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Change of Lipid Peroxide Levels in Mouse Organs after Adriamycin Administration¹⁾

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The influence of adriamycin (ADR), an anthracycline antitumor antibiotic, on lipid peroxide levels in tissues of male CDF₁ strain mice was investigated by using thiobarbituric acid fluorophotometry, as reported in a previous paper (Tanizawa *et al.*, *Chem. Pharm. Bull.*, **29**, 2910 (1980)).

The body weight of mice decreased markedly after intraperitoneal injection of ADR at a dose of 15 mg/kg. The loss reached 25% of the initial weight on the 6th day. The relative weight of organs such as the heart, liver, kidney and spleen also decreased, with a minimum on the 4th day. In contrast, the lipid peroxide levels in the heart, liver, kidney and spleen increased rapidly, reaching a peak on the 4th day. The lipid peroxide level in the heart increased by 2.6-fold on the 4th day and the level was maintained, despite subsequent decreases in the liver and kidney. No increase of lipid peroxide levels in the serum and lung was observed after ADR injection in mice. ADR had a greater lipid peroxide-increasing effect in mice than daunomycin at the same dose.

Keywords—adriamycin; daunomycin; lipid peroxide; cardiotoxicity; fluorophotometry; malonaldehyde; body weight; thiobarbituric acid

Adriamycin (ADR), a representative anthracycline antibiotic, is one of the most effective antitumor agents currently available for cancer chemotherapy. Unfortunately, its clinical use is severely limited by a dose-dependent cardiotoxicity.²⁾ Electron micrographs of ADR-induced cardiac damage show degenerations of plasma membranes, mitochondria and myofibrils.³⁾ The true pathogenesis of ADR-induced cardiomyopathy is unknown. Recently, Myers *et al.*⁴⁾ demonstrated that ADR-induced cardiotoxicity in mice was associated with an increase of lipid peroxide in the myocardium.

We⁵⁾ previously investigated the optimum conditions for thiobarbituric acid fluorophotometry of lipid peroxide and succeeded in applying the method to determine the lipid peroxide level in the myocardium of ADR-treated mice. However, more detailed data on the elevation of lipid peroxide levels in ADR-treated mice are still required. Although the elevation of lipid peroxide levels in serum^{6,7)} and liver⁷⁾ of ADR-treated mice has been reported after Myers *et al.*,⁴⁾ details on the time courses in the serum, liver, heart, kidney, lung and spleen of ADR-treated mice remain to be determined.

In this report, we describe the changes of lipid peroxide levels in mouse tissues after ADR administration, and compare the effects of ADR and daunomycin (DAM), which has a similar chemical structure but weaker antitumor activity and cardiotoxicity compared to ADR, on the lipid peroxide levels in mouse tissues.

Experimental

Materials—ADR injection, 10 mg/vial (ADRIACIN), was purchased from Kyowa Fermentation Inc. (Tokyo, Japan), and DAM injection, 20 mg/vial, from Meiji Seika Co. Ltd. (Tokyo, Japan). These agents were thawed and diluted with sterile isotonic saline to obtain 1.0 mg/ml solution. The other chemicals used in this study were of the highest purity available.

Animals—Male CDF₁ strain mice, 6 weeks old and weighing 20–25 g, obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Shizuoka, Japan), were used. Animals were housed in a room maintained at 25 ± 1°C with 55% relative humidity and given free access to standard laboratory food and water. The room was illuminated for 12 h a day starting at 7:00 a.m.

Animal Experiments—Male CDF₁ strain mice were classified into several groups each consisting of

5–6 mice. ADR or DAM at a dose of 15 mg/kg was intraperitoneally injected into the mice. Control animals were injected with the same volume of sterile isotonic saline alone. The animals were killed by cervical dislocation at a definite time during the 6 d after ADR or DAM administration. After sampling of blood from the heart, organs such as the liver, heart, kidney, lung and spleen were dissected out rapidly, washed in ice-cold isotonic saline, blotted and weighed to obtain the wet weight. The tissue samples were homogenized in 100 vol (v/w) of isotonic saline at 4°C in a glass Potter–Elvehjem type homogenizer with a Teflon pestle. Determinations of lipid peroxide level in the serum and tissue samples were carried out according to Yagi *et al.*⁸⁾ and us,⁵⁾ respectively.

Results

Effect of ADR on Body and Organ Weights of Mice

Fig. 1 shows the time variation of body weight after the administration of ADR or isotonic saline to mice. The body weight of ADR-treated mice gradually decreased and the loss of body weight was 6.30 ± 1.15 g on the 6th day after ADR injection. In contrast, the body weight of control mice which had received isotonic saline increased normally, and the gain was 3.00 ± 0.71 g through the same observation period. The food and water intakes of ADR-treated mice were obviously less than those of control mice.

Fig. 2 shows the changes of relative heart weight after the medication. The relative heart weight of ADR-treated mice decreased in a similar manner to the body weight, though the decrease after the 4th day was very slight.

The relative weights of the liver, kidney and spleen also changed similarly after ADR injection; the relative organ weights on the 4th day are shown in Table I. The maximum decrease was observed in the spleen (60.4% decrease).

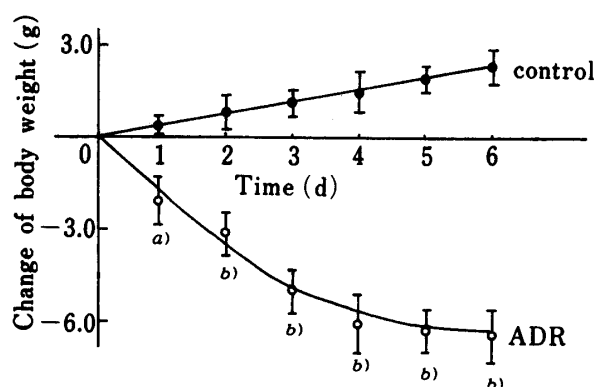


Fig. 1. Time Course of Body Weight Change after ADR Administration

Male CDF₁ mice (6 weeks old, 20–25 g) received an intraperitoneal injection of ADR (15 mg/kg). Each point represents the mean \pm S.D. of 5–6 mice. Significantly different from control (a) $p < 0.01$, b) $p < 0.001$.

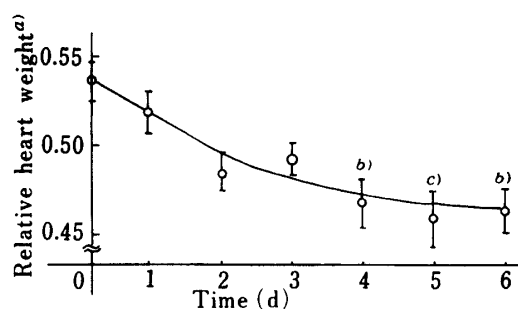


Fig. 2. Time Course of Relative Heart Weight (g/100 g B.W.) after ADR Administration

Male CDF₁ mice (6 weeks old, 20–25 g) received an intraperitoneal injection of ADR (15 mg/kg).

a) Each point represents the mean \pm S.D. of 5–6 mice.

b) $p < 0.05$: Significantly different from the 0 th day.

c) $p < 0.01$: Significantly different from the 0 th day.

TABLE I. Relative Organ Weights (g/100g B.W.) after ADR Administration

	Control	ADR ^{a)}
Heart	0.535 ± 0.042	$0.473 \pm 0.036^{b)}$
Liver	5.513 ± 0.296	$4.017 \pm 0.236^{d)}$
Kidney	0.709 ± 0.034	$0.643 \pm 0.018^{c)}$
Spleen	0.341 ± 0.072	$0.135 \pm 0.027^{d)}$

a) Male mice received an intraperitoneal injection of ADR (15 mg/kg). The mice were killed by cervical dislocation 4 days after of drug administration. Each value represents the mean \pm S.D. of 5–6 mice.

b) $p < 0.05$: Significantly different from control.

c) $p < 0.01$: Significantly different from control.

d) $p < 0.001$: Significantly different from control.

Effects of ADR and DAM on Lipid Peroxide Levels in Mice

Lipid peroxide levels in the serum, lung, heart, liver, kidney and spleen of mice on the 4th day after ADR or DAM injection were determined, and the results are shown in Table II. There was no increase of lipid peroxide level in the serum or lung of ADR- or DAM-treated mice. In contrast, marked increases of the levels in the heart, liver, kidney, and spleen were observed. The largest increase was obtained in the heart (256.8%) of ADR-treated mice and in the liver (161.4%) of DAM-treated mice. The increase of lipid peroxide levels in the observed organs except for the lung was larger in ADR-treated mice than in DAM-treated mice.

TABLE II. Lipid Peroxide Levels in Tissues of Mice Treated with ADR or DAM

Tissue	Lipid peroxide level		
	Control	ADR ^{a)}	DAM ^{a)}
Serum	5.48±0.81 nmol/ml	5.71±0.73 (104.2) ^{b)}	5.25±0.78 (95.8)
Heart	185.31±24.13 nmol/g	475.79±23.04 ^{c)} (256.8)	265.21±18.04 ^{c)} (143.1)
Lung	164.98±17.18	149.97±13.99 (90.9)	160.51±14.97 (97.3)
Liver	243.56±48.30	510.14±57.17 ^{c)} (209.5)	393.03±25.32 ^{c)} (161.4)
Spleen	267.26±31.36	376.82±44.58 ^{d)} (141.0)	314.42±20.50 ^{c)} (117.6)
Kidney	262.26±28.13	499.83±46.50 ^{c)} (190.6)	398.97±23.36 ^{c)} (152.1)

- a) Male mice received an intraperitoneal injection of ADR or DAM (15 mg/kg). The mice were killed by cervical dislocation 4d after drug administration. Each value represents the mean ± S.D. of 5–6 mice.
b) Figures in parentheses show % change from control.
c) $p < 0.001$: Significantly different from control.
d) $p < 0.01$: Significantly different from control.
e) $p < 0.05$: Significantly different from control.

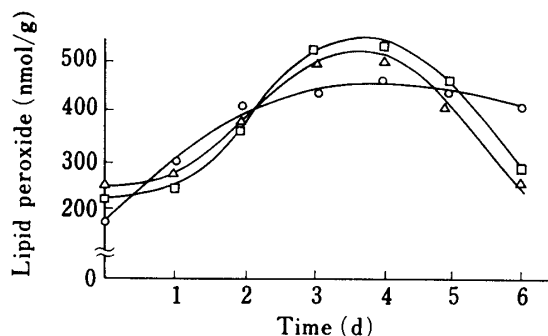


Fig. 3. Changes of Lipid Peroxide Levels in the Heart, Liver and Kidney after ADR Administration to Mice.

Male CDF₁ mice (6 weeks old, 20–25 g) received an intraperitoneal injection of ADR (15 mg/kg). One percent homogenates of the heart, liver and kidney were prepared, and 0.1 ml was used for determination of lipid peroxide level. Each point represents the mean ± S.D. of 5–6 mice.

○—○, heart; □—□, liver; △—△, kidney.

Time Course of Lipid Peroxide Levels in the Heart, Liver and Kidney after ADR Administration

As shown in Fig. 3, an increase of lipid peroxide level in the heart was observed on the first day after ADR injection. The level in the heart peaked on the 4th day and then declined very slowly. On the other hand, the initial increases of lipid peroxide levels in the liver and kidney of ADR-treated mice were slower than that in the heart. However, the lipid peroxide levels of these organs also peaked on the 4th day, and then declined quickly compared with that of the heart.

Discussion

Myers *et al.*⁴⁾ have suggested that the cardiotoxicity of ADR is related to the increase of lipid peroxide in the mouse myocardium. However, they determined the lipid peroxide level by using a trichloroacetic acid–thiobarbituric acid method that is considered unsuitable,⁸⁾ and

furthermore they expressed the results in a rather unusual form; their results cannot be converted easily to lipid peroxide amount (mol) per heart weight (g) from their reported figures. Subsequently, several reports dealt with the increase of lipid peroxide induced by ADR, but there is no detailed report on the changes of lipid peroxide levels in mouse tissues after ADR administration. Therefore, we examined the increase of lipid peroxide in ADR-treated mice by using our method, as described in a previous paper.⁵⁾

No mice died up to the 6th day after intraperitoneal injection of ADR at the dose of 15 mg/kg used by Myers *et al.*, even though the LD₅₀ value is 13.7 mg/kg,⁹⁾ but the body weight of ADR-treated mice decreased rapidly (the loss reached 25% of the initial weight on the 6th day after injection). As the body weight changed only very slightly in the period from the 4th day to the 6th day after ADR injection, the decrease of body weight might have approached the limit for remaining alive. In fact, the acute toxicity of ADR in mice appeared slowly, and most of the mice died during the period from the 7th day to the 13th day after ADR injection at a dose of 15 mg/kg. The decreases of relative weight of organs such as the heart, liver, kidney and spleen in ADR-treated mice were greater than that of body weight, indicating that ADR is rather toxic to the main organs of mice. Further, the greatest decrease of relative weight was observed in the spleen. This seems to be related to the suppressive effect of ADR on hematogenesis through the bone marrow, in addition to its inhibitory action on protein synthesis in the spleen.

The lipid peroxide level in the heart was significantly increased by ADR injection, in agreement with the report of Myers *et al.*⁴⁾ The percentage change from the control was greatest in the heart on the 4th day, among all the tissues, and the level in the heart maintained a higher level thereafter. On the other hand, the levels in the liver and kidney rose and then decreased rapidly. The different behavior of lipid peroxide levels in the heart, liver and kidney does not seem to be caused by a difference in the tissue distribution of ADR, because the concentration of ADR in the heart was reported to be lower than in the liver and kidney.¹⁰⁾ We have confirmed this in CDE₁ mice given ADR intraperitoneally. Therefore, it seems that the liver and kidney, which are principal metabolic and excretory organs, have relatively good resistance to lipid peroxidation. This might be relevant to the cardiotoxicity of ADR.

The lipid peroxide induction by ADR (DAM) is thought to be initiated by free radicals generated through the redox cycling of ADR (DAM) *in vitro*.^{11,12)} If this is the case *in vivo*, the peaks of lipid peroxide levels in mouse tissues after ADR administration might be expected on the first day, when the tissue concentration of ADR were higher than on the 4th day. However, the peak lipid peroxide levels in mice were obtained on the 4th day. Therefore, some other mechanism of lipid peroxide generation may be important in mice after ADR administration. Further investigation seems necessary.

It is said that some agents such as carbon tetrachloride¹³⁾ and paraquat,¹⁴⁾ which possess a strong lipid peroxide-inducing activity, have no effect on the lipid peroxide level of the serum and lung *in vivo*. A similar phenomenon was observed in this experiment with ADR and DAM, while Yamanaka *et al.*⁶⁾ and Fujita *et al.*⁷⁾ reported the elevation of serum lipid peroxide level by ADR in mice. The difference between their results and ours may be at least partly due to the higher dose (20 mg/kg) of ADR used by them. The reason for the absence of an increase of lipid peroxide level in serum in our experiment is not clear, but may related to the properties of ADR and DAM, which bind tightly to tissue protein.¹⁵⁾ It seems clear that measurement of the lipid peroxide level in the serum is not useful in clinical practice in connection with monitoring the cardiotoxicity of ADR. It is still necessary to take electrocardiograms for this purpose.

Finally, ADR increased the levels of lipid peroxide of mouse tissues, more than DAM. We suggest that this may be closely related to the rapid metabolism of DAM by the mouse,¹⁰⁾ as well as to the lower toxicity of DAM compared with ADR.

References and Notes

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