Effect of aspirin on lipid peroxidation in experimental myocardial infarction in rats

T.S. Manjula, R. Deepa, and C.S. Shyamala Devi

Department of Biochemistry, University of Madras, Madras, India

In myocardial necrosis induced by isoproterenol, a marked increase in serum iron content and significant decrease in plasma iron binding capacity, ceruloplasmin activity, and glutathione level were observed. The increase in phospholipase activity and lipid peroxides level, with lowering of glutathione peroxidase, glutathione-S-transferase, catalase, and superoxide dismutase activities observed in isoproterenol treatment may lead to the excessive formation of free radicals, resulting in cardiac cell damage. Aspirin, an antithrombotic agent, showed a marked reversal of these metabolic changes related to ischaemia induced by isoproterenol. (J. Nutr. Biochem. 5:95–98, 1994.)

Keywords: aspirin; isoproterenol; myocardial infarction; lipid peroxides

Introduction

Isoproterenol-induced myocardial infarction in rats has been shown to be accompanied by hyperglycemia, hyperlipidemia, and increased levels of myocardial lipid peroxides.[1] Excessive formation of free radicals may result in the loss of function and integrity of myocardial membranes.[2] Recently, it has been indicated that pretreatment with aspirin, an inhibitor of cyclooxygenase, provides protection against ischemia injury.[3]

The aim of the present study was to understand the mechanism of cardiac damage in relation to lipid peroxides, serum iron content, plasma iron binding capacity, ceruloplasmin activity, glutathione level (GSH), and activities of phospholipase, glutathione peroxidase (GPD), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) to study the effect of aspirin against isoproterenol-induced myocardial ischemia.

Methods and materials

Isoproterenol, epinephrine, 1,1'3,3' tetramethoxy propane, and bovine serum albumin were purchased from Sigma Chemical Company (St. Louis, MO USA). Aspirin was obtained from R.R. Pharmacy, Madras, India. All other chemicals used were of analytical grade.

Adult male Wistar rats weighing 100 to 150 g were obtained from Veterinary College, Madras, India. They were fed with a commercial pelleted rat chow (from M/s. Hindustan Lever Limited, Bombay, India) and provided water ad libitum. The rats were divided into four groups. Group 1 served as controls, group 2 rats were injected with isoproterenol, 200 mg/kg., Sc twice at an interval of 24 hr. Group 3 rats were fed aspirin, 1.0 mg/100 g, orally by intubation daily for 3 months.[4] Group 4 rats received isoproterenol, 200 mg/kg, Sc twice at an interval of 24 hr after treatment of aspirin 1.0 mg/100 g orally by intubation daily for 3 months.

After the experimental period, the rats were killed by cervical dislocation. The heart tissues, plasma, and serum were taken for analysis of biochemical parameters. Estimation of serum iron,[5] plasma iron binding capacity,[6] and ceruloplasmin activity[7] were done by standard methods. Estimation of cardiac necrosis,[8] levels of glutathione (GSH),[9] and activities of glutathione peroxidase (GPD),[10] glutathione-S-transferase (GST),[11] catalase (CAT),[12] superoxide dismutase (SOD),[13] and phospholipase[14] in the heart were also estimated. The level of lipid peroxides in terms of "TBA reactants"[15] were determined using 1,1'3,3' tetramethoxy propane as the standard. Protein was determined by the method of Lowry et al.[16]

The results were analyzed by applying Students *t* test.

Results

Survival rate

The rate of survival in rats given aspirin was 100%, as compared with control rats. The percentages of animals

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Address reprint requests to C.S. Shyamala Devi at the Department of Biochemistry, University of Madras, Guindy Campus, Madras - 600 025, India.
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surviving was more in the group 4 animals (given aspirin and isoproterenol (85 to 90%)) than in group 2 animals (given isoproterenol alone) (50 to 55%).

Maximal cardiac necrosis and enlargement occurred in group 2 animals. In group 4 animals the cardiac necrosis and enlargement was much less than in group 2 animals. Aspirin-treated rats did not show any significant change when compared with control rats *(Figure 1)*.

Table 1 presents the levels of serum iron content, plasma iron binding capacity, ceruloplasmin activity, and GSH of control and test rats. In isoproterenol-treated animals the serum iron content increased significantly with a decrease in GSH level, ceruloplasmin activity, and iron binding capacity. Rats receiving both aspirin and isoproterenol minimized these changes.

Levels of GSH, lipid peroxides, GST, GPD, CAT, SOD, and phospholipase in heart are shown in *Table 2*. Significant decreases in the level of GSH and in GST, GPD, SOD, and CAT activities with an increase in phospholipase activity and lipid peroxides level were observed in isoproterenol treatment (group 2). The alterations were minimal in rats receiving both aspirin and isoproterenol. Group 3 rats did not show any significant change when compared with control rats.

Discussion

Stanton et al. suggested that the cardiovascular actions of isoproterenol may lead to cardiac necrosis, and the results obtained in the present study demonstrate these actions.[8] Aspirin treatment protected rats from cardiac necrosis and enlargement induced by isoproterenol.

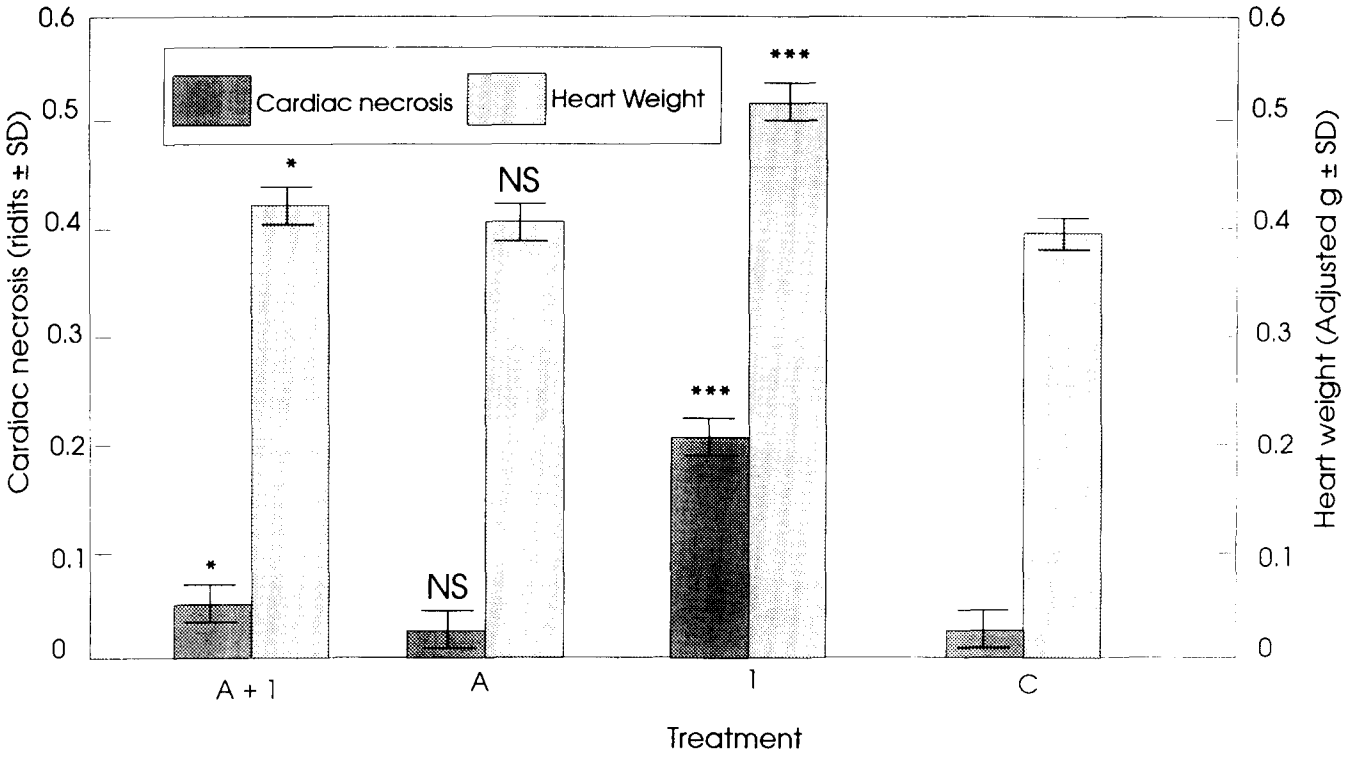

Figure 1 Effect of aspirin pretreatment on the cardiac necrosis (riditis) induced by isoproterenol. C, control rats; I, isoproterenol (200 mg/Kg, Sc) twice at an interval of 24 hr; A, aspirin (1.0 mg/100 g, orally by intubation) daily for 3 months. A+I, isoproterenol (200 mg/kg, Sc) twice at an interval of 24 hr after treatment of aspirin (1.0 mg/100 g, orally by intubation) daily for 3 months. Mean ± SD of six rats per group. As compared with control: ***$P < 0.001$; *$P < 0.05$; NS, not significant.

Table 1 Effect of isoproterenol and aspirin on serum iron, plasma iron binding capacity, blood glutathione, and ceruloplasmin activity

Group	Treatment	Serum iron (mcg/dl)	Plasma iron binding capacity (mcg/dl)	Glutathione (mg/dl)	Ceruloplasmin activity (Units/ml)
1.	None	44.0 ± 2.3	45.4 ± 1.6	57.7 ± 1.6	0.927 ± 0.08
2.	Isoproterenol	71.0 ± 2.2***	31.6 ± 3.9***	42.7 ± 3.9***	0.636 ± 0.03***
3.	Aspirin	47.9 ± 3.7^{NS}	44.9 ± 2.3^{NS}	55.9 ± 3.7^{NS}	0.906 ± 0.08^{NS}
4.	Aspirin + isoproterenol	48.7 ± 3.5^{NS}	40.5 ± 3.7^{NS}	53.5 ± 5.1^{NS}	0.871 ± 0.07^{NS}

Mean ± SD of six rats per group.
As compared with control: ***$P < 0.001$; NS, not significant.

Table 2 Effect of isoproterenol and aspirin on lipid peroxides, glutathione, glutathione peroxidase, glutathione-S-transferase superoxide dismutase catalase, and phospholipase level in the rat heart

Group	Treatment	Lipid peroxides	Glutathione	Glutathione peroxidase	Glutathione-S-transferase	Superoxide dismutase	Catalase	Phospholipase
1.	None	3.3 ± 0.21	4.9 ± 0.32	2.5 ± 0.71	923 ± 15.2	3.1 ± 0.27	4.7 ± 0.15	0.21 ± 0.01
2.	Isoproterenol	4.2 ± 0.17***	2.3 ± 0.21***	1.8 ± 0.11***	722 ± 22.5***	1.6 ± 0.13***	1.9 ± 0.12	0.31 ± 0.02***
3.	Aspirin	3.4 ± 0.31^{NS}	5.0 ± 0.41^{NS}	2.6 ± 0.02^{NS}	901 ± 31.8^{NS}	3.0 ± 0.19^{NS}	4.8 ± 0.23^{NS}	0.20 ± 0.01^{NS}
4.	Aspirin + isoproterenol	3.5 ± 0.16^{NS}	4.3 ± 0.34^{NS}	2.3 ± 0.19^{NS}	887 ± 26.7^{NS}	2.5 ± 0.24^{NS}	4.0 ± 0.30*	0.26 ± 0.01*

Level of lipid peroxides is expressed as n moles of "TBA reactants"/mg protein; level of glutathione is expressed as n moles of GSH/g tissue. Activity of glutathione peroxidase is expressed as mcg of GSH utilized/min/mg protein. Activity of glutathione-S-transferase is expressed as n moles of CDNB conjugated/min/mg protein. Activity of catalase is expressed as n moles of H_2O_2 decomposed/min/mg protein. Activity of superoxide dismutase expressed as units/mg protein. Activity of phospholipase is expressed as micromoles of FFA/mg protein.
Mean ± SD of six rats per group. As compared with control; ***$P < 0.001$; *$P < 0.05$; NS, not significant.

The increased levels of free iron are usually associated with decreased iron binding capacity, and decreased ceruloplasmin activity was seen in isoproterenol treated rats. During ischemia the free iron released from hem-dependent proteins, such as hemoglobin and myoglobin, due to decreased iron binding capacity increases prostaglandin metabolism and in vivo lipid peroxidation by producing hydroxy and superoxide radicals.[17,18] Aspirin treatment decreases the level of serum iron by increasing the iron binding capacity and also inhibits prostaglandin production by inactivating the enzyme cyclooxygenase, a hem-dependent enzyme,[19] and thereby preventing iron-catalyzed lipid peroxidation.

Ceruloplasmin (free iron scavenger) activity is found to be decreased during isoproterenol treatment.[20] Aspirin treatment increases the ceruloplasmin activity, resulting in the decreased production of free iron.

Isoproterenol-treated rats showed a decrease in the level of heart GSH with a decrease in the activities of GPD and GST. During infarction the myocardium, which is exposed to a large flux of hydrogen peroxide and hydroxy radical, leads to the accumulation of oxidized glutathione (GSSG) the oxidized product of GSH.[21] GSSG inactivates many enzymes containing the SH group and inhibits protein synthesis.[22] GPD, an SH-containing protein, is inactivated by peroxides and hydroxy radicals in the absence of reduced glutathione, resulting in the production of hydrogen peroxides.[23] Aspirin treatment maintains the GSH level and increases the activity of GPD and GST. The decreased GSH depletion in aspirin-treated rats may be due to the decreased production of the hydroxy radical. The availability of GSH maintains the activities of GPD and GST.

Heart SOD and CAT activities were significantly reduced in isoproterenol-treated rats. In myocardial infarction, the production of superoxide radicals modulates SOD and CAT, resulting in the loss of activity and accumulation of superoxide anion, thus damaging the myocardial cell.[24] Aspirin treatment increases the level of SOD and CAT. Similar results have been reported by Sushamakumari et al.[25] Aspirin directly scavenges superoxide radicals and reduces the myocardial damage caused by free radicals.[26]

Isoproterenol treatment showed increases in the activity of phospholipase and an increase in the level of lipid peroxides in serum and heart. During infarction the increase in phospholipase activity leads to the accumulation of polyunsaturated fatty acids, which undergo peroxidation catalyzed by the enzyme cyclooxygenase and lipoxygenase, resulting in the formation of lipid peroxides.[27] High level of lipid peroxides injure blood vessels, causing increased adherence and aggregation of platelets to the injured sites.[28] Aspirin treatment decreases the activity of phospholipase and also decreases the level of lipid peroxides by inactivating the enzyme cyclooxygenase.[29]

The results suggest that the biochemical lesion due to the activation of lipid peroxidation and decrease in the antioxidant status are significantly implicated in experimental myocardial infarction induced by isoproterenol. The protective effect of aspirin is achieved by decreasing the peroxide concentration and by the normalization of the antioxidant defense enzymes such as GPD, GST, SOD, and CAT.

References

1 Sushamakumari, S. and Menon, P.V.G. (1987). Effect of carnitine on malondialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. *Indian J. Exp. Biol.* **27,** 134–137

2 Biemond, P., Swaak, A.J.G., Beindroff, C.M., and Koster, J.F. (1986). Superoxide-dependent and independent mechanisms of iron mobilization from ferritin by xanthine oxidase. Implications for oxygen radical induced tissue, destruction during ischemia and inflammation. *Biochem. J.* **239,** 169–173

3 Weissmann, G. (1991). Aspirin. *Sci. Amer.* **264,** 58–64

4 Coronary Drug Project Research Group (1976). Aspirin in Coronary Heart disease. *J. Chron. Dis.* **29,** 625–642

5 Ramsay, W.N.M. (1969). Plasma iron. In *Advances in Clinical Chemistry,* (H. Sobotka and C.P. Stewart, eds.), p. 1–3 Academic Press, New York, NY USA

6 Ramsay, W.N.M. (1969). Ramsay's dipyridyl method for iron-binding capacity. In *Practical Clinical Biochemistry,* (H. Varley, ed.), p. 475–476. Heinemann, London, UK

7 Reinhold, J.G. (1953). Total protein, albumin and globulin. In *Standard Methods of Clinical Chemistry,* (M. Reiner, ed.), p. 88–90, Academic Press, New York, NY USA

8 Stanton, H.C. and Schwartz, A. (1967). Effects of a hydrazine monoamine oxidase inhibitor (Phenelzine) on isoproterenol-induced myocardiopathies in the rat. *J. Pharm. Exp. Therap.* **157,** 649–658

9 Moron, M.S., Depierre, J.W., and Mannerwick, B. (1979). Levels of glutathione, glutathione reductase and glutathione-S-transferase in rat lung and liver. *Biochem. Biophys. Acta* **582,** 67–78

10 Rotruck, J.T., Pope, A.L., Ganther, H., Swanson, A.B., Hafeman, D.G., and Hoeckstra, W.G. (1979). Selenium: Biochemical role as a component of glutathione peroxidase. *Science* **179,** 588–589

11 Habig, W.H., Papst, M.J., and Jacoby, W.B. (1974). Glutathione-S-transferase the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249,** 7130–7139

12 Beers, R.F. and Sizer, I.W. (1952). A spectrophotometric method for measuring the break down of the hydrogen peroxide by catalase. *J. Biol. Chem.* **195,** 133–140

13 Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. *J. Biol. Chem.* **247,** 3170–3285

14 Porumb, H. and King, E.J. (1984). Phospholipases. In *Method in Enzymology,* (J.M. Lowenstein, eds.), p. 192–193. Academic Press, New York, NY USA

15 Okhawa, H., Ohishi, N., and Yagi, K. (1979). Reactions of linoleic acid hydroperoxides and thiobarbituric acids. *Anal. Biochem.* **95,** 351–354

16 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R. (1951). Protein determination with the folin reagent. *J. Biol. Chem.* **193,** 265–275

17 Peperson, P.A., Gerrard, J.M., Rao, G.H., Mills, E.L., and White, J.G. (1980). Interaction of archidonic acid and heme iron in the synthesis of Prostaglandin. *Adv. Prostaglandin Thromboxane Res.* **6,** 157–161

18 Halliwell, B. and Gutteridge, J.M.C. (1986). Oxygen free radicals and iron in relation to biology and medicine some problems and concepts. *Arch. Biochem. Biophys.* **246,** 501–514

19 Weksler, B.B., Pett, B.S., Alonso, D., et al (1983). Differential inhibition by aspirin of vascular and platelet prostaglandin synthesis in atherosclerotic patients. *New Engl. J. Med.* **308,** 800–805

20 Altimini, D.J. and Dormandy, T.L. (1977). The inhibition of lipid autoxidation by human cervloplasmin. *Bio. Chem. J.* **168,** 283–288

21 Ferrari, R., Ceconi, C., Curello, S., Guarnieri, Caldarera, C.M., Albertini, A., and Visioli, D. (1985). Oxygen mediated myocardial damage using ischaemia and reperfusion. Role of the cellular defenses against oxygen toxicity. *J. Mol. Cell Cardiol.* **17,** 937–945

22 Lil, J.L., Startman, F.W., and Lardy, H.A. (1988). Antioxidant enzyme systems in rat liver and skeletal muscle. *Arch. Biochem. Biophys.* **263,** 150–160

23 Maccay, P.B., Gibson, D.D., Fong, K.L., and Hornbrock, K.R. (1976). The effect of glutathione peroxidase activity on lipid peroxidation in biological membranes. *Biochem. Biophys. Acta* **431,** 459–468

24 Sinet, P.M. and Gasber, P. (1981). Inactivation of the human CuZn superoxide dismutase during exposure to superoxide radical and hydrogen peroxide. *Arch. Biochem. Biophys.* **212,** 411–416

25 Sushamakumari, S., Varghese, A., Muraleedharan, D., and Menon, P.V.G. (1989). Protective action of aspirin in experimental myocardial infarction induced by isoproterenol in rats and its effect on lipid peroxidation. *Indian J. Exp. Biol.* **28,** 480–485

26 Kaplan, H., Edelson, H., Karchak, H., Giver, W., Abramson, S., and Weismann, G. (1984). Effect of NSAIDS on human neutrophil functions in vitro and in vivo. *Biochem. Pharmacol.* **33,** 371–375

27 Gutteridge, J.M.C. and Halliwell, B. (1990). The measurement and mechanism of lipid peroxidation in biological systems. *Trends Biochem. Sci.* **15,** 129–135

28 Gryglewski, R.J. (1980). Prostaglandins, platelets and atherosclerosis. *Crit. Rev. Biochem.* **7,** 291–338.

29 Andrez, S., Gryglewski, R., Grodzinska, L., Musial, J., Serwonska, M., and Ewamarcinkie, W. (1979). Platelet aggregation, thromboxane A_2 and MDA formation following administration of aspirin to man. *Thromb. Res.* **15,** 405–414