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Effect of Food Components on Transport of Cadmium Across Rat Small Intestine *in Vitro*

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The effect of various proteins and amino acids and of the extracts from some foods on the tissue accumulation and the transport of cadmium in the *in vitro* rat small intestine was studied. The proteins and amino acids used decreased considerably the accumulation of cadmium in the intestinal tissue. Ovalbumin and glycinin decreased significantly the transport of cadmium. In contrast with such proteins, L-cysteine and L-histidine, which form relatively stable complexes with cadmium, increased the transport of the metal. However, other amino acids, which have a relatively weak affinity for cadmium, had little effect on the transport of the metal. The extracts from soybeans, ivory-shells, and oysters depressed remarkably the accumulation of cadmium in the intestinal tissue. The transport of cadmium considerably decreased by both extracts from soybeans and ivory-shells and, on the contrary, significantly promoted by the oyster extract.

These results suggest that the transport of cadmium across the rat small intestine is considerably influenced by the food components such as proteins and amino acids.

1. Introduction

It has been reported that dietary factors influence the tissue accumulation and the toxicity of cadmium in animals. Fitzhugh and Meiller¹⁾ reported that the toxicity of cadmium in rabbits was increased by a low protein diet. Ishizaki *et al.*²⁾ mentioned that in mice fed on low or high protein diet containing cadmium, the low protein diet gave considerably higher levels of cadmium in the kidney, liver, and whole body. A low calcium diet increased the accumulation in the liver and kidney of cadmium administered orally to mice and rats.^{3),4)} These findings suggest that various components in foods play an important role in influencing the intestinal absorption of cadmium.

The present study is concerned with the effect of various proteins and amino acids and of the extracts from some foods, which have been known to have relatively high activity in accumulating cadmium, on the transport of cadmium across the rat small

intestine *in vitro*.

2. Experimental

2.1. Materials and equipment

Ovalbumin and β -lactoglobulin were purchased from Sigma Chemical Co. and Miles Laboratories, Inc., respectively. Glycinin was isolated from soybeans according to the method of Briggs and Mann.⁵⁾ Gelatin, amino acids, cadmium chloride, and all other chemicals were of reagent grade.

A Shimadzu AA-610S atomic absorption spectrophotometer, a Shimadzu QV-50 spectrophotometer, and Hitachi-Horiba F-7 pH meter were utilized.

2.2. Preparation procedures of food extracts

1) Soybean extract

About 150 g of soybeans was immersed in water for about 15 hr. After the swollen soybeans were ground with a coffee mill, about 500 ml of water was added. The mixture was heated for 1.5 hr in a boiling water

bath with stirring and filtered with a gauze. After centrifuging the filtrate at 3000 rpm for 20 min at 5°C the supernatant was diluted twice with water, allowed to stand overnight in a refrigerator, and then centrifuged at 3000 rpm for 20 min at 20°C. The resulting supernatant was diluted twice with 1.8% sodium chloride solution.

2) Ivory-shell and oyster extracts

About 100 g wet weight of the internal organs of ivory-shells, *Babylonia japonica*, or about 120 g wet weight of oysters was homogenized with about 300 ml of physiological saline at pH 6.0, stirred at 5°C for 1 hr, and centrifuged at 3000 rpm for 20 min at 10°C. The supernatant was allowed to stand overnight in the refrigerator and centrifuged at 3000 rpm for 20 min at 10°C. The resulting supernatant was diluted to 300 ml with the physiological saline.

2.3. *In vitro* intestinal transport experiment

The procedure for studying the transport of cadmium across the everted rat jejunum was carried out according to the circulation method reported in the paper from our laboratory.⁶⁾ The transport of cadmium across the intestinal wall was investigated by measuring the concentration of the metal appearing in the serosal solution. At the termination of the experiment, the intestine was removed, washed on both sides with physiological saline, and its cadmium content was measured. Cadmium content in this tissue was designated as accumulation of the metal.

2.4. Analytical procedures

1) Cadmium

The cadmium content in aqueous sample was analyzed by an atomic absorption spectrophotometer at 2288 Å and compared to fresh dilutions of 1000 ppm reference standard. The correlation between absorbance and concentration was linear to 0.5 µg cadmium per ml.

2) Protein

The protein content in the food extracts was determined by the method of Lowry *et al.*⁷⁾ with crystalline bovine serum albumin as standard.

3) Phospholipid

Phospholipids were extracted from the food

extracts according to the modified procedure⁸⁾ of the method of Folch *et al.*,⁹⁾ and lipid phosphorus content in the extracted lipids was assayed by the method of Feldman *et al.*¹⁰⁾

3. Results and Discussion

3.1. Effect of various proteins on tissue accumulation and transport of cadmium

Cadmium has been known to have a relatively strong affinity for the intestinal wall,^{11)~13)} suggesting that the accumulation of cadmium in the intestinal tissue plays a role in the transport of the metal. We investigated simultaneously the accumulation of cadmium in the intestinal wall and the transport of the metal across it in the presence of the proteins such as ovalbumin, β-lactoglobulin, glycinin, and gelatin (Table 1). The presence of 0.1 mg/ml each protein in the mucosal solution decreased slightly the accumulation of cadmium in the intestinal mucosa. In addition, the tissue accumulation of cadmium was decreased to more extent in the higher concentrations of 2~10 mg/ml ovalbumin, β-lactoglobulin, or glycinin. In contrast with such proteins, gelatin showed a slight inhibitory effect on the accumulation of cadmium. In previous paper,⁶⁾ we reported that the transport of cadmium across the rat small intestine would occur by passive diffusion. This finding suggests the inhibitory effect of these proteins on the mucosal accumulation of cadmium results in a decrease in the transport of the metal. In fact, the transport of cadmium somewhat decreased when 10 mg/ml glycinin, which decreased remarkably the accumulation of the metal, was present in the mucosal solution but little did when other proteins were placed on the mucosal side of the intestine. Furthermore, in the experiment that the period of circulation was extended up to 3 hr, the presence of 10 mg/ml ovalbumin or glycinin in the mucosal solution resulted in the significant decrease in the transport of cadmium, as well as the accumulation of the metal. It is presumed that the proteins, which are little transported across the intestine, decrease the amount of cadmium which is taken up into

the intestinal wall by the formation of inhibitory effect of the proteins on the cadmium—protein complex, resulting in the accumulation and the transport of cadmium decrease in the accumulation and the transport of the metal, and that the extent of the is related to a difference in the binding affinity of the metal with the proteins.

Table 1. Effect of Various Proteins on Intestinal Tissue Accumulation and Transport of Cadmium

Protein	Circulation time (hr)	Concn. (mg/ml)	Cadmium ($\mu\text{g/g}$ wet tissue) ^{a)}	
			Tissue accumulation	Transport
Control	2	—	114.3 $\pm$ 27.8	0.67 $\pm$ 0.37
	3	—	124.4 $\pm$ 12.0	2.63 $\pm$ 0.22
Ovalbumin	2	0.10	100.2 $\pm$ 9.1	0.61 $\pm$ 0.07
	2	2.00	74.6 $\pm$ 13.1	0.55 $\pm$ 0.17
	2	10.00	67.7 $\pm$ 8.0 ^{b)}	0.61 $\pm$ 0.13
	3	10.00	98.1 $\pm$ 4.1 ^{b)}	1.50 $\pm$ 0.25 ^{c)}
β -Lactoglobulin	2	0.10	78.8 $\pm$ 9.2	0.75 $\pm$ 0.28
	2	2.00	75.7 $\pm$ 6.6	0.83 $\pm$ 0.03
Glycinin	2	10.00	76.5 $\pm$ 3.9 ^{b)}	0.54 $\pm$ 0.09
	2	0.10	90.4 $\pm$ 6.5	0.65 $\pm$ 0.16
	2	10.00	27.2 $\pm$ 1.5 ^{d)}	0.42 $\pm$ 0.22
Gelatin	3	10.00	37.8 $\pm$ 2.8 ^{c)}	0.91 $\pm$ 0.29 ^{c)}
	2	0.10	90.0 $\pm$ 11.8	0.58 $\pm$ 0.21
	2	1.00	101.3 $\pm$ 10.6	0.69 $\pm$ 0.17
	2	10.00	92.9 $\pm$ 7.9	0.94 $\pm$ 0.06

Initial mucosal concentration of cadmium was 10 $\mu\text{g/ml}$.

^{a)} The values represent the mean $\pm$ standard deviation for 3 to 7 animals.

Significantly different (Student's *t* test) from control values ^{b)} $p < 0.05$; ^{c)} $p < 0.01$; ^{d)} $p < 0.001$

3.2. Effect of various amino acids on tissue accumulation and transport of cadmium

In a similar manner as above, the effect of various L-amino acids on the tissue accumulation and the transport of cadmium was investigated (Table 2). The accumulation of cadmium in the intestinal tissue was decreased by these amino acids. In particular, L-cysteine and L-histidine, in which the stability constants¹⁴⁾ of their complexes with cadmium are relatively large, decreased remarkably the accumulation of the metal. In contrast with the proteins described above, however, L-cysteine and L-histidine increased significantly the transport of cadmium across the intestinal membrane, although other amino acids had little effect on the transport of the metal. Also similar phenomenon was distinctly observed in the 3 hr circulation experiment which was performed by the use of

10 mM concentration of L-cysteine or L-valine. In addition, the stimulatory effect of L-cysteine and L-histidine on the transport of cadmium was appreciably greater at 30 mM concentration of them than at 10 mM. However, in the cases of amino acids such as L-glutamic acid and L-alanine which have relatively weak affinity for cadmium, no significant stimulatory effect on the transport of the metal was observed even when the concentration of them was increased to 30 mM.

The correlation between the stability constant^{14),15)} of amino acid—cadmium complex and the accumulation or transport of the metal is shown in Fig. 1. It is evident from the results that the accumulation of cadmium in the intestine decreases with increasing the stability constant of the complex and, on the contrary, that the transport of the metal increases with increasing the stability constant.

Table 2. Effect of Various Amino Acids on Intestinal Tissue Accumulation and Transport of Cadmium in 2-Hour Circulation

Amino acid	Stability constant (log K) ^{a)}	Concn. (mM)	Cadmium ($\mu\text{g/g}$ wet tissue) ^{b)}	
			Tissue accumulation	Transport
Control	—	—	114.3 $\pm$ 27.8	0.67 $\pm$ 0.37
L-Cysteine	(18.70) ^{c)}	10	21.4 $\pm$ 1.2 ^{d)}	3.60 $\pm$ 0.33 ^{e)}
		30	15.6 $\pm$ 1.4 ^{f)}	7.68 $\pm$ 1.61 ^{e)}
L-Histidine	9.79	10	70.1 $\pm$ 10.1 ^{g)}	1.46 $\pm$ 0.35 ^{e)}
		30	53.7 $\pm$ 8.7 ^{d)}	2.21 $\pm$ 0.11 ^{e)}
L-Proline	8.70	10	83.0 $\pm$ 3.3	1.05 $\pm$ 0.45
		30	82.4 $\pm$ 10.2 ^{d)}	1.00 $\pm$ 1.13
L-Valine	8.24	10	85.9 $\pm$ 6.3	0.57 $\pm$ 0.23
L-Tryptophane	8.10	10	80.6 $\pm$ 11.7	0.69 $\pm$ 0.29
L-Glutamic acid	7.90	10	88.9 $\pm$ 5.2	0.53 $\pm$ 0.05
		30	88.0 $\pm$ 3.7	0.69 $\pm$ 0.11
L-Alanine	7.60	10	98.1 $\pm$ 7.9	0.72 $\pm$ 0.18
		30	78.9 $\pm$ 1.3	0.66 $\pm$ 0.05
L-Aspartic acid	7.48	10	87.7 $\pm$ 5.2	0.58 $\pm$ 0.13
L-Leucine	7.37	10	76.6 $\pm$ 7.3	0.48 $\pm$ 0.25
L-Methionine	6.99	10	96.7 $\pm$ 10.5	0.48 $\pm$ 0.20

Initial mucosal concentration of cadmium was 10 $\mu\text{g/ml}$.

^{a)} See refs. 11 and 12.

^{b)} The values represent the mean $\pm$ standard deviation for 3 or 4 animals.

^{c)} Indicated the stability constant of L-cysteine—zinc complex. Significantly different (Student's *t* test) from control values

^{d)} $p < 0.01$; ^{e)} $p < 0.001$; ^{f)} $p < 0.02$; ^{g)} $p < 0.05$

Fig. 1. Correlation between stability constant of amino acid-cadmium complex and tissue accumulation or transport of cadmium in everted rat jejunum

The stability constant for each amino acid was indicated in Table 2. The concentration of each amino acid was 10 mM

Δ , L-methionine; $\odot$, L-leucine; $\times$, L-aspartic acid; $\oplus$, L-alanine; $\bullet$, L-glutamic acid; $\circ$, L-tryptophane; $+$, L-valine; $\blacksquare$, L-proline; $\boxtimes$, L-histidine; $\square$, L-cysteine

From these results, it is suggested that these amino acids decrease the accumulation of cadmium in the intestinal tissue by the formation of their complexes with the metal, and that L-cysteine and L-histidine accelerate significantly the intestinal transport of the metal through the permeation of the relatively stable complexes formed between these amino acids and cadmium, in contrast to the inhibitory effect of the proteins on the transport of the metal.

3.3. Effect of food extracts on tissue accumulation and transport of cadmium

The effect of natural food components on the tissue accumulation and the transport of cadmium was examined by the use of the extracts from soybeans, the internal organs of ivory-shells, and oysters, which have been known to contain relatively high levels of cadmium (Table 3). The contents of protein, phospholipid, and cadmium in each extract are shown in Table 4. The addition of the soybean extract to the mucosal solution resulted in a remarkable depression in the accumulation of cadmium in the intestinal tissue

and a considerable decrease in the transport of the metal across the intestinal membrane. Such a transport characteristic of cadmium in the presence of the soybean extract containing 8.6 mg/ml protein was in fair agreement with that at 10 mg/ml concentration of glycine which is a major protein in soybeans.

In the case of the ivory-shell extract, the accumulation of cadmium was remarkably depressed and the transport of the metal decreased to less extent by the extract.

The above-mentioned results suggest that the effect of the extracts from soybeans and ivory-shells on the transport of cadmium may be caused by the proteins which are contained to relatively large amount in the extracts. However, the question of whether the lipids contained in the extracts influence the transport of cadmium remains unanswered.

Moreover, the oyster extract decreased conspicuously the accumulation of cadmium in the intestinal tissue and, contrary to the extracts from soybeans and ivory-shells, enhanced significantly the transport of the metal across the intestine. This phenomenon was similar to that of the transport of cad-

Table 3. Effect of the Extracts from Soybean, Oyster, and Ivory-shell on Intestinal Tissue Accumulation and Transport of Cadmium in 2-Hour Circulation

Food extract	Initial mucosal concn. of cadmium ($\mu\text{g/ml}$) ^{a)}	Cadmium ($\mu\text{g/g}$ wet tissue) ^{b)}	
		Tissue accumulation	Transport
Control (I)	10.00	114.3 $\pm$ 27.8 (19.05)	0.67 $\pm$ 0.37 (0.11)
(II)	20.00	163.5 $\pm$ 11.0 (13.63)	3.54 $\pm$ 0.38 (0.30)
Soybean	10.01 (0.01)	24.2 $\pm$ 2.5 (4.20)	0.31 $\pm$ 0.08 ^{c)} (0.05)
Oyster	10.03 (0.03)	37.8 $\pm$ 1.5 ^{c)} (6.30)	1.53 $\pm$ 0.17 ^{c)} (0.25)
Ivory-shell (A)	10.05 (0.05)	25.3 $\pm$ 2.4 ^{c)} (4.20)	0.54 $\pm$ 0.15 (0.09)
(B)	9.60 (9.60)	15.2 $\pm$ 1.5 ^{d)} (2.64)	0.38 $\pm$ 0.03 (0.07)
(B)	19.60 (9.60)	35.1 $\pm$ 0.3 ^{e)} (2.98)	1.01 $\pm$ 0.22 ^{b)} (0.09)

^{a)} The values in parentheses represent the concentration of cadmium naturally present.

^{b)} The values represent the mean $\pm$ standard deviation for 3 to 7 animals. The values in parentheses represent the percent of dose. Significantly different (Student's *t* test) from control (I)

^{c)} $p < 0.001$; ^{d)} $p < 0.01$; ^{e)} $p < 0.02$; ^{f)} $p < 0.05$ Significantly different from control (II) ^{g)} $p < 0.001$;

^{h)} $p < 0.01$

Table 4. Contents of Protein, Phospholipid, and Cadmium in the Extracts from Soybean, Oyster, and Ivory-Shell

Food extract	Protein (mg/ml) ^{a)}	Phospholipid as lecithin (μg/ml) ^{a)}	Cadmium (μg/ml) ^{a)}
Soybean	8.6	119	0.01
Oyster	4.9	88	0.03
Ivory-shell (A)	8.1	13	0.05
(B)	23.2	566	9.60

^{a)} Indicated the concentration in the mucosal solution.

mium in the presence of the amino acids as previously described. We have obtained the data* that the oyster contains appreciable quantities of several free amino acids, namely, cysteine, histidine, proline, valine, etc. Accordingly, it is presumed that the effect of the oyster extract on the transport of cadmium is attributable to some amino acids, which are contained in the extract and have a relatively strong affinity for the metal.

From these findings, it became evident that the transport of cadmium across the rat small intestine *in vitro* is considerably influenced by the food components such as proteins and amino acids. The experiments are in progress to study in detail a mechanism for the effect of proteins and amino acids on the intestinal absorption of cadmium.

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