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Detoxification Effect of Red Ginseng Extract on Toxicity of Methylmercury Chloride to LDH in the Liver, Kidney, and Serum of Mouse

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생쥐의 肝臟, 腎臟 및 血淸內 LDH에 미치는 염화 메틸水銀 毒性에 대한 紅蔘抽出物의 解毒效果

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摘 要

생쥐의 肝臟, 腎臟 및 血淸內 LDH 活性에 있어서 肝臟과 腎臟에서는 염화 메틸水銀 投與群과 염화 메틸水銀-紅蔘抽出物 投與群의 總 LDH 活性値가 對照群에 비하여 減少되었으나 염화 메틸水銀-紅蔘抽出物 投與群의 活性値는 염화메틸水銀 投與群 보다 약간 減少되었다. 血淸에서는 염화 메틸水銀 投與群의 LDH 活性値가 對照群에 비하여 2倍로 크게 增加되었으며 염화 메틸水銀-紅蔘抽出物 投與群의 活性値는 對照群과 類似하였다.

LDH isozyme의 電氣泳動像에 있어서 肝臟의 對照群과 염화 메틸水銀-紅蔘抽出物 投與群에서는 5가지 LDH isozyme이 모두 나타났으나 염화 메틸水銀 投與群에서는 LDH_1을 제외한 4가지 LDH isozyme이 나타났다. 腎臟과 血淸에서는 3群 모두에서 5가지 LDH isozyme이 나타났다.

INTRODUCTION

Mercury reacts with a variety of protein binding sites including sulfhydryl groups and passes easily across cell membranes due to its lipid solubility (Fang and Fallin, 1976; Lau and Sarkar, 1979). Mercury compounds absorbed usually through respiratory tract, gastro-intestinal tract, and skin are transported to the various organs of the body by the circulating blood (Tunnicliff and Wood, 1973; Vallee and Ulmer, 1972). Methyl mercury mainly transported to the liver, kidney, and central nervous system has toxic effects on these organs (Donaldson and Guber, 1978) and also inhibits the function of enzymes in the glycolytic pathway and protein synthesis(Klaassen, 1980). The accumulation of toxic methyl mercury

induces hepatic injuries and changes in several hepatic enzyme systems (Ware *et al.*, 1974) and tubular function disturbance in the kidney (Valek, 1965; Klaassen, 1980).

Lactate dehydrogenase (LDH) is one of the important enzymes in diagnosis of specific organ diseases. Vertebrate tissues and serum contain the characteristic distributions of the LDH isozymes (Richterich *et al.*, 1963). The LDH activities and electrophoretic patterns of LDH isozymes may be altered in various disease states (Wroblewski and Gregory, 1961; Gelderman *et al.*, 1965).

Meanwhile, the ginseng (*Panax ginseng*) well known as one of the medical herbs increases resistance of organism to various unfavorable influences such as stress and diseases nonspecifically (Brekhman and Dardymov, 1969). Ginseng promotes basic metabolic rate remarkably (Oura and Hiai, 1974) and tends to activate various enzymes (Bae, 1978). Furthermore, ginseng has the possibility of preventing the accumulation of toxic heavy metals by speeding up the metabolic rate of toxic materials (Hong, *et al.*, 1979). However, the detailed mechanisms for antitoxic reaction of ginseng are scarcely known.

The present paper deals with the total LDH activities and electrophoretic patterns of LDH isozymes in order to study the detoxifying effects of red ginseng extract on toxicity of methylmercury chloride in mouse tissues.

MATERIALS AND METHODS

Experimental animals

Female ICR mice weighing 20~30g were used. The animals were divided into three groups (control, group I, and group II). Ten animals per group were housed in cages at 20~25°C and allowed free access to food (Samyang Laboratory Chow) and water.

Treatment

Control group was orally administered with distilled water only for 23 days. Group I was treated with methylmercury chloride (MMC) of 15mg/kg b.w./day dissolved in distilled water by oral administration with animal feeding needle for 23 days. Group II was orally pretreated with red ginseng extract (RGE) of 200mg/kg b.w./day for 7 days and then administered with methylmercury chloride of 15mg/kg b.w./day and red ginseng extract of 200mg/kg b.w./day for 23 days. The red ginseng extract containing crude saponin of 150 mg/g was obtained from the Korean Office of Monopoly.

Total LDH activity measurement

Each tissue homogenate diluted to 1,000 times with distilled water was centrifuged(4,000 rpm, 30 minutes, 4°C) and then each supernatant was used as sample. Serum sample was diluted to 6 times before use. The total LDH activity of each sample was assayed by the spectrophotometric method of Kornberg *et al.* (1955). A unit of activity is that amount of enzyme causing a decrease of 0.04 O.D. per minute at 340 nm.

LDH isozyme electrophoretic pattern analysis

Each tissue homogenate was centrifuged at 4,000 rpm for 30 minutes at 4°C, then each supernatant was used as sample. Each sample of 3μl was applied to agarose gel plate (High Resolution Helena Lab.) and performed electrophoresis with Sodium barbital buffer (pH 8.7~9.1) at 120 V for 90 minutes at 4°C. The staining was carried out by LDH isozyme reagents (Helena Co.) for 40 minutes at 45°C. When LDH isozyme bands appeared apparently, the plate was destained in 5% acetate for 5 minutes and placed in a drying oven at 70°C until dry completely. The dried plate was scanned by Quick Scan densitometer (Helena Co.) at 595 nm, from which densitometric scan and percentage of each LDH isozyme band were obtained.

RESULTS AND DISCUSSION

Total LDH activity

The total LDH activities in the liver, kidney, and serum of three experimental groups of mice were shown in Table 1.

The total LDH activity of control in the liver was 1,500 units and that of group I was 1,027.9 units which was 68.5% of the control. This decrease of total LDH activity of group I would seem due to the decrease of pyruvate content following after the decrease of glycogen content during hepatocyte damage partly caused by methylmercury chloride poisoning. Trump *et al.* (1965) reported that toxic material administered to mouse induces the decrease of glycogen content on the beginning of hepatocyte necrosis. However, the total LDH activity of group II showed the rather less decrease than group I. On the basis of the roles of red ginseng extract having the detoxication effect on toxic heavy metal and the facilitation of glycogen increase (Bae, 1978), it is reasonable that the total LDH activity of group II was decreased less than that of group I.

In the kidney, the total LDH activity of control was 111.7 units and that of group I

Table 1. The total and relative LDH activity in the liver, kidney, and serum of mouse treated with methylmercury chloride and/or red ginseng extract

Tissue	Group	Total activity (unit/g, unit/dL)	Relative activity (%)
Liver	Control	1,500	100
	Group I	1,028	69
	Group II	1,072	72
Kidney	Control	112	100
	Group I	79	71
	Group II	109	97
Serum	Control	252	100
	Group I	440	175
	Group II	224	89

was 79 units which was 70.7 % of the control. It seems that this decrease of the activity in group I was caused by the toxicological effect of methylmercury chloride on enzyme activity. This assumption is suggested by the report of Ware (1974) that methyl mercury necrotizes renal cells partly, breaks mitochondria containing a large amount of detoxifying enzymes, and finally lowers normal cell functions. The total LDH activity of group II was decreased less than that of group I. This would be caused by the detoxifying functions of red ginseng extract lowering the methylmercury chloride poisoning level.

Meanwhile, the total LDH activity of control in the serum was 252 units. The activity of group I was about 2 times as increase as the control. Zondag and Klein (1968) reported that the LDH isozymes leak out from damaged tissue cells to the circulating blood, which caused by the function of toxic material altering the membrane permeability to produce elevation of serum LDH activities. Judging from this fact, the remarkable increase of LDH activity in group I of serum seems due to the toxic effect of methylmercury chloride. Total LDH activity of group II was decreased slightly which similar value with the control. This phenomenon is assumed that red ginseng extract affected favorably carbohydrate metabolism in some way, considering that the LDH isozymes are concerned primarily with the reduction of pyruvate to lactate (Harper *et al.*, 1979; Stryer, 1975) and ginseng component effects on carbohydrate, lipid, and protein metabolism (Oura and Hiai, 1974).

LDH isozyme electrophoretic patterns

The electrophoretic patterns of LDH isozymes in the liver, kidney, and serum of three experimental groups of mice were shown in Fig. 1. Table 2 illustrated the distribution of percentage of LDH isozymes.

In the liver, the LDH isozymes of control and group II showed 5 bands, while 4 bands except LDH_1 were fractionated in group I (Fig. 1A). The disappearance of LDH_1 in group I would be caused by the effect of methylmercury chloride on mitochondria. This assumption is suggested by the report that the LDH_1 isozyme concerned with high oxidation metabolism is decreased by the toxic material destroying the mitochondria containing a great deal of oxidation enzymes (Lindsay, 1963). The appearance of LDH_1 in group II would seem caused by the detoxifying function of red ginseng on the toxic effect of methylmercury chloride.

The percentage of LDH isozymes of group I showed the slight increase of $LDH_{2,3,5}$ and the decrease of LDH_4 in comparison to the control (Table 2). That of group II showed the slight increase of $LDH_{2,3,4}$, and the decrease of $LDH_{1,5}$. The increase of LDH_5 in group I would be caused by the toxic effect of methylmercury chloride inducing liver disease and enhancing anaerobic metabolism. This is suggested by the report of Dawson *et al.* (1964) that LDH_5 predominates in anaerobic tissues.

All the 5 LDH isozyme bands were fractionated in three groups of the kidney (Fig. 1B). The percentage of LLD isozymes of group I showed the decrease of $LDH_{1,2}$ and the increase of $LDH_{3,4}$ compared with the normal group. The percentage of $LDH_{1,5}$ was decreased and

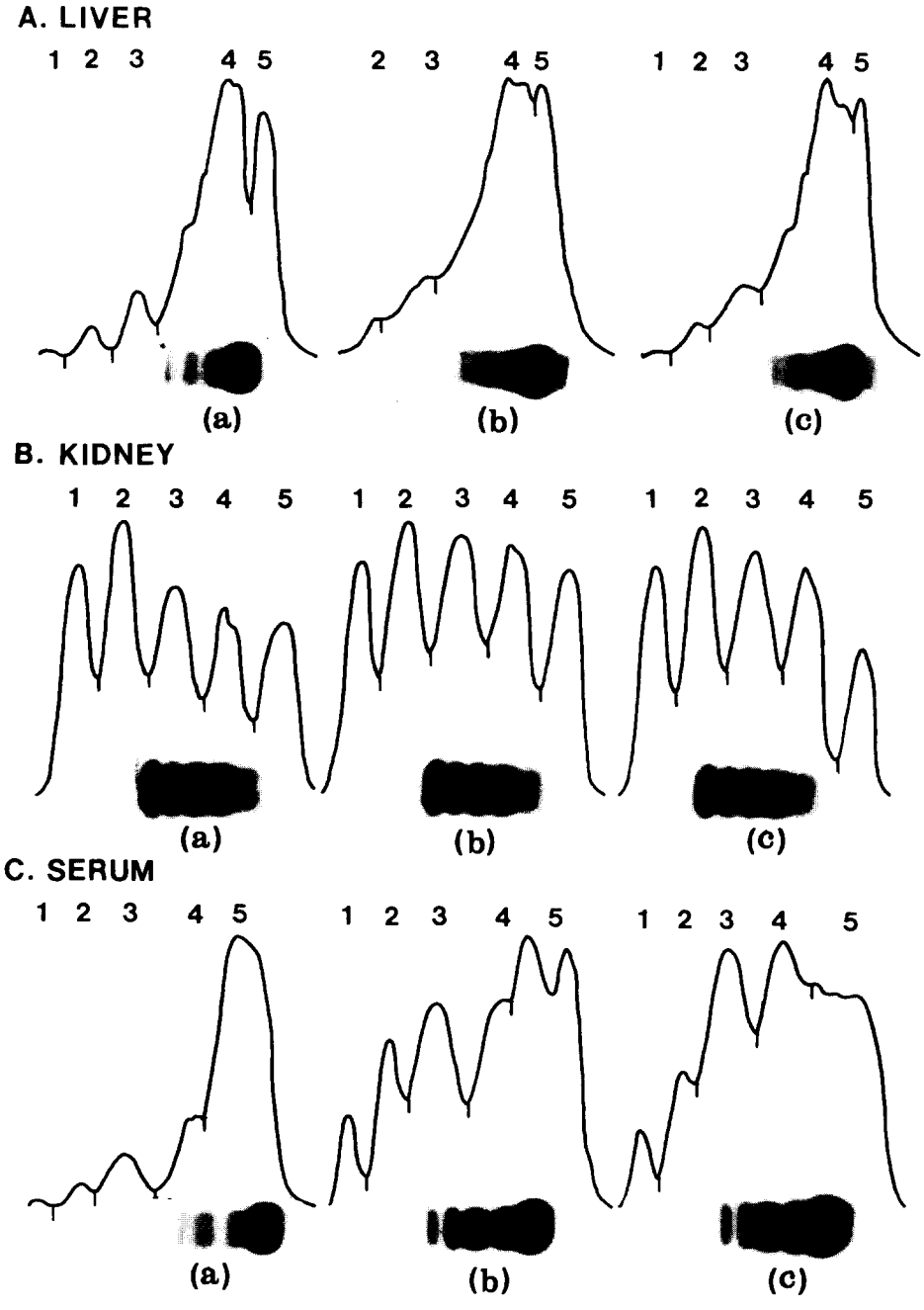

Fig. 1. Densitometric scans and electrophoretic patterns of LDH isozymes in the liver, kidney, and serum of mouse. (a), Control; (b), Group I treated with MMC; (c), Group II treated with MMC and RGE.

Table 2. The distribution of percentage of each LDH isozyme band in the liver, kidney, and serum of mouse treated with methylmercury chloride and/or red ginseng extract

Tissue	Group	LDH isozyme distribution (%)				
		LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Liver	Control	0.5	2.9	7.7	62.7	26.2
	Group I	—	3.4	10.2	57.2	29.1
	Group II	0.4	3.0	10.9	60.4	25.3
Kidney	Control	19.1	24.5	22.5	16.6	17.3
	Group I	15.4	21.1	24.2	21.7	17.6
	Group II	17.9	24.0	26.2	20.9	11.0
Serum	Control	1.1	3.0	10.7	13.9	71.3
	Group I	4.8	11.7	26.9	22.0	34.6
	Group II	3.9	8.3	23.2	22.3	42.3

that of $LDH_{3,4}$ was increased in group II. However, there was almost no difference between control and group II (Table 2). This little difference might be occurred by the detoxifying role of red ginseng on toxicity of methylmercury chloride and then the renal function would be recovered to the normal states in some extent.

In the serum, all the 5 LDH isozymes appeared in three groups (Fig. 1C). Table 2 showed the same patterns of the increase of $LDH_{1,2,3,4}$, and decrease of LDH_5 in both groups I and II. The reason of increase of $LDH_{1,2,3,4}$ in groups I and II is explained by the fact that the tissues leak some of LDH isozymes into the blood during the methyl mercury poisoning (Nerenberg and pogojeff, 1969).

Generally, the pathological findings of the change of LDH isozyme perentage in serum are as follows: the increase of LDH_1 level indicates the kidney infarction, the increase of $LDH_{2,3}$ indicates the pulmonary infarction, and the increase of LDH_4 suggests acute liver disease. The percentage of LDH_5 is increased during the destruction of liver and skeletal muscle.

SUMMARY

The detoxication effects of red ginseng extract against the toxicity of methylmercury chloride on the total LDH activities and electrophoretic patterns of LDH isozymes in the liver, kidney, and serum of mice were studied.

Animals were divided into three groups of the control, group I treated with methylmercury chloride only, and group II treated with methylmercury chloride and red ginseng extract.

The total LDH activities of groups I and II in the liver and kidney were decreased compared with the control. However, the activity of group II was decreased less than that

of group I. The LDH activity of group I in the serum was remarkably increased to 2 times and that of group II was similar to the control.

In the electrophoretic patterns of LDH isozymes, all the 5 bands were fractionated in both control and group II of the liver, while 4 bands except LDH_1 appeared in group I. In the kidney and serum, 5 LDH isozyme bands appeared in the all experimental groups.

REFERENCES

Bae, H.W., 1978. Korean ginseng, 2nd ed. Korea Ginseng Research Instituted Publication pp. 148-151.

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

Dawson, D.M., T.L. Goodfriend and N.O. Kaplan, 1964. Lactic dehydrogenase: functions of the two types. *Science* **143**:929-933.

Donaldson, M.L. and C.J. Gubler, 1978. Biochemical effects of mercury poisoning in rats. Am. J. Clin. Nutr. **31**:859-864.

Fang, S.C. and E. Fallin, 1976. The binding of various mercurial compounds to serum proteins. *Bull. Environ. Contam. Toxicol.* **15**:110-117.

Gelderman, A.H., H.V. Gelboin and A.C. Peacock, 1965. Lactic dehydrogenase isozymes in urine from patients with malignancies of the urinary bladder. *J. Lab. Clin. Med.* **65**:132-142.

Gritzka, T.L. and B.F. Trump, 1968. Renal tubular lesions caused by mercuric chloride. *Am. J. Path.* **52**:1225-1278.

Harper, H.A., V.W. Rodwell and P.A. Mayer, 1979. Review of physiological chemistry. Lange Medical Publications, Canada, pp. 294-298.

Hong, S.A., J.K. Lim an 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 poisons, 2nd ed. Macmillan Publishing Co., New York, pp. 421-428.

Kornberg, A., I.P. Colowick and N.O. Kaplan, 1955. *Methods Enzymol.* **1**:441.

Lau, S. and B. Sarkar, 1979. Inorganic mercury(II)-binding components in normal human blood serum. *J. Toxicol. Environ. Health* **5**:907-916.

Lindsay, L.T., 1963. Isozymic patterns and properties of lactate dehydrogenase from developing tissues of the chicken. *J. Exp. Zool.* **152**:75-89.

Nerenberg, S.T. and G. Pogojeff, 1969. Laboratory diagnosis of specific organ diseases by means of combined serum isoenzyme patterns. *Am. J. Clin. Path.* **51**:429-439.

Cura, H. and S. Hiai, 1974. Biochemical action *Panax ginseng* principle. *Proc. Int. Ginseng Symp.* pp. 23-26.

Richterich, R., P. Schafroth and H. Aebe, 1963. A study of lactic dehydrogenase isozyme pattern of human tissues by absorption-elution on sephadex-DEAE. *Clin. Chim. Acta* **8**:178-181.

Stryer, L., 1975. Biochemistry. Freeman Co., San Francisco, pp. 293-333.

Trump, B.F., P.I. Glodbatt and R.E. Stowell, 1965. Studies of necrosis *in vitro* of mouse hepatic parenchymal cells. *Lab. Invest.* **14**:1946-1986.

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.

Valek, A., 1965. Acute renal insufficiency in toxication with mercury compounds. I. Aetiology, clinical picture, renal function. *Acta Med. Scand.* **177**:63-67.

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 methylmercury. *Nature* **251**:236-237.

Wroblewski, F and K.F. Gregory, 1961. LDH isozymes and their distribution in normal tissue and plasma in disease states. *Ann. N.Y. Acad. Sci.* **94**:869-871.

Zondag, H.A. and F. Klein, 1968. Clinical applications of lactate dehydrogenase isozymes: alteration in malignancy. *Ann. N.Y. Acd. Sci.* **91**:578-586.