Effect of Aclacinomycin on Lipid Peroxide Levels in Tissues of Mice

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We have examined the lipid peroxide levels in aclacinomycin (ACM)-treated mice by using adriamycin (ADR) as a comparative drug. There was no increase in the lipid peroxide level of the heart at either 3 h or 4 d after ACM administration (15 mg/kg, i.p.), although the level in the heart of ADR-treated mice was elevated to 257% of that in normal mice. The effect of ACM and its glycoside-type metabolites on the increase of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent microsomal lipid peroxidation (*in vitro*) was weaker than that of ADR. Then, we examined the tissue concentrations of ACM. The $AUC_{0-24\,h}$ of ACM was the lowest in the heart among the tissues examined, being only 29.3% of that obtained with ADR. However, the concentrations of the glycoside-type metabolites of ACM in all tissues determined were higher than the concentration of ACM. In the heart, the $T_{1/2}$ and $AUC_{0-24\,h}$ of ACM glycosides were somewhat higher than those of ADR. In conclusion, ACM and its metabolites do not lead to an increase in lipid peroxide level in the heart of mouse, and the difference in lipid peroxide increment in the mouse heart induced by ADR and ACM is independent of the tissue concentration of the drugs.

Keywords aclacinomycin; adriamycin; lipid peroxide; pharmacokinetics; cardiotoxicity

Aclacinomycin (ACM), an antitumor anthracycline antibiotic, has been developed for the purpose of reducing the cardiotoxicity of anthracyclines. The cardiotoxicity of adriamycin (ADR), which is one of most widely used anthracyclines, is well known due to its severity. In animal experiments, the cardiotoxicity induced by ACM has been reported to be one-fifteenth that by ADR,[1] although it has been stated to possess lower cardiotoxicity in clinical use.[1] We have been interested in the difference in the cardiotoxicity between ADR and ACM, for which there is no clear explanation at present. However, Myers *et al.*[2,3] have shown that the cardiotoxicity induced by ADR in mice is associated with an increase in the lipid peroxide level of the heart. There is no report describing the lipid peroxide level in the heart of ACM-treated mouse. The present study was designed to examine the lipid peroxide levels in ACM-treated mice by using ADR as a comparative drug.

Experimental

Materials ACM injection, 10 mg/vial (ACLACINON®), from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan), and ADR injection, 10 mg/vial (ADRIACIN®), were purchased from Kyowa Fermentation Inc. (Tokyo, Japan). These agents were thawed and diluted with sterile isotonic saline to obtain a 1.0 mg/ml solution. MA144M1 (4‴-hydroxy-ACM containing L-amicetose), MA144N1 (4‴-hydroxy-ACM containing L-rhodinose) and 7-deoxyaklavinone, ACM metabolites, were obtained from Sanraku Co., Ltd. The chemicals used for lipid peroxide determination were similar to those in a previous paper.[4] The other chemicals used were of the highest purity available.

Animal Experiments Animal experiments were carried out according to a previous paper.[5]

Reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-Dependent Lipid Peroxidation Test NADPH-dependent lipid peroxidation in the microsomal fraction of mouse liver was assayed according to Aust *et al.*[6]

Determination of Lipid Peroxide Determination of lipid peroxide in samples was carried out according to previous papers.[4,7]

Determination of ACM and Its Metabolites ACM and its metabolites in serum and tissue samples were analyzed by thin layer chromatography (TLC), as described by Oki *et al.*[8] As a developing solvent for TLC, the following mixture was used: chloroform–methanol–acetic acid–water (30:4:1:0.1). TLC densitograms were obtained on a Shimadzu dual-wavelength TLC scanner (CS-910) under the following conditions: excitation wavelength, 420 nm; emission wavelength, 585 nm; scanning mode, linear. The peak areas were calculated using an equipped integrator fitted to the scanner. There was a good linear relationship between the amount (0.1—250 ng) of ACM or its metabolites applied and the integration values. The mean recovery percentages of ACM or its metabolites added to biological samples of mice were more than 90%, and standard deviations were generally below 2.0%.

Pharmacokinetic and Statistical Analysis The peak serum or tissue concentration (C_{max}), the peak concentration time (T_{max}), the area under the serum or tissue concentration–time curve (AUC) and the half-life ($T_{1/2}$) were calculated according to a previous paper.[9] Statistical analysis of data was also done according to that paper.[9]

Results

Effect of ACM, ACM Metabolites and ADR on NADPH-Dependent Lipid Peroxidation in Mouse Liver Microsomes (*in Vitro*) The lipid peroxide concentrations of the control group with no ADR or ACM increased about 8-fold during a 60 min incubation, and the presence of ADR or ACM led to an even greater increase (Table I). ADR had a stronger effect on lipid peroxidation than ACM.

The effects of ACM metabolites on the NADPH-dependent lipid peroxidation in liver microsomes were then examined. 7-Deoxyaklavinone (ACMone), an aglycone-type ACM metabolite, did not show any effect on lipid peroxidation. However, both MA144M1 (ACM-M1) and MA144N1 (ACM-N1) glycoside-type ACM metabolites, showed increasing effects on lipid peroxidation to the same degree as ACM itself.

TABLE I. Effect of ADR or ACM or ACM Metabolites on NADPH-Dependent Lipid Peroxidation in Microsomes of Mouse Liver

Group	Concentration (μmol/ml)	Increase (%)
Control	0	100.0 ± 1.8[a]
ADR	1	136.9 ± 2.9[b,c]
	10	144.5 ± 3.1[b,c]
ACM	1	113.8 ± 2.1[b]
	10	119.3 ± 2.6[b]
ACMone	1	98.6 ± 0.9
	10	101.4 ± 1.8
ACM-M1	1	109.0 ± 1.7[b]
	10	122.5 ± 2.3[b]
ACM-N1	1	108.6 ± 1.1[b]
	10	120.5 ± 1.5[b]

Each value represents the mean ± S.D. of 5 experiments. *a*) LPO: 0.910→7.008 nmol/ml. *b*) $p<0.001$: significantly different from control. *c*) $p<0.001$: significantly different from ACM group of the same concentration.

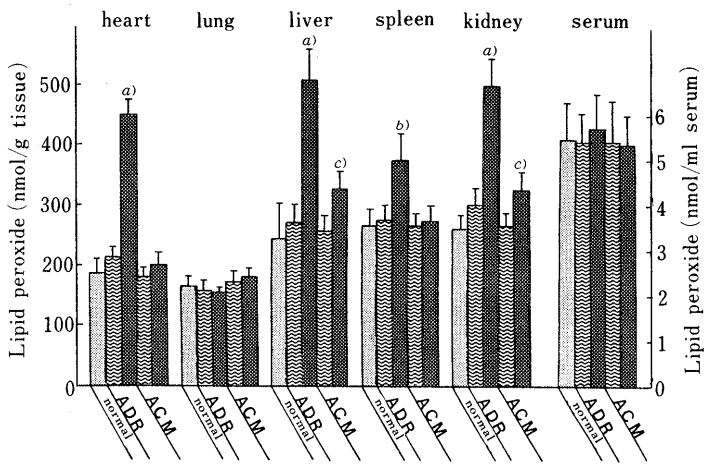

Fig. 1. Lipid Peroxide Levels in Tissues of Mice Treated with ADR or ACM

Male CDF_1 mice received an intraperitoneal injection of ADR or ACM (15 mg/kg). The mice were killed by cervical dislocation at 3 h or 4 d after drug administration. Each value represents the mean ± S.D. for 5 mice. *a*) $p<0.001$: significantly different from control. *b*) $p<0.01$: significantly different from control. *c*) $p<0.05$: significantly different from control. ▒, 3 h; ▓, 4 d.

Effects of ACM and ADR on Lipid Peroxide Levels (*in Vivo*) Lipid peroxide levels in the serum and tissues of mice at 3 h and 4 d after ADR or ACM injection were determined, and the results are shown in Fig. 1.

i) Serum: There was no significant difference between the lipid peroxide level in control mice (5.48 nmol/ml) and in mice at 3 h and 4 d after ACM or ADR injection.

ii) Heart: The lipid peroxide level at 3 h and 4 d after ACM injection did not change as much as that in control mice (185.3 nmol/g). In contrast, lipid peroxide levels in mice at 3 h and 4 d after ADR injection were elevated 1.2-fold (not significant) and 2.6 fold ($p<0.001$), respectively.

iii) Lung: In no case was there a significant change in lipid peroxide levels between mice treated with ACM or ADR and control mice (165.0 nmol/g).

iv) Liver: In ACM treated mice, there was no increase in the lipid peroxide level after 3 h in comparison with the control, but after 4 d it was 1.3 fold ($p<0.05$) greater than in the control mice (243.6 nmol/g).

v) Spleen: In ACM treated mice, there was no significant change from the control value (267.3 nmol/g).

vi) Kidney: The lipid peroxide level on the 4th d after ACM administration was elevated 1.2 fold ($p<0.05$) compared to control mice (262.3 nmol/g).

Tissue Concentrations and Pharmacokinetics of ACM Time courses of ACM concentrations in tissues after intraperitoneal injection were examined. Time courses of ACM concentrations in the heart, liver and kidney are shown in Fig. 2 and the pharmacokinetic data for ACM and its metabolites in tissues are presented in Table II.

ACM concentration in the heart decreased immediately after the injection, but showed a small rebound peak 2.5 h later. ACM in the heart was then eliminated rapidly and was hardly detectable 24 h later. This rebound peak obtained in the heart was also found in all other tissues examined. The elimination of ACM from other tissues after the rebound peak was also rapid.

Metabolites of ACM were found in all tissues examined. Two spots with extremely close *Rf* values were detected as metabolites in the heart. By direct comparison with authentic samples on TLC, these metabolites were identified as MA144M1 and MA144N1, glycosides of ACM. It was

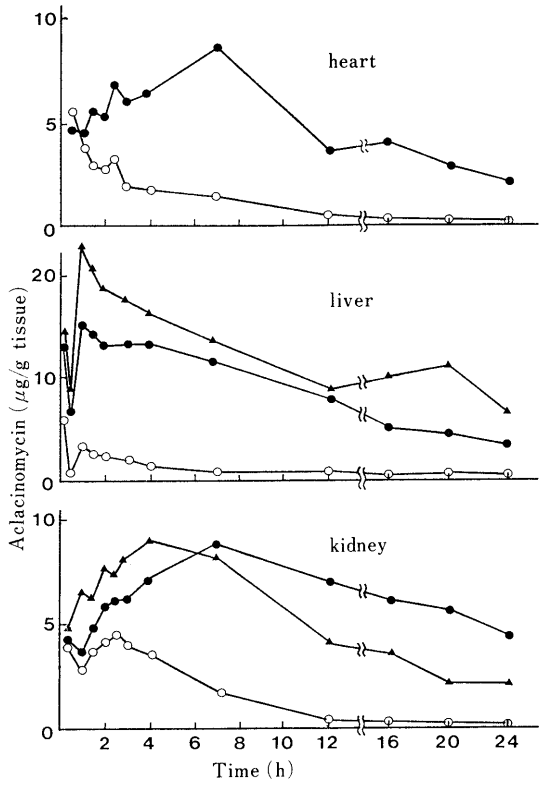

Fig. 2. Time Course of Tissue Levels of ACM after Intraperitoneal Administration of ACM (15 mg/kg) to Male CDF_1 Mice

○—○, ACM; ●—●, glycosides; ▲—▲, aglycone. Each point represents the mean for three animals.

difficult to determine separately the two metabolites by using the TLC scanner because of the proximity of the spots. These metabolites were determined and expressed as glycoside-type metabolites. Concentrations of glycoside-type metabolites of ACM exceeded those of the mother compound in all tissues measured. The glycoside-type metabolites in tissues were eliminated more slowly than the mother compound. The $T_{1/2}$ of glycoside-type metabolites in all tissues except for the spleen was more than twice the value for ACM, reaching 2.7 times in the heart. $AUC_{0-24\,h}$ of ACM in the heart was 22.0 μg/g·tissue·h, while that of

TABLE II. Analysis of Distribution of ACM and ACM Metabolites in Tissues of Normal Mice

	T_{max} (h)	C_{max} (μg/g)	$T_{1/2}$ (h)	$AUC^{a)}$ (0→24 h)
ACM				
Liver	1.0	3.6	4.38	29.0
Kidney	2.5	4.5	3.27	30.1
Heart	0.5	5.5	3.66	22.0
Spleen	1.0	23.5	7.34	228.3
Lung	1.0	16.8	2.43	74.3
ACM glycoside				
Liver	1.0	15.2	11.44	199.6
Kidney	7.0	8.9	17.18	154.1
Heart	7.0	8.7	10.06	113.2
Spleen	7.0	41.5	6.44	696.0
Lung	1.5	25.5	25.79	416.5
ACM aglycone				
Liver	1.0	23.5	12.17	285
Kidney	4.0	9.1	8.38	117.7

a) μg/g·tissue·h.

glycoside-type metabolites in the heart was 113.2 μg/g·tissue·h. AUC_{0-24h} of glycoside-type metabolites in other tissues was about 5 times that of ACM.

Another metabolite of ACM different from glycoside-type metabolites was found in the liver and kidney of ACM-treated mice. Since the *Rf* value of the metabolite was 0.83 and the infrared (IR) spectrum showed strong absorptions at 3550, 1730 and 1620 cm^{-1} (KBr), this metabolite was identified as 7-deoxyaklavinone (ACMone), the aglycone of ACM, and this was further confirmed by direct comparison with an authentic sample. All values for pharmacokinetic parameters of ACMone in the liver were higher than those of glycoside-type metabolites. For example, C_{max} and AUC_{0-24h} of ACMone were 1.5 times and 1.4 times those of glycoside-type metabolites, respectively. However, the value for AUC_{0-24h} of ACMone in the kidney was 76% of that of the glycoside-type metabolites.

Discussion

During the use of antitumor anthracycline antibiotics, several derivatives have been developed to take the place of ADR, because of the severe side-effects of ADR. ACM was one of the candidates and indeed has been in clinical use for about the last decade. ACM has been reported to possess weaker antitumor activity[10] and cardiotoxicity[1] compared to ADR. Myers *et al.*[2,3] demonstrated that ADR-induced cardiotoxicity in mice was associated with an increased lipid peroxide level in the heart. We also confirmed that the lipid peroxide level in the heart of the mouse is specifically increased after intraperitoneal injection of ADR.[5] However, there has been no study of the changes in lipid peroxide levels in mouse tissues following ACM administration.

The effects of ADR and ACM on NADPH-dependent microsomal lipid peroxidation in mice liver were first examined *in vitro*. The increasing effect of ACM on lipid peroxide was only about 40% compared to that of ADR, when similar concentrations (1, 10 μM) were tested. Therefore, it was clear that the effect of ACM on the increment of lipid peroxide *in vitro* was considerable weaker than that of ADR. However, the result *in vitro* does not always correlate with the result obtained *in vivo*. We reported previously[9] that the increase due to ADR of lipid peroxide levels in mouse tissues was more than that due to daunomycin (DAM), in spite of a similar effect on NADPH-dependent lipid peroxidation *in vitro*. Therefore, lipid peroxide levels of tissues in ACM-treated mice were examined. The lipid peroxide levels in mouse tissues after 3 h, the time when the highest tissue concentration of ACM is maintained, and after 4 d, the time when the peak lipid peroxide level is reached after ADR treatment, were determined after ACM (15 mg/kg) intraperitoneal injection. Lipid peroxide levels in the heart of ACM-treated mice were not increased after 3 h, although they were already elevated in ADR-treated mice. Furthermore, there was no increase in lipid peroxide level in the heart on the 4th d after ACM administration. The effect of ACM on lipid peroxide levels in mouse heart was clearly negligible, compared to that of ADR. Considering our previous result,[11] the order of increasing effect on lipid peroxide level in mouse heart was ADR > DAM ≫ ACM = 0. This order agrees completely with that of the severity of clinical cardiotoxicity.[1] This result supports the hypothesis of Myers *et al.*,[2,3] namely, that the increase in lipid peroxide in the heart is related closely to the cardiotoxicity of ADR.

We have shown previously that the difference in effect on lipid peroxide levels between ADR and DAM *in vivo* is related closely to the tissue concentrations of these anthracyclines.[9] The tissue concentrations of ACM in mice were therefore compared in detail with those of ADR. Detailed studies on tissue concentrations of ACM in mouse, rabbit and dog have been reported by Oki *et al.*[8,12-15] However, there has been no report on tissue concentrations of ACM after intraperitoneal injection into mice, which would be necessary in order to discuss the relationship with the lipid peroxide level. After administration of ACM (15 mg/kg, i.p.) to mice, ACM concentrations in all tissues examined reached their C_{max} within 1 h and were then eliminated rapidly, becoming hardly detectable 24 h later. The AUC_{0-24h} of ACM was the lowest in the heart among the tissues examined, being only 29.3% of that obtained with ADR. Consequently, the low distribution of ACM to the heart was proved. It was also confirmed that ACM concentrations in both the lung and spleen were higher than those in other tissues, as reported by Oki *et al.*[8] However, the concentrations of glycoside-type metabolites of ACM were at a high level in all tissues examined. Furthermore, it was also found that there was a low elimination of these metabolites of ACM from tissues. In the pharmacokinetics of glycoside-type metabolites in the heart, $T_{1/2}$ and AUC_{0-24h} of ACM glycosides (10.06 h and 113.2 μg/g·tissue·h, respectively) were higher than these of ADR (2.95 h and 75.2 μg/g·tissue·h, respectively). A similar tendency was seen in other tissues. The effect of these glycoside-type metabolites on NADPH-dependent lipid peroxidation in mouse liver microsomes was equal to that of ACM, though the effect of ACM was weaker than that of ADR. Therefore, it is difficult to explain the difference in lipid peroxide increment induced by ADR and ACM simply from the standpoint of their tissue concentrations.

This study suggests that the lack of effect of ACM on the lipid peroxide levels in the heart of mouse *in vivo* may depend on complicated mechanisms. In brief, not only the

lower ability of ACM to promote lipid peroxidation *in vitro*, but also the mutual interaction between ACM and tissue resistance factor such as protective enzymes against lipid peroxidation, seems to play a major role in determining the lipid peroxide level in the heart of mouse after ACM injection.[16] We have found that there is no change of these enzymes activities after ACM injection.[16] In conclusion, it is clear that there is no increase in the lipid peroxide level of the heart of mouse treated with ACM, and that the difference in lipid peroxide increment after treatment with ADR or ACM is independent of the tissue concentrations of the drugs.

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References

1) K. Yamada, *Jpn. J. Cancer Chemother.*, **10**, 1030 (1983).
2) C. E. Myers, W. P. McGuire, R. H. Liss, I. Ifrim, K. Grotzinger and R. C. Young, *Science*, **197**, 165 (1977).
3) C. E. Myers, W. P. McGuire and R. C. Young, *Cancer Treat. Rep.*, **60**, 961 (1976).
4) H. Tanizawa, Y. Sazuka and Y. Takino, *Chem. Pharm. Bull.*, **29**, 2910 (1981).
5) Y. Sazuka, T. Hirose, J. Hashimoto, H. Tanizawa and Y. Takino, *Chem. Pharm. Bull.*, **32**, 4110 (1984).
6) B. A. Svingen, J. A. Buege, F. O. O'Neal and S. D. Aust, *J. Biol. Chem.*, **254**, 5892 (1979).
7) K. Yagi, *Biochem. Med.*, **15**, 212 (1976).
8) T. Oki, T. Takeuchi, S. Oka and H. Umezawa, *Recent Results Cancer Res.*, **28**, 21 (1981).
9) Y. Sazuka, Y. Yoshikawa, H. Tanizawa and Y. Takino, *Jpn. J. Cancer Res.*, **78**, 1281 (1987).
10) H. Furue, *Jpn. J. Cancer Chemother.*, **8**, 329 (1981).
11) H. Tanizawa, Y. Sazuka and Y. Takino, *Chem. Pharm. Bull.*, **31**, 1714 (1983).
12) I. Kitamura, T. Oki and T. Inui, *J. Antibiotics*, **31**, 919 (1978).
13) T. Ogasawara, Y. Masuda, S. Goto, S. Mori and T. Oki, *J. Antibiotics*, **34**, 52 (1981).
14) T. Oki, N. Shibamoto, H. Iguchi and M. Shirai, *Jpn. J. Antibiotics*, **33**, 163 (1980).
15) H. Iguchi, Y. Matsushita, K. Ohmori, S. Hirano, T. Kiyosaki, S. Hori, H. Tone and T. Oki, *Jpn. J. Antibiotics*, **33**, 179 (1980).
16) Y. Sazuka and H. Tanizawa, Proceeding of 47th Annual Meeting of Japanese Cancer Association, Tokyo, September 1988, p. 550.