

Acta Medica Okayama

Volume 42, Issue 5

1988

Article 2

OCTOBER 1988

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Abstract

The effect of alpha-tocopherol acetate (VE) on the toxicity and tissue distribution of adriamycin (ADM) in mice was studied. After the administration of ADM in 2 doses of 15 mg/kg, mice pretreated with olive oil survived 7.1 +/- 1.0 days, while mice pretreated with VE in ten doses of 500 mg/kg/day (subcutaneously) survived 5.5 +/- 1.7 days (p less than 0.01). The total concentration of ADM and its major metabolite, aglycone I in the liver (1, 3, 5 h), kidneys (1, 3 h), and heart (3 h), as determined by high performance liquid chromatography was significantly higher in the VE-pretreated group (four doses of 500 mg/kg/day) than in the olive oil-pretreated group. The aglycone levels of the VE-pretreated group were significantly higher than those of the olive oil-pretreated group in the liver, kidney and heart, but there was no significant difference between the groups in the levels of the unmetabolized form. Considering these results, the administration of VE concomitant with anti-tumor drugs, including ADM, requires great caution.

KEYWORDS: adriamycin, doxorubicin, toxicity, ?-tocopherol acetate, aglycone, tissue concentration

*PMID: 3223337 [PubMed - indexed for MEDLINE]

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Effect of High Dose α -Tocopherol Acetate on the Toxicity and Tissue Distribution of Adriamycin (Doxorubicin)

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The effect of α -tocopherol acetate (VE) on the toxicity and tissue distribution of adriamycin (ADM) in mice was studied. After the administration of ADM in 2 doses of 15 mg/kg, mice pretreated with olive oil survived 7.1 ± 1.0 days, while mice pretreated with VE in ten doses of 500 mg/kg/day (subcutaneously) survived 5.5 ± 1.7 days ($p < 0.01$). The total concentration of ADM and its major metabolite, aglycone I in the liver (1,3,5 h), kidneys (1,3 h), and heart (3 h), as determined by high performance liquid chromatography was significantly higher in the VE-pretreated group (four doses of 500 mg/kg/day) than in the olive oil-pretreated group. The aglycone levels of the VE-pretreated group were significantly higher than those of the olive oil-pretreated group in the liver, kidney and heart, but there was no significant difference between the groups in the levels of the unmetabolized form. Considering these results, the administration of VE concomitant with anti-tumor drugs, including ADM, requires great caution.

Key words : adriamycin, doxorubicin, toxicity, α -tocopherol acetate, aglycone, tissue concentration

Adriamycin (ADM) has shown marked activity against a wide range of human neoplasms, but its clinical use has been limited because of the risk of severe dose-dependent toxicity to the heart, liver, kidney, and bone marrow (1). The mechanism of ADM-induced toxicity has been discussed from various points of view (2-6), but the precise mechanism is not completely understood. In 1977, Myers *et al.* (4) reported that ADM-induced toxicity was closely related to lipid peroxidation resulting from cell membrane

damage, and was reduced by pretreating animals with α -tocopherol (VE). Thereafter, both the usefulness (7-10) and, conversely, the uselessness (11-13) of VE in the amelioration of the toxicity have been reported. In the present study, we examined the effect of high dose VE on ADM-induced toxicity.

Materials and Methods

Animals. Five-week-old male ICR mice weighing between 25 and 30 g each were used in all experiments. They were fed a standard rat and mouse diet (MF, Oriental Yeast Co., Ltd., Tokyo,

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Japan) and given water *ad libitum*. They were housed in plastic cages with a 12-h cycle of light and dark maintained automatically.

Chemicals. Adriamycin hydrochloride was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo and was used by dissolving it in sterilized saline solution (10 mg/ml). α -Tocopherol acetate (VE) was kindly donated by Eisai Co., Ltd., Tokyo and was used by dissolving it in olive oil (50 mg/ml). Olive oil was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Chloroform, isopropanol, acetic acid and sodium acetate were purchased from Nakarai Chemical Co., Ltd., Kyoto, Japan. All these chemicals were of analytical grade.

Survival experiments. All mice (10 animals/group) were injected intraperitoneally with ADM in doses of 15 mg/kg on days 0 and 4 (day 0 indicates the day of the first ADM administration). VE was administered to mice of the VE-treated group once a day from day -3 to 6, and in addition 2 h before ADM administration on days 0 and 4. Saline or olive oil was administered to mice of the control group according to the same schedule. Body weight and deaths of the mice were checked daily for 30 days.

Determination of ADM and its metabolites in mouse tissues. The mice were divided into ten groups (3 mice in each group) and injected subcutaneously with VE (500 mg/kg) or olive oil (10 ml/kg) from day -3 to day 0. Two hours after the last administration of VE or olive oil, the mice were intraperitoneally injected 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, adriamycinone, adriamycinol and other aglycones were determined by high performance liquid chromatography (HPLC) as reported previously (14). A high-performance liquid chromatograph (Waters, Milford, MA, U.S.A., model 440) was connected to a Hitachi high-sensitivity fluorescence spectrophotometric detector (model 650-10S) equipped with a WISP-710B automatic sample processor. 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 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.

The procedure for extraction of ADM and its metabolites from the tissues has been described previously (15). 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. Values of *p* less than 0.05 were considered to represent significant differences between means.

Results

After an intraperitoneal injection of ADM in doses of 15 mg/kg on days 0 and 4, the mice treated with saline alone survived 7.3 ± 0.8 days. The mice pretreated with VE in doses of 100 mg/kg/day survived (8.0 ± 3.2 days) slightly longer than those treated with saline. The mice pretreated with VE in doses of 500 mg/kg/day showed a significantly shorter survival time (5.5 ± 1.7 days) than the olive oil-administered

Table 1 Effect of α -tocopherol acetate (VE) on the survival time of adriamycin hydrochloride (ADM)-administered mice^a

Group	n ^b	VE dose (mg/kg)	Survival time ^c (Days, mean $\pm$ S.D.)
ADM+Saline	10	0	7.3 ± 0.8
ADM+Olive oil	10	0	7.1 ± 1.0
ADM+VE	10	100	8.0 ± 3.2
	10	500	$5.5 \pm 1.7^*$

a: All mice were injected intraperitoneally with 15 mg/kg of ADM on days 0 and 4 (day 0 = day of first ADM administration). Saline, olive oil (10 ml/kg of body weight/day) or VE was administered subcutaneously from day -3 to 6 once a day. On days 0 and 4, these agents were given 2 h before ADM administration.

b: Number of animals.

c: The value with an asterisk is significantly different from the value obtained in the ADM+olive oil group ($p < 0.01$).

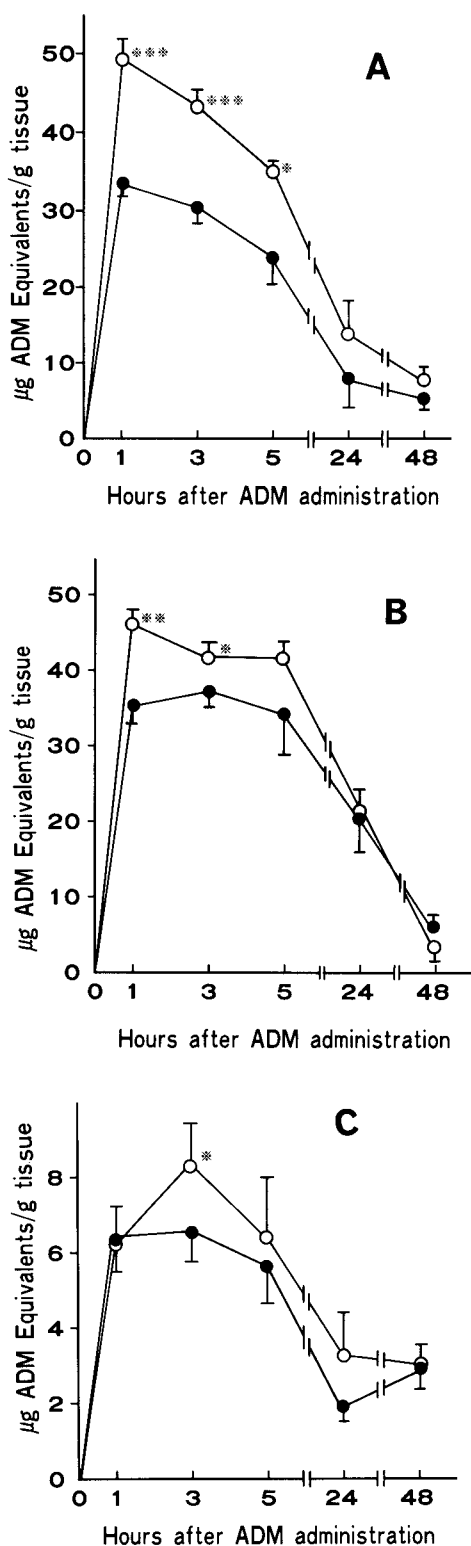


Fig. 1 Effect of α -tocopherol acetate (VE) on the concentration of adriamycin (ADM) equivalents (total concentrations of ADM and its major metabolite, aglycone I) in the liver (A), kidney (B) and heart (C) of ADM-administered mice. —○—, ADM+VE; —●—, ADM+Olive oil; g tissue, gram wet weight of tissue. Significantly different from the values of ADM+Olive oil groups (*, $p < 0.05$, **, $p < 0.02$, ***, $p < 0.01$)

group (7.1 ± 1.0 days) (Table 1). The effects of VE on the tissue concentrations of ADM equivalents (total concentrations of ADM and its major metabolite, aglycone I) are shown in Fig. 1. Higher concentrations of ADM equivalents were detected in the livers, kidneys, and hearts of the mice pretreated with VE than in those of the mice pretreated with olive oil. Livers and kidneys of the VE-treated group 1, 3, and 5 h after ADM administration contained higher concentrations of ADM equivalents than those of the olive oil-pretreated group. In the heart, the concentrations of ADM equivalents in the VE-treated group were significantly higher than those of the olive oil-pretreated group 3 h after ADM administration.

Table 2 shows the concentrations of ADM and its major metabolite, aglycone I, which is considered to be adriamycinone (14), in ADM-administered mouse tissues after pretreatment with VE or olive oil. The concentrations of aglycone I in the livers, kidneys, and hearts of the VE-pretreated group were higher than in those of the olive oil-pretreated group almost every time examined. The differences in the aglycone I concentrations were significant in the livers 1, 3, and 5 h, kidneys 1 and 3 h, and hearts 3 h after the administration of ADM.

Discussion

VE has been used widely in the clinical treatment of cardiovascular disease, arte-

Table 2 Effect of α -tocopherol treatment on the concentrations of adriamycin (ADM) and its major metabolite, aglycone I, in tissues of ADM-administered mice^a

Tissue	ADM and metabolite determined	Hours after ADM administration ^b									
		1		3		5		24		48	
		Olive O.	VE	Olive O.	VE	Olive O.	VE	Olive O.	VE	Olive O.	VE
Liver	Aglycone I	29.3±1.4	45.1±2.2 ^{c*}	27.3±1.5	39.9±1.9 ^{c**}	20.4±2.6	33.1±0.4 ^{c*}	6.5±3.6	12.3±4.1	4.6±0.2	5.9±1.2
	ADM	3.8±0.4	3.1±0.5	2.4±0.4	2.4±0.3	2.3±0.7	1.2±0.2	0.8±0.6	0.9±0.1	0.2±0.1	0.6±0.1
Kidney	Aglycone I	28.7±1.3	36.9±0.4 ^{c*}	30.2±1.0	34.8±0.4 ^{c*}	27.7±3.5	33.3±1.3	14.1±3.2	15.1±2.4	3.8±1.1	2.2±0.7
	ADM	3.9±0.4	5.8±1.0	4.0±0.4	4.2±0.5	4.2±1.7	5.4±0.5	3.7±0.9	3.8±0.5	2.2±0.2	1.3±0.1
Heart	Aglycone I	2.4±0.6	2.8±0.3	2.6±0.4	4.7±0.8 ^{c***}	2.2±0.4	2.9±0.9	0.3±0.1	1.1±0.1	0.4±0.1	0.8±0.2
	ADM	1.7±0.2	1.7±0.2	1.4±0.1	1.9±0.2	1.0±0.4	0.6±0.3	0.1±0.1	0.5±0.1	0.2±0.1	0.4±0.2

a: The ICR male mice were divided into ten groups (3 mice in each group) and injected subcutaneously with α -tocopherol acetate (VE, 500 mg/kg) or olive oil (Olive O., 10 ml/kg) (control) from day -3 to day 0. Two hours after the last administration of VE or olive oil, the mice were injected intraperitoneally with ADM (5 mg/kg).

b: Values are expressed as the mean $\pm$ S.E. in μ g per g wet weight of tissue.

c: Significantly different from olive oil control. *, $p < 0.01$; **, $p < 0.02$; ***, $p < 0.05$.

riosclerosis, and muscular dystrophy (16), as an antioxidant agent (17) and as a membrane stabilizer (18). Since Myers *et al.* (4) reported that ADM-induced cardiotoxicity was reduced by pretreatment of mice with VE, a free radical scavenger, many authors have discussed the value of VE treatment. The results of our investigation showed that when the mice pretreated with VE were dosed with ADM, a slight prolongation of the survival time was noted at a VE dose of 100 mg/kg/day, but conversely, the survival time was significantly reduced at a VE dose of 500 mg/kg/day. One possible explanation of these findings is that ADM, particularly the aglycone, increases in tissue due to dosing with VE. Reich and Bachur (19) have reported that when mice pretreated with phenobarbital were dosed with ADM, tissue aglycone levels increased, and the survival time of the mice was significantly shortened. We also found a significant increase in aglycone in tissue due to VE administration, and when considering the strong inhibitory action of aglycone on cardiac function and the damage to biomembranes (20), compared to that of ADM, the tissue aglycone concentration must be assumed to play an important role in the manifestation of the toxicity. Another possible explanation of the present findings is that stabilizes the cell membrane by interacting with arachidonic acid to decrease the membrane fluidity (21) and regulates membrane permeability to materials (22). Therefore, interactions of VE with membrane components due to the high dose of VE administered in this experiment may have resulted in decreased release of ADM and aglycone from the membrane. Since synthetic isoprenoids have been reported to interact with the cell membrane by decreasing the efflux of anticancer agents (23), the isoprenoid side chain of VE may act in the same way. *In vitro*, VE has been reported

to enhance the growth inhibitory effect of ADM on human prostatic carcinoma (24), neuroblastoma and glioma cells (25). The cause of this enhancement is assumed to be related to the balance between uptake of VE by the cell membrane and regulation of its release. If malignant or tumor cells are the target, the reinforcement of the anti-tumor drug by VE is advantageous, but side effects occur and toxicity is reinforced if normal cells are the target. Albert *et al.* (26) have studied the effect of ADM on mouse myelocyte proliferation. When jointly used with ADM, VE significantly inhibited myelocyte proliferation. Taken together with the experimental results of the present study, these findings indicate that the clinical application of VE concomitant with anti-tumor drugs, including ADM, requires great caution.

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Received March 11, 1988; accepted August 2, 1988