大韓獸醫學會誌(1997) 第37卷 第2號
Korean J Vet Res(1997) 37(2) : 383~388

Effect of cadmium on immune responses and enzyme activities in *BALB/c* mice

3. Enzyme activities

Chang-yong Yoon*, Tae-joong Kim, Jeong-gon Cho, Hee-jong Song

*Korean Food and Drug Administration, Eunpyung-ku, Seoul, 122-020, Korea**
Bio-Safety Research Institute, Chonbuk National University, Chonju, 561-756, Korea
(Received Feb 27, 1997)

카드뮴이 *BALB/c* 마우스의 면역반응 및 효소활성에 미치는 영향

3. 효소활성

윤창용* · 김태중 · 조정곤 · 송희종

국립식품의약품 안전본부 세균제제과* · 전북대학교 생체안전성연구소
(1997년 2월 27일 접수)

초 록 : 카드뮴이 마우스의 효소활성에 미치는 영향을 평가하고자 *BALB/c* 마우스를 대상으로 0, 25, 50, 100 및 200 ppm의 $CdCl_2$가 첨가된 음료를 7주동안 자유급식한 후 간 및 신장에서 카드뮴(Cd)의 축적정도 및 효소(LDH 및 SOD)활성 변화를 조사하여 다음과 같은 결과를 얻었다.

1. 간 및 신장에서의 Cd축적도는 투여량이 증가될 수록 높았으며 특히 신장에서 더욱 높았다.

2. 간장과 신장의 lactate dehydrogenase(LDH)활성치는 신장의 경우 농도가 증가할 수록 활성치도 증가(25 ppm ; $p<0.05$, 50, 100 및 200 ppm; $p<0.01$)하는 경향을 보였으나 간에서는 100 ppm까지는 농도에 비례하여 활성치도 증가(50 및 100 ppm; $p<0.05$)하였다가 200 ppm 투여군에서는 25 ppm 투여군 수준으로 감소되었다.

3. Superoxide dismutase(SOD)활성은 25 ppm 투여군의 간장에서 유의한 상승치($p<0.05$)를 보인 것을 제외하고는 대조군의 간 및 신장의 활성치와 유사한 결과를 보였다.

이상의 결과는 카드뮴이 농도에 따라서 생체내의 LDH 및 SOD와 같은 효소계의 활성에 영향을 미칠 수 있음을 시사한다.

Key words : cadmium, lactate dehydrogenase(LDH), superoxide dismutase(SOD), cadmium concentration, *BALB/c* mouse.

Address reprint requests to Dr. Chang-yong Yoon, Korean Food and Drug Administration, Eunpyung-ku, Seoul, 122-020, Republic of Korea.

Introduction

In recent years, cadmium(Cd) has been recognized as one of the most toxic environmental and industrial pollutants due to its ability to induce severe alterations in various organs and tissues following either acute or chronic exposure[1,2]. Consequently, extensive studies have been carried out to identify the mechanisms of Cd toxicity. Although the mechanisms of Cd toxicity are still not understood completely, several pathways for its toxicity have been proposed including DNA damage resulting from free radical generation[3], alteration in membrane structure and permeability which may be caused by lipid peroxidation[4], impairment of energy metabolism[1], inhibition of adenosine triphosphate-based membrane transport[5] and interaction with thiol ligands[5] etc. Moreover, a variety of accompanying changes in antioxidant defense system were reported[1,2,6-8].

On the other hand, cytotoxicity could be assessed by measuring the release of lactate dehydrogenase(LDH) from the cells[5] and increased activity of this enzyme was indicative of biologic stress on the tissues[9]. Recently, Cd-induced increase of superoxide anion radical(O_2^-) production and lipid peroxidation were reported[1,2,6]. O_2^- has been proposed to play a crucial role in cell neoplastic transformation and causes extensive strand breakage of DNA, protein-DNA crosslinks, oxidative modification of DNA bases and oxidation of thiol groups in proteins[10,11]. And also, lipid peroxidation constitutes a free radical oxidation process in which polyunsaturated fatty acids of the cell membrane decompose to yield, among others, highly reactive oxygen species and malondialdehyde[11]. Malondialdehyde is the most abundant individual aldehyde resulting from lipid peroxidation and causes cross-linking and polymerization of membrane components and may contribute to the mutagenesis, genotoxicity and carcinogenesis[11]. In biological system, there exist powerful antioxidant enzymes which are capable of controlling the cytotoxic effects of active oxygen species[10,11]. Among these enzymes, superoxide dismutase (SOD) is thought to play a very important role in protecting living cells, against toxic oxygen derivatives[12]. However, there were conflicting data concerning the activity of SOD in animals treated with Cd[1,7,8].

Hence, the purpose of the present study was to investigate the effects of varying concentrations of Cd on the enzyme(LDH and SOD) activities related with the concentrations of Cd in liver and kidney which are the main target organs of systemic Cd.

Materials and Methods

Animals : Male *BALB/c* mice, 6 to 8 weeks of age and weighing 17 to 25g, were obtained from the Korea research institute of chemical technology(Taejeon). Animals were housed five to six per cage, maintained at ambient temperatures of 20 to 23℃, and fed commercial rodent chow pellet(Samyang Co.) *ad libitum*.

Cadmium treatment : Mice received distilled water alone or water supplemented with 25, 50, 100 or 200 ppm of $CdCl_2$(Cd, Sigma) *ad libitum* for 7 weeks.

Lactate dehydrogenase(LDH) : Cytotoxicity by Cd was evaluated by measuring the activity of extracellular LDH[13]. One day following Cd treatment, liver and kidney were obtained, weighed and irrigated. And then, these organs were placed into 4-fold volume of LDH buffer(pH 7.4) and homogenized by polytron tissue homogenizer at 13,500 rpm for 5 minutes. The homogenized cell suspension was centrifuged at 2,500 rpm for 10 minutes. The supernatant was recentrifuged at 15,000 rpm for 10 minutes, and then, the supernatant was diluted 1/250 with LDH buffer. And 0.1 ml of this diluted sample was mixed with 0.2 ml of 200μM β-nicotinamide adenine dinucleotide, reduced form(β-NADH) and 2.5 ml LDH buffer, and stored at 37℃ for 5 minutes, and then, placed in 3 ml cuvettes. The reaction was initiated by adding 0.2 ml of 0.6 mM pyruvic acid. After 10 minutes, the rate of decrease of absorbance at 340 nm was recorded. LDH activity was calculated as follows;

$$\text{LDH activity} = \frac{\triangle A \times 1{,}000 \times 3.0}{10 \times 6.23 \times 0.2} = A \times 241 \text{ (IU/L)}$$

Superoxide dismutase(SOD)[14] : The organs obtained from mice one day after Cd-treatment were irrigated by sa-

line, and placed into 4-fold volume of 1 mM EDTA, 10 mM potassium phosphate buffer(pH 7.8) and homogenized. The final supernatant was obtained by the method described above. And 0.8 ml of buffer containing sample was mixed with 0.1 ml of 0.1 mM ferricytochrome C and 0.1 ml of 0.5 mM xanthine, and then, this mixture was placed in 3 ml cuvette at 25℃.

The reaction was initiated by adding 25μl of xanthine oxidase(0.025 U/ml) and the rate of increase of absorbance at 550nm was recorded. SOD activity was expressed as unit/mg protein, where one unit of SOD was defined as the amount of enzyme required to inhibit cytochrome C reduction by 50%.

Cd analysis : Cd in tissues obtained from each mouse was determined by flame atomic absorption spectrometer (AAS, 551 SpectrAA-400) after wet ashing with HNO_3, H_2SO_4 and $HClO_4$. Its detail procedure was shown in Fig 1.

Sample
- ⇦ HNO_3 25 ml
- ⇦ H_2SO_4 2 ml, $HClO_4$ 5 ml

Heating
- ⇦ H_2O 50ml
- ⇦ Ammonium citrate 10ml(25%)
- ⇦ BTB sol 4 drops

NH_4OH(1+1) : yellow → green
- ⇦ Ammonium sulfate 10ml(40%)

Separatory funnel
- ⇦ DDTC 6 ml(5%)
- ⇦ MIBK 20 ml

Shaking for 10 minutes

M.I.B.K. separation

Volatilization
- ⇦ HNO_3 3 ml
- ⇦ $HClO_4$ 2 ml

Volatilization
- ⇦ 0.1N HCl

Make-up to 5 ml

Flame A.A.S.

Fig 1. Method for determination of cadmium concentrations in liver and kidney in mice.

Protein determination : Protein contents in samples were determined by the method of Lawry *et al*[20] using bovine serum albumin(BSA) as a standard.

Statistical analysis : The statistical significance of data between unexposed control group and Cd-exposed groups was estimated by Student's paired *t*-test.

Results

Accumulation of cadmium(Cd) in liver and kidney : Mice were orally administered with varying concentrations of Cd for 7 weeks. One day following Cd exposure, these organs were removed and frozen at -72℃ for residual Cd analysis. As shown in Table 1, Cd was accumulated in a

Table 1. The cadmium concentrations in liver and kidney of mice administered with varying concentrations of $CdCl_2$ for 7 weeks

Groups	Liver	Kidney
25	10.50±0.79[a]	15.73±4.19
50	15.06±3.09	26.82±12.67
100	23.40±4.50	51.82±13.42
200	45.22±10.4	74.94±18.17
Control	1.83±0.22	2.01±0.48

[a]: Mean±SD.
Each group was composed of 5 male mice.
Cadmium contents were expressed as μg/g wet weight.

Table 2. The LDH activities in liver and kidney of mice administered with varying concentrations of $CdCl_2$ for 7 weeks

Groups	Activities of LDH(IU/L)[a]	
	Liver	Kidney
25	51.72±4.00[b]	63.91±4.68*
50	57.26±6.50*	65.36±7.47**
100	59.29±5.67*	65.56±1.47**
200	51.77±6.75	67.14±4.87**
Control	49.77±4.67	55.76±2.53

[a]: International unit/liter. [b]: Mean±SD.
Each group was composed of 5 male mice.
LDH activities of 250×diluted-homogenates of liver and kidney were measured by Mauck and Davis's method and unit was expressed as IU/L.
*$p<0.05$, **$p<0.01$ for the differences between control and experimental groups.

Table 3. The SOD activities in liver and kidney of mice administered with varying concentrations of $CdCl_2$ for 7 weeks

Groups	Activities of SOD(U/mg)[a]	
	Liver	Kidney
25	15.59±1.97[a,*]	9.10±0.94
50	11.39±2.82	9.52±1.25
100	12.65±3.31	9.11±0.57
200	12.63±3.97	9.11±0.60
Control	12.41±1.36	8.77±1.48

[a]: Mean±SD.
Each group was composed of 5 male mice.
SOD activities were expressed as unit/mg protein.
*: $p<0.05$ for the differences between control and experimental groups.

dose dependent fashion. And also, this pattern was more conspicuous in kidney than that in liver.

LDH and SOD activities of liver and kidney : After Cd administration for 7 weeks, changes of LDH and SOD activities by Cd were examined. LDH activities of liver and kidney were increased in a dose dependent fashion except for that of liver in 200 ppm Cd-fed group(Table 2). SOD activities of liver and kidney in Cd exposed mice were similar to that of control except for elevation of SOD activity in liver of 25 ppm Cd administered group(Table 3).

Discussion

Liver and kidney have been shown to be major target organs by Cd toxicity[15]. Therefore, in this study these organs were selected for investigation of Cd toxicity.

Our results indicate that Cd exposure at a higher dose causes significant accumulation in these organs(Table 1). Thus, Cd may have a dose dependent effect on its accumulation. And also, this pattern was more conspicuous in kidney than that in liver. It was explained by several reports that in chronic Cd feeding Cd was accumulated in the liver initially and gradually transported to the kidney in the form of Cd + metallothionein, thereby increasing the Cd deposition in the kidney[16,17].

Meanwhile, it is well-known that the activity and relative amount of LDH reflect toxic effects of pollutants, including Cd, under unfavorable conditions[18]. In the present study, LDH activities of liver and kidney were increased in a dose dependent fashion except for that of liver in 200 ppm Cd administered group which was similar to that of 25 ppm Cd group(Table 2). This assertion was supported by the reports that lysosomal activities of liver were increased against Hg invasion thereby promoting the cellular digestion and excretion of damaged organelles[19] and that Cd in liver increased linearly for the first duration of administration, but thereafter hepatic concentrations of Cd decreased gradually[15]. These cited reports suggest that the activity of hepatocytes can be recovered from Cd-induced damaged condition.

Recently, Cd-induced increase of O_2^- production and lipid peroxidation were reported although the mechanisms responsible for their formation were not well understood[1,2,6]. To protect the organisms from this cytotoxic O_2 metabolite, the enzyme SOD has involved[1,12]. SOD catalyzes breakdown of the superoxide radicals according to the reaction: $2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$. Thus, it is play a major role in protecting living cells, against deposition of endogeneous cytotoxic superoxide radicals[7,14]. However, Cd exposure has been reported variously to stimulate[1], to suppress[7], and to have no effect on the SOD activity[6,8]. Kostic *et al*[1] reported that it was reasonable to expect as a biological response an increased activity of SOD in RBCs of Cd-treated animals since Cd led to increased production of superoxide radicals. Whereas, Hussain *et al*[7] said that Cd inhibited SOD activity by directly interacting with the SOD molecules and several authors reported[6,8] that Cd did not produce any changes in SOD activities of various organs. And also, it was reported that activities of liver and kidney SOD could be fluctuated at various times after injection or administration of nickel[11] or mercury[19]. These results suggest that SOD activity can be shown differently by dose and route etc. of Cd exposure. In this study, SOD activities of Cd exposed liver and kidney were similar to that of control except for elevation of SOD activity in liver of 25 ppm Cd treated group. Namely, our results indicate that SOD activity can be elevated at low dose of Cd exposure but this increased activity can be reduced with the increase of Cd accumulation and tissue damage.

In conclusion, the activities of various enzymes can be

modulated by Cd intoxication. However, further study is needed to correlate Cd metabolism and cytotoxicity.

Summary

This study was designed to investigate the effects of varying concentrations of $CdCl_2$ on the enzyme activities such as LDH and SOD related with the accumulations of cadmium(Cd) in liver and kidney of *BALB/c* mice.

1. Cd accumulations in liver and kidey were increased in a dose dependent fashion. And also, this pattern was more conspicuous in kidney than that in liver.

2. LDH activities of liver and kidney were increased in a dose dependent fashion except for that of liver in 200 ppm Cd group which was similar to that of 25 ppm Cd group.

3. SOD activities of Cd exposed liver and kidney in Cd-fed mice similar to those of controls except for elevation of SOD activity of liver in 25 ppm Cd group.

The results of this study suggest that the activities of various enzymes can be modulated by Cd intoxication.

Acknowledgement: This study was financially supported in part by a Research Grant from Bio-Safty Research Institute, Chonbuk National University in 1997(CNU-BSRI, No. 97-03).

References

1. Kostic MM, Ognjanovic B, Dimitrijevics, *et al.* Cadmum-induced changes of antioxidant and metabolic status in red blood cells of rats : *in vivo* effects. *Eur J Haematol*, 51:86-92, 1993.
2. Manca D, Ricard AC, Trottier B, *et al.* Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. *Toxicology*, 67:303-323, 1991.
3. Coogan TP, Bare RM, Waalkes MP. Cadmium-induced DNA strand damage in cultured liver cells: Reduction in cadmium genotoxicity following zinc pretreatment. *Toxicol Appl Pharmacol*, 113:227-233, 1992.
4. Stacey NH, Catilena LR, Klaassen CD. Cadmium toxicity and lipid peroxidation in isolated rat hepatocytes. *Toxicol Appl Pharmacol*, 53:470-480, 1980.
5. Beattie JH, Marion M, Schmit JP, *et al.* The cytotoxic effects of cadmium chloride and mercuric chloride mixtures in rat primary hepatocyte cultures. *Toxicology*, 62:161-173, 1990.
6. Gill KD, Pal R, Nath R. Effect of cadmium on lipid peroxidation and antioxidant enzymes in undernourished weanling rat brain. *Pharmacol Toxicol*, 65:73-77, 1989.
7. Hussain T, Shukla GS, Chandra SV. Effects of cadmium on superoxide dismutase and lipid peroxidation in liver and kidney of growing rats : *in vivo* and *in vitro* studies. *Pharmacol Toxicol*, 60:355-358, 1987.
8. Park BY, Park JD, Chung KC. Dose-response relationship between catalase and superoxide dismutase activity in testes of acutely intoxicated rats by cadmium. *Kor J Preventive Med*, 21:295-306, 1988.
9. Hagadorn JE, Bloor CM, Yang MS. Elevated plasma activity of lactate dehydrogenase isoenzyme-3(LDH_3) in experimentally induced immunologic lung injury. *Am J Pathol*, 64: 575-584, 1971.
10. Kappus H, Sies H. Toxic drug effects associated with oxygen metabolism : Redox cycling and lipid peroxidation. *Experientia*, 37:1223-1241, 1981.
11. Misra M, Rodriguez RE, Kasprzak KS. Nickel induced lipid peroxidation in the rat: Correlation with nickel effect on antioxidant defense systems. *Toxicology*, 64:1-17, 1990
12. Sun Y, Oberley LW, Li Y. A Simple method for clinical assay of superoxide dismutase. *Clin Chem*, 34: 497-500, 1988.
13. Mauck JC, Davis JE. Clinical enzymology. In: SonnenwirthAC, JaretteL(eds.), *Gradwohl's Clinical Laboratory Methods and Diagnosis*, 8th ed., CY Mosby Co, St Louis 1:318-321, 1980.
14. Crapo JD, McCord JM, Fridovich I. Preparation and assay of superoxide dismutase. In: Fleischer S and Packer L (eds.), *Methods in enzymology*, Biomembranes part D: Biological oxidants mitochondrial and microbial system. Academic Press, New York 53:382-387, 1978.
15. Dudley RE, Gammal LM, Klaassen CD. Cadmium-in-

duced hepatic and renal injury in chronically exposed rats: Likely role of hepatic cadmium-metallothionein in nephrotoxicity. *Toxicol Appl Pharmacol*, 77:414-426, 1985.

16. Probst GS, Bousquet WF, Miya TS. Kinetics of cadmium-induced hepatic and renal metallothionein synthesis in the mouse. *Toxicol Appl Pharmacol*, 39:51-60, 1977.
17. Webb M, Etienne AT. Studies on the toxicity and metabolism of cadmium-thionein. *Biochem Pharmacol*, 26:25-30, 1977.
18. Chung HW, Lee CD, Choi SS. Toxicological effect of methylmercury on lactate dehydrogenase in organs of killifish, *Oryzias latipes. Res Rep Env Sci Tech*, 6:17-22, 1988.
19. Kim JS. A Study on the lactate dehydrogenase and antioxidant enzyme activity in mouse administrated mercury chloride in the drinking water, Ph.D. thesis, Department of Medical Science, Chonbuk National University Graduate School, 1993.
20. Bollag DM, Edelstein *SJ. Protein method*, Wiley-Liss. 56-59, 1991.