with the graphite oven technique by the method of *Stabel-Taucher et al.* (1977). The concentrations of the elements are given as mg/kg of wet weight.

Histological analyses

Two specimens of tissue, 1 cm × 1 cm × 1 cm each, were cut transversely opposite to the hilus area from the cortex of the kidneys. The samples were fixed in buffered 10 % formaldehyde solution. The fixed tissue samples were processed for paraffin sectioning and stained by the following staining methods: hematoxylin-eosin, periodic acid Schiff (PAS), and van Gieson and Goldner's trichrome staining for connective tissues (*Luna* 1968). The prepared samples were coded and analyzed by light microscopy for the occurrence of 5 histopathological parameters: periglomerular fibrosis, tubular proteinuria (PAS-positive casts), interstitial granulocyte infiltration, tubular dilatation and glomerular sclerosis. The histopathological changes were classified relatively from 0 (i.e. no changes) to 3 (i.e. marked changes:.

Statistical methods

The population of 32 horses was divided into different groups according to age and Cd and Se contents of the kidneys for data analysis (Table 1). The statistical calculations were performed using the Survo 76 statistical program (*Mustonen & Mellin* 1982).

Results

The age, Cd and Se concentrations and the histopathological changes in the kidney cortex of the horses are presented in Table 2.

The age of the horses varied from 1.5 to 32 years, mean age was 16 years. The Cd levels in the cortex varied from 6.9 to 91.6 mg/kg wet weight, mean level was 31.9 mg/kg. Se concentrations were between 0.5–1.5 mg/kg and mean concentration was 1.0 mg/kg.

The Cd concentrations in horse kidney cortex in relation to age are shown in Fig. 1. The correlation between Cd concentrations and age was 0.47 for young horses ($\leq$ 16 years old). For all the horses the correlation

Table 1. Sorting of the population for statistical analyses.

Group	Number of horses (= n)	Description of the group
Total population	32	
Young horses	15	≦ 16 years old
Old horses	17	> 16 years old
High Cd group	17	> 30 mg/kg Cd in renal cortex
Low Cd group	15	≦ 30 mg/kg Cd in renal cortex
High Se group	14	> 1 mg/kg Se in renal cortex
Low Se group	18	≦ 1 mg/kg Se in renal cortex

was 0.14. The Cd concentrations were somewhat lower (26.9 ± 13.7 mg/kg) for the horses slaughtered in the Ostrobothnia area than in the Helsinki area (36.3 ± 22.8 mg/kg), but the difference was not statistically significant. The high Cd group (> 30 mg/kg) had slightly more glomerular sclerosis than the low Cd group (≦ 30 mg/kg). The difference was not, however, statistically significant.

The Se concentrations in horse kidney cortex in relation to age are shown in Fig. 2. The Se levels decreased with age, the correlation was –0.50 ($p < 0.01$). The Se concentrations were also statistically lower ($p < 0.05$, Student's t-test) among the old horses than among the young horses when the groups were compared with each other. The Se levels were significantly lower in the horses from Ostrobothnia than in the horses from the Helsinki area ($p < 0.001$, Student's t-test). The horses from Ostrobothnia were also significantly older than the horses from the Helsinki area ($p < 0.01$, Student's t-test).

No statistically significant correlations between Cd and Se concentrations and periglomerular fibrosis, granulocyte infiltration or tubular dilatation could be found. However, when the PAS-positive casts (proteinuria) in relation to the Se concentrations were analyzed (Fig. 3), the correlation was –0.42 ($p < 0.05$). The high Se group (> 1 mg/kg) had significantly less PAS-positive casts than the low Se group (≦ 1 mg/kg) ($p < 0.01$, χ^2-test).

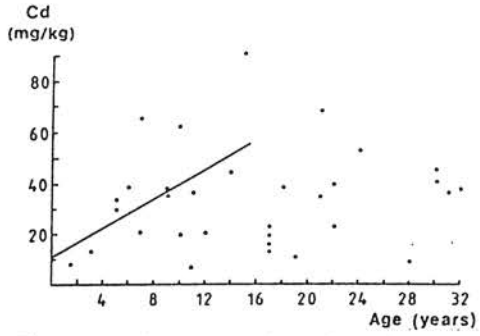

Figure 1. The concentration of cadmium (mg/kg w.w.) in horse kidney cortex as a function of age.

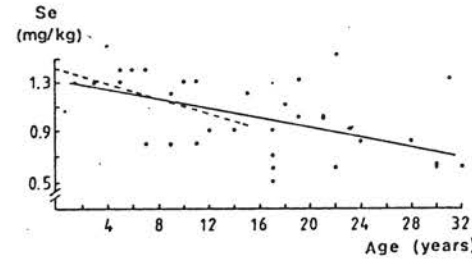

Figure 2. The concentration of selenium (mg/kg w.w.) in horse kidney cortex as a function of age. Solid line = total population, plotted line = young horses.

Table 2. Age, Cd and Se concentrations and the relative histopathological changes in the kidney cortex of the horses.

Horse No.	Age (years)	Cd (mg/kg)	Se (mg/kg)	Histopathological changes[a]				
				Peri-glomerular fibrosis	Granulo-cyte in-filtration	PAS-positive casts	Tubular dilation	Glomerular sclerosis
H6	1.5	7.9	1.3	1	3	3	2	2
H5	3	13.5	1.3	2	2	1	2	2
H28	5	30.0	1.3	1	2	1	2	3
H29	5	34.0	1.4	1	2	1	3	3
H1	6	39.1	1.4	0	3	3	1	2
H2	7	20.2	1.4	0	2	1	3	2
H4	7	65.7	0.8	0	2	1	2	2
H13	9	35.7	0.8	2	1	2	1	1
H30	9	38.4	1.2	1	2	2	3	3
H9	10	19.8	1.3	1	3	2	2	2
H7	11	6.9	1.3	2	2	3	2	2
H22	11	36.7	0.8	1	1	2	3	3
H11	12	20.2	0.9	2	2	2	1	2
H12	14	44.5	0.9	1	1	2	1	2
H32	15	91.6	1.2	2	1	1	2	2
H15	17	16.0	0.5	1	2	2	3	3
H17	17	19.6	0.7	1	1	2	1	1
H21	17	13.1	0.6	1	3	3	2	2
H25	17	22.6	0.9	3	2	2	2	2
H3	18	38.2	1.1	1	2	2	2	2
H14	19	10.3	1.3	3	1	1	2	2
H18	19	10.8	1.0	3	2	2	1	1
H27	21	68.8	1.0	1	2	2	2	3
H31	21	34.0	1.0	2	1	2	2	3
H20	22	39.2	1.5	2	1	2	1	2
H26	22	23.0	0.6	1	2	2	3	2
H8	24	52.7	0.8	2	2	3	2	3
H16	28	7.3	0.8	2	1	2	1	1
H23	30	40.6	0.6	1	1	3	1	2
H24	30	45.7	0.6	2	1	3	2	2
H10	31	36.7	1.3	1	2	1	1	2
H19	32	37.9	0.6	3	2	3	2	2

[a] 0 (= no changes) – 3 (= marked changes).

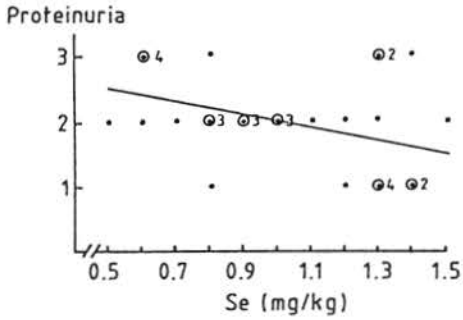

Figure 3. The PAS-positive casts in the tissue samples of horse kidney cortex in relation to the concentration of selenium (mg/kg w.w.). The circled data points include 2, 3 or 4 observations, respectively.

The relationship between selenium and cadmium concentrations in renal cortex is shown in Fig. 4. The correlation for the whole population was –0.05. For the young horses the correlation was 0.33 and for the old horses 0.11. The horses that had more than 40 mg/kg cadmium in the kidney cortex (n = 7) showed a statistically significant ($p < 0.05$) positive correlation (r = 0.86) between Cd and Se concentrations. Among these 7 horses a high correlation (r = 0.90) between age and proteinuria (PAS) was noticed as well ($p < 0.01$). Among the other horses (n = 25) the respective correlations were 0.12 and 0.09.

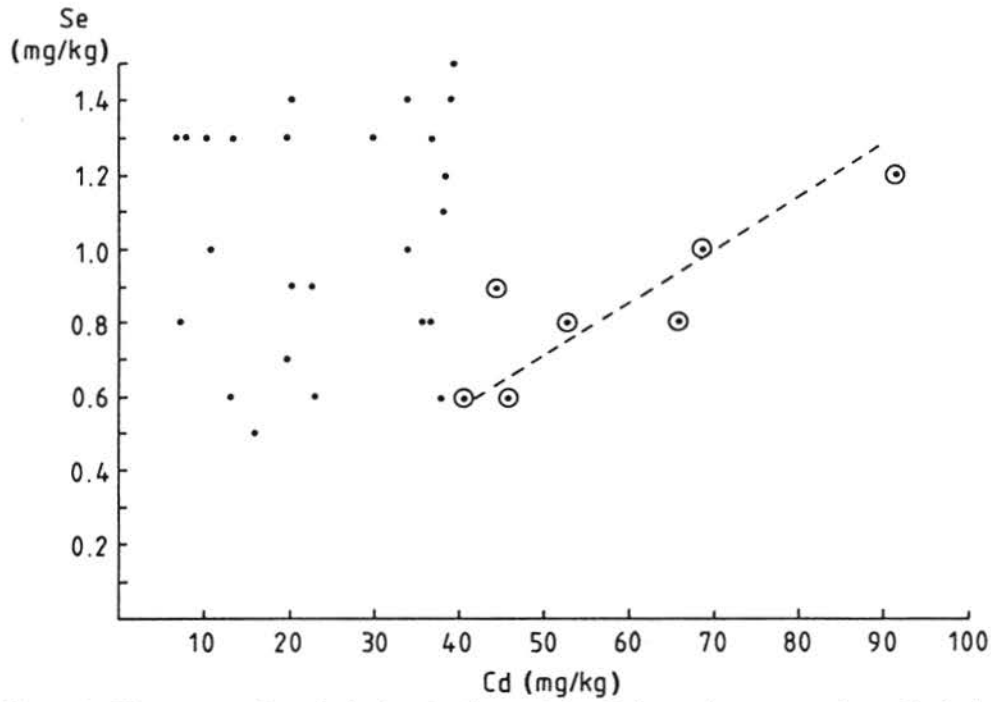

Figure 4. The concentration of selenium (mg/kg w.w.) in relation to the concentrations of cadmium (mg/kg w.w.) in horse kidney cortex of the 32 horses. Plotted line = horses with more than 40 mg/kg Cd in renal cortex (circled data points).

Discussion

The results of the present study show that the Cd concentrations in the kidney cortex of horses increase linearly with age to an age of about 15 years. Horses older than 15 years show a wide variation in kidney Cd levels, but no clear correlation was found. This is in agreement with the results of *Bjorland & Norheim* (1981) and *Teilmann & Hansen* (1984) who found that Cd concentrations in kidney cortex of horses increase in relation to age to a certain level. *Bjorland & Norheim* (1981) also found decreasing levels of Cd in renal cortex of older horses, so that the curve could be described as a parabola. In the present study this is not so apparent. The disappearance of the linear correlation in renal Cd concentration among the horses over 16 years old could be explained as the result of changes in the kidneys because of aging, toxins, kidney diseases, Cd exposure, etc.

Elinder et al. (1981) found that higher levels of cadmium in the kidney cortex of the horses also gave rise to a higher incidence of degenerative histopathological changes in the tissues. In this study no such obvious relationship could be found. The explanation for this might be either the different method of histopathological classification of the samples or the markedly higher levels of cadmium in the kidneys of the Swedish horses.

The observed decreasing trend of Se concentrations in kidney cortex could have been caused by regional factors, e.g. low Se levels in the soil in some parts of the Ostrobothnia area (*Koljonen* 1975). Whether horses came from areas with low Se in the soil or whether the observed phenomenon was independent of it remains open. However, a similar decreasing trend existed also in the group of young horses mainly from the Helsinki area. *Teilmann & Hansen* (1984) found no significant correlation between Se and age in Danish horses nor any regional distribution in Se levels of kidney cortex between different parts of the country. However, they had only 8 horses more than 12 years old in their material. The observed correlation between a low amount of PAS positive casts and high Se levels in the tissue samples in the present study might indicate that Se has a protective effect on tubular cells in the kidneys. The possible role of aging in proteinuria remains obscure.

No correlation between Cd and Se concentrations in horse kidney cortex for the whole material was observed. However, the horses with more than 40 ppm cadmium in their renal cortex seem to show some relationship. Because of the size of the population (N = 7) the finding gives only limited scope to draw conclusions. High Cd concentrations are known to induce proteinuria and losses of Cd in the urine (*Friberg et al.* 1974). Thus there might be interaction between Cd and Se after degenerative changes in the kidneys have been initiated by an excessive amount of Cd in the proximal tubules.

In this connection it is noteworthy that the slight linear relationship between age and Cd levels was lost after the horses had reached 16 years of age. The mean age of the above mentioned 7 horses was 20 years. Thus, the observed linear correlation between Cd and Se was found in that part of the population where the existence of pathological changes was quite obvious. The observed decreasing trend of Se levels in renal cortex and the possible interaction of Cd and Se in the kidneys with more than 40 ppm Cd levels require more research.

It can be concluded on the basis of the data presented that Finnish horses accumulate Cd in their kidney cortex during the early phase of their lives and that Se levels in the

kidney cortex decrease in relation to age. Also less proteinuria seems to exist in the presence of higher levels of Se in the kidney cortex. Some of the data also show a slight interaction between Cd and Se in the kidney cortex. The effect of certain uncontrolled factors (mineral concentrations in the soil, history of diseases of the animals, breed, sex etc.) on the results remains open, and there is a need for new studies to determine the final relevance of the present findings.

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Sammanfattning

Interaktion av kadmium och selen i njurarken hos hästar i relation till histopatologiska förändringar. Njurbarken hos 32 finska hästar analyserades med kemiska metoder rörande förekomsten av kadmium (Cd) och selen (Se) och med ljusmikroskopiska metoder rörande histopatologiska förändringar. Cd-koncentrationen i njurbarken varierade från 6.9 till 91.6 mg/kg våtvikt med ett medel-

värde av 31.9 mg/kg. Se-koncentrationen varierade från 0.5 till 1.5 mg/kg med et medelvärde av 1.0 mg/kg. Hästarnas ålder varierade från 1.5 till 32 år; genomsnittsåldern var 16 år.

Cd-halten föreföll att öka jämnt till 16 års ålder men hos gamla (över 16 år) hästar kunde ingen sådan korrelation påvisas. Se-koncentrationen minskade i relation til åldern. PAS-positiva ackumulationer förekom, lägre grad hos hästarna med hög (över 1 mg/kg) Se-koncentration än hos hästarna med låg Se-halt. I hela hästmaterialet kunde ingen korrelation påvisas mellan koncentrationerna av Cd och Se. Hästarna med mera än 40 mg/kg Cd i njurbarken visade en svag positiv korrelation mellan koncentrationerna av Cd och Se.

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