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Effect of Adriamycin on the Activities of Superoxide Dismutase, Glutathione Peroxidase and Catalase in Tissues of Mice

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The increment of lipid peroxide in the hearts of mice treated with adriamycin (ADR) was examined in relation to the decrease in the activities of superoxide dismutase (SOD), glutathione peroxidase (GSHpx) and catalase. The natural activities of these enzymes in mouse heart are lower than those in the liver. The biggest decrease in enzyme activity observed in the heart after ADR administration was that of GSHpx. Therefore, the increment of lipid peroxide was attributable to the decrease in the activities of these enzymes, especially GSHpx. Subsequently, the effects of antioxidants on the decreases in activities of SOD, GSHpx and catalase in the hearts of mice treated with ADR were examined. However, the decrease in the activities of the enzymes were not accompanied with any increment of lipid peroxide. This result suggests that active oxygen radicals produced by ADR through the agent's redox cycling have no effect on the activities of these enzymes. Therefore, it appears that the decrease in the activities of these enzymes induced by ADR in the mouse results from inhibition of enzyme protein biosynthesis.

Key words: Adriamycin — Superoxide dismutase — Glutathione peroxidase — Catalase — Antioxidant

The severe cardiotoxicity of anthracycline antitumor antibiotics, such as adriamycin (ADR) and daunomycin (DAM), is well known and is thought to be due to[1]: 1) interaction with DNA, 2) production of free radicals, 3) membrane lipid damage, 4) an effect on the mitochondrial membrane and 5) alteration of calcium transport. In connection with 2), 3) and 4), Myers *et al.*[2,3] proposed that ADR-induced cardiotoxicity in mice was associated with an increase in lipid peroxide level in the myocardium, because the cardiac lesion produced by ADR administration to mice is strikingly similar to the myocardial pathology produced by α-tocopherol deficiency in this animal. Our previous studies[4] showed that the lipid peroxide levels in the heart, liver and kidney of the mouse increased after ADR administration, and that the increase in the heart lipid peroxide level was maintained at a high level. We also confirmed that NADPH-dependent lipid peroxidation in microsomes of mouse liver was promoted by ADR *in vitro.*[5] Therefore, studies on the mechanism of this increment of lipid peroxides appear to be important for clarifying the cause of the cardiotoxicity induced by ADR.

Currently, the mechanism of the increment of lipid peroxides induced by ADR is considered to be active oxygen radical production by the redox cycle of ADR, on the basis of *in vitro* experiments.[6] However, as there was a time lag between the lipid peroxide increment in the mouse tissues and the tissue concentrations of ADR after its administration in our experiments,[7,8] we though that the *in vitro* results were not directly applicable to situation *in vivo.*

The living body contains antioxidative enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GSHpx, EC 1.11.1.9) and catalase (EC 1.11.1.6), which protect against lipid peroxidation. In the present study, we examined the changes in the activities of these enzymes in tissues of mice after ADR administration, in order to clarify whether this lipid peroxide increment is induced by decreases in these antioxidative enzyme activities.

MATERIALS AND METHODS

ADR injection, 10 mg/vial (Adriacin), was purchased from Kyowa Fermentation Inc., Tokyo. The drug was dissolved in sterile isotonic saline to obtain a 1.0 mg/ml solution. α-Tocopherol (Wako Pure Chemical Industries, Ltd., Tokyo) was emulsified with sterile isotonic saline using Tween 80.

Xanthine oxidase (EC 1.2.3.2), peroxidase (EC 1.11.1.7), *o*-dianisidine 2HCl and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, MO. Nitroblue tetrazolium was obtained from Tokyo Kasei Kogyo, Tokyo, and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) was purchased from Wako Pure Chemical Industries, Ltd. The other chemicals used in this study were of the highest purity available.

Animals Male CDF_1 mice, 5 weeks old and weighing 20–25 g, obtained from Shizuoka Laboratory Animal Center, Hamamatsu, were used. The animals were housed in a room maintained at 25 ± 1°C with 55 ± 5%

relative humidity and given free access to standard laboratory feed and water for one week.

Animal experiments ADR at a dose of 15 mg/kg was injected intraperitoneally into the mice. Control mice were injected with the same volume of sterile isotonic saline alone. The animals were killed by cervical dislocation at a definite time within 6 days after ADR administration. After bleeding, the lung, heart, liver and kidney were rapidly dissected out. The tissue samples were homogenized in a suitable buffer solution at 4°C in a glass Potter-Elvehjem-type homogenizer with a Teflon pestle, according to the method used for the determination of each enzyme activity.

Measurements of antioxidative enzyme activities

SOD activity: The homogenate was prepared with 0.1 *M* phosphate buffer (pH 7.0) and centrifuged at 100,000*g* for 60 min. The supernatant was dialyzed overnight against 0.1 *M* phosphate buffer (pH 7.0) and analyzed for SOD activity by the method of Imanari *et al.*[9] The reaction mixture (2.9 ml) contained 0.043 *M* Na_2CO_3 buffer (pH 10.2), 0.1 m*M* xanthine, 0.1 m*M* EDTA, 0.05 mg/ml bovine serum albumin, 0.025 m*M* nitroblue tetrazolium and the sample. After a 10 min preincubation at 25°C, the reaction was started with 0.1 ml of xanthine oxidase and incubation was performed for 20 min at 25°C. After addition of 0.2 m*M* $CuCl_2$, the absorbance of the solution at 560 nm was measured. The activity of SOD required to inhibit the ratio of NBT reduction by 50% was defined as 1 unit of activity.

GSHpx activity: The homogenate was prepared with 0.15 *M* KCl solution and centrifuged at 10,000*g* for 20 min. The supernatant was analyzed for GSHpx activity by the method of Hafemann *et al.*[10] The enzyme assay tubes, containing 0.2 m*M* GSH, 0.4 *M* phosphate buffer (pH 7.0), 1 m*M* NaN_3, the sample and 0.25 m*M* H_2O_2, a total of 2.5 ml, were incubated at 37°C for 6 min. After addition of 2.0 ml of 1.67% HPO_3, this mixture was centrifuged at 3,000 rpm for 15 min. The supernatant (2.0 ml) was added to a mixture of 2.0 ml of 0.4 *M* Na_2HPO_4 and 1.0 ml 1.0 m*M* DTNB. Soon after a 10-min incubation at 37°C, the absorbance of the reaction mixture was measured at 412 nm. For convenience, one unit of enzyme activity was defined as a decrease in the log[GSH]of 0.001 per minute after subtraction of the decrease in log[GSH] per minute for the non-enzymatic reaction.

Catalase activity: The homogenate was prepared with 0.1 *M* phosphate buffer (pH 7.0) and centrifuged at 100,000*g* for 60 min. The supernatant was analyzed for catalase activity by the method of Okazaki *et al.*[11] The reaction mixture contained 0.45 m*M* H_2O_2 (in 0.1 *M* phosphate buffer, pH 7.0) and the sample. Aliquots of the reaction mixture (0.5 ml) were removed at 20 s intervals and added to 2.0 ml of a solution containing 0.2 mg/ml *o*-dianisidine, 0.0125 mg/ml peroxidase and 0.081 mg/ml NaN_3. After a 10-min incubation at room temperature, 50% (v/v) H_2SO_4 was added to stop the reaction. The absorbance of the reaction mixture was measured at 530 nm. One unit of enzyme activity (k′) was calculated as follows:

$$k' = \frac{2.303}{t} \log \frac{a}{a-x}$$

a, starting concentration of H_2O_2;
$a-x$, H_2O_2 concentration after *t* min.

Lipid peroxide level: Determination of the lipid peroxide level in tissue samples was carried out according to the method described in our previous paper.[12]

Antioxidant administration test Intraperitoneal injection of glutathione (GSH, 10 mg/kg/day) or α-tocopherol (50 mg/kg/day) into mice was started on the day before ADR (15 mg/kg, ip) administration and continued for 4 days. ADR injection was done 3 h after the second administration of each antioxidant. The animals in the control or normal group were injected with the same volume of saline.

The animals were killed on the day following the last administration of each antioxidant to determine the activity of each antioxidative enzyme and the level of lipid peroxide in the heart.

Statistical analysis Statistical significance was evaluated by the use of Student's *t* test.

RESULTS

Time courses of activities of SOD, GSHpx and catalase in tissues after ADR administration

Liver (Fig. 1) SOD activity: At 3 h after ADR administration, SOD activity was transiently decreased to 71% ($P<0.001$) of the normal level (107.4±4.7 U/mg protein), and returned to the normal level by the 1st day after ADR administration. Then, the activity gradually decreased again and was 73% ($P<0.001$) of the normal level at the 3rd day. Subsequently, the activity gradually recovered.

GSHpx activity: At 3 h after ADR administration, the GSHpx activity was also decreased to 93% of the normal level (214.5±18.6 U/mg protein), and after that no significant change occurred. The ratio of decrease of the GSHpx activity was greatest at the 3rd day after ADR administration (84% of the normal level, $P<0.05$).

Catalase activity: The activity was remarkably decreased to 56% ($P<0.001$) of the normal level (4.79± 0.41 k′/mg protein) at 3 h after ADR administration. The activity recovered slightly after 1 day, and then decreased again to 51% ($P<0.001$) and 63% ($P<0.001$) at the 4th and 5th day after ADR administration, respectively.

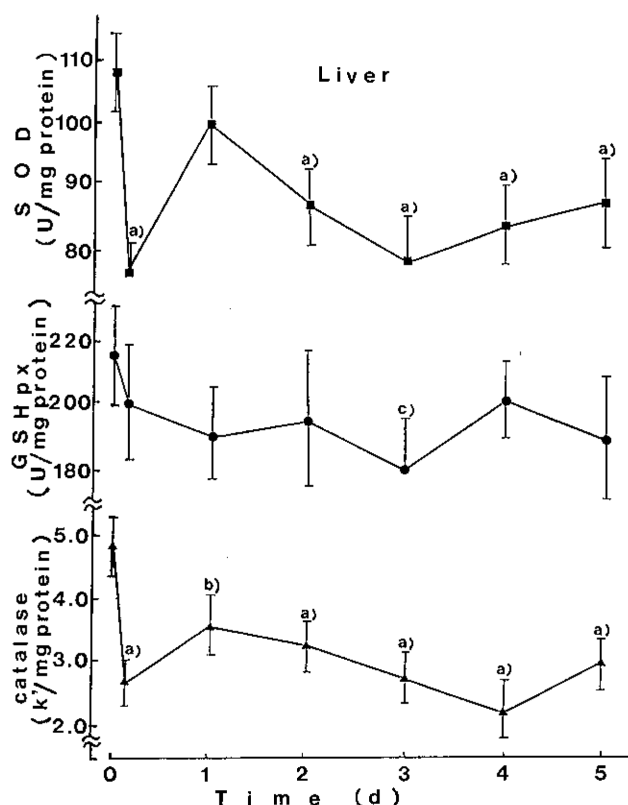

Fig. 1. Time course of the activities of SOD, GSHpx and catalase in the livers of male CDF_1 mice after intraperitoneal administration of ADR (15 mg/kg). Each point represents the mean±SD for 5 mice. Significantly different from the control values, a) $P<0.001$, b) $P<0.01$, and c) $P<0.05$.

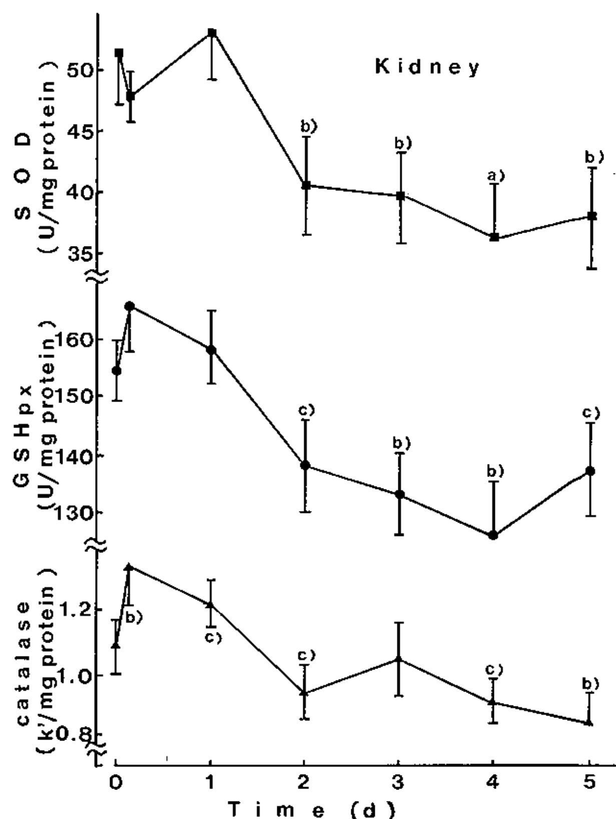

Fig. 2. Time course of the activities of SOD, GSHpx and catalase in the kidneys of male CDF_1 mice after intraperitoneal administration of ADR (15 mg/kg). Each point represents the mean±SD for 5 mice. Significantly different from the control values, a) $P<0.001$, b) $P<0.01$, and c) $P<0.05$.

Kidney (Fig. 2) SOD activity: At 3 h after ADR, the SOD activity was decreased to 93% of the normal level (51.5±4.4 U/mg protein). After that, the activity was quickly regained. However, the SOD activity then decreased rapidly to 72% ($P<0.001$) and 75% ($P<0.01$) at 4 and 5 days, respectively.

GSHpx activity: The activity was 106% of the normal level (153.7±4.9 U/mg protein) at 3 h after ADR administration, shortly folllowed by a gradual decrease. The level at the 4th day was 83% ($P<0.01$) of normal. However, it recovered slightly to 90% ($P<0.001$) of the normal level at the 5th day.

Catalase activity: The activity was elevated to 120% ($P<0.01$) of the normal level (1.82±0.09 k'/mg protein) at 3 h after ADR treatment. Then the activity decreased to 87% of the normal level at the 2nd day. The activity at 5 days was 82% of normal.

Heart (Fig. 3) SOD activity: At 3 h after ADR administration, the SOD activity transiently decreased to 83% ($P<0.01$) of the normal level (22.4±1.2 U/mg protein). From the 1st day, the activity decreased gradually, reaching 73% ($P<0.001$) and 77% ($P<0.001$) of the normal level on the 4th and 5th days after ADR administration, respectively.

GSHpx activity: On the 1st day after ADR administration, the GSHpx activity was 54% ($P<0.001$) of the normal level (11.9±0.6 U/mg protein). Afterwards, the GSHpx activity remained at this low level. Even at the 5th day, the activity was 61% ($P<0.001$) of normal.

Catalase activity: The catalase activity showed a transient 10% increase relative to the normal level (0.25±0.02 k'/mg protein) at 3 h after ADR administration. Then, the activity gradually decreased to 81% ($P<0.05$) and 72% ($P<0.01$) of normal on the 2nd and 5th days, respectively.

Effects of antioxidants The effects of antioxidants on the activity of each enzyme after ADR administration were examined. The results are shown in Table I.

Both GSH and α-tocopherol had no effect on the significant decreases in SOD, GSHpx and catalase activities in the hearts of mice treated with ADR. On the other hand, the lipid peroxide level in mouse heart was significantly increased after ADR administration, as described in a previous paper.[7] Both GSH and α-tocopherol significantly decreased the increase in the lipid peroxide level induced by ADR. The inhibition ratios by GSH and α-tocopherol were 73.6% and 89.4%, respectively.

DISCUSSION

It is certain that the increment of lipid peroxides induced by ADR is closely correlated with the cardiotoxicity of this agent, on the basis of several biochemical and histological findings.[2]

It is well known that SOD, GSHpx and catalase play important roles as protective enzymes against lipid peroxidation in tissues, and several studies[13-17] have examined changes in SOD, GSHpx and catalase activities in tissues of experimental animals after ADR administration. However, no report has described the simultaneous determination of these three enzymes, and only a few[13, 14]

Fig. 3. Time course of the activities of SOD, GSHpx and catalase in the hearts of male CDF$_1$ mice after intraperitoneal administration of ADR (15 mg/kg). Each point represents the mean±SD for 5 mice. Significantly different from the control values, a) $P<0.001$, b) $P<0.01$, and c) $P<0.05$.

Table I. Effect of GSH or α-Tocopherol on ADR-induced Changes in Enzyme Activities and Lipid Peroxide Levels in Mouse Heart

	Saline (15 ml/kg, ip)	ADR (15 mg/kg, ip)		
	Normal (saline, 10 ml/kg)	Control (saline, 10 ml/kg)	+GSH (10 mg/kg)	+ α-Tocopherol (50 mg/kg)
SOD (U/mg protein)	21.51±1.13[a]	16.24±0.83	16.06±1.15	16.90±1.28
GSHpx (U/mg protein)	10.89±1.08[a]	5.96±0.59	6.46±0.62	6.31±0.58
Catalase (k′/mg protein)	0.29±0.04[b]	0.21±0.03	0.22±0.02	0.24±0.03
Lipid Peroxide (nmol/g tissue)	238.65±19.41[b]	514.61±38.97	311.64±29.41[a]	267.81±28.21[a]

Administration schedule: 0 (Drug), 1 (Drug, ADR), 2 (Drug), 3 (Drug), 4 (Slaughter) (days)

Values represent means ±SD for 5–6 animals. Significant differences from the control values are indicated by *a*) $P<0.001$ and *b*) $P<0.01$.

have dealt with the time courses of activities of these enzymes after ADR administration.

In the present study, the activities of SOD and catalase in the liver decreased transiently at an early stage after ADR administration. This decrease in the liver has been thought to be induced by the toxicity of excessively produced active oxygen radicals, including the superoxide anion radical, after ADR administration. It was apparent that the amount of active oxygen radicals was not sufficient to destroy the enzymes, since the activities of both SOD and catalase recovered almost completely within 24 h after ADR administration. Furthermore, there was no significant change in liver GSHpx activity and the ratio of increase in the lipid peroxide level in the liver 24 h after ADR treatment was low.[7] The activities of SOD and catalase on and after the 24th hour and the activity of GSHpx after 3 days in the liver were significantly lower than the respective control levels. Therefore, the increment of lipid peroxide level in the liver within 24 h after ADR administration was mainly attributable to decreases in the activities of SOD and catalase. The bigger increment on and after the 3rd day was a result of decreases in the activities of the three enzymes, since ADR in tissues was scarcely detected from 24 h after administration of the agent.[8] The decreases in the activities of the three enzymes seemed to be induced by the inhibition of enzyme protein biosynthesis, based on the inhibition of DNA synthesis, which is the known antitumor mechanism of ADR.[18] It seems to be wrong to attribute the decreases in the activities of the three enzymes to active oxygen radicals produced by ADR.

In the kidney, the activities of these three enzymes showed the same time courses after ADR administration. Namely, they decreased to 70–80% of the normal levels by 3–4 days later. In other words, the changes in enzyme activities and ADR tissue concentration[8] in the kidney seemed to be in conflict with each other. These results also suggest that the decreases in enzyme activities seen after ADR administration were not caused by active oxygen radicals produced by ADR.

In the heart, where a severe side-effect of ADR occurs, the activities of these enzymes were the lowest among all the tissues examined in normal mice. The activities of SOD, GSHpx and catalase in the heart were only 21%, 5.6% and 5.2% of those in the liver, respectively, in normal mice. When taking into account the maximum ADR concentration in the heart,[8] which was about 56% of that in the liver, the level of each enzyme activity in mouse heart appears to be extremely low for the purpose of protection against increment of ADR-induced lipid peroxide.[8] Thus, it is easy to conclude that the heart is affected readily by ADR, which inhibits the syntheses of DNA, RNA and proteins, thus being linked to the biosyntheses of SOD, GSHpx and catalase. In particular, the cardiac level of GSHpx activity, which was scarcely decreased in the liver and kidney after ADR administration, decreased to about 50% of the normal level at 24 h after ADR administration, and did not recover until the 5th day. The percentage decrease of GSHpx activity was the largest among the three enzymes. Therefore, we may conclude simply that the increment of lipid peroxide in the heart after ADR administration was attributable mainly to the decrease in GSHpx activity. However, it is crucial not to neglect the fact that there was a time lag between lipid peroxide increment[7] and the decrease of the activity of GSHpx in the heart after ADR treatment. It is clear that the increment of lipid peroxide was attributable to decreases in the activities of SOD and catalase, as well. Namely the lipid peroxide level in the liver and heart tissue after ADR administration was the result of the overall activities of three enzymes. Doroshow *et al.*[14] have reported that the activity of GSHpx in the heart of normal rats was 86% of that in the liver. However, the activity of GSHpx in the heart of normal rats was reported to be 5–10% of that in the liver in general,[19] in agreement with our result (5.6%).

In the lung, there is little change in the activities of the three enzymes after ADR administration (data not shown). At present, we can not explain this lack of change. However, a lack of any increment of lipid peroxide in the lung after ADR administration, as described in a previous paper,[9] was thought to be closely related to the stability of these enzymes in the lung, even though their activities in normal mice are not very high. Furthermore, as the lung is the main organ that comes in direct contact with atmospheric oxygen, it might be protected from lipid peroxidation by some mechanism. For example, the amount of unsaturated fatty acids in the lung, which are substrates for lipid peroxidation, is said to be lower than that in other tissues.[20]

Next, the effects of glutathione (GSH) and α-tocopherol, both antioxidants, on the decreases in the activities of SOD, GSHpx and catalase in the hearts of mice treated with ADR were examined. In the heart, which was selected because of the marked ADR-induced cardiotoxicity, both antioxidants significantly depressed the lipid peroxidation induced by ADR. However, neither of them had any effect on the decreases in the activities of the three enzymes that were seen after ADR administration. These enzymes have been speculated to be affected by the increment of lipid peroxide induced by ADR.[21] However, the present results showed that lipid peroxide produced by ADR had no effect on these enzymes. Therefore, the decrease in the activities of these enzymes after ADR administration is considered to be the result of inhibition of DNA, RNA and protein biosyntheses, which are the fundamental antitumor effects

of ADR. The increment of lipid peroxide appears to be a result of the decrease in these enzymes activities. It was also proved that the increment of lipid peroxide induced by ADR could be depressed by antioxidants, although the activities of SOD, GSHpx and catalase were not normalized.

In conclusion, it is clear that the increment of lipid peroxide in the hearts of mice treated with ADR is attributable to a decrease in the activities of SOD, GSHpx and catalase, the natural activities of which are lower than those in the liver. The biggest decrease of enzyme activity seen in the heart after ADR administration was that of GSHpx. It was also strongly suggested that the decrease in the activities of these enzymes was the result of inhibition of enzyme protein biosynthesis. The effects on nucleic acid and protein biosyntheses are the main actions of ADR, and not the direct action of active oxygen radicals generated from the redox cycling of the drug. Further results concerning the effects of ADR on DNA, RNA and protein biosynthesis in mouse heart will be reported in detail in the next paper.

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REFERENCES

1) Politi, P. M. and Rothlin, R. P. Acute effects of doxorubicin on chronotropic and inotropic mechanisms in guinea pig atria. *Cancer Treat. Rep.*, **69**, 859–865 (1985).
2) Myers, C. E., McGuire, W. P., Liss, R. H., Ifrim, I., Grotzinger, K. and Young, R. C. Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response. *Science*, **197**, 165–167 (1977).
3) Myers, C. E., McGuire, W. P. and Young, R. C. Adriamycin: amelioration of toxicity by α-tocopherol. *Cancer Treat. Rep.*, **60**, 961–962 (1976).
4) Sazuka, Y., Hirose, T., Hashimoto, J., Tanizawa, H. and Takino, Y. Species, strain, sex and weekly age differences of lipid peroxide levels in animal tissues before and after adriamycin administration. *Chem. Pharm. Bull.*, **32**, 4110–4116 (1984).
5) Sazuka, Y. and Tanizawa, H. Studies on the mechanism of increment of lipid peroxides induced by anthracyclines — effect of aclacinomycin on lipid peroxide in mice tissues. *Proc. Jpn. Cancer Assoc., 47th Annu. Meet.*, 550 (1988) (in Japanese).
6) Goodman, J. and Hochstein, P. Generation of free radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. *Biochem. Biophys. Res. Commun.*, **77**, 797–803 (1977).
7) Tanizawa, H., Sazuka, Y. and Takino, Y. Change of lipid peroxide levels in mouse organs after adriamycin administration. *Chem. Pharm. Bull.*, **31**, 1714–1718 (1983).
8) Sazuka, Y., Yoshikawa, K., Tanizawa, H. and Takino, Y. Effect of doxorubicin on lipid peroxide levels in tissues of mice. *Jpn. J. Cancer Res.*, **78**, 1281–1286 (1987).
9) Imanari, T., Hirota, M., Miyazaki, M., Hayakawa, K. and Tamura, Z. Improved assay method for superoxide dismutase. *Igaku no Ayumi*, **101**, 496–497 (1977)(in Japanese).
10) Hafemann, D. G., Sunde, R. A. and Hoekstra, W. G. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *J. Nutr.*, **104**, 580–587 (1974).
11) Okazaki, T., Ohkusu, T. and Shukuya, R. An improved photometric assay of catalase activity in biological materials. *J. Nippon Med. Sch.*, **43**, 143–148 (1976) (in Japanese).
12) Tanizawa, H., Sazuka, Y. and Takino, Y. Microdetermination of lipoperoxide in the mouse myocardium by thiobarbituric acid fluorophotometry. *Chem. Pharm. Bull.*, **29**, 2910–2914 (1981).
13) D'Alessandro, N., Dusonchet, L., Crosta, L., Creseimanno, M. and Rausa, L. Does catalase play a role in adriamycin-induced cardiotoxicity? *Pharm. Res. Commun.*, **12**, 441–446 (1980).
14) Doroshow, J. H., Locker, G. Y. and Myers, C. E. Enzymatic defences of the mouse heart against reactive oxygen metabolites. *J. Clin. Invest.*, **65**, 128–135 (1980).
15) Zidenberg, C. S. and Keen, C. Influence of dietary manganese and vitamin E on adriamycin toxicity in mice. *Toxicol. Lett.*, **30**, 79–87 (1986).
16) Revis, N. W. and Marusic, N. Glutathione peroxidase activity and selenium concentration in the hearts of doxorubicin-treated rabbits. *J. Mol. Cell. Cardiol.*, **10**, 945–951 (1978).
17) Facchinetti, T., Delaini, F., Salmona, M., Donati, M. B., Feuerstein, S. and Wendel, A. The influence of selenium intake on chronic adriamycin toxicity and lipid peroxidation in rats. *Toxicol. Lett.*, **15**, 301–307 (1983).
18) Wang, J. J., Chervinsky, D. S. and Rosen, J. M. Comparative biochemical studies of adriamycin and daunomycin in leukemic cells. *Cancer Res.*, **32**, 511–515 (1972).
19) Behne, D. and Wolters, W. Distribution of selenium and glutathione peroxidase in the rat. *J. Nutr.*, **113**, 456–461 (1983).
20) Ohno, K. and Shimojo, S. Phospholipid metabolism in the lung. *Metabolism*, **11**, 1568–1585 (1974)(in Japanese).
21) Gutteridge, J. M. C. Lipid peroxidation and possible hydroxy radical formation stimulated by the self-reduction of a doxorubicin-iron (III) complex. *Biochem. Pharm.*, **33**, 1725–1728 (1984).