Effects of Peroxidised PUFA on Tissue Defense System in Experimentally Induced Myocardial Infarction in Rats

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The effects of peroxidised polyunsaturated fatty acids (PUFA) intake on tissue peroxidation in experimentally induced myocardial infarction in male albino rats were studied. The levels of protein, serum diagnostic marker enzymes, lipid peroxides and reduced glutathione, and the activities catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase in heart tissue were determined. A significant elevation was observed in the level of serum diagnostic marker enzymes in isoproterenol-induced myocardial infarction as compared to control group. The level of protein was significantly increased in serum of isoproterenol-treated rats, while a slight decline was observed in the heart tissue. This might be due to increased release of marker enzymes and proteins from the damaged heart tissue into the blood stream. In the present study, the administration of peroxidised PUFA was found to aggravate the isoproterenol-induced myocardial infarction in rats, which was evident from the increased level protein and enhanced the release of marker enzymes in the serum of rats given PUFA and isoproterenol. It was also observed that oxidized PUFA in the diet resulted in higher levels of peroxidation and lower levels of antiperoxidative enzymes.

Key words : Peroxidised PUFA, myocardial infarction, albino rats

A vast majority of deaths in patients who have suffered an acute myocardial infarction is due to lethal arrhythmias that can develop within hours or days of its onset (Nair *et al.*, 1999). Prevention of arrhythmia in these patients is of vital importance because this would allow them to recover and live many healthy and productive years. Recent in vitro studies (Leaf & Kang 1997) have shown that non-esterified, very long chain polyunsaturated fatty acids (PUFA), such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid, can prevent induced arrhythmias in cardiac myocytes. The dietary supplementation with marine (n-3) polyunsaturated fatty acids (PUFA) has already been proved to prevent ventricular

arrhythmias in experimental animals such as rats, marmoset monkeys and dogs. Mechanisms responsible for the anti-arrhythmic properties of (n-3) PUFA are not clear at present.

PUFA are highly unstable in nature and easily undergo peroxidative changes at normal temperatures. Hence it is necessary to store PUFA at very low temperature in the absence of oxygen. PUFA in the capsule forms are readily available in the market and the chances of peroxide formation of PUFA are not taken care off. Since the consumption of peroxides is highly deleterious to our normal health, hence it is necessary to assess the effects of peroxidised PUFA intake on condition like myocardial infarction.

Myocardial infarction induced by isoproterenol, a synthetic catecholamine and ß-adrenergic agonist, shows many metabolic and morphological aberrations in the heart tissue of experimental animals similar to those observed in human myocardial ischemia induces severe stress in the myocardium resulting in infarct-like necrosis of heart muscle in laboratory animals, an experimental model for the induction of myocardial ischemia of human beings (Sreepriya, *et al.*, 1998). Isoproterenol-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membranes (Todd *et al.*, 1980). It generates free radicals and stimulates lipids peroxidation. The formation of free radicals as well as accumulation of lipid peroxides has thus been recognized as one of the possible mechanisms for the myocardial damage caused by this catecholamine. During isoproterenol-induced myocardial infarction, there is a significant reduction in the level of antioxidant status with a reflection on the activities of antiperoxidative enzymes and glutathione-dependent antioxidant enzymes (Ithayrasi *et al.*, 1997). Hence is the present study it is attempted to assess the effect of peroxidised PUFA administration and compared it with isoproterenol-induced peroxidative changes on tissue defense system in male albino rats.

Materials and methods: Isoproterenol and epinephrine were obtained from M/s. Sigma Chemical Company, St. Louis, MO, USA. PUFA concentrate used in this study was prepared in our laboratory from commercially available fish oil by removing the major part of saturated and monounsaturated fractions as urea inclusion compounds (Ackman *et al.*, 1988) Adult male Wistar strain albino rats weighing about 100-120 g were used for the study. Twenty four rats were divided into two groups of 12 rats each. The rats were housed individually in polypropylene cages.

Group I and Group II rats were fed on commercial feed with added coconut oil at 1% level and PUFA at 1% level respectively. The rats were fed on this diet. Feed and water were given *ad libitum*. After 90 days feeding, the animals were divided into four subgroups of six animals each i.e., Group I, Group Ia, Group II and Group IIa.

Group Ia and Group IIa animals were given i.p. injection of isoproterenol (11mg.100g^{-1} body wt.day^{-1}) for 2 days. After the experimental period, the animals were sacrificed and blood was collected with EDTA as anticoagulant. The plasma separated was used for the determination of alanine aminotransferase (ALT) (Mohur & Cook, 1957), aspartate aminotransferase (AST) (Mhur & cook, 1957), lactate dehydrogenase (LDH) (King, 1965) and creatinine phosphokinase (CPK) (Okinaka *et al.*, 1961). The heart tissue was excised immediately, homogenised in 0.1M Tris HCl buffer, pH 7.4 and the homogenate was used for the estimation of lipid peroxides (Okhava *et al.*, 1979), glutathione (GSH) (Ellman, 1959), catalase (CAT) (EC 1.11.1.6) (Takahara *et al.*, 1960), superoxide dismutase (SOD) (EC 1.15.1.1) (Misra & Fridovich, 1972), glutathione peroxidase (GPx) (EC 1.11.1.19) (Pagila *et al.*, 1967) and glutathione-S-transferase (GST) (EC 2.5.1.18) (Habig *et al.*, 1974).

Results and Discussion

Increased levels of AST, ALT, LDH and CPK in serum are well known diagnostic indicators of isoproterenol-induced myocardial infarction (Mitra *et al.*, 1999). In the present study, the levels of serum diagnostic marker enzymes (ALT, AST, LDH and CPK) were significantly increased in Group Ia and Group IIa rats as compared to that of Group I and Group II rats respectively (Table 1). In cases of cardiac damage with myocyte lesions and parenchymal cell necrosis, these marker enzymes are released from damaged tissues into the blood stream. There was a slight increase in the levels of these marker enzymes in the serum of rats given peroxidised PUFA alone (Group II). The increased observed in the levels of these enzymes in the serum of Group IIa rats indicated that peroxidised PUFA administration might have enhanced the susceptibility of myocardial cells to isoproterenol-induced peroxidative damage. The level of protein was significantly increased in serum of Group Ia and Group IIa rats as compared to that of control animals. A slight decline in the protein was also observed in the heart tissue of these animals. This might be due to increased release of marker enzymes and proteins from the damaged heart tissue into the blood stream.

Biological membranes are sensitive to lipid peroxidation induced by reactive oxygen species. Lipid peroxidation of membranes is regulated by the availability of substrate in the form of PUFA, the availability of inducers such as free radicals and excited-state molecules to initiate propagation, the antioxidant defense status of environment, and the physical status of the membrane lipids (Anandan *et al.*, 1998). The level of lipid peroxidation was significantly increased in Group Ia and Group Iia rats as compared to that of Group I and Group II rats, respectively. A significant increase was also observed in the heart of Group II animals. The reaction of -OH with polyunsaturated lipids leads to the formation of lipid radicals and eventually to short chain aldehydes and hydroxy alkenols. Lipid peroxidation worsens tissue injury. The level of lipid peroxidation is significantly higher in the heart of Group Iia rats as compared to that of Group II animals. This indicates that peroxidised PUFA administration further adds to the oxidative stress and makes the cells more vulnerable to oxidative damage.

Activities of antiperoxidative enzymes (SOD and CAT) and glutathione-dependent antioxidant enzymes (GPx and GST) and the level of reduced glutathione were significantly decreased in Group Ia and Group Iia rats, which indicates that antioxidant defense mechanism is being operated at a diminished level. SOD, CAT and GPx, enzymes responsible for the destruction of peroxides, have a specific role in protecting tissues against oxidative damage (Wohaieb & Godin, 1987). Reduction in the activities of these enzymes can lead to the formation of O^{2-} and H_2O_2, which in turn can bring about a number of reactions harmful to the cellular and subcellular membranes (Kalra *et al.*, 1988).

Table 1. Levels of serum diagnostic marker enzymes ALT, AST, LDH, CPK and protein in normal and experimental groups of rats (mean± SD for six animals in each group)

Groups	Group I	Group Ia	Group II	Group IIa
ALT	112±8.3	398±31.4*	132±9.7	453±37.5*
AST	77.5±6.2	324±25.9*	103±8.1	357±28.4*
LDH	203±15.6	409±32.4*	210±14.6	489±39.1*
CPK	428±37.8	743±65.9*	491±38.1	910±76.7*
Protein	6.52±0.43	8.41±0.52**	7.18±0.48	8.96±0.58**

Values expressed: ALT, AST and LDH- µmoles of pyruvate liberated.$h^{-1}.l^{-1}$; Protein-$g.dl^{-1}$
As compared with respective controls, i.e. Group Ia vs. Group I, Group IIa vs. Group II: *$p<0.001$; **$p<0.01$

Table 2. Levels of protein, lipid peroxides and GSH and the activities of CAT, SOD, GPx and GST in normal and experimental groups of rats (mean± SD for six animals in each group)

Groups	Group I	Group Ia	Group II	Group IIa
Protein	223±18.2	196±15.9***	208±15.1	187±14.6***
LPO	121±8.3	198±10.7*	158±12.4	243±17.3*
GSH	63.5±4.7	36.9±2.4*	74.2±5.1	25.5±1.94*
CAT	8.9±0.54	4.8±0.21*	12.6±0.97	4.08±0.17*
SOD	3.7±0.5	1.93±0.17*	4.65±0.25	1.02±0.09*
GPx	3.41±0.3	1.63±0.10*	4.21±0.22	0.95±0.05*
GST	1323±118	874±71*	1579±+132	718±64*

Values expressed: Protein-mg.g^{-1} of wet tissue; LPO - n moles of MDA.mg protein^{-1}; GSH-n moles.g^{-1} tissue; CAT- moles of H_2O_2 consumed/mi/mg protein; SOD- units min^{-1}.mg protein^{-1}; GPx-n moles of GSH oxidized.min^{-1}.mg protein^{-1}; GST- n moles of CDNB conjugated min^{-1}.mg protein^{-1}

As compared with respective controls, i.e. Group Ia vs. Group I, Group IIa vs. Group II: *p<0.001; ***p<0.05

Depletion of GSH results in enhanced lipid peroxidation (Younes *et al.*, 1981), and excessive lipid peroxidation can cause increased GSH consumption (Comporti, 1985), as observed in the present study (Table 2). Gpx, an antioxidant enzyme, offers protection to the myocardial membrane from peroxidative damage (Umalakshmi & Devaky, 1992). A decrease in the activity of GPx makes myocytes sensitive to oxidative damage, which leads to a change in the cell composition and function. GST, another scavenging enzymes, binds to many lipophilic compounds (Seishi *et al.*, 1992); so it is expected to bind with lipid peroxidative radicals and acts as an enzyme for GSH conjugation reactions. The significant reduction in its activity noted in this study (Table 2) might have been due to the decreased availability of GSH. But a significant increase was observed in the activities of these enzymes in Group II animals. This might be to counter the effects of elevated level of lipid peroxidation. These findings lead to the conclusion that GSH and GSH-dependent enzyme systems may be directly related to the pathogenic mechanism of myocardial infarction.

The present observation indicates that the peroxidised PUFA intake is highly deleterious to health by virtue of its ability to damage myocardial membrane. However a detailed study has to be carried out to determine the exact nature and mechanism of action of peroxidised PUFA.

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