Bull. Environ. Contam. Toxicol. (1987) 38:969–974

Environmental Contamination and Toxicology

Effect of Iron Upon Cadmium–Manganese and Cadmium–Iron Interaction

Nevenka Gruden and Snježana Munić

Institute for Medical Research and Occupational Health, 41001 Zagreb, Yugoslavia

Increased cadmium production (Sherlock 1984) has enhanced the potential danger of this toxic metal including its effect upon the metab olism of some essential elements as, for instance, manganese and iron. Relevant data about the cadmium-manganese interaction are rather scanty (Sahagian et al. 1966, 1967; Doyle and Pfander 1975). In a study of the effect of cadmium on manganese metabolism we have found that a three-day treatment with a daily dose of 0.02 mg or more cadmium decreased significantly the manganese transfer and intestinal retention in five--week-old rats (Gruden 1985). Since there is more data of the effect of iron on the metabolism of either of these ions independently (Valberg et al. 1976; Gruden 1977a; Flanagan et al. 1980; Khandelwal et al. 1984; Leon and Johnson 1985) we decided to investigate how the presence of iron affected the interaction between cadmium and manganese and how cadmium alone or in combination with the additional iron affected iron transfer and retention in the intestinal wall.

MATERIALS AND METHODS

Five-week-old albino rats of 110 to 125 g body weight were used. Three types of experiments were performed in which experimental procedure was slightly different:
1/ The animals received the following doses of cadmium by gastric intubation: 0 (control), 0.002, 0.02, 0.2 or 2.0 mg Cd/d/rat for three days. For this period all animals were fed cow´s milk instead of stock diet.
2/ For three days the animals received the same cadmium doses as in Experiment 1. All experimental animals were given iron-fortified cow´s milk (10.0 mg Fe/100 ml) in the form of sulphate while the controls were fed plain cow´s milk.
3/ The animals were placed into five groups and fed 0.05 (control), 1.3, 2.5, 5.0 and 15.0 mg Fe/100 ml cow´s milk for three days. All animals, except the controls, received 0.2 mg Cd/rat daily by gastric intubation.

Send reprint requests to Nevenka Gruden at the above address.

In all cases, the animals had no solid food. Their milk consumption and body weight were recorded.

On the 4th day of the experiment the rats were killed and a segment of the duodenum was processed by the "everted gut sac" method (Wilson and Wiseman 1954). Samples were incubated in a modified Krebs-Ringer solution to which either $MnCl_2$ labelled with Mn-54 or Fe Cl_3 labelled with Fe-59 and sodium ascorbate were added. The same K. R. solution without manganese, iron or ascorbate was inside the tied duodenal segment. The manganese-54 or iron-59 content in the serosal and mucosal solutions and their retention in the intestinal wall were determined after incubation. The results were calculated as S/M (serosal over mucosal) activity ratios for ion transport, and as percentages of the initial mucosal solution activity for their intestinal retention. Student's t test was used to calculate the statistical significance of the differences between the groups within the same experiment.

To facilitate comparison between the control and experimental groups, the results for the latter are presented as percentages of the corresponding control values taken as 100 per cent.

RESULTS AND DISCUSSION

The results in Table 1/a show that when milk contained 10.0 mg Fe/100 ml both manganese transfer through and its retention within the intestinal wall were markedly inhibited in animals which had received cadmium irrespective of the dose. Cadmium doses from 0.002 up to 0.2 mg per day per animal lowered the manganese transfer by 85-90 per cent and its retention by 45 per cent. At the highest dose of 2.0 mg Cd these parameters of manganese metabolism were depressed only a little further.

When the cadmium dose was kept constant at 0.2 mg and the iron concentration in the milk varied from 1.3 to 15.0 mg Fe/100 ml, the transport and retention of manganese were also significantly depressed (Table 1/b). Again, manganese transport was inhibited more (by 75-84 per cent) than the retention (by 23-40 per cent).

Both cadmium and iron, administered separately, inhibit the transport and intestinal retention of manganese (Gruden 1977a; Gruden 1985). Since the two ions also inhibit each other's absorption (Schäfer and Forth 1984; Leon and Johnson 1985) cadmium and iron in combination can act upon manganese either synergistically, or by mutual interaction.

Table 1. Duodenal manganese-54 transfer and intestinal retention in cadmium and iron treated rats*

No of rats	Cd/day (mg)	Fe/100 ml (mg)	Transfer $\bar{x}$±S.E.	Retention
1/a.	Different cadmium doses, one iron dose			
9	0.002	10.0	13.10±1.06^{S}	56.27±1.50^{S}
9	0.02	10.0	13.02±0.87^{S}	54.55±2.45^{S}
8	0.2	10.0	11.47±1.05^{S}	55.81±2.16^{S}
9	2.0	10.0	5.97±0.54^{S}	48.23±2.22^{S}
1/b	Different iron doses, one cadmium dose			
8	0.2	1.3	23.93±2.75^{S}	77.13±5.95^{S}
8	0.2	2.5	24.55±2.07^{S}	74.53±3.23^{S}
9	0.2	5.0	15.45±0.97^{S}	60.73±2.64^{S}
7	0.2	15.0	16.26±0.78^{S}	62.76±1.99^{S}

*The transfer and retention data for experimental animals presented as percentages of the corresponding controls - taken as 100 per cent (Mean±S.E.)

^{S}Values bearing the superscript letter are significantly different from the corresponding control values ($P<0.05$).

The synergistic action of cadmium and iron on manganese is supported by the observation that the inhibitory effect of cadmium alone (Gruden 1985) is enhanced in the presence of iron (Table 1/a) and this is detectable even at the smallest doses of cadmium (0.002 mg Cd per animal, per day - Table 1/a) at which cadmium otherwise does not affect the manganese transport (Gruden 1985). A saturation (cadmium-iron) effect is observed when 10.0 mg, Fe/100 ml of milk is administered at all levels of cadmium. It seems that low doses of cadmium (up to 0.2 mg) leave some free sites for action of iron. Also, at two lower iron doses (1.3 and 2.5 mg Fe/100 ml - Table 1/b) the earlier observed (Gruden 1985) effect of 0.2 mg Cd (alone) was unchanged while at two higher doses (5.0 and 15.0 mg Fe/100 ml) there was an enhanced inhibitory effect of cadmium (Table 1/b and Gruden 1985). Additionally, within the range 5.0-15.0 mg Fe/100 ml milk the iron effect upon cadmium was unchanged (Tables 1/a and 1/b). This would appear to be another indication of iron saturation, as observed earlier (Gruden 1977a) at iron levels above 5.0 mg Fe/100 ml milk.

The effect of cadmium on iron transport and intestinal

retention appears to be somewhat different. For instance, the iron transport and retention are appreciably less sensitive to the presence of cadmium than is manganese (Gruden 1985; Table 1 and Table 2). In animals treated by cadmium alone (Table 2/a) only the highest cadmium dose (2.0 mg daily) decreased iron transfer significantly (by 72 per cent). There was no inhibitory effect on the iron intestinal retention. Moreover, when cadmium was administered alone in a dose of 0.02 mg, the iron transfer and intestinal retention were even stimulated. This stimulative effect of cadmium observed on the different ions (Sahagian et al. 1967; Gruden 1977b, 1985; Mas and Arola 1985) is of special interest and deserves further investigation.

Table 2. Duodenal iron-59 transfer and intestinal retention in cadmium (iron) treated rats*

No of rats	Cd/day (mg)	Fe/100 ml (mg)	Transfer $\bar{x}$±S.E.	Retention
2/a.	Different cadmium doses, no iron			
9	0.002	0	105.78±14.97^{s}	101.18±0.92^{s}
10	0.02	0	155.23±15.00^{s}	105.56±1.01^{s}
8	0.2	0	109.56±15.84^{s}	100.83±1.85
8	2.0	0	24.02± 4.26^{s}	99.75±1.65
2/b.	Different cadmium doses, one iron dose			
15	0.002	10.0	43.08 ± 5.26^{s}	103.29±6.35
16	0.02	10.0	35.48 ± 3.13^{s}	97.60±5.22
16	0.2	10.0	40.31 ± 4.40^{s}	97.47±5.07
2/c.	Different iron doses, one cadmium dose			
10	0.2	1.3	32.02 ± 3.80^{s}	97.11±1.20^{s}
9	0.2	2.5	37.69 ± 5.05^{s}	92.87±1.69^{s}
8	0.2	5.0	29.74 ± 3.42^{s}	92.93±1.78^{s}
8	0.2	15.0	26.03 ± 2.25^{s}	89.06±1.50^{s}

*,^{s}See the same note in Table 1.

Iron transfer was inhibited markedly and to the same level (by 57-65 per cent) in all animals which had received cadmium, irrespective of its dose, and were fed milk containing 10.0 mg Fe/100 ml (Table 2/b). When the cadmium dose was unchanged (0.2 mg daily) and iron concentration in the milk varied (from 1.3 to 15.0 mg/100 ml), the transfer of iron was also significantly decreased

(by 67-75 per cent) and always to the same level, irrespective of the iron concentration (Table 2/c). All this indicates a saturation effect by iron alone independent of the synergistic action of both cadmium and iron.

The variance in response of iron and manganese to either cadmium alone or to the cadmium-iron combination might be explained by differences in their metabolism. The intestinal mucosa is predominant in the regulation of iron homeostasis by adapting iron absorption to the body's need (Nathanson et al. 1985; Finch and Huebers 1986). This does not apply to manganese and cadmium.

It should be noted that the effect of cadmium (alone or in combination with iron) is much less on manganese retention than on its transfer (Table 1 and Gruden 1985), and that it has practically no effect on iron intestinal retention (Table 2). This suggests that the binding sites for the transport of these ions are not the same as those for their retention in the intestinal mucosa.

These studies showed that the administration of 5.0-15.0 mg iron to 100 ml dietary milk either enhanced the already present inhibitory effect of cadmium upon manganese absorption or, at the lowest cadmium doses, inhibition was provoked by the iron. Iron itself exhibited a higher sensitivity to its own presence than to that of cadmium. A simple rationalization of our results could be that there is a competition for the transport route through the intestinal wall between cadmium and iron on one side, and between iron and manganese on the other. Their competition, i.e. absorption from the intestine probably depends on their relative concentration and the kinetics of and affinity for their interaction with the binding sites in the mucosa.

Acknowledgments. Supported by the Scientific Research Council of S.R. Croatia. The authors thank Dr. G.E. Harrison for his help in the final stage of the preparation of the manuscript, Mrs. K. Pribić for her technical assistance and Mrs. K. Ferčec and D. Vasiljević for their care and handling of animals.

REFERENCES

Doyle JJ, Pfander WH (1975) Interaction of cadmium with copper, iron, zinc and manganese in ovine tissues. J Nutr 105:599-606

Finch CA, Huebers HA (1986) Iron metabolism. Clin Physiol Biochem 4:5-10

Flanagan PR, Haist J, Valberg LS (1980) Comparative effects of iron deficiency induced by bleeding and a low-iron diet on the intestinal absorptive interactions of iron, cobalt, manganese, zinc, lead and cadmium. J Nutr 110:1754-1763
Gruden N (1977a) Suppression of transduodenal manganese transport by milk diet supplemented with iron. Nutr Metab 21:305-309
Gruden N (1977b) Influence of cadmium on calcium transfer through the duodenal wall in rats. Arch Toxicol 37:149-154
Gruden N (1985) Cadmium-manganese interaction in the rat's intestinal tract. In: Lekkas TD (ed) Heavy metals in the environment, vol. 1. Athens, p 676
Khandelwal S, Ashquin M, Tandon SK (1984) Influence of essential elements on manganese intoxication. Bull Environ Contam Toxicol 32:10-19
Leon L, Johnson DR (1985) Role of iron in jejunal uptake of cadmium in the newborn rat. J Toxicol Environ Health 15:687-696
Mas A, Arola LL (1985) Cadmium and lead toxicity effects on zinc, copper, nickel and iron distribution in the developing chick embryo. Comp Biochem Physiol 80:185-188
Nathanson MH, Muir A, McLaren GD (1985) Iron absorption in normal and iron-deficient beagle dogs: mucosal iron kinetics. Am J Physiol 249:G439-G448
Sahagian BM, Harding-Barlow I, Mitchell Perry H Jr (1966) Uptakes of zinc, manganese, cadmium and mercury by intact strips of rat intestine. J Nutr 90:259-267
Sahagian BM, Harding-Barlow I, Mitchell Perry H Jr (1967) Transmural movement of zinc, manganese, cadmium and mercury by rat small intestine. J Nutr 93:291-300
Schäfer SG, Forth W (1984) Effect of acute and subchronic exposure to cadmium on the retention of iron in rats. J Nutr 114:1989-1996
Sherlock JC (1984) Cadmium in foods and the diet. Experientia 40:152-156
Valberg LS, Sorbie J, Hamilton DL (1976) Gastrointestinal metabolism of cadmium in experimental iron deficiency. Am J Physiol 231:462-467
Wilson TH, Wiseman G (1954) The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J Physiol 123:116-125
Received December 15, 1986; accepted February 5, 1987.