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Influence of Cadmium on Calcium Transfer through the Duodenal Wall in Rats

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Abstract. Five-week-old female albino rats were given different doses of cadmium chloride by gastric intubation daily for 1 (or 2) weeks. They were killed on 8th (or 15th) day of the experiment. Calcium-45 was used as marker to assess calcium transfer through and its retention in the duodenal wall by the everted gut sac method of Wilson and Wiseman. In all animals liver, kidney and femur wet weight was also determined.

There was no significant difference in any of the parameters between rats receiving no cadmium and those who daily received 0.002 or 0.02 mg cadmium. Significantly increased calcium transfer was observed in rats given daily 0.2 mg of cadmium.

A daily dose of 2.0 mg cadmium significantly decreased calcium transfer, an effect which was independent of whether the rats were killed on 8th or 15th day of the experiment. The same effect, with a significantly decreased uptake of ^{45}Ca in the duodenal wall and heavier liver was a result of administration of 15 mg of cadmium (divided in two doses).

Kidneys and femurs were lighter after 7 days of treatment with 2.0 mg of cadmium, but the same cadmium dose over a fortnight produced a significant increase in the weight of kidneys.

The results are discussed in the light of direct cadmium-induced membrane alterations and those which are due to inhibited activation of vitamin D.

Key words: Cadmium – Calcium-transfer – Duodenum.

Zusammenfassung. Fünf Wochen alte Weibchen der weißen Ratte erhielten täglich in der Dauer von einer (oder zwei) Wochen mittels der Magensonde verschiedene Dosen von Kadmiumchlorid und wurden am 8. (oder 15.) Tage des Versuches getötet. Kalzium-45 wurde zum Kennzeichnen zwecks Einsicht des Transportes von Kalzium in der Darmwand und sein Verbleiben in der Wand des Zwölffingerdarmes mittels der Methode des „umgestülpten Darmsackes" verwendet. Allen Versuchstieren wurde das Naßgewicht der Leber, Niere und Femur bestimmt.

In keinem von diesen Parametern waren bedeutende Unterschiede zwischen den Ratten, die kein Kadmium und jenen, die täglich 0,002–0,02 mg Kadmium erhielten. Der Transport des Kalziums durch die Duodenalwand wurde bei den Ratten, die innerhalb von 7 Tagen 0,2 mg Kadmium erhielten, erheblich vergrößert.

Die Dosis von 2,0 mg Kadmium täglich verminderte den Transport des Kalziums erheblich, unabhängig davon, ob die Tiere am 8. oder 15. Tag der Probe getötet wurden. Derselbe Effekt bei bedeutend verminderter Aufnahme von ^{45}Ca durch die Zwölffingerdarmwand und schwerere Leber wurden auch nach einer Gesamtdosis von 15 mg Kadmium (aufgeteilt in zwei Dosen) beobachtet.

Die Nieren und Femur waren bei den Tieren leichter, die während 7 Tagen je 2,0 mg Kadmium erhielten, während die gleiche Dosis 14 Tage hindurch angewandt bedeutend schwerere Nieren verursachte.

Die Resultate wurden im Aspekt der Veränderungen in der Membrane diskutiert, welche durch Anwendung von Kadmium – sei es direkt oder durch die Inhibition der Aktivierung von Vitamin D – entstehen.

Although cadmium is not considered to be as important a health hazard to man as lead it must be nevertheless regarded as a dangerous pollutant. Human beings and domestic animals accumulate considerable amounts of cadmium during their lifetimes, mostly in the kidneys and livers (Webb, 1972; Sugawara and Sugawara, 1974; Nomiyama et al. 1975). The most common effect of cadmium exposure is renal dysfunction (Fassett, 1975; Nomiyama et al., 1975).

Some authors (Larsson and Piscator, 1971; Itokawa et al., 1974; Washko and Cousins, 1975) demonstrated the interrelationship of dietary calcium level and cadmium toxicity, indicating that low dietary calcium could increase the susceptibility to the deleterious effects of environmental cadmium.

Methods

One hundred and sixty-two rats 5 weeks old, with body weights ranging from 90–120 g were fed a standard laboratory diet (1.1% Ca, 0.65% P). They received drinking water ad lib. The animals were divided into 8 groups. The number of animals in each group and the daily dose of cadmium (as cadmium chloride administered in 1 ml redistilled water) are given in Table 1. On 7th or 14th day, after the last gastric intubation of cadmium, the rats were deprived of food as were the controls (no cadmium) and killed the following day by decapitation. The duodenum of each rat was processed by Wilson and Wiseman's method for the everted gut sac (1954). Ten microcuries of carrier-free ^{45}Ca (Radiochemical Centre, Amersham, England) was added to 100 ml of buffer solution which contained: 135 mM NaCl, 11 mM KCl, 0.05 mM $CaCl_2$ and 10 mM sodium phosphate buffer pH 7.4. After 45 min incubation the empty duodenal segments were ashed at 600° C for 18 h and dissolved in warm hydrochloric acid. Calcium-45 was assayed in the same way as in the serosal and mucosal solutions.

In all animals wet weights of the livers, kidneys (both) and femurs (right) (carefully separated from the adjacent tissues) were determined.

In rats treated with cadmium during a fortnight the food consumption and body weight gain were recorded regularly for the control and experimental animals throughout experiment.

Results

A. Seven-Day Treatment with Different Doses of Cadmium

The number of animals in each group and the mean ratio of the serosal to the mucosal content of ^{45}Ca together with ^{45}Ca intestinal content are given in Table 1. There was no significant difference in the transport ratio (S/M) for ^{45}Ca between the control rats (receiving no cadmium) and those receiving 0.002 or 0.02 mg cadmium per day. For the rats given 14 mg of cadmium divided into 7 equal doses or 15 mg of cadmium divided to 2 doses (10 and 5 mg/dose), the calcium transfer was significantly decreased ($P > 0.01$ and $P < 0.001$ respectively).

In animals which received 0.2 mg of cadmium daily through seven days calcium transport was increased ($P < 0.01$).

As for the ^{45}Ca uptake in the duodenal wall (Table 1) only the highest cadmium dose caused a diminution ($P < 0.001$).

Table 1. The mean ratio (S/M) of ^{45}Ca in the serosal, S, and in the mucosal fluid, M, and % of initial mucosal ^{45}Ca activity in the gut wall after cadmium application by gastric intubation

No. of rats	Cd/day (mg)	^{45}Ca S/M ± S.E.	^{45}Ca intestinal content (% ± S.E.)
33	0	2.62 ± 0.13	33.63 ± 0.73
26	0.002	2.26 ± 0.18	32.93 ± 1.15
18	0.02	2.42 ± 0.22	35.09 ± 1.14
20	0.2	3.32 ± 0.19*	33.89 ± 0.57
19	2.0	2.05 ± 0.13*	35.33 ± 1.06
12[a]	15.0	1.45 ± 0.17*	25.95 ± 1.83*

[a] Rats were given cadmium for 2 days: 10 mg the first, 5 mg the second day. They were killed 6 days later. In all other experiments the rats were given cadmium for 7 days and were killed on 8th day

* $0.01 > P > 0.001$

Table 2. Liver, kidney and femur wet weight per 100 g body weight

No. of rats	Cd/day (mg)	Liver (g)	Kidneys (mg)	Femurs (mg)
34	0	3.234 ± 0.030	791.4 ± 8.9	294.2 ± 4.2
28	0.002	3.159 ± 0.030	779.1 ± 7.9	296.9 ± 5.5
18	0.02	3.191 ± 0.045	784.6 ± 11.1	284.6 ± 4.7
20	0.2	3.212 ± 0.060	790.3 ± 18.3	304.1 ± 10.4
19	2.0	3.299 ± 0.038	761.4 ± 7.6*	277.2 ± 2.5*
12[a]	15.0	3.452 ± 0.083**	802.6 ± 14.0	300.8 ± 5.0

[a] Same as under Table 1

* $0.01 > P > 0.001$

** $P < 0.02$

Table 3. Calcium transfer through and content in duodenal wall and liver, kidney and femur wet weight

		Controls	Experimental animals
^{45}Ca transfer (S/M)		2.31 ± 0.19	1.38 ± 0.16*
^{45}Ca content (% of initial mucosal activity)		30.69 ± 0.87	30.50 ± 1.18
Kidneys	(mg/100 g body weight)	759.1 ± 11.5	817.2 ± 25.5**
Femur	(mg/100 g body weight)	304.4 ± 4.2	293.8 ± 9.3
Liver	(g/100 g body weight)	3.269 ± 0.067	3.267 ± 0.073

Experimental animals were given 2.0 mg cadmium daily for 14 days and were killed on 15th day. All results are expressed as arithmetic means with standard errors for groups of 15–19 animals
* $P < 0.001$
** $P < 0.05$

The wet weights of the livers, kidneys and femurs are shown in Table 2 expressed (in mg or g) per 100 g of body weight. The animals which received 7 × 2.0 mg of cadmium had significantly lighter kidneys and femurs ($P > 0.01$ and $P < 0.001$ respectively), while in animals which received 2 higher doses totalling 15 mg Cd, the liver was significantly heavier ($P < 0.02$).

B. A Fortnight Treatment with 2.0 mg Cadmium Daily

On the average, an animal from the control group consumed 11.9 ± 0.3 g food/day throughout the experiment. Experimental animals consumed 7.2 ± 0.2 g food per day during the first four days, 9.5 ± 0.3 g per day from 4th–8th day, and 10.8 ± 0.2 g per day till the end of the experiment.

After a slight (~ 2%) initial drop, the experimental animals showed an increase in their body weight. Nevertheless, at the end of the experiment they were less heavy than the controls ($P > 0.02$). Their weight amounted to the 90% of the body weight of the control group.

From the 10th day onwards the abdomen of experimental animals enlarged, and their movements became sluggish (clumsy). No other change in the condition of cadmium treated animals was noticed during the experiment.

The influence of a 2-week treatment with 2.0 mg of cadmium daily on calcium content and transfer through the duodenal wall, as well as on the wet weight of the kidneys, liver and femurs are given in Table 3. Cadmium treatment significantly decreased calcium transfer ($P < 0.001$), while the kidneys became heavier ($P < 0.05$). Other parameters were not significantly changed.

Discussion

Cadmium may have influenced calcium transport at least in 2 ways: (a) by altering directly the transport properties of the duodenal wall, and/or (b) by inhibiting vitamin D hydroxylation in the kidneys and liver because this vitamin is known to play an

important role in the calcium membrane-transport. It is not possible to separate firmly the two alternatives so we shall discuss them together.

The method used in this study will register, in vitro, alterations in ^{45}Ca transport through the duodenal wall if the in vivo pretreatment with cadmium caused any lasting changes in the membrane. The results from Tables 1 and 2 as well as the behaviour of the animals during the pretreatment period indicate that with doses up to 0.02 mg Cd/day no lasting changes occurred. Former investigators (Sugawara and Sugawara, 1974) studied the cadmium effect during longer periods of time and the accumulation of cadmium in the kidneys and liver was proportional to the applied doses. The total amount of cadmium administered was of the same order of magnitude as in our study up to the threshold-dose of 0.02 mg per day.

It thus appears that below this amount cadmium may freely pass across the intestinal (duodenal) membrane and bind to the cadmium-thionein in the kidneys. It is known that the biosynthesis of this "scavanger"-protein is induced by increased levels of cadmium in the body (Webb, 1975) which thus counteracts the deleterious presence of cadmium. One of its effects (shown in vitro) is the inhibition of the hydroxylation by which vitamin D is activated from its precursor in the kidneys (Feldman and Cousins, 1973). In vivo, the same amount of cadmium exerts no inhibition just because of the protective binding of cadmium to thionein so that in our experiments vitamin D effect on calcium-transport can be operative with low cadmium-doses. With higher doses the trapping action of thionein may not suffice and the cadmium present in excess may block the activation of vitamin D which, in turn, will diminish calcium transport.

All the other results in the present study show convincingly that higher doses of cadmium cause lasting changes in the membrane. The dose of 0.2 mg cadmium/day applied for one week enhanced calcium-transport, but produced no change either in calcium-content in the membrane (see Table 1) or in relative weights of liver, kidneys and femur (Table 2). This we cannot explain. Further experiments may provide a clue as to whether this dose is really a transitory one (with an unknown interplay of several counteracting effects) towards the diminished calcium-transport observed with higher doses.

For instance, the application of 2 mg Cd/day for a week diminished calcium-transport without causing any changes in calcium-content in the gut wall and liver weights, but diminished weights of kidneys and femurs. When the same daily-dose was applied for a fortnight calcium-transfer was even more depressed. Its content in the gut wall and the liver weights did not change, nor did the weight of femurs, while the relative weight of the kidneys increased (Table 3). It seems therefore safe to conclude that daily doses of 2 mg Cd/day up to a fortnight definitely alter the membrane transmittance of ^{45}Ca and cause changes in the relative weight of the kidneys.

Washko and Cousins (1975) showed that more cadmium is accumulated in the intestinal mucosa when calcium content in the food is lower. It is possible that there is a transport route for which cadmium and calcium compete so that at fairly large concentrations (such as here) cadmium will use this route (i.e. the protein binding-sites) at the expense of calcium, hence diminishing S/M ratio for calcium. Supporting evidence for this may be our finding (Table 1) that a large dose of 10 + 5 mg Cd not only drastically supressed calcium-transport but also diminished its content within the duodenal wall.

Finally, cadmium-induced biosynthesis of thionein within the intestinal wall was observed by Sugawara and Sugawara (1975). The content of thionein doubled after 105 days of application of cadmium at a dose comparable to 2.0 mg per day per animal used in the present study. Even the amount of the total protein in the intestinal wall doubled. We do not know to what extent our short pretreatment with cadmium produced the same increase in the membrane. If the same protein inductive effect of cadmium pertains to our experiments too, it would not be surprising that calcium-transport through a membrane with quite a different protein-lipid ratio is changed. However, it is not possible to predict in what direction calcium-transport will change.

The present study has established that only large doses of cadmium suppress calcium transport through the intestinal wall, and thus confirmed the suggestion of Wahko and Cousins (1975). However, the mechanism of this action remains unexplained. We think that although the effect of cadmium on calcium metabolism may be regarded as secondary to the kidney damage there could be some competitive interactions between these ions at the sites of transfer from the intestine into blood.

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