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EFFECT OF α-TOCOPHEROL ON THE MICROSOMAL LIPID PEROXIDATION INDUCED BY DOXORUBICIN : INFLUENCE OF ASCORBIC ACID

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Summary : α-Tocopherol (40 mg/rat/day) was administered, orally, to doxorubicin treated rats (2 mg/kg, twice weekly, for 4 weeks) singly and also in combination with ascorbic acid (1 g/100 ml/day) in drinking water. The vitamin therapy was carried out for a period of 1 month. The microsomal lipid peroxide levels in liver and heart were found to be increased in doxorubicin treated rats. α-tocopherol and ascorbic acid treatment decreased the lipid peroxide level and also NADPH-dependent lipid peroxidation. A significant depletion of glutathione in liver and heart of doxorubicin treated animals was found to be ameliorated by vitamin therapy. Ascorbic acid was found to maintain the level of microsomal α-tocopherol. The activities of the detoxifying enzymes like catalase, superoxide dismutase and glutathione peroxidase were suppressed in doxorubicin treated rats and vitamines coadministration maintained the levels of these enzymes. Ascorbic acid was found to potentiate the antioxidant nature of α-tocopherol.

Key words : doxorubicin lipid peroxidation α-tocopherol ascorbic acid glutathione catalase superoxide dismutase

INTRODUCTION

Doxorubicin (adriamycin is an antitumor antibiotic isolated from cultures of *streptomytes peucetius* (1). This drug is effective against human malignancies such as leukemias lymphomas and many solid tumors (2). However, repetitive administration in patients and in experimental animals causes toxic side effects in liver (3) and heart (4). Various efforts have been made to overcome this toxicity, by supplementing agents which do not interfere with the cytotoxic effect of the drug. Few among them are α-tocopherol, riboflavin butyrate and ubiquinone (5, 6).

The cell killing effects of doxorubicin involve intercalation into double helical DNA and subsequent inhibition of polymerases and of DNA and RNA synthesis (7). Doxorubicin has been shown to be a potent inducer of free radicals. *In vitro*, quinone containing anticancer agents including doxorubicin have been shown to form semiquinone free radical intermediates in the presence of certain flavin enzymes (8). These free radicals abstract hydrogen from polyunsaturated fatty acids which are abundant in membrane phospholipids. These events can initiate a chain reaction in which there is formation of organic peroxides and production of more organic radicals, which are responsible for various deleterious effects. Among subcellular organells, microsomes are labile to lipid peroxidation and concurrent damage (9).

Fortunately, the living cell has defense mechanisms which can provide protection against devastating effects of lipid peroxidation. The most important components in the defense systems are

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vitamin E, glutathione and some glutathione dependent enzymes (10).

α-Tocopherol (Vit E) plays an important role as an antioxidant in microsomes along with glutathione (11) and ascorbic acid has been reported to influence the activity (12).

Since liver is the major organ involved in detoxification and heart is the target organ for this drug, we have studied the effect of the doxorubicin and vitamins on liver and heart microsomes.

MATERIAL AND METHODS

Chemicals : Doxorubicin hydrochloride, bovine serum albumin, NADPH and 1, 1, 3, 3—tetramethoxypropane (Sigma Chemical Company, Missouri), ascorbic acid, sodium pyrophosphate, ferric chloride and potassium chloride (all from E. Merck; analytical grade) were used.

Methods: Adult male Wistar rats were maintained with free access to food and water. The rats were divided into 4 groups. Group I served as control. Group II, III and IV animals received doxorubicin (2 mg/kg, iv) in saline, twice weekly, for 4 weeks (13). Group II animals were left without vitamin therapy. Group III animals were given Vit. E orally (400 mg/kg) and Group IV animals were given ascorbic acid in drinking water (1 g/100 ml/day) along with vit E. The vitamin therapy was carried out for a period of 30 days.

After the experimental period the animals were killed by cervical decapitation. Liver and heart were removed washed in ice-cold saline and homogenised in isotonic potassium chloride. A portion of the homogenate was used for the estimation of GSH (14). GSH peroxidase (15), GSH-reductase (16), GSH transferase (17), catalase (18) and superoxide dismutase (19). Microsomes were separated by differential centrifugation (20) from the remaining portion of the homogenate. The lipid peroxide level was determined by the method of Okhawa *et al* (21). NADPH dependent lipid peroxidation was measured by the method of Hogberg *et al* (22). The method of Tayler *et al* (23) was adopted for the estimation of vit E in microsomes. Protein was estimated by the method of Lowry *et al* (24).

RESULTS AND DISCUSSION

Levels of microsomal lipid peroxides in liver and heart of control and test animals are given in Table I.

TABLE I : Levels of microsomal lipid peroxides in liver and heart of control and doxorubicin treated animals with or without vitamin supplementation. Values are expressed as mean ± SD from 6 animals in each group.

Animals treated with	*Liver*	*Heart*
I. None	500.0±43.3	220.0±11.1
II. DXR	750.1±31.4@	363.1±14.0@@
III. DXR+α-toc	573.9±41.5@	300.4±17.1@
IV. DXR+α-toc+asc	300.2±21.5@@	150.0±11.9@@

Lipid peroxide levels are expressed as n moles of MDA formed/100 mg protein

@ $P < 0.01$ @@ $P < 0.001$

Group I is compared with Group II

Group III & IV are compared with Group II

A significant increase in the level of lipid peroxides is noted in doxorubicin treated animals. Singal *et al* (25) have also demonstrated that lipid peroxide production is enhanced during doxorubicin treatment. But the percentage of increase is high in heart when compared to liver. This could be due to the lesser availability of antioxidants in heart (26). Vitamin E treatment reduced the level of lipid

peroxides significantly. The protection given by Vit E is potentiated by the supplementation cf vitamin C (Group IV).

TABLE II : Effect of doxorubicin and vitamins on NADPH dependent microsomal lipid peroxidation. Values are expressed as Mean ± S.D. from 6 animals in each group.

Animals treated with	*Liver*	*Heart*
I. None	703.4±40.0	285.0±21.0
II. DXR	1120.0±83.1@	724.3±69.0@
III. DXR+α-toc	875.1±64.0@	550.5±31.0@
IV. DXR+α-toc+asc	601.5±49.0@@	325.0±29.0@@

The level of lipid peroxidation is expressed in terms of n moles MDA Formed/100 mg protein/5 min

@ $P < 0.01$; @@ $P < 0.001$

Group I is compared with Group II

Group III & IV are compared with Group II

Table II shows a significant increase (0.01) in NADPH dependent lipid peroxidation in doxorubicin treated animals. The microsomal metabolism of the drug may be by two major pathways, one pathway consists of the reductive cleavage of the sugar moiety to yield anthraquinone aglycone. It involves a semiquinone free radical intermediate produced by Cyt-P_{450} reductase which is mediated by NADPH (27). Bachur *et al* (28) have also demonstrated the oxidation of NADPH during doxorubicin metabolism. The NADPH dependent increase in lipid peroxide level clearly shows the possibility of the production of toxic free radicals during microsomal metabolism of the drug. An effective decrease in the NADPH induced lipid peroxide level is noted in group III and IV animals. This could be either due to the antioxidant effect of these vitamins or the interaction in the NADPH Cyt-P_{450} reductase based metabolism of the drug.

Fig. 1 shows the level of Vit. E in microsomal fractions of liver and heart of control and test

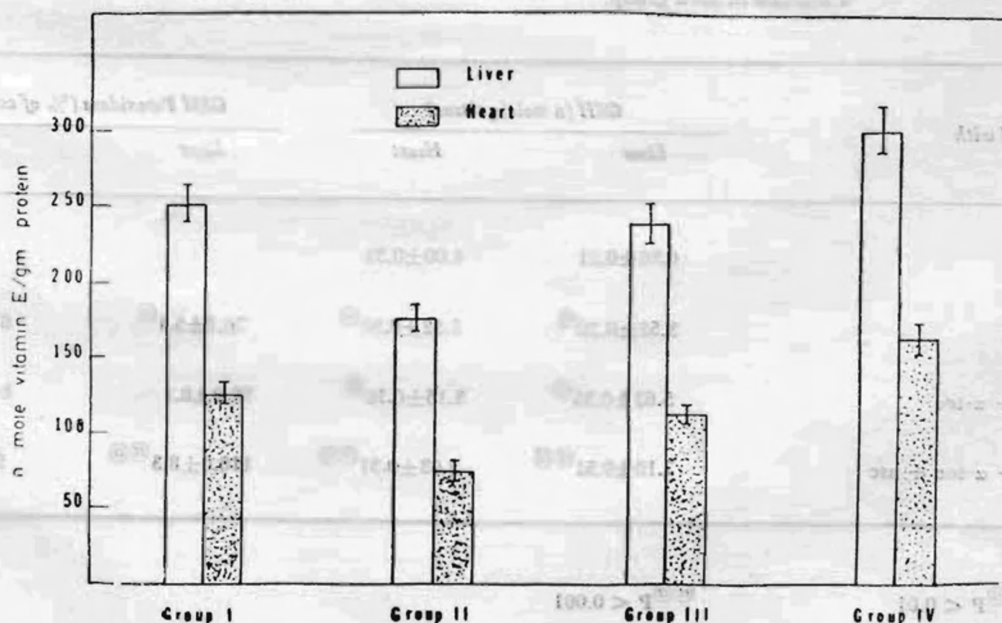

Fig. 1 : Vitamin E levels in microsomal fractions of liver and heart of rats. Group I : control, Groups II, III and IV, doxorubicin treated. Groups III and IV were given Vit. E, Group IV was also given Vit. C (See Methods).

animals. Group III and IV animals which were treated with vitamins show high level of microsomal Vit. E content which is responsible for the low level of lipid peroxides noted in our results (Table I). It has been demonstrated that Vit. E protects the microsomes from peroxidative damage with the combined action of glutathione (11). A greater increase in the Vit. E content of group IV animals when compared with group III might have been due to the regeneration of α-tocopherol by ascorbic acid. It has been reported that Vit C regenerates α-tocopherol from tocopheroxyl radical (12) which is produced during its antioxidant action.

The level of glutathione and the activities of glutathione dependent enzymes are given in Table III and IV. A significant decrease in GSH content is seen in doxorubicin treated animals. Increased consumption of GSH could be due to the interaction with toxic hydroperoxides formed during lipid peroxidation (29) however, vitamins treatment maintains the level of glutathione. It has been proved that vit. E along with cytosolic GSH protects microsomes from lipid peroxidation damage (11).

Glutathione peroxidase activity decreases considerably in doxorubicin treated rats (Group II) which could be due to the inactivation of the enzyme by free radicals (30). The activity of cytosolic glutathione peroxidase which protect microsomes (31) is restored in animals given vitamin therapy.

The activities of GSH-reductase and GSH-transferase which provide continuous supply of GSH, increases significantly in vitamins supplemented animals.

TABLE III : Effect of doxorubicin and vitamins of the level of reduced glutathione and the activity of glutathione peroxidase. Values are expressed as Mean ± SD from 6 animals in each group.

Animals treated with	*GSH (n mole/g tissue)*		*GSH Peroxidase (% of control)*	
	Liver	*Heart*	*Liver*	*Heart*
I. None	6.98±0.21	4.00±0.31		
II. DXR	3.52±0.20@	1.52±0.50@	70.1±5.1@	61±2.3@
III. DXR + α-toc	5.62±0.35@	3.15±0.10@	92.8±8.1	84±3.1
IV. DXR + α-toc + asc	7.10±0.51@@	5.03±0.31@@	110.0±8.3@@	93±7.1@

@ $P < 0.01$ @@ $P < 0.001$

Group I is compared with Group II

Group III & IV are compared with Group II

Table V shows the activities of catalase and superoxide dismutase in liver and heart of experimental animals. These two enzymes are effectively involved in scavenging hydroxyl and superoxide anion radicals (32). Depletion of catalase and SOD activities may accordingly lead to an increase in lipid peroxide level in group II animals. The enzyme activities are restored in vitamins treated animals probably by the alternative utilization of other antioxidants like Vit E and glutathione.

TABLE IV : Effect of doxorubicin and vitamins on GSH-reductase and GSH-transferase activities. Values are expressed as Mean±S.D. from 6 animals in each group.

Animals treated with	*GSH-reductase (% of control)*		*GSH-transferase (% of control)*	
	Liver	*Heart*	*Liver*	*Heart*
II. DXR	66±3.1@	70±5.1@	70±3.1@	49±3.5@@
III. DXR + α-toc	92±6.1@	86±5.2@	99±4.3@	64±5.1@
IV. DXR + α-toc + asc	119±7.1@@	101±5.1@@	131±7.1@@	98±4.9@

@ $P < 0.01$ @@ $P < 0.001$

Group I is compared with Group II

Group III & IV are compared with Group II

TABLE V : Effect of doxorubicin and vitamins on the activities of catalase and superoxide dismutase. Values are expressed as Mean±SD from 6 animals in each group.

Animals treated with	*Catalase m mole H_2O_2 decomposed/mg protein*		*Superoxide dismutase units/mg protein*	
	Liver	*Heart*	*Liver*	*Heart*
I. None	15.01±0.80	5.1±0.1	8.12±0.25	2.12±0.15
II. DXR	9.10±0.61@	2.9±0.1@	5.00±0.31@	1.05±0.08@
III. DXR + α-toc	13.01±0.50@	5.5±0.2@	6.94±0.41@	1.98±0.07@
IV. DXR + α-toc + asc	19.20±0.91@@	7.2±0.5@@	10.79±0.51@@	2.23±0.02@

@ $P < 0.01$ @@ $P < 0.001$

Group I is compared with Group II

Group II & IV are compared with Group II

Hence, it is concluded that Vit E supplementation provides beneficial effects against doxorubicin induced toxicity and ascorbic acid potentiates the role of Vit E as an antioxidant.

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