Acta Medica Okayama

Volume 45, *Issue* 3 1991 *Article* 11

JUNE 1991

Tissue concentration of doxorubicin (adriamycin) in mouse pretreated with alpha-tocopherol or coenzyme Q10.

Shinya Shinozawa[*] Yutakata Gomita[†]

Yasunori Araki[‡]

[*]Okayama University,

[†]Okayama University,

[‡]Okayama University,

Tissue concentration of doxorubicin (adriamycin) in mouse pretreated with alpha-tocopherol or coenzyme Q10.*

Shinya Shinozawa, Yutakata Gomita, and Yasunori Araki

Abstract

The tissue concentration of doxorubicin (adriamycin; ADM) and its major metabolite (aglycone I) was examined in mice pretreated with alpha-tocopherol (VE) or coenzyme Q10 (CoQ). In VE-pretreated group, the concentrations of aglycone I of the liver (1, 3 and 5 h after the administration), kidney (1 and 3h) and heart (3h) were significantly higher than those in the saline group. The clinical application of VE or CoQ concomitant with anti-tumor drugs especially ADM, requires caution.

KEYWORDS: doxorubicin, tissue concentration, ?-tocopherol, coenzymeQ10

---

*PMID: 1891979 [PubMed - indexed for MEDLINE]

Acta Med Okayama 45 (3) 195-199 (1991)

— Brief Note —

Tissue Concentration of Doxorubicin (Adriamycin) in Mouse Pretreated with α-Tocopherol or Coenzyme Q_{10}

Shinya Shinozawa*, Yutaka Gomita and Yasunori Araki

Department of Hospital Pharmacy, Okayama University Medical School, Okayama 700, Japan

The tissue concentration of doxorubicin (adriamycin; ADM) and its major metabolite (aglycone I) was examined in mice pretreated with α-tocopherol (VE) or coenzyme Q_{10} (CoQ). In VE-pretreated group, the concentrations of aglycone I of the liver (1, 3 and 5h after the administration), kidney (1 and 3h) and heart (3h) were significantly higher than those in the olive oil group. In CoQ-pretreated group, the concentrations of aglycone I of the kindey (1 and 3h) and heart (3h) were significantly higher than those in the saline group. The clinical application of VE or CoQ concomitant with anti-tumor drugs especially ADM, requires caution.

Key words : doxorubicin, tissue concentration, α-tocopherol, coenzyme Q_{10}

Doxorubicin (adriamycin: ADM), an anthracycline type of the anti-malignant tumor agent, is widely used in clinical therapy, but it causes severe side effects such as cardiotoxicity and suppression of bone marrow functions. Accordingly, its use is often limited (1, 2). It has been reported that the mechanism of ADM-induced toxicity is closely related to a lipid peroxidation resulting from cell membrane damage (3, 4) and to an insufficient mitochondrial function (5, 6). Therefore, clinical or experimental application of α-tocopherol (VE) and coenzyme Q_{10} (CoQ) as an anti-oxidant agent has often been tried (7-10). It has been also reported that the co-administration of VE or CoQ with anti-tumor drugs enhances the chemotherapeutic effects of these drugs *in vitro* or *in vivo* (11-14). However, the precise mechanism of these enhancements is not well understood. Therefore, in the present study we examined the effects of VE and CoQ on the tissue concentration of ADM in mice.

Animals. Five-week-old male ICR mice weighing between 25 and 30g were used in all experiments. They were fed a standard rat and mouse diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and given water *ad libitum*. They were housed in plastic cages with a 12-h cycle of light and dark maintained automatically.

Chemicals. ADM was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo Japan. ADM was dissolved in sterilized saline solution (10mg/ml). VE acetate and CoQ (kindly donated by Eisai Co., Ltd., Tokyo, Japan) were used by dissolving VE in olive oil (50mg/ml), and by suspending CoQ in saline solution (0.2mg/ml). Olive oil was purchased from Sigma Chemical Co., St. Louis, MO, USA. Chloroform, isopropanol, acetic acid and sodium acetate were purchased from Nakarai Chemical Co., Ltd., Kyoto, Japan. All these chemicals were of

* To whom correspondence should be addressed.

analytical grade.

Determinations of ADM and its metabolites in mouse tissues. The mice were divided into 20 groups (three mice in each group). They were injected subcutaneously with VE (500 mg/kg) or olive oil (10 ml/kg), and administered orally with CoQ (10 mg/kg) or saline solution once a day for 3 days. Two hours after the last administration of these drugs, the mice were injected intraperitoneally with ADM (5 mg/kg), then sacrificed 1, 3, 5, 24 and 48 h later by cutting the cervical artery. The liver, kidney and heart were excised, washed with a sterilized saline solution, and homogenized with 1.15 % KCl using a Polytron homogenizer to make a 5–10 % homogenate. The concentrations of ADM, adriamycinol, adriamycinone and other aglycones were determined by high-performance liquid chromatography (HPLC) as reported previously (15). HPLC apparatus (Waters model 440, Milford, MA, USA.) was connected to a high-sensitivity fluorescence spectrophotometric detector (Hitachi Model 650–10S) equipped with an automatic sample processor (Waters, WISP-710B). HPLC was carried out at a flow rate of 1.0 ml/min using Zorbax Sil as the stationary phase, and chloroform-isopropanol-acetic acid-water-sodium acetate buffer (pH 4.5) (100 : 100 : 14 : 14 : 1) as

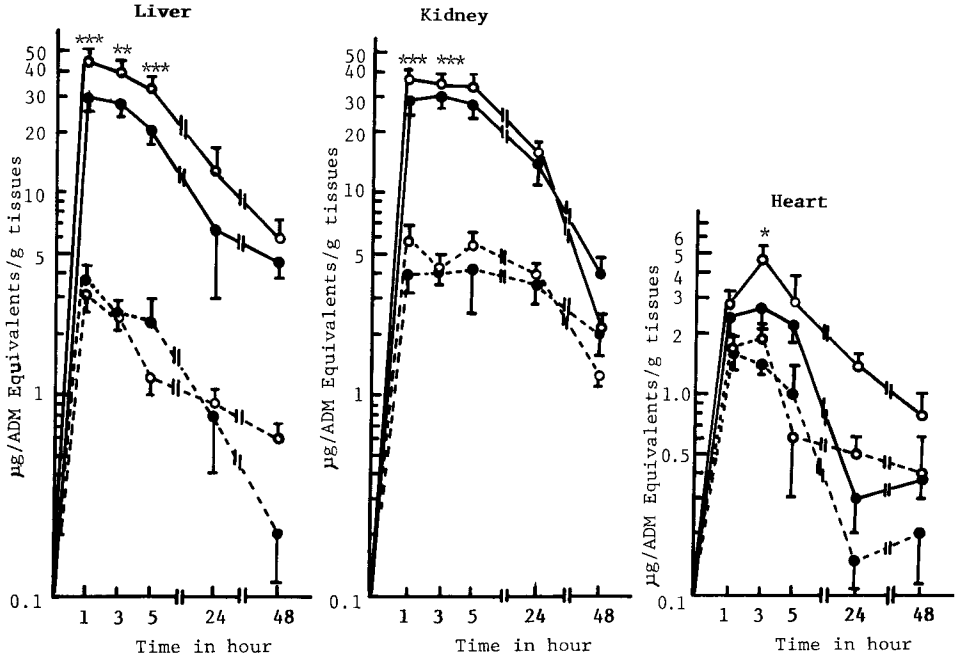

Fig. 1 Effect of α-tocopherol (VE) on the concentration of adriamycin (ADM) equivalents and its major metabolite, aglycone I, in the liver, kidney and heart of ADM-administered mice.
g tissue: gram wet weight of tissue. Significantly different from the value of ADM + olive oil group. (* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$)
o—o, ADM aglycone I of VE-pretreated group; ●—●, ADM aglycone I of olive oil-pretreated group. o- - -o, ADM of VE-pretreated group; ●- - -●, ADM of olive oil-pretreated group.

the mobile phase. The fluorescence spectrophotometric detector was operated at an excitation wavelength of 470 nm and an emission wavelength of 585 nm. The results were calculated by a Data-module 730. All operations with ADM and related fluorescent compounds were carried out in near darkness.

Statistical analysis. Statistical analyses were performed using Student's *t* test. Vaules of p less than 0.05 were considered to represent significant differences between means.

Fig. 1 shows the concentrations of ADM and its major metabolite (aglycone I) in ADM-administered mouse tissues after pretreatment with olive oil or VE. Higher concentrations of aglycone I were detected in the liver, kidney and heart of the mice pretreated with VE than in those of the mice pretreated with olive oil. In the VE-pretreated group, the concentrations of aglycone I of the liver (1, 3 and 5 h), kidney (1 and 3 h) and heart (3 h) were significantly higher than those in the olive oil-pretreated group. But there was no significant difference between the groups in the concentration of ADM. Fig. 2 shows the concentrations of ADM and its major metabolite (aglycone I) in ADM-administered mouse tissues after pretreatment with saline or CoQ. Higher concentrations of aglycone I were detected in the liver, kidney and hearts of the mice pretreated with CoQ than in those of the mice pretreated

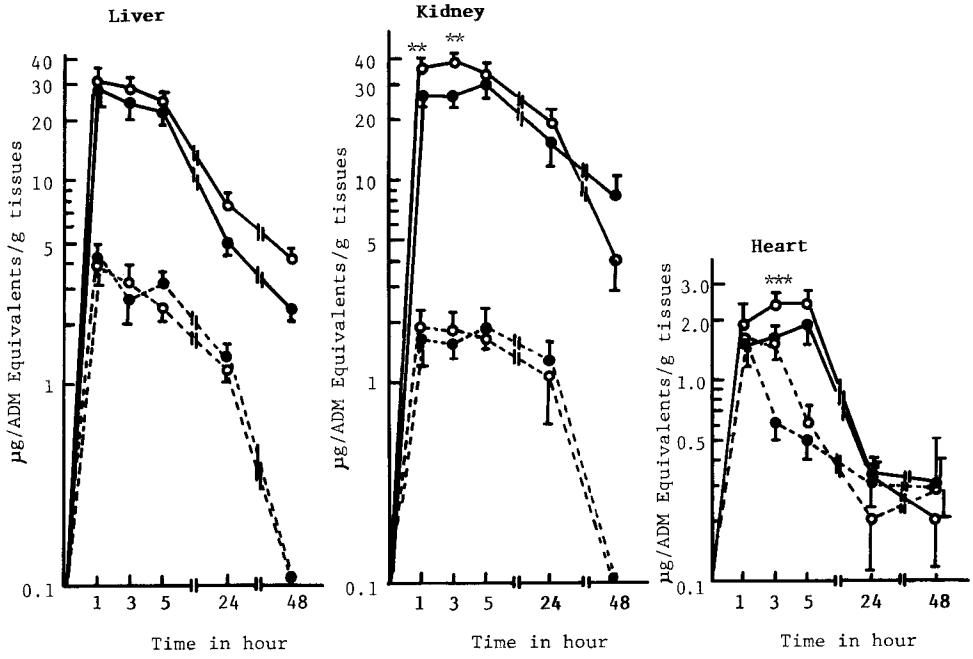

Fig. 2 Effect of coenzyme Q_{10} (CoQ) on the concentration of ADM equivalents and its major metabolite, aglycone I, in the liver, kidney and heart of ADM-administered mice.
g tissue: gram wet weight of tissue. Significantly different from the value of ADM + saline group. (** $p < 0.02$, *** $p < 0.01$)
o—o, ADM aglycone I of CoQ-pretreated group; ●—●, ADM aglycone I of saline-pretreated group.
o- - o, ADM of CoQ-pretreated group; ●- - ●, ADM of saline-pretreated group.

with saline. In the CoQ-pretreated group, the concentration of aglycone I of the kidney (1 and 3h) and heart (3h) was significantly higher than those in the saline group. But there was no significant difference between the groups in the concentration of ADM.

The experimental or clinical use of VE or CoQ as a membrane stabilizer and an anti-oxidant is worthy of note for the prevention of ADM-induced cardiotoxicity (7–10). Therefore, the tissue distributions of ADM and the related metabolites were examined in the mice pretreated with VE or CoQ. The concentration of aglycone I in the VE or CoQ pretreated group was significantly higher than those in the control. Significantly high levels of aglycone I were observed especially in VE-pretreated mouse liver and kidney. On the mechanism of the increased aglycone concentration in this experiment first, promotion of ADM-metabolism may be considered. On the basis of previous reports that the hepatic microsomal drug metabolizing enzyme activity was decreased in VE-deficient rats (16), and that synthetic efficiency of ubiquinone was also decreased in hepatic microsomes injured by carbon tetrachloride (17), it was inferred that VE and CoQ may affect the microsomal activity. In fact, phenobarbital-pretreated mice, subsequently treated with ADM showed higher aglycone concentrations in the liver and significantly lower survival than did controls (18). This fact is closely related to another report that adriamycinone (main component of aglycone I) was about three times as potent an inhibitor of beef heart mitochondrial succinoxidase activity as ADM (8). The reason for possible promotion of ADM-metabolism by the administration of VE or CoQ (as anti-oxidant drugs) is not clear at present, but it may be considered that the stimulation of drug-metabolizing enzyme activity was due to share NADPH-electron donor between lipid peroxidation and drug-metabolizing reactions in microsomes (19). Secondarily, differences in the concentration of membrane uptake between ADM and aglycones may be supposed. Aglycone I may show higher concentration of membrane uptake than ADM because of its more hydrophobic nature. In our *in vitro* experiment on the uptake of ADM and aglycone into mouse mitochondria pretreated with VE, higher concentration of aglycone in the membrane was observed (20). Some isoprenoids were reported to potentiate the cytotoxic effects of anti-cancer agents, particularly ADM, in cultured mammalian cells, because they might increase the intracellular levels of the agents due to enhancement of the uptake and reduction of the efflux (21). The isoprenoid side chain of VE or CoQ may act as a membrane stabilizer interacting with cell membrane and regulating membrane permeability to materials (22). Accordingly, in this experiment interaction of VE or CoQ with membrane components might have resulted in a decrease in the release of aglycone I from the membrane. If malignant or tumor cells are the target, the reinforcement of the anti-tumor drug by VE or CoQ is advantageous, but side effects occur and toxicity is reinforced if normal cells are the target. Therefore, the clinical application of VE or CoQ concomitant with anti-tumor drugs, such as ADM requires caution.

References

1. Minow RA, Benjamin RS and Gottlieb JA: Adriamycin (NSC-123127) cardiomyopathy-An overview with determination of risk factors. Cancer Chemother Rep (1975) **6**, 195–202.
2. Bristow MR, Thompson PD, Martin RP and Mason JW : Early anthracycline cardiotoxicity. Am J Med (1978) **65**, 823–832.
3. Myers CE, McGuire WF, Ifrim I, Liss RH, Grotzinger K and Young RC: Adriamycin, the role of lipid peroxidation in cardiac toxicity and tumor response. Science (Washington, DC) (1977) **197**, 165–167.
4. Mimnaugh EG, Gram TE and Trush MA: Stimulation of mouse heart and liver microsomal lipid peroxidation by anthracycline anticancer drugs: Characterization and effects of reactive oxygen scavengers. J Pharmacol Exp Ther (1983) **226**, 806–816.
5. Ferrero ME, Ferrero E, Gaja G and Bernelli-Zazzera A: Adriamycin: Energy metabolism and mitochondrial oxidation in the heart of treated rabbits. Biochem Pharmacol (1976) **25**, 125–130.

6. Shinozawa S, Fukuda T, Araki Y and Oda T: Effect of dextran sulfate on the survival time and mitochondrial function of adriamycin (Doxorubicin)-treated mice. Toxicol Appl Pharmacol (1985) **79**, 353–357.
7. Van Vleet JF and Ferrans VT: Evaluation of vitamin E and selenium protection against chronic adriamycin toxicity in rabbits. Cancer Treat Rep (1980) 64, 315–317.
8. Kishi T, Watanabe T and Folkers K: Bioenergetics in clinical medicine: Prevention by forms of coenzyme Q of the inhibition by adriamcyin of coenzyme Q10-enzyms in mitochondria of the myocardium. Proc Natl Acad Sci (1967) **73**, 4653–4656.
9. Ghione M and Bertazzoli C: Biochemical and Clinical Aspects of Coenzyme Q eds. Folkers K and Yamanaka Y, Elsevier, Amsterdam (1977) pp 183–189.
10. Shinozawa S, Etowo K, Araki Y and Oda T: Effect of coenzyme Q10 on the survival time and lipid peroxidation of adriamycin (Doxorubicin) treated mice. Acta Med Okayama (1984) **38**, 57–63.
11. Alberts DS, Peng YM and Moon TE: α-Tocopherol pretreatment increases adriamycin bone marrow toxicity. Biomedicine (1978) **29**, 189–191.
12. Prasad KN, Prasad JE, Ramanujam S and Sakamoto A: Vitamin E increases the growth inhibitory and differentiating effects of tumor therapeutic agents on neuroblastoma and glioma cells in culture. Proc Soc Exp Biol Med (1980) **164**, 158–163.
13. Ripoll EAP, Rama BN and Webber MM: Vitamin E enhances the chemotherapeutic effects of adriamycin on human prostatic carcinoma cells *in vitro*. J Urol (1986) **136**, 529–531.
14. 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 Q10 in mouse. Cancer Chemother Pharmacol (1979) **3**, 223–227.
15. Shinozawa S, Araki Y and Oda T: Determination of adriamycin (Doxorubicin) and its related fluorescent compounds in rat lymph and gall by high-performance liquid chromatography. J Chromatogr (1981) **212**, 323–330.
16. Zannoni VG and Sato PH: The effect of certain vitamin deficiences on hepatic drug metabolism. Fed Proc (1976) **35**, 2464–2469.
17. Aiyar AS and Streenivasen A: Intracellular distribution and biosynthesis of ubiquinone in rat liver in carbon tetrachloride liver injury. Biochem J (1962) **82**, 179–182.
18. Steven DR and Bachur NR: Alteration in adriamycin efficacy by phenobarbital. Cancer Res (1976) **36**, 3803–3806.
19. Kamataki T and Kitagawa H: Effects of lipid peroxidation on activities of drug-metabolizing enzymes in liver microsomes of rats. Biochem Pharmac (1973) **22**, 3199–3207.
20. Shinozawa S, Gomita Y and Araki Y: Effect of high dose α-tocopherol and α -tocopherol acetate pretreatment on adriamycin (Doxorubicin) induced toxicity and tissue distribution. Physiol Chem Phys Med NMR (1988) **20**, 329–335.
21. Ikezaki K, Yamaguchi T, Miyazaki C, Ueda H and Kishiye T: Potentiation of anticancer agents by new synthetic isoprenoids. I. Inhibition of the growth of cultured mammalian cells. JNCI (1984) **73**, 895–901.
22. Lucy JA: Functional and structural aspect of biological membranes: A suggested structural role for vitamin E in the cotrol of membrane permeability and stability. Ann NY Acad Sci (1972) **203**, 4–11.

Received November 28, 1990; accepted January 9, 1990.