Acta Medica Okayama

Volume 38, *Issue* 1 1984 *Article* 8

FEBRUARY 1984

Effect of coenzyme Q10 on the survival time and lipid peroxidation of adriamycin (doxorubicin) treated mice.

Shinya Shinozawa* Kouhei Etowo†

Yasunori Araki‡ Takuzo Oda**

*Okayama University,

†Okayama University,

‡Okayama University,

**Okayama University,

Effect of coenzyme Q10 on the survival time and lipid peroxidation of adriamycin (doxorubicin) treated mice.*

Shinya Shinozawa, Kouhei Etowo, Yasunori Araki, and Takuzo Oda

Abstract

The effect of coenzyme Q10 (Co Q10) was examined on the survival time and lipid peroxidation of adriamycin (ADM)-treated ICR mice. Co Q10 showed a protective effect against a subacute toxicity in mice induced by two intraperitoneal administrations of ADM (15 mg/kg). The group treated orally with 10 mg/kg of Co Q10 showed the longest survival time of all the groups studied (16.81 +/- 10.29 days, mean +/- S.D.) and a significantly longer survival time (p less than 0.001) than the ADM-alone group (7.48 +/- 1.99 days). The inhibitory effect of Co Q10 on the plasma and tissue lipid peroxidation levels did not correlate with the effect of prolonging the survival time of mice. Co Q10 tended to inhibit rises in plasma and liver lipid peroxidation levels induced by ADM administration, but there was no statistically significant difference between treatments. There was a statistically significant different inhibitory effect in the kidney lipid peroxidation levels, but was not in those of the heart.

KEYWORDS: coenzymeQ_{10}, adriamycin, doxorubicin, lipid peroxidation, plasma and tissues, toxicity

---

*PMID: 6702487 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **38,** (1), 57-63 (1984)

EFFECT OF COENZYME Q_{10} ON THE SURVIVAL TIME AND LIPID PEROXIDATION OF ADRIAMYCIN (DOXORUBICIN) TREATED MICE

Shinya SHINOZAWA, Kouhei ETOWO, Yasunori ARAKI and Takuzo ODA*

*Department of Hospital Pharmacy, and * Department of Biochemistry, Cancer Institute, Okayama University Medical School, Okayama, 700, Japan*

Received September 11, 1983

Abstract. The effect of coenzyme Q_{10} (Co Q_{10}) was examined on the survival time and lipid peroxidation of adriamycin (ADM)-treated ICR mice. Co Q_{10} showed a protective effect against a subacute toxicity in mice induced by two intraperitoneal administrations of ADM (15 mg/kg). The group treated orally with 10 mg/kg of Co Q_{10} showed the longest survival time of all the groups studied (16.81 $\pm$ 10.29 days, mean $\pm$ S.D.) and a significantly longer survival time ($p < 0.001$) than the ADM-alone group (7.48 $\pm$ 1.99 days). The inhibitory effect of Co Q_{10} on the plasma and tissue lipid peroxidation levels did not correlate with the effect of prolonging the survival time of mice. Co Q_{10} tended to inhibit rises in plasma and liver lipid peroxidation levels induced by ADM administration, but there was no statistically significant difference between treatments. There was a statistically significant different inhibitory effect in the kidney lipid peroxidation levels, but was not in those of the heart.

Key words : coenzyme Q_{10}, adriamycin, doxorubicin, lipid peroxidation, plasma and tissues, toxicity.

Adriamycin (ADM), an anthracycline antibiotic, has shown marked activity against a wide range of human neoplasms, but its clinical use has been limited because of the risk of dose-dependent severe toxicity to the liver, kidney, bone marrow and heart (1-3). ADM-induced toxicity has been considered to result from biomembrane damage (4, 5). Therefore, in this paper we report the effect of coenzyme Q_{10} (Co Q_{10}), which has membrane stabilizing and anti-oxidant activities (6-8), on ADM-induced toxicity.

MATERIALS AND METHODS

Reagents. Adriamycin hydrochloride (Adriacin®, Lot No. 145 AJB) was kindly donated by Kyowa Hakko Co., Ltd. (Tokyo, Japan). Udidecarenone (Co Q_{10} powder), injectable Co Q_{10} (Co Q_{10} E-0216-019) and its solvent (placebo E-0216-119, which contains 1.8 mg phospholipid, 30 mg polyglycol and 45 mg sorbitol/ml of citrate buffer, pH 7.4) were kindly donated by Eisai Co., Ltd. (Tokyo, Japan).

In vivo, survival experiment. Five-week-old male ICR mice (25-30 g body weight) were divided into groups of 10-20 mice each and injected intraperitoneally with ADM solution (2 mg ADM/ml of sterilized saline) at a dose of 15 mg/kg on days 0 and 4. For the Co Q_{10} solution

groups, mice were administered a Co Q_{10} suspension (1 mg Co Q_{10}/5 ml of 0.5 % carboxymethyl cellulose, CMC solution) orally (p.o.) at doses of 2, 10 and 50 mg/kg. Co Q_{10} E-0216-019 was administered p.o. or injected subcutaneously (s.c.) at doses of 2, 10 and 50 mg/kg into mice of the Co Q_{10} E-0216-019 groups. For the placebo groups (placebo E-0216-119 group) the mice were administered 10 mg/kg E-0216-119 p.o. or injected s.c. with the same at doses of 10 and 50 mg/kg. Co Q_{10}, placebo and saline were all administered to mice from day -3 to 6 once a day, two h before ADM administration (day 0 = day of first ADM administration).

In vivo, lipid peroxidation experiments. The animals used and the dosage and method of ADM administration were the same as those used in the survival experiments. Groups of six mice each were administered ADM and saline p.o. (ADM group) or ADM and Co Q_{10} p.o. (Co Q_{10} group) at a dose of 10 mg/kg. From day -1 to day 6, two h after Co Q_{10} or ADM administration, the cervical artery of each mouse was cut at definite intervals. Blood samples were collected in heparinized tubes. Plasma samples were obtained by centrifugation at $2000 \times g$ for 10 min, and the plasma and carcasses were stored at $-20^{\circ}C$ until measurement. Subsequently, the liver, kidney, spleen and heart of each mouse was excised, washed with a sterilized saline solution and cut into small pieces in a 1.15 % KCl solution. Lastly, the pieces were homogenized with a Polytron homogenizer to make a 10 % homogenate.

Determination of lipid peroxidation in plasma and tissues. Determination of lipid peroxidation in plasma was carried out by measuring the formation of 2-thiobarbituric acid (TBA)-reacting substances, supposedly malondialdehyde, using tetraethoxypropan as the standard material in a fluorophotometric assay (excitation wavelength at 515 nm, emission at 553 nm) described by Yagi (9). The lipid peroxidation in the plasma was expressed as n moles malondialdehyde/ml of plasma. Determination of lipid peroxidation in the tissues was carried out with a photometric assay at 534 nm described by Ohkawa *et al.* (10), and the lipid peroxidation was expressed as n moles malondialdehyde/g·wet wt. The significance of the mean was determined with Student's unpaired tests in all experiments.

RESULTS

Effect of Co Q_{10} on the Survival of ADM-Treated Mice. After an intraperitoneal injection of ADM at a dose of 15 mg/kg on days 0 and 4, the control mice treated with saline alone (ADM group) survived 7.48 ± 1.99 days ($n=54$, mean $\pm$ S.D.). In this group no mice survived more than 30 days. Mice of the group treated with 10 mg/kg of Co Q_{10} CMC solution (p.o.) survived the longest of all the mice studied (16.81 ± 10.29 days) and survived significantly longer than the mice of the ADM group ($p<0.001$). Six of the twenty-two mice in the group survived more than 30 days. Most of the groups treated with Co Q_{10} E-0216-019 (except the 2 mg/kg treatment) showed a significantly longer survival time than the ADM group, but not significantly longer than the placebo E-0216-119 groups whose dosage corresponded to each. The placebo E-0216-119 groups showed a significantly longer survival time than the ADM group, as did p.o. group number 11 ($p<0.05$) and s.c. group number 12 ($p<0.001$), as shown in Table 1.

Effect of Co Q_{10} on Lipid Peroxidation in Plasma and Tissues. The plasma lipid peroxidation in the ADM group was most reactive and showed peak levels on days

TABLE 1. EFFECT OF Co Q_{10} ON THE SURVIVAL TIME OF ADM-TREATED ICR MICE[a]

	Group	Co Q_{10} Dose (mg/kg)	Route	Survival time (mean ± S.D.)	No. of mice surviving 30 days	No. of experiments	Significance against (group No.)
1	ADM + Saline	0	p.o.	7.48± 1.99	0	54	
2	ADM + Co Q_{10} sol.[b]	2	p.o.	15.40± 10.66	6	22	$p < 0.001$ (1)
3	〃	10	p.o.	16.81± 10.29	6	22	$p < 0.001$ (1)
4	〃	50	p.o.	9.45± 3.95	0	11	$p < 0.02$(1)
5	ADM + Co Q_{10} E-0216-019[c]	2	p.o.	7.90± 3.14	0	10	N.S.[d](1),(11)
6	〃	10	p.o.	14.81± 9.68	2	10	$p < 0.001$ (1) N.S. (11)
7	〃	50	p.o.	11.30± 6.66	0	10	$p < 0.001$ (1) N.S. (11)
8	〃	2	s.c.	10.00± 6.12	0	10	$p < 0.02$ (1) N.S. (12)
9	〃	10	s.c.	11.60± 8.28	1	10	$p < 0.01$ (1) N.S. (12)
10	〃	50	s.c.	12.31± 9.67	2	16	$p < 0.001$ (1) N.S. (13)
11	ADM + Placebo E-0216-119[e]	0	p.o.[f]	9.40± 4.88	0	10	$p < 0.05$ (1)
12	〃	0	s.c.[g]	11.70± 7.76	1	10	$p < 0.001$ (1)
13	〃	0	s.c.[h]	7.70± 1.05	0	10	N.S. (1)

a The mice were injected with ADM (15 mg/kg) i.p. on days 0 and 4 with 10 times of the administration of saline, Co Q_{10} or its placebo (once a day, from day -3 to 6).

b Co Q_{10} sol.: Ubidecarenone powder in 0.5 % carboxymethyl cellulose sol.

c Co Q_{10} E-0216-019: Injectable Co Q_{10}.

d Not significantly different.

e Placebo E-0216-119: Placebo of Co Q_{10} E-0216-019 (contains phospholipid 1.8 mg, polyglycol 30 mg and sorbitol 45 mg/ml of citrate buffer, pH 7.4).

f,g,h Administered 10 mg/kg placebo E-0216-019 p.o. (*f*), s.c. (*g*) and 50 mg/kg s.c. (*h*).

0, 4 and 6. The Co Q_{10} group showed inhibition of these rises, but did not show a statistically significant difference. In the heart, the peak levels in the ADM group were observed on days 0, 2 and 5. The Co Q_{10} group showed a statistically significant inhibition of peroxidation on day 0, but not on day 5. In the liver, the peak levels in the ADM group were observed on days 2 and 6. The Co Q_{10} group showed same, but not statistically significant inhibition of peroxidation. In the kidney, a delayed response in lipid peroxidation levels was observed, with a shoulder on day 2 and a peak on day 4. The Co Q_{10} group showed a statistically significant inhibitory effect against peroxidation on days 2 and 4. In the spleen, response of the lipid peroxidation levels was slow, and an inhibitory effect of Co Q_{10} was not observed (Table 2).

TABLE 2. EFFECT OF Co Q_{10} ON LIPID PEROXIDATION[a] IN PLASMA AND TISSUES OF ADM-TREATED ICR MICE

Plasma or tissues	Days after Co Q_{10} or ADM administration							
	−1	0	1	2	3	4	5	6
Plasma								
ADM alone	7.8± 2.1	10.0± 2.8	7.4± 0.8	5.9± 0.6	7.7± 1.2	9.7± 2.5	7.3± 1.5	12.4± 3.2
ADM + Co Q_{10}	7.3± 1.0	8.9± 0.8	7.6± 0.7	5.8± 0.7	6.8± 1.2	7.5± 1.9	7.2± 0.2	11.3± 2.3
Liver								
ADM alone	112.9± 18.6	104.0± 34.5	79.1± 12.2	332.8± 125.3	241.7± 76.6	165.2± 44.4	145.3± 61.9	195.3± 51.6
ADM + Co Q_{10}	185.6± 73.4	91.8± 16.4	93.6± 28.9	277.8± 44.0	199.7± 87.5	146.4± 55.9	151.6± 40.5	171.4± 49.8
Heart								
ADM alone	149.1± 24.3	257.3± 21.6	184.5± 12.4	213.3± 44.9	157.1± 15.7	168.0± 19.0	268.9± 21.9	157.9± 13.3
ADM + Co Q_{10}	150.4± 31.2	218.2± 20.2[b]	212.4± 18.2[d]	194.5± 21.2	138.8± 41.9	218.9± 25.2[e]	271.7± 23.3	128.8± 21.4
Kidney								
ADM alone	82.3± 24.1	80.4± 24.0	82.4± 5.4	111.6± 9.0	118.7± 20.8	147.3± 25.2	108.4± 17.3	99.0± 13.7
ADM + Co Q_{10}	80.4± 10.0	85.3± 17.5	70.3± 16.4	94.5± 12.4	106.8± 15.0	101.0± 39.9[c]	110.0± 3.3	102.1± 13.9
Spleen								
ADM alone	35.9± 3.2	43.4± 2.3	47.0± 10.9	31.7± 3.7	46.7± 9.8	33.6± 5.4	29.1± 6.8	46.4± 11.5
ADM + Co Q_{10}	34.5± 3.6	43.9± 3.5	49.9± 7.7	37.9± 6.5	37.1± 5.1	23.2± 10.4	30.9± 3.1	40.6± 4.5

a The values are the mean ± S.D. of duplicate determinations on 6 separate mice.

b,c Significantly different from the value obtained with the group treated with ADM alone (b : $p < 0.02$, c : $p < 0.05$).

d,e Significantly different from the value obtained with the group treated with Co Q_{10} + ADM (d : $p < 0.02$, e : $p < 0.01$).

DISCUSSION

Co Q_{10} showed a protective effect against subacute toxicity induced in mice with an intraperitoneal administration of ADM. Possible mechanisms of the ADM-induced toxicity have been reported to be interference with DNA directed DNA or RNA synthesis (11), inhibition of mitochondrial oxidative phosphorylation (12), inhibition of membrane Na^+-K^+-ATPase (13), calcium accumulation in the myocardium (14) and so forth. ADM, an amphophilic compound, interacts electrostatically with negatively charged lipids, especially cardiolipin (15, 16). ADM has been shown to be distributed in tissues rich in membrane phospholipids, namely, the liver, kidney and heart (17-19), then, to accumulate in these organs (20) and destabilize the cell membrane (21). Therefore, production of lipid peroxidation (22) due to membrane damage was considered to induce toxicity. Co Q_{10} and α-tocopherol, having free radical scavenger (22, 23) and membrane stabilizing effects (6-8, 24) were expected to have a protective effect against ADM-induced toxicity. The effectiveness of α-tocopherol against ADM-induced toxicity has been found in terms of cardiac function (25), survival (22, 26), and biochemistry (27), but experiments showing no effect also have been reported (8, 28). Several reports have been made on the protective effect of Co Q_{10} against ADM-induced toxicity (12, 29-33). Yamanaka *et al.* (32) reported that the plasma levels of lipid peroxidation correlated negatively with the survival time of mice. In our experiments, Co Q_{10} markedly prologed the survival time of mice, but did not inhibit lipid peroxidation. Kishi *et al.* (33) reported that Co Q_{10} prevented the ADM-induced inhibition of succinoxidase and NADH-oxidase enzymes in the mitochondria. Our findings concerning survival agree with those reported by Kishi *et al.* from *in vitro* experiments. Although we examined lipid peroxidation at the tissue homogenate level, further investigation should be done at the mitochondrial and microsomal levels.

Acknowledgement. We thank Dr. T. Fukuda for providing facilities for this work.

REFERENCES

1. Minow, R.A., Benjamin, R.S. and Gottlieb, J.A.: Adriamycin (NSC-123127) cardiomyopathy-An overview with determination of risk factors. *Cancer Chemother. Rep.* Part 3, **6**, 195-202, 1975.
2. *MARTINDALE, The Extra Pharmacopoeia,* Twenty-seven Edition (Wade, A. ed.). Pharmaceutical Press. Pharmaceutical Society of Great Britain, London, pp. 145-147, 1977.
3. Bristow, M.R., Thompson, P.D., Martin, R.P. and Mason, J.W.: Early anthracycline cardiotoxicity. *Am. J. Med.*, **65**, 823-832, 1978.
4. Handa, K. and Sato, S.: Generation of free radicals of quinone group-containing anticancer chemicals in NADPH-microsome system, as evidenced by initiation of sulfite oxidation. *Gann* **66**, 43-47, 1975.
5. Mettler, F.P., Young, D.M. and Ward, J.M.: Adriamycin-induced cardiotoxicity (cardiomyopathy and congestive heart failure) in rats. *Cancer Res.* **37**, 2705-2713, 1977.

6. Maggio, B., Diplock, A.T. and Lucy, J.A.: Interactions of tocopherols and ubiquinones with monolayers of phospholipids. *Biochem. J.*, **161**, 111-121, 1977.
7. Shinozawa, S., Araki, Y. and Oda, T.: Stabilizing effects of coenzyme Q_{10} on potassium ion release, membrane potential and fluidity of rabbit red blood cells. *Acta Med. Okayama* **34**, 255-261, 1980.
8. Takeshige, K., Takayanagi, R. and Minakami, S.: Reduced coenzyme Q_{10} as an antioxidant of lipid peroxidation in bovine heart mitochondria. ed. Y. Yamamura, K. Folkers and Y. Ito, In *Biomedical and Clinical Aspects of coenzyme Q*, vol. 2, Elsevier/North-Holland Biomedical Press. Amsterdam, pp. 15-25, 1980.
9. Yagi, K.: A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem. Med.* **15**, 212-216, 1976.
10. Ohkawa, H. Ohishi, N. and Yagi, K.: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351-358, 1979.
11. Merski, J., Daskal, Y. and Busch, H.: Comparison of adriamycin-induced nucleolar segregation in skeletal muscle, cardiac muscle and liver cells. *Cancer Treat. Rep.* **62**, 771-778, 1978.
12. Iwamoto, Y., Hansen, I.L., Porter, T.H. and Folkers, K.: Inhibition of coenzyme 'Q_{10} enzymes, succinoxidase and NADH-oxidase, by adriamycin and other quinones having antitumor activity. *Biochem. Biophys. Res. Commun.* **58**, 633-638, 1974.
13. Gosalvez, M., van Rossum, G.D.V. and Blanco, M.F.: Inhibition of sodium-potassium-activated adenosine 5'-triphosphatase and ion transport by adriamycin. *Cancer Res*, **39**, 257-261, 1979.
14. Olson, H.M., Young, D.M., Prieur, D.J., LeRoy, A.F. and Reagan, R.L.: Electrolyte and morphologic alterations of myocardium in adriamycin-treated rabbits. *Am. J. Pathol.* **77**, 439-453, 1974.
15. Goormaghtigh, E., Chatelain, P., Caspers, J. and Ruysschaert, J.M.: Evidence of a specific complex between adriamycin and negatively charged phospholipids. *Biochim. Biophys. Acta* **597**, 1-14, 1978.
16. Karczmar, G.S. and Tritton, T.R.: The interaction of adriamycin with small unilamellar vesicle liposomes. A fluorescence study. *Biochim. Biophys. Acta* **557**, 306-319, 1979.
17. Yesair, D.W., Schwartzbach, E., Shuck, D., Denine, E.P. and Asbell, M.A.: Comparative pharmacokinetics of daunomycin and adriamycin in several animal species. *Cancer Res.* **32**, 1177-1183, 1972.
18. Loveless, H., Arena, E., Felsted, R.L. and Bachur, N.R.: Comparative mammalian metabolism of adriamycin and daunorubicin. *Cancer Res.* **38**, 593-598, 1978.
19. Shinozawa, S., Mimaki, Y. and Araki, Y.: Determination of the concentration of adriamycin and its metabolites in the serum and tissues of Ehrlich carcinoma-bearing mice by high-performance liquid chromatography. *J. Chromatogr*, **196**, 463-469, 1980.
20. Shinozawa, S., Fukuda, T., Araki, Y. and Oda, T.: Pharmacokinetic analysis of adriamycin (Doxorubicin) and related fluorescent compounds in Ehrlich tumor-bearing mouse plasma and tissues. *Acta Med. Okayama* **36**, 125-132, 1982.
21. Tritton, T.R., Murphree, S.A. and Sartorelli, A.C.: Adriamycin : A proposal on the specificity of drug action. *Biochem. Biophys. Res. Commun.* **84**, 802-808, 1978.
22. 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.
23. Tappel, A.L. and Zalkin, H.: Inhibition of lipid peroxidation in microsomes by vitamin E. *Nature (London)* **185**, 35, 1960.
24. Kibata, M., Shinozawa, S., Miyake, K., Shimizu, Y., Shoji, K. and Miyahara, K.: Studies

on "abnormal spectrin" detected in vitamin E deficient rat erythrocyte membrane by SDS-polyacrylamide gel electrophoresis. *Rinsho Kagaku* **5**, 326-329, 1977 (in Japanese).

25. Sonneveld, P.: Effect of α-tocopherol on the cardiotoxicity of adriamycin in the rat. *Cancer Treat, Rep.* **62**, 1033-1036, 1978.
26. Myers, C.E., McGuire, W. and Young, R.: Adriamycin : Amelioration of toxicity by α-tocopherol. *Cancer Treat. Rep.* **60**, 961-962, 1976.
27. Wang, Y.M., Madanat, F.F., Kimball, J.C., Gliser, C.A., Ali, M.K., Kaufman, M.W. and van Eys, J.: Effect of vitamin E against adriamycin-induced toxicity in rabbits. *Cancer Res.* **40**, 1022-1027, 1980.
28. Breed, J.G.S., Zimmerman, A.N.E., Dormans, J.A.M.A. and Pinedo, H.M.: Failure of the antioxidant vitamin E to protect against adriamycin-induced cardiotoxicity in the rabbit. *Cancer Res.* **40**, 2033-2038, 1980.
29. Kishi, T. and Folkers, K.: Prevention by coenzyme Q_{10} (NCS-140865) of the inhibition by adriamycin (NSC-123127) of coenzyme Q_{10} enzymes. *Cancer Treat. Rep.* **60**, 223-224, 1976.
30. Bertazzoli, C. and Ghione, M.: Adriamycin associated cardiotoxicity : Research on prevention with coenzyme Q. *Pharmacol. Res. Commun.* **9**, 235-250, 1977.
31. Folkers, K., Liu, M., Watanabe, T. and Porter, T.H.: Inhibition by adriamycin of the mitochondrial biosynthesis of coenzyme Q_{10} and implication for the cardiotoxicity of adriamycin in cancer patients. *Biochem. Biophys. Res. Commun.* **77**, 1536-1542, 1977.
32. Yamanaka, N., Kato. T., Nishida. K., Fujikawa, T., Fukushima, M. and Ota, K.: Elevation of serum lipid peroxide level associated with doxorubicin toxicity and its amelioration by [dl]-α-tocopheryl acetate or coenzyme Q_{10} in mouse. *Cancer Chemother. Pharmacol.* **3**, 223-227, 1979.
33. Kishi, T., Watanabe, T. and Folkers, K.: Bioenergetics in clinical medicine : prevention by forms of coenzyme Q of the inhibition by adriamycin of coenzyme Q_{10}-enzymes in mitochondria of the myocardium, *Proc. Natl. Acad. Sci., U.S.A.* **73**, 4653-4656, 1976.