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# REVERSAL OF CHANGES OF MYOCARDIAL LIPIDS BY CHRONIC ADMINISTRATION OF ASPIRIN IN ISOPROTERENOL-INDUCED MYOCARDIAL DAMAGE IN RATS

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**Abstract** : The effect of aspirin on isoproterenol-induced changes related to myocardial damage was studied in rats. Rats were treated with aspirin (1.2 mg/100 g/day) orally, daily for a period of one month. Isoproterenol (20 mg/100 g, sc, twice at an interval of 24 hr) was administered. In isoproterenol treated rats marked increase in cholesterol, free fatty acids and triglycerides in both serum and heart were observed. The phospholipid level was lowered in heart with significant increase in serum in isoproterenol treatment. Serum LDL cholesterol was found to be increased with a significant decrease in the level of HDL cholesterol with enchanced level of lipid peroxides in heart. Aspirin showed marked reversal of these metabolic changes induced by isoproterenol.

**Key words** : aspirin, isoproterenol, myocardial damage, cholesterol, myocardial infarction, serum lipids

## INTRODUCTION

The β-adrenergic agonist, isoproterenol yielded useful information on experimental myocardial metabolic changes (1). During myocardial infarction induced by isoproterenol, there is increased level of myocardial lipids (2).

Isoproterenol is known to generate free radicals and to stimulate lipid peroxidation (3). The formation of free radicals as well as accumulation of lipid peroxides has been recognised as one of the possible biochemical mechanisms for the myocardial damage (3).

Aspirin is found effective in reducing incidence of acute myocardial infarction in patients with unstable angina and morbidity in patients with previous myocardial infarction (4).

Aspirin has action on platelet aggregation, and inhibits the endoperoxide formation by blocking the conversion of arachidonic acid to cyclic endoperoxides (5, 6).

The aim of the present study was to understand isoproterenol-induced myocardial damage in relation to lipid peroxides and myocardial lipids and to study the overall effect of aspirin on such parameters.

## METHODS

Adult male Wistar rats weighing 150-200 g were used for the study. The rats were fed with commercial pelleted rat chow and water given *ad libitum*. The rats were divided into two groups (1, control group and 2, aspirin treated group).

To the animals of group (2), aspirin was administered daily (1.2 mg/100 g) for a period of one month. Control rats were given saline.

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At the end of one month the animals were again grouped as follows : 1) normal control groups, 2) control group administered isoproterenol, 3) aspirin treated group, 4) aspirin treated group administered isoproterenol.

Isoproterenol (Sigma, USA : 200 mg/kg, sc) was administered twice at an interval of 24 hr. Aspirin was continued to be administered to the animals of group 3 and 4 till the end of experiment.

After the experimental period the rats were killed by cervical decapitation. HDL and LDL fractions were separated from serum according to the dual precipitation method (7). The heart was dissected out immediately washed in ice-cold saline and 0.1% homogenate was prepared in 0.1 M tris-Hcl buffer (pH 7.4). This was used for the estimation of lipid peroxides in terms of "TBA reactants" (8). 1, 1', 3, 3' tetra methoxy propane was used as the standard.

Cholesterol (9), phospholipid (10), triglycerides (11) and free fatty acids (12) were estimated after extracting the total lipid by the method of Folsch et al (13).

Students 't' test used for the statistical analysis.

## RESULTS

Table I shows the levels of cholesterol, phospholipid, triglyceride and free fatty acids in the serum of control and experimental animals. Levels of these lipids are elevated in isoproterenol treated rats (Group 2). But the elevation is significantly attenuated in group 4 rats. Group 3 rats did not show any significant change when compared to control rats.

A significant increase was observed in Serum LDL cholesterol in isoproterenol treated rats (Table II). The alterations were minimum in rats pretreated with aspirin.

TABLE II : Levels of cholesterol in lipoprotein fractions isolated from control and experimental animals. Values are expressed as mean ± SD for 6 animals in each group.

| *Group* | *Rats treated with* | *HDL Cholesterol* | *LDL Cholesterol* | *Risk factor* $LDL_c/HDL_c$ |
|---|---|---|---|---|
| 1. | None | 20.63±1.10 | 42.01±1.03. | 2.03±0.11 |
| 2. | Isoproterenol | 10.57±0.81*** | 56.31±3.80*** | 5.32±0.19*** |
| 3. | Aspirin | 20.43±1.05$^{NS}$ | 42.51±3.84$^{NS}$ | 2.08±0.06$^{NS}$ |
| 4. | Aspirin + Isoproterenol | 19.93±0.96$^{NS}$ | 43.51±3.33$^{NS}$ | 2.18±0.18$^{NS}$ |

Values are expressed as mg/dl serum. Difference statistically significant variations. *** $P < 0.001$, NS = Not significant.

Stastistically significant increase was observed in TBA reactants, total lipid, cholesterol, triglycerides, free fatty acid levels and significant decrease in phospholipid levels in isoproternol treated animals (Table III). Aspirin + isoproterenol treated rats showed low levels of TBA reactants, total lipid, cholesterol, triglycerides, free fatty acid and increase in phospholipid when compared to that which received isoproterenol alone.

TABLE I : Levels of serum cholesterol, phospholipid, triglyceride and free fatty acid in control and experimental animals. Values are expressed as mean ± SD for 6 animals in each group.

| *Group* | *Rats treated with* | *Cholesterol* | *Phospholipid* | *Triglyceride* | *Free fatty acid* |
|---|---|---|---|---|---|
| 1. | None | 74.46±2.58 | 104.26±1.47 | 36.31±1.14 | 23.54±1.20 |
| 2. | Isoproterenol | 91.81±2.76*** | 177.14±5.63*** | 58.78±4.97*** | 31.78±1.65*** |
| 3. | Aspirin | 75.05±2.92$^{NS}$ | 105.46±9.11$^{NS}$ | 36.81±2.09$^{NS}$ | 23.66±2.05$^{NS}$ |
| 4. | Aspirin + Isoproterenol | 78.43±6.84$^{NS}$ | 108.09±9.23$^{NS}$ | 41.81±3.65* | 7.52±2.25* |

Values are expressed as mg/dl serum. Difference statistically significant. ***P<0.001, *P <0.05, NS = Not significant.

TABLE III : Levels of lipid peroxides, total lipid, cholesterol, phospholipid, triglycerides and free fatty acids in heart of control and experimental rats. Values are expressed as mean ± SD for 6 animals in each group.

| *Particulars* | *None* | *Isoproterenol* | *Aspirin* | *Aspirin + Isoproterenol* |
|---|---|---|---|---|
| Lipid peroxides (nmoles of "TBA reactants"/g tissue) | 112.59±9.25 | 189.87±2.92*** | 114.42±5.76$^{NS}$ | 116.14±11.48$^{NS}$ |
| Total lipid | 50.25±2.50 | 63.07±5.65*** | 51.35±3.95$^{NS}$ | 58.29±4.20* |
| Cholesterol | 4.17±0.03 | 6.63±0.04*** | 4.20±0.03$^{NS}$ | 5.03±0.09$^{NS}$ |
| Phospholipid | 25.41±1.81 | 15.25±1.00*** | 25.05±1.17$^{NS}$ | 22.52±2.09* |
| Triglycerides | 3.16±0.05 | 5.12±0.32*** | 3.17±0.19$^{NS}$ | 4.14±0.03* |
| Free fatty acids | 0.14±0.00 | 0.22±0.00*** | 0.14±0.00$^{NS}$ | 0.15±0.00$^{NS}$ |

Values are expressed as mg/g of wet tissue. Difference statistically significant.

***P<0.001, P<0.05 NS = Not significant.

## DISCUSSION

That isoproterenol increased the level of serum lipid is an evidence for its known hyperlipidemic effect (14). High level of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage (15). It will be seen that isoproterenol mainly raised LDL Cholesterol. There is a positive correlation between the risk of developing ischemic heart disease and serum LDL cholesterol level and a negative one with that of HDL cholesterol (16). Aspirin treatment elevates HDL cholesterol significantly. So the protective action of aspirin seems to be mediated through the maintenance of a favourable risk factor ($LDL_C/HDL_C$, ratio).

A significant increase in free fatty acid and a decrease in phospholipid in isoproterenol-treated rats might have been due to the breakdown of membrane phospholipids. The increased peroxidation of polyunsaturated fatty acids is recognised as one of the possible biochemical mechanisms for the genesis of membrane injury in the myocardium (17).

The increased peroxidation of the membrane phospholipids release the free fatty acids by the action on phospholipase $A_2$ (18). $Ca^{2+}$ ions have been reported to one of the inducers of phospholipase $A_2$. So the observed increase in free fatty acid concentration could have been due to the indirect effect of calcium level which was reported to be altered in isoproterenol-treatment (19).

Accelerated membrane phospholipid degradation resulting in cell injury and cell death has been well known (20). This is probably due to the defects in the membrane system which regulate $Ca^{2+}$ availability. Pretreatment with aspirin was observed to increase the levels of phospholipid probably due to reduced degradation and increase in the level of free fatty acid in the heart.

Hypertriglyceridemia was seen in isoproterenol treated rats; such a state is also associated with cardiovascular disturbances (22). In aspirin treated rats there is decreased levels of triglycerides.

The increased level of lipid peroxides is a casuative factor in the irreversible damage to the myocardial membrane, which is usually observed in myocardial infarction (23). Aspirin decreased the levels of lipid peroxides probably by blocking the formation of lipid peroxides from unsaturated fatty acids (24). Lipoxygenase catalyses the oxidation of polyunsaturated fatty acids to 5-hydroxy - 6, 8, 11, 14 eicosatertraenoic acid (5 HETE) and 12 hydroxy 6,8, 11, 14 eicosatetranoic acid (12 HETE) which are potent vasoactive and inflamogenic substance that produce free radicals (25). Aspirin inhibits the conversion of arachidonic acid to 5 HETE and 12 HETE and

reduce the production of free oxygen radicals (26). Aspirin serves as an effective free radical scavanger and the salicylate itself has been found to react with free radicals (27).

The results obtained in our work with isoproterenol thus indicates that aspirin offers protection in experimental myocardial infarction by preventing the activation of lipid peroxidation system. This is probably by decreasing the level of myocardial lipids (to near control valve) preventing overloading of the myocardium with lipids, which in turn maintains the normal property and function of the myocardium.

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