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Species, Strain, Sex and Weekly Age Differences of Lipid Peroxide Levels in Animals Tissues before and after Adriamycin Administration[1)]

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We investigated which laboratory animal would be the most suitable for studies on the mechanism of adriamycin (ADR)-induced lipid peroxidation. Lipid peroxide (LPO) levels of the main organs on the 3rd day after ADR administration (when a remarkable increase of LPO level in mouse heart was seen) were determined. Both the normal LPO levels and the increase ratios of LPO levels after ADR administration in all the studied tissues of mice, except for the serum, were higher than those in rats and guinea pigs. Next, strain differences in 9 strains of male mice (DDY, ICR, C3H, BALB/c, C57BL, DBA/2, BDF_1, CDF_1 and $B6C3F_1$) were examined, and clear strain differences were recognized. Further, sex and weekly age differences of LPO tissue levels in CDF_1 strain mice were shown. It was concluded that DBA/2 and CDF_1 strains of male mice were the most suitable laboratory animals for studies of the mechanism of the lipid peroxidation induced by ADR.

Keywords——adriamycin; tissues lipid peroxide level; cardiotoxicity; lipid peroxidation; species difference; strain difference; sex difference; age difference

The anthracyclic antibiotic adriamycin (ADR) is one of the most clinically useful cancer chemotherapeutic agents against human tumor.[2,3)] The mechanism of antitumor action is said to be based on the inhibition of deoxyribonucleic acid (DNA) biosynthesis.[4)] However, its administration to humans and animals is known to cause a severe cardiotoxic side-effect which is a major clinical handicap limiting its cumulative dosage.[5)] Myers *et al.*[6,7)] demonstrated that this ADR-induced cardiotoxicity in mice was associated with an increase of lipid peroxide (LPO) level in the myocardium, and we[8)] confirmed a specific increase of LPO level in the myocardium of mouse. However, Adachi *et al.*[9)] reported that administration of ADR induced a very small increase of LPO level in the myocardium of mouse. This discrepancy might be a consequence of differences of species, strain, sex and/or weekly age of the animals. However, there is no report on such differences. Therefore, this study was designed to examine the effects of these difference on the increase of LPO levels in animal organs induced by ADR administration.

Experimental

Animals——Nine strains (DDY, ICR, C3H, BALB/c, C57BL, DBA/2, BDF_1, CDF_1 and $B6C3F_1$) of male mice and female CDF_1 strain mice (5 weeks old and weighing 18—20 g), male Sprague–Dawley strain rats (5 weeks old and weighing 120—150 g) and Hartley strain guinea pigs (weighing 300—350 g) were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Shizuoka, Japan). Animals were used after being housed in a room at 25 ± 1 °C with 55% relative humidity and with free access to standard laboratory diet and water for over a week. Male CDF_1 strain mice (3, 5, 7 and 9 weeks old) for the experiment on weekly age difference were used immediately after purchase.

Apparatus——Fluorescence intensities were measured with an SPF-125 fluorescence spectrophotometer (AMINCO).

Reagents——ADR injection, 10 mg/vial (ADRIACIN®), was purchased from Kyowa Fermentation Inc.,

Tokyo, Japan. This agent was thawed and diluted with sterile isotonic saline to obtain a 1.0 mg/ml solution. The other chemicals used in this study were of the highest purity available.

Methods——ADR at a dose of 15 mg/kg was intraperitoneally injected into the animals. Control animals were injected with the same volume of sterile isotonic saline alone. The animals were killed by cervical dislocation on the 4th day after ADR administration. After sampling of blood from the heart, organs such as the liver, heart, kidney, lung and spleen were dissected out rapidly and washed in ice-cold isotonic saline. Each organ were 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 LPO level in the serum and tissue samples were carried out according to Yagi *et al.*[10] and Tanizawa *et al.*,[11] respectively.

The results shown in figures are mean values ± S.D. Statistical analyses were done by using Student's *t*-test.

Results and Discussion

Biological membranes are made up of double layers of phospholipids. The phospholipids contained large amounts of highly unsaturated fatty acids such as linoleic acid, linoleinic acid and arachidonic acid. Consequently, the membranes are easily peroxidized by radical or non-radical mechanisms through the actions of superoxide radical, hydrogen peroxide, singlet oxygen and hydroxy radical. Lipid peroxidation in the membrane is usually prevented because several protective systems exist. However, once the protective systems are damaged by drugs, diseases and aging, excessive peroxidation can take place. This may be followed by membrane damage, changes of enzyme activities, and loss of function. However, it is not yet clear which laboratory animal is the most suitable for studies on these processes.

We[8] previously described the time course of LPO levels in tissues of CDF_1 male mice after ADR intraperitoneal administration. However, we did not investigate the effects of differences of species, strain, sex and weekly age of animals on the LPO levels before and after ADR administration. Therefore, this report complements our previous paper.[8]

Species Difference

LPO levels in the tissues of normal or ADR-treated male CDF_1 strain mice, Sprague–Dawley strain rats and Hartley strain guinea pigs are shown in Table I.

i) Serum——LPO levels in normal rats and mice were twice as high as that in normal guinea pigs. The increase ratio of LPO level in ADR-treated rats was the highest (144%

Table I. Effect of Species Differences on the Increase of Lipid Peroxide Levels in Tissues of CDF_1 Mice on the 3rd Day after ADR Administration

		Lipid peroxide[a]		
		Mice	Rats	Guinea pigs
Serum	Normal	14.8 ± 0.8	15.7 ± 2.4	8.2 ± 0.9
	ADR	16.2 ± 1.0[b]	22.6 ± 1.4[c]	10.2 ± 1.0[b]
Heart	Normal	195.3 ± 22.8	123.5 ± 11.5	98.6 ± 9.2
	ADR	425.8 ± 25.5[d]	145.7 ± 13.3[b]	133.2 ± 14.1[c]
Lung	Normal	164.9 ± 17.2	96.0 ± 5.3	76.4 ± 7.5
	ADR	184.7 ± 14.8	115.8 ± 8.2[c]	114.5 ± 12.3[d]
Liver	Normal	215.6 ± 25.5	119.3 ± 12.1	126.8 ± 10.9
	ADR	495.9 ± 31.1[d]	149.1 ± 15.0[c]	135.2 ± 14.3
Spleen	Normal	274.1 ± 12.8	100.4 ± 4.4	145.8 ± 10.2
	ADR	381.0 ± 21.6[d]	91.8 ± 7.2	180.8 ± 18.3[c]
Kidney	Normal	284.1 ± 15.7	111.0 ± 10.4	91.4 ± 9.2
	ADR	409.1 ± 38.6[d]	158.7 ± 13.1[d]	106.0 ± 12.0[b]

a) Serum, (nmol/ml serum); tissue, (nmol/g tissue).
Values are expressed as means ± S.D. of 5—6 animals. Significant differences from the normal value are indicated by *b*) $p<0.05$, *c*) $p<0.01$ and *d*) $p<0.001$.

compared to normal rats; $p<0.01$) among the 3 species.

ii) Heart——The normal LPO level in mice (195.3 nmol/g) was the highest, corresponding to 156 and 198% of those in rats and guinea pigs, respectively. The increase ratio of LPO level in ADR-treated mice was 218% ($p<0.001$). In contrast, those in ADR-treated rats and guinea pigs were 118 and 135%, respectively.

iii) Lung——The LPO level in normal mice (165 nmol/g) was about twice as high as those in rats and guinea pigs. The increase ratios of LPO levels in ADR-treated mice, rats and guinea pigs were 112, 120 and 150%, respectively. These figures are lower than those obtained in other organs.

iv) Liver——The normal LPO level in mice (215.6 nmol/g) was the highest, corresponding to 192 and 170% of those in rats and guinea pigs, respectively. The LPO level in ADR-treated mice was remarkably elevated to 230% ($p<0.001$) compared to the normal state. On the other hand, that in ADR-treated rats was elevated to only 125% and no elevation was observed in guinea pigs.

v) Spleen——The normal level of LPO in mice (274.1 nmol/g) was the highest, corresponding to 273% in rats and 188% in guinea pigs. The increase ratios of LPO levels in ADR-treated mice and guinea pigs were 139% ($p<0.001$) and 124% ($p<0.01$). On the other hand, no increase of LPO level was observed in ADR-treated rats.

vi) Kidney——The normal LPO level in mice (284.1 nmol/g) was 256 and 311% of those in normal rats and guinea pigs, respectively. The LPO levels in ADR-treated mice, rats and guinea pigs were elevated to 144% ($p<0.001$), 143% ($p<0.001$) and 116% ($p<0.05$), respectively.

The normal LPO levels in all the studied tissues of mice, except for the serum, were higher than those in rats and guinea pigs. The increase ratios of LPO levels in tissues of mice on the 3rd day after ADR administration, when the LPO levels had reached approximately their peaks, were also generally higher than those of rats and guinea pigs. From these results, it was concluded that the mouse is the most suitable animal for studies on the mechanism of ADR-induced lipid peroxidation *in vivo*.

Strain Differences in Mice

LPO levels in the tissues of normal or ADR-treated male DDY, ICR, C3H, BALB/c, C57BL, DBA/2, BDF_1, CDF_1 and $B6C3F_1$ strain mice were determined. The results are shown in Table II.

i) Serum——The normal LPO level in C57BL strain (20.6 nmol/ml) was the highest, followed by $B6C3F_1$ and CDF_1 strains in that order. The LPO level in ADR-treated C57BL strain was elevated to 124% ($p<0.001$) and was the highest among the 9 strains of mice used. The increase ratios of LPO level in ADR-treated DBA/2, ICR and CDF_1 strains were about 110% ($p<0.01$), and no increase was observed in the other strains.

ii) Heart——The normal LPO level in BALB/c strain (482.1 nmol/g) was the highest, followed by $B6C3F_1$ and CDF_1 strains in that order. However, those in DBA/2, BDF_1 and DDY strains were below 100 nmol/g. The increase ratios of LPO level in ADR-treated DBA/2, CDF_1 and $B6C3F_1$ strains were over 200% ($p<0.001$), but no increase was observed in BDF_1 and C3H strains.

iii) Lung——The normal LPO levels in 7 strains (excluding CDF_1 and C3H) were about 100 nmol/g, lower than in other tissues. The increases of LPO level after ADR administration in these 7 strains were also lower than obtained in other tissues. On the other hand, the normal LPO level in CDF_1 strain was relatively high (164.9 nmol/g) but the increase of LPO level after ADR administration was not significant.

iv) Liver——There was no significant difference among the normal LPO levels of the 9 strains. However, the increase ratios of LPO level after ADR treatment differed greatly and

TABLE II. Effect of Strain Differences on the Increase of Lipid Peroxide Levels in Tissues of Mice on the 3rd Day after ADR Administration (15 mg/kg, *i.p.*)

		Lipid peroxide[a]								
		DDY	ICR	C3H	BALB/c	C57BL	DBA/2	BDF_1	CDF_1	$B6C3F_1$
Serum	Normal	9.3± 1.7	8.9± 1.0	10.4± 1.2	12.6± 1.2	20.6± 0.7	11.9± 0.7	12.1± 0.8	15.0± 0.9	17.4± 0.4
	ADR	9.9± 1.0	10.4± 1.0	10.7± 1.4	11.2± 1.8	25.6± 1.7[d]	13.3± 0.9[b]	9.7± 1.5	16.5± 0.5[b]	16.8± 0.5
Heart	Normal	94.4± 8.3	132.8± 6.1	168.5±17.0	482.1±24.7	107.3± 8.1	61.7± 5.2	96.9± 5.0	195.3±22.8	318.9±17.5
	ADR	119.3±19.7[b]	154.3± 7.2[c]	152.8±12.6	591.4±82.1[b]	123.2± 5.2[c]	160.3±10.7[d]	101.6± 4.7	410.1±21.8[d]	675.1±12.3[d]
Lung	Normal	64.9±10.9	106.7± 8.9	138.9± 4.1	79.2± 8.8	101.6± 6.4	103.9± 7.5	117.9± 7.3	164.9±17.2	167.3± 6.1
	ADR	105.7±10.6[d]	113.6±10.7	141.9± 4.8	96.4±10.1[b]	126.5± 9.1[d]	134.3± 9.9[d]	115.6±11.3	181.5±15.1	156.1± 5.5[b]
Liver	Normal	198.1±20.1	153.9±17.4	169.8±16.9	223.3±19.3	212.2±17.9	190.8±17.8	155.6±12.8	215.6±25.5	186.4±19.5
	ADR	205.3±21.2	426.8±26.9[d]	261.0±17.2[d]	557.7±63.8[d]	231.4±23.9	530.2±16.2[d]	240.6±19.6[c]	527.2±35.2[d]	236.9±23.4[c]
Spleen	Normal	190.2±28.6	185.7±17.3	209.7± 5.4	183.6±18.1	171.6± 7.7	136.2± 7.2	161.6± 5.5	274.1±12.8	182.8± 3.6
	ADR	311.8±15.1[d]	204.2±16.5	289.1±21.4[d]	160.4±12.1	205.0±11.9[d]	165.4± 7.2[d]	238.4±19.2[d]	367.7±12.3[d]	202.3± 5.9[d]
Kidney	Normal	200.2±21.2	232.6±17.6	229.4±18.5	218.6±23.2	251.3±17.3	195.5±17.9	165.9±12.5	284.7±15.7	212.4±12.2
	ADR	207.6±19.3	251.3±19.8	294.7±18.2[d]	301.3±25.1[d]	284.8±23.5[b]	403.2±38.2[d]	163.8±11.7	415.8±41.5[d]	246.0±18.8[c]

a) Serum, (nmol/ml serum); tissue, (nmol/g tissue).
Values are expressed as means±S.D. of 5—6 animals. Significant differences from the normal value are indicated by *b*) $p<0.05$, *c*) $p<0.01$ and *d*) $p<0.001$.

were greater than those obtained in other organs. In particular, those for DBA/2 (278%), ICR (277%), BALB/c (250%) and CDF_1 (244%) strains were over 200%. On the other hand, there was no increase in the case of DDY or C57BL strain.

v) **Spleen**——The normal LPO levels in 9 strains were all about 200 nmol/g. On the other hand, the increase ratios of LPO level after ADR treatment were over 130% ($p<0.001$) in DDY, BDF_1, C3H and CDF_1 strains, whereas no increase was observed in BALB/c and ICR strains.

vi) **Kidney**——The normal LPO level in CDF_1 strain was the highest (284.7 nmol/g) and that in BDF_1 strain was the lowest (165.9 nmol/g). The increase ratios of LPO level after ADR treatment were higher in C3H, DBA/2, BALB/c and CDF_1 strains. Among these strains, that in DBA/2 strain was the highest (207%).

Marked strain differences in normal LPO levels of mice existed. Broadly speaking normal LPO levels in CDF_1, C3H and $B6C3F_1$ were higher and those in BDF_1 were lower than in the other strains. As regards LPO levels in tissues, the highest levels in heart and liver were obtained with BALB/c strain, in lung, kidney and spleen with CDF_1 strain and in the serum with C57BL strain. It is interesting that the normal LPO level in the heart of BALB/c,[12-14] which suffers from the development of arteriosclerosis and cardiomyopathy in old age, was higher than those of the other strains. BALB/c might be useful for studies on the relationship between heart disease and LPO.

After the administration of ADR, there were clear strain differences in the increases of tissue and serum LPO levels of mice. In summary, the increase ratios in BALB/c, CDF_1, DBA/2 and C3H strains were larger than those in the other strains. Therefore, it was concluded that DBA/2 and CDF_1 strains of mice are most suitable for studies on the mechanism of the lipid peroxidation induced by ADR. The validity of the use of CDF_1 strain mice in our previous experiments, as in the work of Myers *et al.*[6,7] was supported by this result. CDF_1 strain mice are the first filial generation between male BALB/c and female DBA/2 inbred strains. Therefore, the results obtained in this paper are not unexpected. On the other hand, increase ratios of LPO levels after ADR administration in DDY, ICR and BDF_1 strains of mice were lower than those in the other strains. Adachi *et al.*[9] previously reported that increase ratios of LPO levels in DDY mice after ADR administration were small. Our result is consistent with their report.

Sex Difference in CDF_1 Strain Mice

The sex difference in LPO levels in CDF_1 strain mice is shown in Table III.

Male mice showed higher normal LPO levels than female mice in all tissues measured. Further, as regards the increase ratio of LPO level after ADR treatment, male mice gave higher values than female mice generally. In particular, that in the liver of male mice was 239% ($p<0.001$) against 109% ($p<0.01$) in female mice. On the other hand, no increase of LPO level after ADR administration was observed in the lung in male or female mice. A clear sex difference in CDF_1 strain mice thus exists, and male CDF_1 mice are the most suitable for experimental use.

Weekly Age Differences in CDF_1 Strain Mice

Weekly age differences in LPO levels in male CDF_1 strain mice (3—9 weeks old) were examined. The results are shown in Table IV.

i) **Serum**——The normal LPO levels increased with age as follows: 146% ($p<0.05$) at 5 weeks old, 140% ($p<0.05$) at 7 weeks old and 170% ($p<0.001$) at 9 weeks old compared to that at 3 weeks old. However, the LPO level was not elevated after ADR administration at any age.

ii) **Heart**——The normal LPO level increased with age as follows: 186% ($p<0.001$) at 5 weeks old and 181% at 7 weeks old compared to that at 3 weeks old. Increase ratios of

Table III. Effect of Sex Difference on the Increase of Lipid Peroxide Levels in Tissues of CDF_1 Mice on the 3rd Day after ADR Administration

		Lipid peroxide[a]	
		Female	Male
Serum	Normal	14.6± 0.9	15.1± 0.8
	ADR	15.6± 0.8	16.1± 0.5[b]
Heart	Normal	151.1± 5.4[e]	195.3±22.8
	ADR	174.0± 9.4[c]	410.1±21.8[b]
Lung	Normal	148.4± 8.4	165.0±17.2
	ADR	155.3± 5.8	181.5±15.1
Liver	Normal	200.4±12.8	210.8±20.5
	ADR	220.5± 6.2[c]	503.8±32.5[d]
Spleen	Normal	222.0±11.9[f]	274.7±12.8
	ADR	221.2±10.3	367.7±12.3[d]
Kidney	Normal	230.0± 9.7[f]	284.7±15.7
	ADR	238.2± 8.7	415.8±41.5[d]

a) Serum, (nmol/ml serum); tissue, (nmol/g tissue).
Values are expressed as means±S.D. of 5—6 animals. Significant differences from the normal values are indicated by *b*) $p<0.05$, *c*) $p<0.01$ and *d*) $p<0.001$. Significant differences from male are indicated by *e*) $p<0.01$ and *f*) $p<0.001$.

Table IV. Effect of Weekly Age Differences on the Increase of Lipid Peroxide Levels in Tissues of CDF_1 Mice on the 3rd Day after ADR Administration

		Lipid peroxide[a]			
		3 weeks	5 weeks	7 weeks	9 weeks
Serum	Normal	11.1± 0.5	16.2± 2.1	15.5± 0.6	18.9± 1.4
	ADR	11.6± 1.0	17.5± 1.1	14.9± 0.7	18.6± 1.6
Heart	Normal	105.0± 7.5	195.3±10.1	190.1±10.0	199.5±13.8
	ADR	102.5± 7.0	238.7±18.3[b]	315.6±32.0[b]	312.5±18.3[b]
Lung	Normal	61.8± 4.2	61.6± 7.1	152.2±11.6	119.9±11.0
	ADR	64.4± 3.9	73.4± 7.9	160.3±15.3	150.6± 6.2[b]
Liver	Normal	82.0± 5.8	130.4±14.2	216.7±15.9	234.5±23.7
	ADR	189.6±12.3[b]	318.4±27.4[b]	533.0±37.2[b]	538.7±30.5[b]
Spleen	Normal	172.6±18.1	195.0±10.3	250.2±21.6	321.0±28.6
	ADR	189.1±15.4	201.9±18.7	665.5±41.8[b]	532.9±39.9[b]
Kidney	Normal	120.8±15.1	193.7±18.9	310.3±21.5	308.6±30.1
	ADR	150.9±18.5	275.0±25.1[b]	546.1±35.0[b]	518.5±28.9[b]

a) Serum, (nmol/ml serum); tissue, (nmol/g tissue).
Values are expressed as means±S.D. of 5—6 animals. Significant differences from the normal values are indicated by *b*) $p<0.001$.

LPO level after ADR treatment were 122% at 5 weeks old and 166% at 7 weeks old; the 3-week-old mice did not show any increase.

iii) Lung——The normal LPO level at 9 weeks old was 196% ($p<0.001$) compared to that at 3 weeks old. Increase of LPO level after the ADR treatment was observed only in 9-week-old mice.

iv) Liver——The normal LPO level increased with age as follows: 159, 264 and 286% ($p<0.001$) at 5, 7 and 9 weeks old against that at 3 weeks old, respectively. The increase ratios of LPO level after ADR treatment reached the maximum (246%) at 7 weeks old and

maintained that level. Thus, the increase ratio of LPO level in the liver with aging was greater than that in other tissues. The increase of LPO level in the liver of ADR-treated mice was also greater than that in other tissues.

v) Spleen——The normal LPO level increased with age as follows: 113% ($p<0.05$) at 5 weeks old, 145% ($p<0.01$) at 7 weeks old and 186% ($p<0.001$) at 9 weeks old compared to that at 3 weeks old. The increase ratios of LPO level after ADR treatment were 266 and 166% at 7 and 9 weeks old, respectively, while at 3 and 5 weeks old no increase was observed.

vi) Kidney——The normal LPO level increased with age, and the levels at 7 and 9 weeks old were about 280% ($p<0.001$) compared to that at 3 weeks old. Increase ratios of LPO level in ADR-treated mice were 142% ($p<0.01$) at 5 weeks old, 176% ($p<0.001$) at 7 weeks old and 168% ($p<0.001$) at 9 weeks old, while there was no increase at 3 weeks old.

This result is consistent with surum data for man, whose normal LPO level in serum increased with age up to 30 years old.[15] The increase ratios of LPO levels tissues except for lung and serum after ADR treatment also increased with age.

Though the reasons for the differences in tissue LPO levels are unclear, the activities of enzymes associated with LPO metabolism, such as superoxide dismutase, glutathione peroxidase and catalase, in animal tissues might be important. In fact, we found that the activities of the enzymes mentioned above in male CDF_1 strain mice were lower than those in male DDY strain mice.[1] Other possible causes of these differences in tissue LPO levels after ADR treatment are differences in distribution, excretion and metabolism of ADR. Further detailed studies are in progress.

From the data presented above, it is clear that CDF_1 strain male mice are suitable for studies on the mechanism of the lipid peroxidation induced by ADR, though it is necessary to take account of their age.

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