Indian J Physiol Pharmacol 2013; 57(2) : 132–137

PROTECTIVE EFFECT OF GLUTATHIONE AGAINST ISOPROTERENOL INDUCED MYOCARDIAL INJURY IN RATS

SUDHA M[1*], RAJKUMAR D[1] AND JOHN WILLAMS FELIX[2]

Departments of [1]Physiology and [2]Community Medicine,
Raja Muthiah Medical College,
Annamalai University,
Annamalai Nagar – 608 002,
Tamil Nadu, India

(Received on December 9, 2012)

Abstract : Though there are studies to show protective effect of glutathione against neurotoxicity and nephrotoxicity, the present study was designed to investigate the cardio protective effect of glutathione against isoproterenol induced myocardial infarction in rats by demonstrating the changes in serum cardiac markers, antioxidant enzymes and ECG changes. Wistar male rats were randomly divided into four groups namely control (GI), glutathione (GII), isoproterenol (GIII) and glutathione + isoproterenol treated (GIV). Glutathione treated group-received glutathione (200 mg/kg body wt) orally for 30 days. Myocardial infarction was induced in rats by isoproterenol administration (100mg/kg) subcutaneously (sc) at an interval of 24 hrs on 31st and 32nd day cardiac marker enzymes, ECG, lipid peroxidation and antioxidant enzymes were assessed 24 hrs after the last dose of isoproterenol. Isoproterenol showed changes in ECG pattern, increase in serum level of cardiac marker, increased lipid per oxidation and decreased antioxidant defense system in heart. Glutathione pretreatment brings almost all the parameters to near normal level in isoproterenol-induced myocardial infarction in rats. The present study revealed that glutathione ameliorates cardiac damage in isoproterenol induced myocardial infarction in rats due to potent antioxidant, free radical scavenging effect, myocardial adaptation at cellular and organ levels.

Key words : reduced glutathione myocardial infarction rats

INTRODUCTION

Ischemic heart disease continues to be the major cause of death in Asia, Europe and USA (1). The incidence of myocardial infarction is also high among people with Indian origin who are living abroad (2). Epidemiological studies and randomized clinical trials have provided compelling evidence that occurrence of myocardial infarction is largely preventable (3). Hence high incidence of Myocardial infarction

*Corresponding author : Dr. Sudha M, Reader in Physiology, RMMC, Annamalai University, Email: drsudhabaskaran@gmail.com

required new ways of prevention. Free radical generations have been established as an important etiological mechanism for myocardial ischemia reperfusion injury (4). Reduced glutathione is a non-enzymatic antioxidant in heart and has been reported to play a vital role in myocardial protection against ischemia reperfusion injury (5).

Myocardial infarction induced by isoproterenol (Isoproterenol; L- β - (3, 4 dihydroxy phenyl) α Isopropylamino ethanol hydrochloride), β adrenergic agonist has been reported to exhibit many metabolic and morphological aberration in heart tissue of experimental animal (6), similar to that observed in human myocardial infarction.

Isoprenaline challenge induces myocardial necrosis by a multiple step mechanism. The primary disturbance of isoprenaline induced myocardial infarction has been reported to enhance adenylate cyclase activity resulting in increased C-AMP formation which in turn leads to increased lipid accumulation in the myocardium. It is also well known to generate free radicals and to stimulate lipid per oxidation, which may be a causative factor for irreversible damage to the myocardial membrane (7). Isoprenaline induced myocardial infarction thereby serve as a well standardized model to standby the beneficial effects of many drugs and cardiac function (8).

Glutathione (L gamma-L glutamyl-L cysteine) a sulphur containing amino acid composed of 3 different amino acid cysteine, glutamine and glycine. It is primarily synthesized in liver. It is present in fruits, vegetables and meat products (9).

It has many biological action including myocardial antioxidant defense, alternate oxidative change induced by I/R. Ischemia/ Reperfusion (10) by important myocardial adaptations at cellular and organ levels like enhanced high energy phosphate reserve, coronary collateral blood flow, Ca^{2+} homeostasis, antioxidant defense, peripheral adaptation such as vascular proliferation and hormonal changes (11, 12).

MATERIALS AND METHODS

Wistar strain male albino rats weighing 150-200 g were selected for the study. The animals were allowed a standard diet and water ad libitum. The experiment was carried out after the institutional ethical committee approval No 457 in Raja Muthiah Medical College, Annamalai University.

Induction of myocardial infarction:

Myocardial infarction was induced in experimental animals by subcutaneous injection of isoproterenol 100 mg/kg body weight per day for 2 consecutive days.

Experimental protocol:

Animals were divided into 4 groups comprising of 6 rats each. Group I (normal control) rats received standard diet for a period of 30 days. Group II rats were orally administered with glutathione 200 mg/kg/ body weight dissolved in distilled water by intragastric intubation for 30 days. Group III rats were injected with isoproterenol 100 mg/kg body weight per day subcutaneously for 2 consecutive days at an interval of 24 hrs for induction of myocardial infarction. Group IV rats were pretreated

with glutathione 200 mg/kg body weight orally for 30 days and myocardial infarction was induced with isoproterenol at a dose of 100mg/kg body weight at an interval of 24 hrs on 31st and 32nd day.

At the end of experimental period i.e. 24hrs after last injection of isoproterenol the experimental animals were sacrificed, blood was collected using heparin as an anticoagulant and plasma was separated for the determination of cardiac marker enzymes (AST, ALT, LDH and CKMB), plasma and lipid peroxidation, and heart antioxidant enzymes (SOD, glutathione peroxides, catalase) and reduced glutathione. Lipid peroxidation in heart tissue and plasma were assayed as thiobarbuteric acid reactive substances TBARS in heart tissue.

ECG was recorded before and after administration of isoproterenol in all groups of rats. Male wistar rats (weight 150 to 260 gm) were anaesthetized by intraperitoneal injection (i.p) of Nembutal (pentobarbital sodium); 40 mg/kg body weight, this injection was sufficient to maintain anesthesia until the end of experiment. ECG was recorded by means of subcutaneous electrodes. The transducers were connected to ECG machine CARDIART 108T/MK-V1. Lead I recorded by placing subcutaneous electrodes in both axilla and over xiphoid process. Heart rate was determined by using R-R interval. ST segment elevation was taken as indicator for myocardial infarction.

Biochemical assays:

A portion of heart tissue was homogenized in 0.1 M TrisHcl buffer pH 7.4 and was used for the estimation of antioxidant enzyme activities. Lipid per oxidation assayed as thiobarbituric acid reactive substances (TBARS) in heart tissue were assayed according to the methods of Yagi (13) and Ohkawa et al (14) respectively. Heart tissue antioxidant enzymatic activities viz, SOD catalase, glutathione peroxides and nonenzymatic antioxidant reduced glutathione were assayed by Kakkar et al (15), Sinha (16) and Beutler and Kelley (17) respectively.

Statistical analysis:

The data were expressed as mean±SD. Statistical comparisons were performed by one-way ANOVA. If it was significant, Bonferroni multiple comparison tests have been applied by to find out which groups are statistically different. The results were considered as significant if P values were 0.05 or less.

RESULTS

Table I depicts the level of heart tissue TBARS, reduced glutathione, and the activities of antioxidant enzymes (Gpx, catalase & SOD) in the heart tissue of experimental rats. Isoproterenol induced a significant increase in the TBARS in-group III rats as compared with group I rats. A marked decrease in the level of GSH and the antioxidant enzymes were also observed. Glutathione pretreatment significantly reduced the TBARS level and preserved the antioxidant enzyme activities to near normal.

Table II depicts the levels of cardiac marker enzymes (CK-MB, ALT and LDH) in the plasma of normal and experimental groups of rats. Subcutaneous administration

TABLE I: Antioxidant enzyme levels in normal and experimental group of rat's heart.

Parameters	*Control (GI) (n=6)*	*Glutathione (GII) (n=6)*	*Isoproterenol (GIII) (n=6)*	*Glutathione+ Isoproterenol (GIV) (n=6)*	*P value*
Catalaseμmoles of H_2O_2 utilized/min/mg of Hb	51.93±2.8	51.8±2.5	34.8±3.5$^{***\#\#\#}$	38.4±3.0$^{***\#\#\#}$	<0.0001
GPXμg of GSH consumed/ min/mg of Hb	3.37±0.2	3.5±0.04	2.7±0.04$^{***\#\#\#}$	5.8±0.02$^{***\#\#\#fff}$	<0.0001
GSHmg/dl of RBC	5.3±0.02	5.4±0.03***	2.7±0.03$^{***\#\#\#}$	3.8±0.02$^{***\#\#\#fff}$	<0.0001
SODUnits/mg of Hb	5.34±0.7	5.4±0.5	3.6±0.6$^{***\#\#\#}$	4.4±0.7	0.0002
TBARSnmoles/mg of protein	0.42±0.03	0.4±0.02	0.7±0.03$^{***\#\#\#}$	0.6±0.02$^{***\#\#\#fff}$	<0.0001

Data expressed as mean±SD. The * depicts comparison with Group I, # depicts comparison with Group II and f depicts comparison with Group III. *P<0.05, **P<0.01, ***P<0.001, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, ^{f}P<0.05, ^{ff}P<0.01, ^{fff}P<0.001. GPX: Glutathione peroxidase, GSH: Reduced glutathione, SOD: Superoxide dismutase & TBARS: Thiobarbuteric acid reactive substances.

TABLE II: Level of cardiac markers in normal and experimental group of rats.

Parameters IU/L	*Control (GI) (n=6)*	*Glutathione (GII) (n=6)*	*Isoproterenol (GIII) (n=6)*	*Glutathione Isoproterenol (GIV) (n=6)*	*P value*
AST	96.7±8.5	92.2±6.8	386±17.4$^{***\#\#\#}$	130±5.01$^{***\#\#\#fff}$	<0.0001
ALT	84.5±4.6	80.5±6.9	344±12.5$^{***\#\#\#}$	110±7.02$^{***\#\#\#fff}$	<0.0001
LDH	158.3±9.4	152.3±7.4	310±15.6$^{***\#\#\#}$	180±6.04$^{**\#\#\#fff}$	<0.0001
CPK-MB	131.4±8.0	126.4±6.6	303±24.2$^{***\#\#\#}$	150±8.03$^{\#fff}$	<0.0001

Data expressed as mean±SD. The * depicts comparison with Group I, # depicts comparison with Group II and f depicts comparison with Group III. *P<0.05, **P<0.01, ***P<0.001, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, ^{f}P<0.05, ^{ff}P<0.01, ^{fff}P<0.001. AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactic dehydrogenase, CPK-MB: Creatinine phosphokinase - MB.

of isoproterenol caused a significant (p=0.001) elevation in the levels of cardiac enzymes in the plasma of group III rats compared with that of group I rats. In this study, oral pretreatment with glutathione 200 mg/kg body weight for a period of 30 days significantly reduced the cardiac enzymes to near normal level.

Table III shows the ECG changes in the experimental group of rats. There was increase in heart rate; prolonged QRS complex and prolonged QT interval and ST segment elevation in-group III rats compared to control rats. Glutathione prior administration significantly maintained the ECG normal.

DISCUSSION

In present study there is significant increase in levels of lipid peroxide in heart tissue and serum during isoproterenol administration. Isoproterenol administration produces free radicals via beta adrenoceptor mechanism affects the cell metabolism such that toxic free radical are formed producing myocardial cell necrosis (18). This is shown

TABLE III: ECG changes in normal and experimental group of rats.

ECG changes	*Control (GI) (n=6)*	*Glutathione treated group (GII) (n=6)*	*Isoproterenol (GIII) (n=6)*	*Glutathione Isoproterenol (GIV) (n=6)*	*P value*
HR beats/min	369.1±25	400.5±39	543±83.1$^{***###}$	386.6±48.6 ^{fff}	<0.0001
QRS Complex (sec)	0.06±0.02	0.06±0.02	0.13±0.03$^{***###}$	0.04±0.01 ^{fff}	<0.0001
QT interval (sec)	0.1±0.07	0.1±0.03	0.2±0.03$^{**##}$	0.15±0.03	0.0018

Data expressed as mean±SD. The * depicts comparison with Group I, # depicts comparison with Group II and f depicts comparison with Group III. *P<0.05, **P<0.01, ***P<0.001, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, ^{f}P<0.05, ^{ff}P<0.01, ^{fff}P<0.001. HR: Heart rate.

TABLE IV: ST segment elevation in normal and experimental group of rats.

ST Segment	*1*	*2*	*3*	*4*	*5*	*6*
Control (n=6)	0.0	0.0	0.0	0.0	0.0	0.0
Glutathione treated (n=6)	0.0	0.0	0.0	0.0	0.0	0.0
Isoprenaline (n=6)	0.1 mv	0.2 mv	0.1 mv	0.2 mv	0.1mv	0.1 mv
Isoprenaline + Glutathione (n=6)	0.0	0.0	0.0	0.0	0.0	0.0

There is ST segment elevation in GIII rats compared to GI and GII rats Glutathione pretreatment brought ST segment to isoelectric line.

by increasing TBARS (Thiobarbutric acid reactive substances) in heart of experimental group of rats.

Glutathione treatment significantly reduces the level of lipid peroxide and increase enzyme levels in both heart and serum by its myocardial antioxidant defense mechanism, alternate oxidative change induced I/R by important myocardial adaptation at cellular and organ level. Cardiac enzymes are significantly increased in isoproterenol treated rats as reported by earlier studies. ECG changes were also observed (19). This might be due to generation of free radicals released from damaged myocardial tissue. Reperfusion of ischemic areas may itself be associated with reperfusion injury mediated by free radicals mediated oxidation (20). Pretreatment with glutathione prevents the rise in level of cardiac marker enzymes in G IV rats compared to GIII rats and also restored ECG changes.

Glutathione protects cardiac cells from I/R injury by important myocardial adaptation at cellular and organ levels like enhanced high energy phosphate reserve, coronary collateral blood flow, Ca^{2+} homeostasis, peripheral adaptations like vascular proliferation and hormonal changes. In summary data from present study indicates that glutathione act as a potent antioxidant and enhance myocardial resistance to I/R injury induced damage in anaesthetized rats. This important adaptation result from preservation of heart GSH Homeostasis and increased antioxidant defense mechanism, which improved myocardial functional recovery from initial I/R result. There are studies to show effect of oral glutathione

supplementation for 17 days on anti oxidant defense, oxidative damage in response to ischemia reperfusion (I/R) in rat's heart. So we made a study by supplementing oral glutathione for 30 days to have better effects (21).

REFERENCES

1. Rosamond W, Flegal K. American Heart Association Statistics Committee. *Circulation* 2008; 117: 25–146.
2. Gupta R and Gupta VP. Meta analysis of coronary heart disease prevalence in India. *Ind Heart J* 1996; 48: 241–345.
3. Gupta R. Recent trends in Coronary heart disease epidemiology in India. *Ind Heart J* 2008; 60: 34–18.
4. Simpson PJ, Lucchesi BR. Free radicals and myocardial ischemia and reperfusion injury. *J Lab Clin Med* 1987; 110: 13–30.
5. Ramires PR. and Ji LL. Glutathione supplementation and training increases myocardial resistance to ischemia reperfusion in vivo. *Am J Physiol Heart Circphysiol* 2001; 281: 679–688.
6. Yan Jun Xu, Amarjit SA, Paramjit ST and Naranjan SD. The potential health benefits of taurine in cardiovascular disease. *Expelin Cardiol* 2008; 13(2): 57–65.
7. Crlg SA. Betaine in human nutrition. *Am Clin Nutrition* 2004; 80: 539–549.
8. Park ET and Garrow TA. Interaction between dietary methionine and methyl donor intake on rat liver betaine-homocysteine methyltransferase gene expression and organization of the human gene. *J Biol Chem* 1999; 274: 7816–7824.
9. White AC, Thannickal VJ, Fan Burg BL. Glutathione deficiency in human disease. *J Nutr Biochem* 1994; 5: 218–226.
10. Ce Coni, Curello S, Cargnoni A, Ferrari R, Albertini A and Visiolio. The role of glutathione status in the protection against ischemic and reperfusion damage effects of N-acetyleystenine. *Cell Cordial* 1998; 20: 5–13.
11. Moore RL, and Kubrick DH. Cellular adaptations of myocardium to chromic exercise. *Prog Cardiovac* 1995; 37: 37–396.
12. Shuible T, and Scheuer J. Cardiac adaptation to chronic exercise. *Prog Cardiovac* 1985; 27: 297–324.
13. Yagi K. Lipid peroxides and human a disease. *Chem Phy Lipids* 1987; 45: 337–351.
14. Donnan SK. The thiobarbituric acid test applied to tissues from rats treated in various ways. *Biochem J* 1950; 40: 182–415.
15. Kakkar P, Das B, and Viswanatham PR. A modified spectrophotometric assay of SOD. *Ind J Biochem Biophy* 1978; 21: 130–132.
16. Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972; 47: 389–394.
17. Beutler E and Kelley. In: Red cell metabolism. A manual of biochemical methods. 3rd edition. Orlando, Grune Stratton. 2002; 68–70.
18. Noronha Dutra AA, Steen EM, WoolFN. The correlation between catecholamine and lipid per oxidation induced damage in heart cells. *Basic Res Cardiol* 1985; 80: 133–136.
19. Farvin KHS, Anandham R, Kumar SHS, et al. Effect of squalene on tissue defense system in isoproterenol-induced myocardial infarction in rats. *Pharmacol* 2004; 50: 231–236.
20. Balaraman G, Rangasamy R, Rangasamy A, Nanjappan D. Biochemical studies on protective effect of Betaine on mitochondrial function in experimentally induced myocardial infarction in rats. *Health Sci* 2007; 53: 671–681.
21. Sunmonu TO, Afolayan AJ. Protective effect of Artemisia afra jacq on isoproterenol Induced myocardial injury in wistar rats. *Food Chem Toxicol* 2010; 48: 1969–1972.