Korean J. Ecol. 10(4):205~213, 1987

Effects of Red Ginseng on Methyl Mercury Toxicities to Lipoprotein and Tissue Protein in Mouse

Chung, Hee Won, Soo Kyoung Shin and Choon Koo Lee

(*Dept. of Biology, Sookmyung Women's University*)

생쥐의 脂蛋白質과 組織蛋白質에 미치는 메틸水銀 毒性에 대한 紅蔘의 影響

鄭喜媛·辛秀景·李春九

(淑明女子大學校 生物學科)

ABSTRACT

In order to investigate the effects of red ginseng extract to methyl mercury toxicities in mice, the serum lipoproteins, tissue protein patterns, and growth rates were studied. Animals were divided into 3 groups of the control, group I treated with methyl mercury chloride only, and group II treated together with methyl mercury chloride and red ginseng extract. In serum lipoprotein fractions of group I, beta lipoprotein fraction was increased and pre-beta lipoprotein fraction was decreased in comparison to those of the control. However, there was almost no difference in quantities of serum lipoprotein fractions between the control and group II. Total protein contents of groups I and II were increased in the liver and those of groups I and II in the kidney were decreased. However, in comparison to group I, total protein contents of group II in the liver and kidney were similar values with those of the control. Percentage of tissue protein fractions between control and group I in the liver and kidney showed considerable difference. On the other hand, the percentage of protein fractions of group II approximated to that of the control. Daily average growth rate of body weight in group II was similar to the control, but that of group I was decreased significantly in comparison to the other 2 groups.

INTRODUCTION

Mercury-made agrichemicals and mercuric wastes from various industries contaminate terrestrial and aquatic environments (Klein and Goldberg, 1970). The danger of mercury-contaminated ecosystems has been tragically emphasized by the Minamata disease, which occurred in the 1950s among Japanese who ingested fish contaminated with methyl mercury discharged into the water (Smith, 1980).

Mercury compounds in polluted environments, extremely toxic to all animals, are absorbed usually through respiratory, gastrointestinal tracts, and skin etc. And they are transported to various organs of the body by circulatory system(Tunnicliff and Wood, 1973; Vallee and Ulmer, 1972). Usually methyl mercury transported to the liver, kidney, and central nervous systems has toxic influences on these organs or inhibits the functions of several enzyme systems and protein synthesis (Klaassen, 1980; Ware *et al.*, 1974).

Serum lipoproteins play an important roles in the transport of lipids to the various body tissues and provide informations useful for the diagnosis and treatment of lipoprotein abnormalities in health and diseases of animals (Lee and Puppione, 1978).

Meanwhile, ginseng extracts promote the basic metabolic rates and synthesis of proteins, and then prevent the progressions of pathological changes and functions of toxic materials (Oura and Hiai, 1974; Bae, 1978; Hong *et al.*, 1979). By these functions, ginseng extracts increase resistance of organisms under unfavorable conditions such as physical and chemical stresses nonspecifically (Brekhman and Dardymov, 1969).

This study reports the influences of detoxifying functions of red ginseng extracts on methyl mercury, being increased daily in ecosystems, in the aspects of serum lipoproteins, tissue proteins, and growth rates in mice.

MATERIAL AND METHODS

Animals and treatment

Experimental animals were female ICR mice weighing 20-30 grams.

Thirty mice were divided into 3 groups: control, group I, and group II. Ten animals per group were housed in the cage at 20-25°C and permitted free access to food and water. Animals of control group were orally administered with distilled water for 23 days. Animals of group I were treated with methyl mercury chloride of 15 mg/kg b.w./day by oral administration with animal feeding needle for 23 days. Animals of group II were treated together with methyl mercury chloride of 15 mg/kg b.w./day and red ginseng extract of 200 mg/kg b.w./day for 23 days after pretreatment of red ginseng extract of 200 mg/kg b.w./day for 7 days. At 24 hours after final treatment of all 3 groups, animals were decapitated for blood withdrawal, and liver and kidney removal. The red ginseng extract containing crude saponin of 150 mg/g was obtained from the Korean Office of Monopoly.

Serum lipoprotein electrophoresis

Serum lipoprotein fractions were separated with the agarose gel lipoprotein kit by the method of Helena laboratory, U.S.A. The percentage of lipoprotein bands was computed automatically by the Quick Scan densitometer (Helena Co.) at 525 nm.

Total protein content measurement

Each liver and kidney tissue homogenate diluted with potassium phosphate buffer (pH 8.7) was centrifuged (4,000 rpm, 30 minutes, 4°C) and then each supernatant was used

as sample. The total protein content of each sample was assayed by the method of Lowry *et al.* (1951).

Tissue protein electrophoresis

Each liver and kidney tissue was homogenized and centrifuged, then each supernatant was used as sample. Electrophoresis was performed by the use of agarose gel high resolution protein kit and the method of Helena company, U.S.A. Densitometric scans and percentage of separated protein bands were obtained by a densitometer at 595 nm.

Absolute value of each tissue protein fraction was obtained by the following formula:

Absolute value=Total tissue protein × Relative percentage

The number of protein fractions were given in turn from the fraction which was located closest to the anode for convenience.

Body weight measurement

Each mouse was weighed just before treatment daily. Data were given as mean±S.E. for the 5 animals in each group. Statistical comparisons were based on Student's t test and the level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Serum lipoprotein electrophoretic pattern

Electrophoretic patterns of serum lipoproteins from 3 experimental groups were shown in Figure 1. The distribution of percentages of lipoprotein fractions was given in Table 1.

Alpha, pre-beta, and beta lipoprotein fractions appeared in all 3 groups (Fig. 1). Alpha lipoprotein fraction was moved closest to the anode, although, percentages of serum lipoproteins among 3 groups were different (Table 1).

Group I showed the significant increase of beta lipoprotein fraction and decrease of pre-beta lipoprotein fraction in comparison to those of the control. The same result is observed in mice affected by disorder of generalized lysosome lipid storage (Morris *et al.*, 1982) and in patients with liver cirrhosis (Song *et al.*, 1982). The amount of beta lipoprotein fraction increases and pre-beta lipoprotein fraction decreases in Nieman-Pick disease of man generally (Fredrickson and Sloan, 1972). This lipoproteins change in group I would be caused by the methyl mercury toxicity inducing hepatic metabolism impairment. And this assumption is supported by the studies that liver synthesizes and secretes several kinds of lipoproteins, and is the active site of lipid metabolism (Bae, 1978).

There was almost no difference in quantities of lipoprotein fractions between control and group II. This little difference might be explained by the detoxication of red ginseng extract on methyl mercury toxicity as the report of Oura and Hiai (1974).

Total protein content

The total protein contents in the liver and kidney of each experimental group of mice were shown in Table 2. The total protein content in the liver of control mice was 64.3

Table 1. Distribution of the percentages of serum lipoprotein fractions of mouse treated with methyl mercury and/or red ginseng extract

Group	Lipoprotein fraction (%)		
	Alpha lipoprotein	Pre-beta lipoprotein	Beta lipoprotein
Control	65.8	19.9	14.3
Group I	61.9	8.5	29.6
Group II	66.8	16.7	16.5

Table 2. Total protein content and percentage in the liver and kidney of mouse treated with methyl mercury and/or red ginseng extract

Tissue	Group	Total protein (mg/g)	Percentage (%)
Liver	Control	64.3	100
	Group I	94.3	147
	Group II	77.8	121
Kidney	Control	50.8	100
	Group I	30.2	60
	Group II	42.8	84

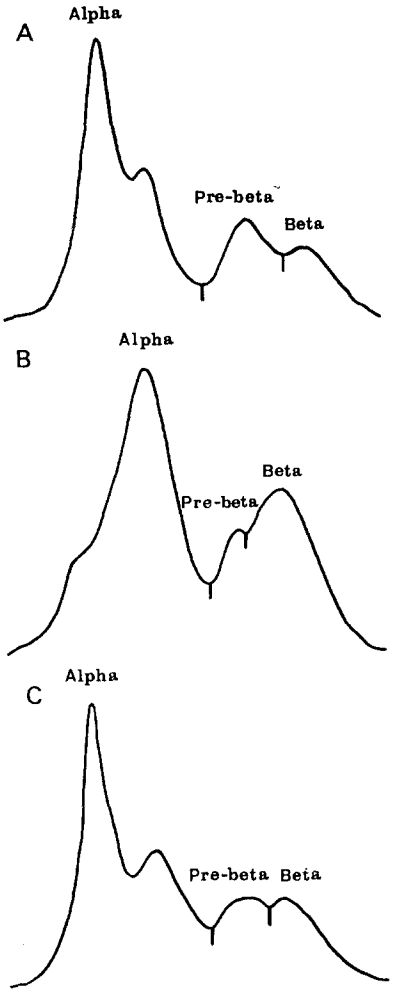

Fig. 1. Densitometric scans of serum lipoprotein fractions separated by agarose gel electrophoresis in mouse. A, Control; B, Group I; C, Group II. Left is anode.

mg/g and that of group I was 94.3mg/g which was 147% of the control. This remarkable increase of total protein content of group I is considered to be due to the facilitation of protein synthesis in the hepatocytes during mercury poisoning. This assumption is supported by the report of Klein and Goldberg (1970) that protein synthesis is promoted at the early and last stages of mercury toxication. The total protein content of group II was increased slightly which was similar value with the control. This phenomenon in mouse would be caused by the detoxifying functions of red ginseng extract on the toxic effect of methyl mercury.

The total protein content in the kidney of control was 50.8 mg/g and that of group I was 30.2 mg/g which was 60% of the control. Metallothionein in the kidney has a high affinity for mercury and serves a protective role for the kidney by sequestering mercury. But, excretion of albumin and low molecular weight proteins resulted from glomerular and

tubular damage is induced when mercury is concentrated more than critical concentration level of injury (Klaassen, 1980). Considering from this fact, the decrease of protein content in group I seems due to the disturbance of the kidney tissue affected by methyl mercury. The total protein content of group II was decreased less than that of group I. This would be explained by the detoxication effect of red ginseng extract lowering the methyl mercury poisoning level. It seems that alteration of total protein content caused by methyl mercury toxicity might be occurred also in the kidney of field mice.

Tissue protein electrophoretic pattern

The electrophoretic patterns of water soluble protein in the liver and kidney tissues were showen in Figs. 2 and 3. Table 3 and 4 illustrated the distribution of percentage and absolute value of each tissue protein fraction.

In the liver, protein fractions of control was 9 and those of groups I and II were 8, respectively (Fig. 2). The number 9 fraction showed the largest percentage in all 3 groups (Table 3). Compared with control, the new fractions appeared in groups I and II were number 17 and number 14, respectively. But, number 2 and number 13 bands in group I disappeared and numbers 13 and 16 in group II also disappeared.

The protein fractions in the kidney of control were 8 and those of groups I and II were 9 and 8, respectively (Fig. 3).

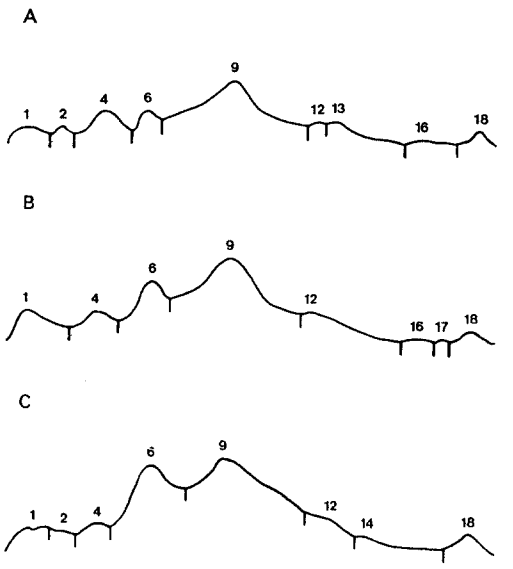

Fig. 2. Densitometric scans of tissue protein fractions in the liver of mouse. A, Control; B, Group I; C, Group II. Left is anode.

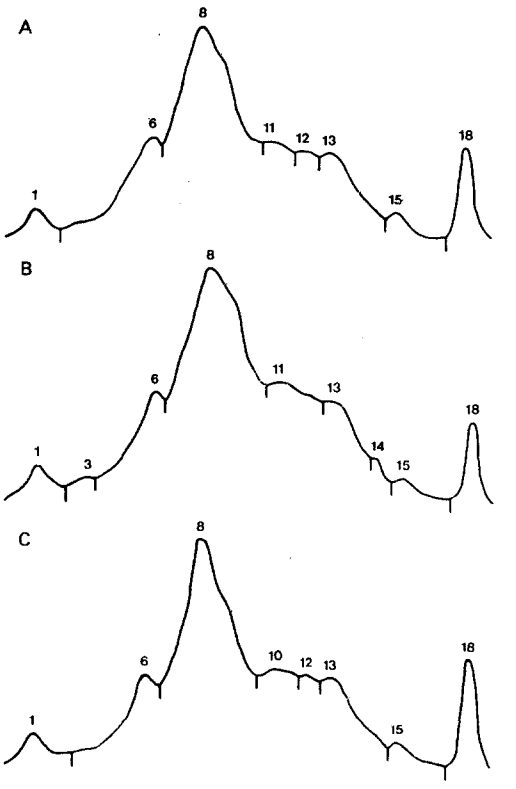

Fig. 3. Densitometric scans of tissue protein fractions in the kidney of mouse. A, Control; B, Group I; C, Group II. Left is anode.

Table 3. Distribution of the percentages of tissue protein fractions in the liver and kidney of mouse treated with methyl mercury and/or red ginseng extract

Tissue	Group	Serial number of protein fraction								
		1	2	3	4	5	6	7	8	9
Liver	Control	5.7	2.3	—	14.7	—	12.5	—	—	46.9
	Group I	9.3	—	—	7.2	—	14.4	—	—	54.1
	Group II	3.0	6.1	—	5.1	—	25.9	—	—	45.5
Kidney	Control	2.7	—	—	—	—	15.0	—	47.9	—
	Group I	2.6	—	1.6	—	—	12.3	—	49.5	—
	Group II	2.8	—	—	—	—	13.3	—	47.2	—

Tissue	Group	Serial number of protein fraction								
		10	11	12	13	14	15	16	17	18
Liver	Control	—	—	3.0	9.3	—	—	1.9	—	3.7
	Group I	—	—	12.1	—	—	—	0.5	0.2	2.2
	Group II	—	—	9.1	—	3.0	—	—	—	2.3
Kidney	Control	—	10.3	5.6	11.9	—	2.1	—	—	4.5
	Group I	—	17.6	—	9.7	1.6	1.9	—	—	3.2
	Group II	11.0	—	6.5	11.8	—	2.0	—	—	5.4

Table 4. Distribution of the absolute values (mg/g) of tissue protein fractions in the liver and kidney of mouse treated with methyl mercury and/or red ginseng extract

Tissue	Group	Serial number of protein fraction								
		1	2	3	4	5	6	7	8	9
Liver	Control	3.67	1.47	—	9.45	—	8.04	—	—	30.16
	Group I	8.77	—	—	6.79	—	13.58	—	—	51.02
	Group II	2.33	4.75	—	3.97	—	20.15	—	—	35.40
Kidney	Control	1.37	—	—	—	—	7.62	—	24.33	—
	Group I	0.79	—	0.48	—	—	3.72	—	14.95	—
	Group II	1.19	—	—	—	—	5.69	—	20.20	—

Tissue	Group	Serial number of protein fraction								
		10	11	12	13	14	15	16	17	18
Liver	Control	—	—	1.93	5.98	—	—	1.22	—	2.38
	Group I	—	—	11.41	—	—	—	0.47	0.19	2.08
	Group II	—	—	7.08	—	2.33	—	—	—	1.79
Kidney	Control	—	5.23	2.85	6.05	—	1.07	—	—	2.29
	Group I	—	5.32	—	2.93	0.48	0.57	—	—	0.97
	Group II	4.71	—	2.78	5.05	—	0.86	—	—	2.31

The fraction of number 8 showed the largest percentage among all 3 groups (Table 3). The fractions appeared newly in the group I were numbers 3 and 14, and fraction of number 10 appeared newly in group II. The protein fractions disappeared in groups I and

Table 5. Growth rates of body weight (g) in each experimental group of mice for the period of 23 days

Growing period (Day)	Control		Group I		Group II	
	Body weight (Mean±S.E.)	Growth rate (%)b	Body weight (Mean±S.E.)	Growth rate (%)b	Body weight (Mean±S.E.)	Growth rate (%)b
0[a]	18.8±1.16		17.6±0.63		19.0±1.14	
		7.5		4.5		8.4
1	20.2±1.34		18.4±0.77		20.6±1.41	
		−3.9		0.0		−2.9
2	19.4±1.20		18.4±0.55		20.0±1.25	
		9.3		5.4		6.0
3	21.2±1.20		19.4±0.73		21.2±1.16	
		1.9		4.1		5.6
4	21.6±1.45		20.2±0.76		22.4±0.89	
		−2.7		0.0		−2.6
5	21.0±1.48		20.2±0.81		21.8±0.87	
		4.8		0.0		4.5
6	22.0±1.87		20.2±0.87		22.8±0.81	
		−2.7		0.0		−0.8
7	21.4±1.70		20.2±0.67		22.6±0.84	
		3.7		3.9		0.8
8	22.2±1.80		21.0±0.71		22.8±1.12	
		6.4		3.8		4.3
9	23.6±1.61		21.8±0.81		23.8±0.87	
		−5.1		−5.5		−4.2
10	22.4±1.70		20.6±0.77		22.8±1.02	
		5.4		−0.9		3.5
11	23.6±1.64		20.4±0.71		23.6±0.95	
		4.2		3.9		4.2
12	24.6±1.82		21.2±0.67		24.6±1.26	
		3.3		7.5		4.0
13	25.4±1.89		22.8±0.59		25.6±1.05	
		0.8		0.0		0.8
14	25.6±1.82		22.8±0.87		25.8±1.07	
		−0.8		3.5		2.3
15	25.4±1.70		23.6±0.95		26.4±1.22	
		−0.8		−0.8		−0.8
16	25.2±1.98		23.4±0.95		26.2±1.16	
		1.6		−0.8		3.0
17	25.6±1.82		23.2±1.02		27.0±1.14	
		0.0		−0.8		−1.5
18	25.6±2.02		23.0±0.71		26.6±1.05	
		−0.8		−0.9		−0.8
19	25.4±1.92		22.8±0.81		26.4±0.95	
		1.6		0.9		2.2
20	25.8±1.87		23.0±0.95		27.0±1.09	
		−0.8		0.0		−3.7
21	25.6±2.07		23.0±0.84		26.0±1.22	
		0.8		−1.7		1.5
22	25.8±2.16		22.6±0.77		26.4±1.00	
Total		33.7		26.1		33.8
(Mean±S.E.)	1.5318±0.7956		1.1863±0.6346		1.5363±0.7414	
			c		c	

a, Before treatment; b, Percentage compared with the weight of previous day;
c, Significant at 5% level. by *t* test

II were numbers 12 and 14, respectively.

Absolute values of protein fractions in the liver and kidney between the control and group I showed considerable difference (Table 4). This difference might be caused by toxicity of methyl mercury. However, most absolute values of protein fractions in group II approximated to those of the control. This occurrence probably might be due to detoxifying effects of red ginseng extract.

Growth rate

The change of body weight in each experimental group was given in Table 5.

Daily average growth rates of the control and group II showed similar values, which were 1.5318%±0.7956 and 1.5363%±0.7414, respectively. That of group I was 1.1863%±0.6346, which was statistically significant decrease in comparison to the other 2 groups. This decrease of growth rates is typical symptoms induced by methyl mercury poisoning (Herman *et al.*, 1973; Turner *et al.*, 1981). The reason why group II showed more significant increase than group I probably might be effects of red ginseng extract on detoxication and weight increase.

摘 要

生態系의 汚染物質로서 점증적으로 증가되고 있는 메틸水銀이 생쥐의 血淸脂蛋白質, 組織蛋白質 및 成長率에 미치는 毒性的 影響과 紅蔘抽出物의 解毒效果를 연구하였다. 實驗群으로서는 對照群, 메틸水銀을 투여한 處理Ⅰ群, 및 메틸水銀과 紅蔘抽出物을 병행 투여한 處理Ⅱ群으로 나누었다. 本 實驗에서 얻어진 결과는 다음과 같다.

1. 三群의 血淸脂蛋白質은 alpha, pre-beta, 그리고 beta lipoprotein 分劃으로 분리되었다. 각 분획의 百分率에 있어서 對照群과 處理Ⅱ群에서는 비슷하였고 이들 두 群에 비하여 處理Ⅰ群에서는 beta lipoprotein 分劃이 증가되었고 pre-beta lipoprotein 分劃은 감소되었다.

2. 肝臟의 總蛋白質量은 處理Ⅰ群과 Ⅱ群에서는 對照群에서 보다 약간 증가되고 腎臟의 총 단백질량은 處理Ⅰ群과 Ⅱ群에서는 감소되었다. 그러나 處理Ⅱ群에서는 對照群에서와 거의 비슷한 수치로 나타났다.

3. 肝臟과 腎臟의 組織蛋白質 分劃의 百分率에 있어서 處理Ⅰ群에서는 對照群에서와 상당한 差異가 있었고 處理Ⅱ群에서는 對照群에서와 類似하였다.

4. 日別 평균 成長率은 對照群과 處理Ⅱ群에서는 거의 같았으며 處理Ⅰ群에서는 다른 두 群에서 보다 有意하게 감소되었다.

LITERATURES CITED

Bae, H.W. (1978). Korean ginseng, 2nd ed. Korea Ginseng Research Institute Publication, p. 317.

Brekhman, I.I. and I.V. Dardymov. (1969). New substance of plant origin which increase nonspecific resistance. Ann. Rev. Pharmacol., 9 : 419~430.

Fredrickson, D.S. and H.R. Sloan. (1972). Sphingomyelin lipidases: Niemann-Pick disease, the metabolic basis of inherited diseases, 3rd ed. MacGraw-Hill, New York, pp. 783~807.

Herman, M.R., R. Klein, F.A. Talley and M.R. Krigman. (1973). An ultrastructural study of methyl mercury-induced primary sensory neuropathy in the rat. Lab. Invest., **28** : 104~118.

Hong, S.A., J.K. Lim and C.W. Park. (1979). Pharmacological action of ginseng. J. Ginseng Sci., **3** : 66~93.

Klaassen, C.D., J. Doull and M.O. Amdur. (1980). Casarett and Doull's toxicology: The basic science of poison. 2nd ed. Macmillan Publishing Co., New York, p. 723.

Klein, D.H. and E.D. Goldberg. (1970). Mercury in the marine environment. Environ. Sci. Technol., **4** : 765~768.

Klein, R., S.P. Herman, P.E. Brubaker and G.W. Lucier. (1972). A model of acute methyl mercury intoxication in rats. Arch. Pathol., **93** : 408~418.

Lee, R.F. and D.L. Puppione. (1978). Serum lipoproteins in the spiny lobster, ***Panulirus interruptus***. Comp. Biochem. Physiol., **59** : 239~243.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., **193** : 265~275.

Morris, M.M., C.B. Bhunaneswaran, H.S. Ma and S. Fowler. (1982). Lysosome lipid storage disorder in NCTR-BALB/c mice. Am. J. Pathol., **108** : 140~149.

Oura, H. and S. Hiai. (1974). Biochemical action of ***Panax ginseng*** principle. Proc. International Ginseng Symp., pp. 23~26.

Smith, R.L (1980). Ecology and field biology. 3rd ed. Harper and Row Publisher, New York, pp. 183~185.

Song, H.G., C.Y. Chung, T.W. Lee and H.K. Yeum. (1982). Study on serum lipoprotein in normal and patient with liver disease. Choson Univ., Medical Study, **82** : 141~153.

Tunnicliff, G. and J.D. Wood. (1973). The inhibition of mouse brain neurotransmitter enzymes by mercury compounds and a comparison with the effects of hyperbaric oxygen. Comp. Gen. Pharmacol., **4** : 101~105.

Turner, C.J., M.K. Bhatnagar and S. Yamashiro. (1981). Ethanol potentiation of methyl mercury toxicity: A preliminary report. J. Toxicol. Environ. Health, **7** : 665~668.

Vallee, B.T. and D.D. Ulmer. (1972). Biochemical effects of mercury, cadmium and lead. Ann. Rev. Biochem., **41** : 91~128.

Ware, R.A., L.W. Chang and P.M. Burkholder. (1974). Ultrastructural evidence for foetal liver injury induced by in utero exposure to small doses of methyl mercury. Nature, **251** : 236~237.

(*Received August 12, 1987*)