COMPARISON BETWEEN ANTIOXIDANT EFFECT OF VITAMIN-C ALONE AND ITS COMBINATION TO VITAMIN-E IN LONG EVANS NORWEGIAN RAT

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ABSTRACT

In rat myocardial damage was produced the administration of adrenaline in a dose of 2 mg/kg body weight (b.w.) subcutaneously for 2 consecutive mornings. This damage was assessed indirectly by significant increase in serum aspertate transminase (AST) and directly by microscopic changes in the myocardium.

Pretreatment with the vitamin-C in a dose 10mg/kg body weight subcutaneously and preteatment with the combination of vitamin-C in a dose of 10mg/kg b.w. subcutaneously and vitamin-E in a dose of 50mg/kg b.w. orally for 10 days prevented the adrenaline induced myocadial damage equally which was evidenced by the equal prevention in the rise of serum AST & LDH levels as well as equal prevention of microscopic changes of the myocardium by the adrenaline.

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INTRODUCTION

Heart disease is one of the major health problems throughout the world. Among the heart diseases, the ischemic heart disease (IHD) is the most important cause (about 80%) of cardiac death. Ischemia occurs as a result of critical imbalance between coronary blood flow and myocardial demand[1]. The myocardial damage produced by adrenaline is identical to that of human myocardial infarction[2, 3].

In producing myocardial damage during ischemia, generation of oxygen derived free radicals are also involved besides other mechanisms. Free radicals are the chemical species that possess unpaired electron. During ischemia ATP level is depleted. It causes uncoupled mitochondrial respiratory burst chain which is responsible for the generation of oxygen-derived free radicals[4]. These free radicals then interact with the membrane phospholipid and causes peroxidation of polyunsaturated lipids in the membrane. Antioxidants can trap these free radicals. Vitamin-C also acts as an antioxidant by reacting directly with the aqueous free radicals. Vitamin-E is also a lipophilic radical scavenging antioxidant[5].

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MATERIALS AND METHODS

The experiment was carried out on a total number of 60 Long Evans Norwegian rats of both sexes. They were 3-5 months old, weighing between 150-250 gm. They fed on standard laboratory diet and were allowed to drink water ad.libitum. The rats were divided into 6 groups. Total duration of experiment was 10 days. First two groups received distilled water (vehicle) and adrenaline (2mg/kg b.w. subcutaneously) for 2 consecutive days i.e. - on the 9th and 10th day of the experiment respectively. Third group received vitamin-C (10mg/kg b.w. s/c) for 10 days and adrenaline (2mg/kg b.w. subcutaneously) for 2 days i.e. - on the 9th and 10th day. Fourth group received vitamin-C (10gm/kg b.w. s/c) for 10 days and adrenaline (2mg/kg b.w. subcutaneously) for 2 consecutive days i.e. - on the 9th and 10th day. Fifth group received combination of vitamin-C (10mg/kg b.w. subcutaneously) and vitamin-E (50gm/kg b.w. orally) for 10 days. Sixth group received combination of vitamin-C (10mg/kg b.w. s/c) and vitamin-E (50mg/kg b.w. orally) for 10 days and adrenaline (2mg/kg b.w. s/c) for 2 days i.e. - on the 9th of 10 day.

All the rats were sacrificed on the 11th day i.e. -48 hours after 1st adrenaline injection under light anesthesia with chloroform. By cutting neck blood was collected and the serum was separated for estimation of aspertate transminase (AST) and lactate dehydrogenase (LDH) by standard kits supplied by Boehringer, Germany. The hearts were preserved in formalin for histopathological

examination. Haematoxylin and eosin stain was done for Mythological study of the myocardium. Statistical analysis of result was done by unpaired student's "T" test.

RESULT

There were highly significant ($p < 0.001$) rise in serum AST and significant ($p < 0.01$) rise in serum LDH levels following adrenaline injection as compared to those of control group (Table -I).

In the histological study of the myocardial tissue 60% of the animals showed severe degree of myocardial damage as evidence by the mononuclear cellular infiltration, interstitial oedema formation, fragmentation of muscle fibres and vacuolar degeneration as compared to those of control group where normal architecture of the myocardiam was maintained (Table - II).

The group which received vitamin-C (Group III) there were no rises in serum AST and LDH levels like adrenaline-only treated group. So the results were highly significant ($p<0.001$) in serum AST and LDH levels as compared to those of adrenaline treated group (Table-1). Normal architecture of the myocardium was also maintained (Table-II).

In the group pretreated with vitamin -C followed by adrenaline (Group-IV) there were also no rise in serum AST and LDH levels like adrenaline treated group. So the results were significant in serum AST ($p<0.05$) and LDH ($p<0.01$) levels as compared to those of adrenaline treated group (Table-I). Normal architecture of the myocardium was also maintained (Table-II)

The group which received vitamin -C and -E combination there were no rise in serum AST and LDH levels like adrenaline only treated group. So the result were significant ($p < 0.01$) in serum AST and highly significant ($p < 0.001$) in serum LDH levels as compared to those of adrenaline treated group (Table-I). Normal architecture of the myocardium was also maintained (Table - II).

In the group pretreated with vitamin-C and -E combination followed by adrenaline there were also no rise in the serum AST and LDH levels like adrenaline treated group. So the result were significant ($p<0.01$) in serum AST and highly significant ($p<0.001$) in serum LDH levels as compared to those of adrenaline treated group (Table-I). Myocardial histology showed no microscopic changes (Table-II).

Table - I

Comparison of the effects of vitamin-C alone and vitamin-C and -E combination in serum AST and LDH levels in adrenaline treated rats

Groups		Enzyme	Mean ± SE (U/L)	95% confidencelimit
I		AST	14.97± 2.8	(9.48-20.46)
	(Control)	LDH	60.92±12.97	(35.5-86.34)
II	Adrenaline	AST	44.07±3.98***	(36.27-51.87)
	(2 mg/kg b.w. orally)	LDH	124.2±7.03**	(110.42-137.98)
III	Vitamin-C	AST	16.88±4.16***	(8.73-25.03)
	(10 mg/kg. b.w. s/c)	LDH	39.36±8.36***	(22.97-55.75)
IV	Adrenaline (2 mg/kg b.w. s/c)	AST	22.7±6.4*	(10.16-35.24)
	+ Vitamin-C (10 mg/kg b.w. s/c)	LDH	85.3±6.14**	(73.27-97.33)
V	Vitamin-C (10 mg/kg b.w. s/c)+	AST	22.7±3.24**	(16.35-29.05)
	Vitamin-E (50 mg/kg b.w. orally)	LDH	31.98±7.61***	(17.06-46.9)
VI	Adrenaline (2 mg/kg b.w. s/c) +	AST	25.61±2.97**	(22.64-31.43)
	Vitamin-C (10mg/kg b.w. s/c)+	LDH	59.04±12.28***	(34.47-83.11)
	Vitamin-E (50mg/kg b.w. orally)			

Comparison made: I vs II, II vs III, II vs IV, II vs V, II vs VI,
*=p<0.05, **=p<0.01, ***=p<0.001

Table II

Comparison of the effects of vitamin-C alone and vitamin-C & E combination on myocardial histology in adrenaline treated rats.

Lesions	Control	Adrenaline (2mg/kg b.ws/c)	Vit-C (10mg/ kg b.w s/c)	Adrenaline (2mg/kgb.w s/c)+ Vit-C(10mg/ kgb.w s/c)	Vit-C(10mg /kgb.w. s/c)+ Vit-E(50mg/ kgb.w orally)	Adrenaline (2mg/kg b.ws/c+ Vit-C(10mg/ kgb.w. s/c + Vit-E(50mg/kg b.w orally)
Mononuclear cellular infiltration	_	+ (1) + + (3) + + + (6)	_	+ (2) + + (1)	_	+ (2) + + (1)
Interstitial	_	+ + (3)	_	+ (2)	+ (2)	+ (2
Oedema		+ + + (5)		+ + (1)		+ + (1)
Fragmentation of muscle fibers	_	+ (1) + + (2) + + + (6)	_	_	_	+ (3) + + (1)
Vacuolar degeneration	_	+ + (4) +++ (3)	_	+ (1)	_	+ (1)

+ = Mild degree of lesion

+ + = Moderate degree of lesion

+ + + = Severe degree of lesion

Figure in the Parentheses denote the numbers of heart examined.

DISCUSSION

In the present study, adrenaline was administered to produce myocardial lesion in experimental animals such as in rats. This was evidenced by the fact that serum AST and LDH levels were significantly elevated in adrenaline induced rats as compared to those of control group. The results are consistent with the finding of other investigators who conduct similar types of studies[4,6,7]. The increased enzyme levels were associated with severe degree of microscopical changes supporting myocardial damage. These are also in well agreement with others [4,8-10]. Vitamin-C has no significant effect on normal myocardium which was evidenced by the fact that there were no rise in serum AST and LDH levels like adrenaline alone treated group (Gr-II). Adrenaline induces histological changes were also not observed in the vitamin-C pretreated group (Table-II).

Vitamin-C and -E combination has no significant effect on normal myocardium which was evidenced by the facts that there were no rise in serum AST and LDH levels like adrenaline. Normal architecture of the myocardium was also maintained by vitamin-C and -E combination. But adrenaline induced myocardial damage was prevented by vitamin-C and -E combination. So, in the group pretreated with vitamin-C and -E combination followed by adrenaline (group-VI) there were no rise in serum AST and LDH levels like adrenaline alone treated group. Adrenaline induced microscopical changes were also not observed in the vitamin-C and -E combination pretreated group (Table-II).

Ischemia is a condition in which blood flow is reduced to a level inadequate to meet the metabolic requirements of the tissue. During ischemia the condition may be suitable for the production of free radical entities. Oxygen-derived free radical may initiate or extend cellular injury during reperfusion of oxygen radicals suggested that once these compounds are produced they could readily interact with the membrane phospolipids. Myocardial membrane phospolipids contain substantial levels of unsaturated fatty acids. The diene bodies in these lipids represent reactive sites which could interact with oxygen radicals. Such radical lipid interactions would in turn cause structural rearrangement of unsaturated lipids and altered membrane integrity. The oxygen molecule is capable of producing reactions in the cell, forming highly reactive free radicals and inducing lipid peroxidation of membranes, altering their integrity and increasing their fluidity and permeability. The ischemic cardiac cell is the prime candidate for this reaction sequence and explain the molecular mechanism underlying the pathologic events related to membrane dysfunction[11].

Vitamin-C can react directly with the aqueous free radicals and quench their reactivity. Since free radicals

species have been associated with damaging effects to intracellular and extracellular structures, the antioxidant function of vitamin-C is important in the protection of cellular functions[12].

As cardioprotctive agent, the use of vitamin -E is based on the assumption that vitamin-E acts primarily as a lipohilic radical scavenging antioxidant and suppresses the chain initiation and for chain propagation by donating its phenolic hydrogen to the oxygen radicals. It is generally accepted to be an antioxidant that inhibit membrane lipid peroxidantion and also an important structural component of biological membranes that stabilizes them. The tocopherol molecule consists of two functional domains, a hydrocarbon chain that is necessary for proper orientation of the molecule in the membranes and a chromanol nucleus that is responsible for its antioxidant properties. The ability of vitamin -E to attenuate myocardial injury result from the reduction of the accumulation of non-esterified fatty acids in the myocardium, preserve cardiac functional parameters and decrease tissue calcium and LDH release[13].

Since vitamin-E can halt lipid peroxidantion by acting as a chain breaking antioxidant and is a prominent membrane constituent in cardiac muscle, the possibilities exists that vitamin-E could have both protective and therapeutic roles against cardiac ischemic reperfusion injury[14].

There is synergism between vitamin-E and -C. This synergism may primarily due to facts that vitamin-C probably has an exclusive function for the generation of vitamin-E at the interphase[15]. A low plasma level of lipid standardized vitamin-E and -C is a risk factor in early angina pectoris and arteriographically standardized IHD may be inversely related to plasma vitamin-C[16].

The patients with IHD have increased plasma levels of thiobarbituric acid-reactive material i.e. an indicator of increased susceptibility of IDL towards lipid peroxidation, which can be decreased by vitamin-C and / or vitamin-E[17].

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