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Protective role of L-carnitine on liver and heart lipid peroxidation in atherosclerotic rats

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Abstract

Lipid peroxides are considered to be the initiation factor for atherosclerosis. Present study depicts that L-carnitine treatment (300 mg/kg body weight/day) for 7 and 14 days caused significant reduction in the tissue lipid peroxidations. It also shows marked improvement in the antioxidant status. By this way carnitine maintain the normal function of the cells.

Keywords: Lipid peroxides; Hyperlipidemia; Carnitine; Vitamins; Antioxidants

1. Introduction

Organ, cell and subcellular injury due to ischemia are common causes of morbidity, and possibly mortality. Oxidative stress and the resulting generation of oxidants have been implicated as putative mediators of injury in diseases like hepatic ischemia and myocardial infarction [1,2]. Lipid peroxides are themselves considered to be the initiating factor for atherosclerosis [3]. Cardiovascular diseases including atherosclerosis and cardiac tissue injury after myocardial infarction have been shown to result in part from over production of free radicals generated at the site of damage [4,5].

Free radical attack on poly unsaturated fatty acids (PUFA) may result in the loss of PUFA [5] which plays an important role in cell membrane structure; and formation of lipidperoxides and related compounds which can cause damage to other cellular constituents. These are precisely controlled by several antioxidants which serve as protective mechanism [6].

Carnitine is a vital component in lipid metabolism for the production of adenosine triphosphate through β-oxidation and subsequent oxidative phosphorylation [7]. Carnitine restores high energy phosphate pools in myocardial cells [8]. There is a decrease in the concentration of carnitine in blood and tissues in hyperlipidemic condition [9]. Carnitine derivatives have been shown to protect ischemic myocardium [10].

2. Materials and methods

Male albino rats of Wistar strain weighing between 180–200 (3 months aged) were divided into 4 groups Group I comprised control (normal rats) and Group II saline treated atherosclerotic rats. (To induce atherosclerosis the cholesterol rich diet was fed to the animals. This diet was based on the formula of Hahn et al. [11] Groups III and IV were the atherosclerotic animals treated with carnitine for 7 and 14 days respectively.

The basal diet used was a commercial rat feed manufactured by M/s.Hindustan Liver Ltd., Bombay, India. Animals were accommodated in well ventilated spacious cages, food and water were provided ad libitum.

L-carnitine (Sigma Chemical Company, St. Louis, MO USA) 300 mg/kg body weight/day was dissolved in physiological saline and administered intraperitoneally to the experimental animals for 7 and 14 days. This procedure was adopted from Bardy et al. [12] with a slight modification in the dosage of carnitine. After the experimental period, rats were decapitated and tissues were collected for Biochemical investigations.

The estimations were carried out in tissues of normal and experimental rats. Liver and heart lipid peroxidation [13]. Vitamin C [14], Vitamin E [15], Vitamin B_6 [16], catalase

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Table 1
Levels of lipid peroxidation and antioxidants in liver of normal and experimental animals

Investigation	Normal rats treated with saline (I)	Atherosclerotic rats treated with saline (II)	Atherosclerotic rats treated with carnitine	
			7 days (III)	14 days (IV)
Liver lipid peroxidation (η moles of MDA released/mg protein)	2.4 ± 0.20	3.3 ± 0.21$^{\#\#\#}$	3.0 ± 0.16*	2.9 ± 0.18**
SOD (units**/min/mg protein)	5.9 ± 0.43	4.1 ± 0.39$^{\#\#\#}$	4.6 ± 0.37*	4.9 ± 0.41**
CATALASE (μmoles of GSH utilised/min/mg protein)	59.4 ± 4.31	43.3 ± 3.91$^{\#\#\#}$	47.9 ± 3.12*	51.3 ± 3.94**
GPx (μmoles of GSH utilised/ min/mg protein)	8.1 ± 0.71	6.3 ± 0.58$^{\#\#\#}$	7.2 ± 0.73*	7.8 ± 0.70**
G6PD (μg/mg protein)	15.8 ± 1.4	8.8 ± 0.80$^{\#\#\#}$	10.2 ± 1.23*	12.4 ± 1.31***
Vitamin C (μg/mg protein)	2.1 ± 0.17	1.5 ± 0.13$^{\#\#\#}$	1.8 ± 0.17**	1.9 ± 0.12***
Vitamin E (μg/mg protein)	1.4 ± 0.12	1.1 ± 0.07$^{\#\#\#}$	1.2 ± 0.08*	1.3 ± 0.11**
Vitamin B_6 (μg/mg protein)	152.0 ± 15.0	112.0 ± 13.0$^{\#\#\#}$	132.0 ± 14*	135.0 ± 10**

** Unit of superoxide dismutase is the amount of enzyme required to inhibit the autoxidation of pyrogallol by 50% in a standard assay system of 3 ml. Values are expressed as mean ± SD for six animals in each group.

For statistical evaluation, saline treated atherosclerotic rats were compared with saline treated normal $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ and on comparing saline treated atherosclerotic rats with carnitine treated atherosclerotic rats * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

[17], SOD [18], glutathione peroxide [19], glucose-6-phosphate dehydrogenase [20] were assayed.

Statistical significance of difference was determined by one-way analysis of variance and least significant difference test or independent students t-test [21] $P < 0.05$ was regarded as significant.

3. Results

Table 1 demonstrated that the lipid peroxidation level was greatly increased ($p < 0.001$) in liver of the atherosclerotic rats compared to control. Carnitine treatment significantly reduces this level ($p < 0.001$). The levels of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glucose 6 phosphate dehydrogenase (SOD, Cat, GPx and G6PD) and antioxidant vitamins (Vitamin C, E and B_6) were significantly declined in atherosclerotic rats compared to control. Treatment with L-carnitine increased these levels to almost normal.

As regards the levels of heart lipid peroxidation and antioxidants, a marked elevation in TBARS was observed but antioxidant levels were significantly declined in atherosclerotic rats (Group II) compared to controls. Carnitine treatment significantly reduced the TBARS levels and the antioxidants were markedly improved (Table 2) which was similar to the trend observed in the case of liver.

4. Discussion

High level of lipid peroxides injure blood vessels causing increased adherence and aggregation of platelets to the

Table 2
Levels of lipid peroxidation and antioxidants in heart of normal and experimental animals

Investigation	Normal rats treated with saline (I)	Atherosclerotic rats treated with saline (II)	Atherosclerotic rats treated with carnitine	
			7 days (III)	14 days (IV)
Heart lipid peroxidation (η moles of MDA released/mg protein)	1.8 ± 0.12	2.3 ± 0.19$^{\#\#\#}$	2.0 ± 0.16*	1.9 ± 0.14**
SOD (units** min/mg protein)	8.4 ± 0.71	5.3 ± 0.58$^{\#\#\#}$	6.2 ± 0.71*	6.7 ± 0.63**
CATALASE (μmoles of GSH utilised/min/mg protein)	49.1 ± 3.61	37.3 ± 3.10$^{\#\#\#}$	43.1 ± 3.72*	45.7 ± 3.70**
GPx (μmoles of GSH utilised/ min/mg protein)	5.8 ± 0.48	4.1 ± 0.32$^{\#\#\#}$	4.8 ± 0.48*	5.4 ± 0.45***
G6PD (μg/mg protein)	1.8 ± 0.18	1.5 ± 0.14$^{\#\#}$	1.7 ± 0.16*	1.8 ± 0.17**
Vitamin C (μg/mg protein)	2.1 ± 0.13	1.4 ± 0.14$^{\#\#\#}$	1.7 ± 0.17**	1.9 ± 0.18***
Vitamin E (μg/mg protein)	1.3 ± 0.16	1.0 ± 0.09$^{\#\#}$	1.1 ± 0.07*	1.2 ± 0.09**
Vitamin B_6 (μg/mg protein)	133.6 ± 12.0	93.7 ± 11.0$^{\#\#\#}$	112.4 ± 12.1*	120.2 ± 13.3**

** Unit of superoxide dismutase is the amount of enzyme required to inhibit the autoxidation of pyrogallol by 50% in a standard assay system of 3 ml. Values are expressed as mean ± SD for six animals in each group.

For statistical evaluation, saline treated atherosclerotic rats were compared with saline treated normal $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ and on comparing saline treated atherosclerotic rats with carnitine treated atherosclerotic rats * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

injured sites. This is the initial event in the process of atherogenesis. A marked enhancement in levels of TBARS in liver and heart of atherosclerotic rats was observed in this study. In atherosclerotic condition the increased level of lipids was responsible for the higher level of lipid peroxidation and tissue injury. Lipid peroxides accelerate the incorporation of LDL, into arterial smooth muscle cells and promote the formation of lipid laden foam cells which contribute to the development of atheromatous plaques [22].

The increased levels of serum lipid peroxides in atherosclerotic rats is in good agreement with the studies reported earlier [23,24]. Elevated TBARS levels were found in ageing [25] hypertension [26], hyperlipidemia [27].

In atherosclerotic condition there was a depletion of myocardial carnitine, resulting in a decrease in the transport of fatty acid into mitochondria. L-carnitine treatment reduces the TBARS levels. This is may be due to the enhancement of transport of fatty acids by carnitine into mitochondria for energy production. Carnitine inhibits the microsomal peroxidation [28] and it has a role in chelating free Fe^{2+} ions and by this way it reduces free radical generation [10]. Vitamin C sparing activity of the carnitine subsequently reduces the TBARS levels.

In tissues, the balance between free radicals and the multilevel defense system is maintained. In the case of inflammatory responses like macrophage necrosis by hyperlipidemia following the release of intracellular free radicals, the protective system is overwhelmed. The antioxidant enzymes and vitamins were significantly reduced in atherosclerosis. This was in conformity with other studies [29,30].

Carnitine administration increases antioxidants level significantly. The enzyme GPx requires glutathione as a cofactor. Elevation in GSH levels has been observed upon carnitine supplementation in rats and this in turn, increases activity of GPx as reported earlier [25]. Vitamin E inhibits oxidative endothelial damage [31]. Ascorbate stimulates Vitamin E [32]. It is a chain-breaking antioxidant capable of preventing the oxidative modification of proteins. England and Seifter [33] reported vitamin C is a cofactor for biosynthesis of carnitine. Lipid lowering effect of Vitamin C exerts a protective role against the peroxidative damage [34].

Reduced glutathione and vitamin B_6 increases the lipid peroxidation in hyperlipidic condition [35,36]. Carnitine treatment increases the levels of vitamin B_6. Pyridoxyl phosphate (PLP) acts as a cofactor for β-hydroxy-Σ-N-trimethyllysine aldolase which catalyses the cleavage of β-hydroxy-N-trimethyllysine, to glysine and r-trimethyl aminobutryaldehyde which is an important step in carnitine biosynthesis. Carnitine exerts thiol and methionine sparing activity [37]. Increased levels of these antioxidants by carnitine treatment maintain normal function of cells.

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