Effect of Tetrahydropyranyladriamycin (THP-ADR) and 4′-Epiadriamycin (4′-Epi-ADR) on Lipid Peroxide Levels in Mice

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Lipid peroxide levels were measured in mouse tissues to study their relation to the cardiotoxicity of anthracyclines. The effects of tetrahydropyranyladriamycin (THP-ADR) and 4′-epiadriamycin (4′-epi-ADR), which are less cardiotoxic than adriamycin (ADR), were examined. Neither THP-ADR nor 4′-epi-ADR increased the lipid peroxide levels in the heart, however, both increased reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent lipid peroxidation in mouse liver and lung microsomes, to the same degree as ADR. Therefore, the results obtained *in vitro* were not the same as those obtained *in vivo*. Of all of the anthracyclines tested, only THP-ADR increased the lipid peroxide levels in the lung. Thus, THP-ADR may be pulmotoxic. Differences in the activities of glutathione peroxidase and glutathione S-transferase reflected the differences in lipid peroxide levels.

Keywords lipid peroxide; tetrahydropyranyladriamycin; 4′-epiadriamycin; adriamycin; glutathione peroxidase; glutathione S-transferase

Adriamycin (ADR), an anthracycline antitumor antibiotic, is important in cancer chemotherapy because of its wide spectrum and potent antitumor activity.[1,2] Its clinical use, however, is severely limited by dose-dependent cardiotoxicity.[3,4] Myers *et al.* demonstrated that ADR-induced cardiotoxicity in mice was associated with increased lipid peroxide levels in the myocardium.[5,6] We reported that the increase in lipid peroxide levels in the hearts of mice treated with ADR is attributable to a decrease in the cardiac activity of enzymes which prevent lipid peroxidation, particularly glutathione peroxidase (GSHpx), and also to inhibition of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein biosyntheses in the heart.[7-10] Because of the usefulness of ADR, new anthracyclines have been developed. Tetrahydropyranyladriamycin (THP-ADR)[11] and 4′-epiadriamycin (4′-epi-ADR)[12] are now commercially available. However, there are few fundamental or clinical studies of their toxicity in noncardiac tissues. Thus, there is no data on the changes in lipid peroxide levels in mouse tissues after THP-ADR or 4′-epi-ADR administration. In this study, the effects of these drugs on reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent microsomal lipid peroxidation in mouse liver and lung *in vitro*, and on the lipid peroxide levels in mouse tissues *in vivo*, were examined and compared with the effects of ADR. We also measured GSHpx activity, which is decreased after ADR administration,[9] and glutathione S-transferase (GST) activity in these mice in order to clarify the relations between lipid peroxide levels and the activities of these enzymes.

Experimental

Materials ADR injection, 10 mg/vial (Adriacin®) and 4′-epi-ADR injection, 20 mg/vial (Farmorbicin®), were obtained from Kyowa Fermentation Inc. (Tokyo, Japan). THP-ADR injection, 10 mg/vial (Pinorubin®), was purchased from Nihon Kayaku Co., Ltd. (Tokyo, Japan). These agents were thawed and diluted with sterile isotonic saline to obtain 1.0 mg/ml solutions. The chemicals used to determine lipid peroxide levels were similar to those used previously.[13] The other chemicals used were of the highest purity available.

Animal Experiment Animal experiments were carried out as previously described.[14] Male CDF_1 mice were divided into several groups of 5—6 mice each, then ADR, THP-ADR or 4′-epi-ADR at 15 mg/kg was intraperitoneally injected. Control animals were injected with the same volume of sterile isotonic saline. The animals were killed by cervical dislocation on the 4th day after drug administration. The lungs, heart, liver and kidneys were rapidly dissected out. 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 to determine lipid peroxide level[13] and the activity of each enzyme. Methods for measurement of GSHpx and GST activities have been described.[15]

NADPH-Dependent Lipid Peroxidation Test NADPH-dependent lipid peroxidation in the microsomal fraction of the mouse liver was assayed according to Svingen *et al.*[16]

Results

Effect of THP-ADR and 4′-Epi-ADR on Lipid Peroxide Levels (*in Vivo*) Lipid peroxide levels in the tissues of mice on the 4th day following ADR, THP-ADR or 4′-epi-ADR injection (15 mg/kg, i.p.) are shown in Fig. 1.

Lipid peroxide levels in hearts of mice given ADR were 1.9 ($p<0.001$) times greater than in hearts of control mice (0.529 ± 0.161 nmol/mg protein). In contrast, there were no significant differences between hearts of control mice and of those given THP-ADR or 4′-epi-ADR. The lipid peroxide levels in the livers of mice given ADR, THP-ADR and 4′-epi-ADR were 1.5 ($p<0.001$), 1.3 ($p<0.001$) and 1.2 ($p<0.05$) times higher, respectively, than the control values. There was no significant difference between lipid peroxide levels in the lungs of mice given ADR or 4′-epi-ADR, and the corresponding control values. In contrast, the lung lipid peroxide level after THP-ADR administration was 1.4 times ($p<0.01$) the level in control mice.

The activities of GSHpx and GST in the lungs of mice on the 4th day after administration of ADR or 4′-epi-ADR did not differ from the activities in control mice (Table I).

However, in the lungs of mice given THP-ADR, GSHpx activity was 1.3 times ($p<0.001$) greater than in control mice and GST activity was 58.3% ($p<0.001$) of the control level. In all tissues except the lungs, the activities of GSHpx and GST in animals given THP-ADR or 4′-epi-ADR were not significantly different from those in control mice.

Effect of THP-ADR and 4′-Epi-ADR on NADPH-Dependent Lipid Peroxidation in Mouse Liver and Lung Microsomes (*in Vitro*) The lipid peroxide concentrations of the control group in the liver and lung microsomes increased about 8 times and 5 times after 60 min of incubation, respectively, and the presence of ADR, THP-ADR or 4′-epi-ADR led to closely dependent increases (Table II).

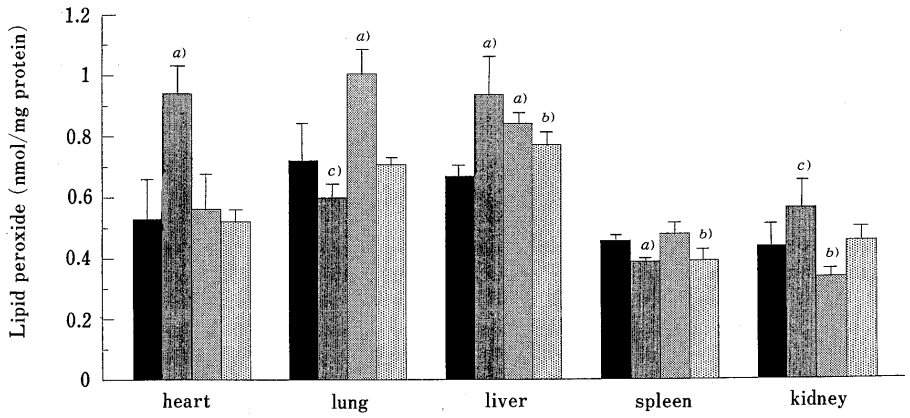

Fig. 1. Lipid Peroxide Levels in Mouse Tissues after ADR, THP-ADR or 4′-Epi-ADR Administration

Male CDF_1 mice received an intraperitoneal injection of ADR, THP-ADR or 4′-epi-ADR (15 mg/kg). The mice were killed by cervical dislocation on the 4th day after drug administration. Heights of the bars represent means ± S.D. for 5—6 mice in each group. *a*) $p<0.001$, *b*) $p<0.01$ and *c*) $p<0.05$: significantly different from the control value. ■, control; ▩, ADR; ▨, THP-ADR; ⊞, 4′-epi-ADR.

TABLE I. Lipid Peroxide (LPO) Levels and the Activities of GSHpx and GST in Lungs of Mice after ADR or THP-ADR or 4′-Epi-ADR Administration

	Control	ADR	THP-ADR	4′-Epi-ADR
LPO[a]	0.719±0.142	0.597±0.059 (93.0)	1.002±0.085[c] (139.2)	0.681±0.093 (94.7)
GSHpx[b]	31.10 ±1.80	34.20 ±3.00 (110.0)	41.75 ±3.69[c] (134.3)	32.48 ±2.88 (104.4)
GST[b]	23.80 ±1.68	21.60 ±0.74 (90.8)	13.88 ±2.18[c] (58.3)	21.94 ±1.57 (92.2)

Values in parentheses are percents of the corresponding control values. *a*) nmol/mg protein. *b*) unit/mg protein. Significantly different from the corresponding control value, *c*) $p<0.001$.

TABLE II. Effect of ADR, THP-ADR or 4′-Epi-ADR on NADPH-Dependent Lipid Peroxidation in Microsomes of Mouse Liver or Lung

Drug	Concentration (μmol/ml)	Lipid peroxide (%)[a]	
		Liver	Lung
Control	0	100.0±3.9[b]	100.0±1.7[c]
ADR	1	108.9±2.9[d]	119.8±2.3[d]
	10	123.7±6.6[d]	127.8±3.1[d]
	100	138.3±3.1[d]	139.5±6.8[d]
THP-ADR	1	101.6±2.0	116.0±4.6[d]
	10	114.4±4.3[d]	125.5±5.6[d]
	100	140.8±5.8[d]	149.4±5.2[d]
4′-Epi-ADR	1	122.1±4.7[d]	120.4±3.8[d]
	10	131.8±3.2[d]	129.9±4.3[d]
	100	138.9±3.0[d]	135.6±5.5[d]

Each value is a mean ± S.D. of 5 experiments. *a*) Percent of control value. Lipid peroxide concentration after 1 h incubation: *b*) 4.04→31.48 nmol/mg protein, *c*) 2.88→14.62 nmol/mg protein. *d*) Significantly different from the control value, $p<0.001$.

THP-ADR and 4′-epi-ADR increased lipid peroxidation to the same extent as ADR in both tissues.

Discussion

We previously reported[7-10] on the mechanism responsible for the increase in lipid peroxides induced by ADR, in relation to its cardiotoxicity.[5,6] In this study, we examined the changes in lipid peroxide levels in tissues of mice given THP-ADR or 4′-epi-ADR, which are less cardiotoxic than ADR.

In the heart, ADR significantly increased lipid peroxide levels; however, these levels did not increase in the hearts of mice given THP-ADR or 4′-epi-ADR. THP-ADR and 4′-epi-ADR have been reported to be less cardiotoxic than ADR.[17,18] Therefore, these results support the hypothesis of Myers *et al.*[5,6] that the increase in lipid peroxide levels in the heart is closely related to the cardiotoxicity of anthracyclines. GSHpx activity in the hearts of mice given ADR was 64% ($p<0.001$)[9] of the control level. However, there was no such change after THP-ADR or 4′-epi-ADR administration. We have proposed that the increase in lipid peroxide level in the hearts of mice given ADR is attributable to a decrease in GSHpx activity.[9] This hypothesis is supported by the finding that GSHpx activity did not decrease in mice given THP-ADR or 4′-epi-ADR, and there was no increase in the lipid peroxide level.

In the liver, all of the drugs significantly increased the lipid peroxide level with the order of increasing effect ADR > THP-ADR > 4′-epi-ADR. The relative weight (g/100 g body weight) of the liver decreased after these drugs were given. This order agrees with that of the effect on lipid peroxide level.

Unlike the liver, in the lung, the lipid peroxide levels were not changed by ADR or 4′-epi-ADR, however, the level was significantly higher ($p<0.01$) in the mice given THP-ADR. Other anthracycline drugs (ADR, daunomycin and aclacinomycin) do not increase lipid peroxide levels in the lung.[7,19] This may reflect the high concentration of THP-ADR in the mouse lung after this drug is given.[20] The maximum concentration of THP-ADR in the lung can be twice the concentration of ADR.[20] These results are suggestive, but it is still not clear whether THP-ADR is, in fact, pulmotoxic. The increase in lipid peroxide level in the lung appears to be related to the increase in GSHpx activity and the decrease in GST activity after THP-ADR administration.

Each of the drugs increased NADPH-dependent microsomal lipid peroxidation in the mouse liver and lung *in vitro*, but there were no significant differences between the effect of ADR, THP-ADR and 4′-epi-ADR in the microsomes of the two tissues. The results obtained *in vitro* could therefore not explain why of all the drug tested, only

THP-ADR increased the lipid peroxide levels in the lung. On the basis of *in vitro* experiments, the increase in lipid peroxide level caused by anthracyclines *in vivo* is generally thought to result from production of active oxygen radicals through the redox cycle of anthracyclines.[21-24] However, we found[7-9] that the results obtained *in vitro* did not agree with our findings[14] obtained *in vivo*. The results of the present study also support earlier findings.[7-9]

In conclusion, the order of the magnitude of effect on lipid peroxide level *in vivo* was ADR > THP-ADR > 4′-epi-ADR. This completely agrees with the order of the clinical severity of cardiotoxicity.[25] These results suggest that the increase in lipid peroxide level is closely related to the cardiotoxicity of anthracyclines. Furthermore, THP-ADR may be pulmotoxic, since it was the only anthracycline tested with increased the lipid peroxide level in the lung.

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