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EFFECT OF N-2-MERCAPTOPROPIONYLGLYCINE IN LIMITING MYOCARDIAL REPERFUSION INJURY FOLLOWING 90 MINUTES OF ISCHEMIA IN DOGS

YOGESH TRIPATHI*, B. M HEGDE**, Y. S. RAI*** AND C. V RAGHUVEER****

*Departments of *Physiology,*
***Medicine, ***Surgery and ****Pathology*
Kasturba Medical College,
Mangalore – 575 001

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Abstract : Present study was designed to examine the effectiveness of N-2-mercaptopropionyl glycine (MPG) on oxygen free radical (OFR) mediated reperfusion injury. Twenty dogs underwent 90 min of left anterior descending (LAD) coronary artery occlusion followed by 4 h of reperfusion. In control animals (n = 12), 115 ml of saline was infused through left atrium at the onset of reperfusion whereas treated animals (n =8) received loading dose of MPG (40 mg/kg) infused through left atrium for 1 h followed by maintenance dose (25 mg/kg) for remaining 3 hours. Percentage area of necrosis vis-a-vis area at risk and percentage necrosis in left ventricular mass in MPG treated animals was significantly lower in comparison to control animals. Reperfusion in control group increased the lipid peroxidation and lowered glutathione (GSH) and superoxide dismutase (SOD) activity. MPG treatment significantly lowered the lipid peroxidation whereas GSH and SOD levels in necrotic zone were higher than in control. The above results suggest that MPG can offer a significant cardioprotection against oxidative stress in canine model.

Key words : cardioprotection, free radical, glutathione, lipid peroxidation, N-2-mercaptopropionyl glycine, superoxide dismutase

INTRODUCTION

It is well known that thrombolytics can reduce mortality in the patients with acute myocardial infarction. On the contrary, experimental studies in animals show that reperfusion in ischemic myocardium can be detrimental as it can kill myocytes which are reversibly injured at the termination of ischemia (1–3). Oxygen free radicals have been implicated as one of the major cause in the development of reperfusion injury (4). Reperfusion in ischemic myocardium can result in increased formation of superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2) (5, 6). Studies examining the role of free radicals have shown conflicting results (4, 7–12). N-2

*Corresponding Author

mercaptopropionyl glycine which contains sulfhydryl group, is known to offer protection against inflammation (13) and hyperoxygenation (14). Therefore, in the present study, we examined the role of MPG in limiting myocardial reperfusion injury in canine model.

METHODS

Chemicals: N-2-mercaptopropionyl glycine, triphenyl tetrazolium chloride (TTC), glutathione (reduced), nitroblue tetrazolium (NBT), riboflavin and bovine serum albumin were purchased from Sigma Chemical Company (St Louis, M O; USA). 2-thiobarbituric acid was purchased from Loba Chemicals, India. All other chemicals used were of analytical grade.

Animals, surgical preparation and instrumentation

Adult male mongrel dogs weighing 10 to 15 kg were anesthetised with 30 mg/kg intravenous pentobarbital sodium, intubated and ventilated with room air by INCO (India) positive pressure ventilator. Heart was suspended in pericardial cradle after left side thoracotomy at fourth intercostal space. A polythene catheter (1.5 mm inner diameter) was placed in the left ventricle to record ventricular pressure changes on Grass 16 channel polygraph (Model 78 D, USA) by using Gould P 231 pressure transducer (Gould, Cardiovascular Product Division, Oxnard, California). Saline or drug was infused through left atrium. Polythene catheter was placed in left atrium through a small incision on ventral surface. Incision was closed by purse-string sutures. Heart rate and ST segment changes were monitored in limb lead II on BPL (India) electrocardiograph. Animals with basal heart rate $\geq$ 180 beats/min were excluded from the study.

Coronary artery occlusion and reperfusion

LAD coronary artery was dissected free above first diagonal branch and below the origin of left circumflex artery. Coronary artery was occluded for 90 min with a vessel occluder. Visual inspection of the coronary artery, ST elevation and appearance of epicardial cyanosis confirmed occlusion. Ninety minutes following occlusion, reperfusion was initiated by removing the occluder. Onset of reflow was confirmed by observing full reactive hyperemia over earlier cyanotic area (15). No attempt was made to resuscitate the animals which developed ventricular fibrillation at the time of reperfusion and these animals were excluded from the study.

Experimental groups and treatments

Group I (Control, saline treated animals): Twelve animals underwent 90 win of LAD coronary artery occlusion followed by reperfusion for 4 h. Normal saline (0.9% Nacl, 115 ml) was infused through left atrium at the end of 90 min ischemia for 4 h.

Group II (MPG treated animals): MPG was dissolved in 115 ml of saline. Eight animals received loading dose (40 mg/kg) for an hour

followed by maintenance dose (25 mg/kg) for three hours through left atrium at the time of reperfusion. Duration of ischemia was similar to Gr. I.

Quantification of infarct size

LAD coronary artery was completely re-occluded at the end of reperfusion. Evans blue dye (5%, 15 ml) was infused through left atrium to identify zone at risk. Animals were killed by injecting 2.56 M potassium chloride directly into the ventricle. Zone of infarction in zone at risk was identified by incubating 1 mm thick sections of myocardium in 1% TTC prepared in phosphate buffer (pH 7.4) for 30 minutes (16, 17). Myocardial tissue unstained by Evans blue represented myocardial tissue at risk and unstained portions by TTC showed infarcted zone.

Biochemical estimations

Myocardial tissue lipid peroxidation, GSH & SOD levels in non ischemic zones, zone at risk and infarction were measured by standard methods (18–21). Tissue samples for non ischemic zones were taken at the end of reperfusion from right ventricle not supplied by LAD coronary artery. Results of lipid peroxidation were expressed in terms of MDA (malondialdehyde/g wet tissue) after taking molar extinction coefficient 1.56×10^5 for MDA. One unit of SOD was defined as amount required to inhibit reduction of NBT by 50% under specific condition. Protein was estimated by Lowry's method (22).

Data analysis

All values were presented as mean ± SEM. Statistical comparisons were made by using Student 't' test. Statistical significance was considered as $P<0.05$.

RESULTS

Two dogs which had heart rate ≥ 180 beats/min were excluded from the study. During reperfusion of saline (Gr. I), four of the twelve animals died due to ventricular fibrillation within first 15 min of reperfusion. Sixteen animals were included

TABLE I: Infarct size, % left ventricular necrosis and % myocardial preservation following MPG treatment.

Experimental groups	*% area at risk*	*% infarction in area at risk*	*% left ventricular necrosis*	*% myocardial preservation*
I (Saline reperfused control, n = 8)	30.70±4.26	39.24±5.2	10.75±3.4	62.1±5.86
II (MPG treated n = 8)	36.5±2.87	5.86±2.28*	5.60±0.8*	92.34±6.47*

Values are mean ± SEM

*P<0.001 as compared to Gr. I

MPG; N-2-mercaptopropionyl glycine

TABLE II: LVEDP and LVSP preceding and following ischemia and reperfusion.

Experimental groups	Left ventricular pressure (mm of Hg)	Pre-CAO	Post CAO (min)		Post reperfusion (h)			
			30	60	90	1	2	3	4
I (saline treated control, n = 8)	LVEDP	2.4±1.2	1.6±1.1	8.3±2.4*	19.62 ±3.6**	12.3±2.74	14.55±2.6	16.84± 2.46	14.3±3.4
	LVSP	156.6±3.7	150.7±2.66	158.4±4.24	154.9 ±2.29	160.4±3.52	156.42±6.88	156.8± 4.32	158.8±2.45
II (MPG treated) n = 8	LVEDP	1.9±0.6	2.4±1.2	8.6±1.4*	19.00 ±1.6**	10.22±1.8***	9.4±2.2***	12.6± 2.2***	10.46±2.4***
	LVSP	156.2±4.2	152.7±3.4	158.4±2.88	160.2 ±3.6	158.4±2.4	150.24±3.6	156.34± 4.8	159.8±3.6

Data expressed as mean ± SEM
*P<0.001 as compared to Pre-CAO, **P<0.001 as compared to 60 min, ***P<0.001 as compared to 90 min.
LVEDP: Left ventricular end diastolic pressure; LVSP: Left ventricular systolic pressure, CAO: Coronary artery occlusion, MPG: N-2-mercaptopropionyl glycine.

in the final analysis (8 in each group). Ventricular fibrillation was not observed in MPG treated animals. Results presented in Table I show that infusion of MPG at the onset of reperfusion significantly reduced the percentage necrosis in the zone at risk. Further, percent preservation of ischemic myocardium in MPG treated animals was higher in comparison to saline reperfused animals (92.34 ± 6.47 VS 62.10 ± 5.86, P<0.001). A significant increase in LVEDP was observed in saline reperfused dogs after 90 min of ischemia (Table II). In the same group, 4 h reperfusion failed to decrease LVEDP in comparison to the pressure recorded after 90 min of ischemia. In contrast, LVEDP in MPG treated animals after 4 h reperfusion was lower (10.46 ± 2.4 VS 19.62 ± 3.6, P<0.001). Changes in MDA, GSH and SOD level in non-ischemic zone, zone at risk and infarction is presented in Table III. It is evident that MDA level in zone at risk in Gr. I was significantly higher in comparison to non-ischemic zone (7.4 ± 1.22 VS 2.8 ± 0.32, <0.001) whereas GSH and SOD levels showed significant decrease. In zone of infarction, MDA level was significantly higher in comparison to the level in zone at risk. In contrast, SOD & GSH levels were lower. MPG treatment significantly increased the myocardial tissue GSH and SOD levels as compared to saline reperfused animals. MDA concentration was lower in infarcted zone. Tissue levels of MDA, GSH and SOD in area at risk were similar in both the groups (Gr. I & II).

TABLE III : MDA, GSH & SOD levels in non-ischemic zone, zone at risk and infarction.

Experimental groups	*Biochemical parameters*	*Non-ischemic zone*	*Zone at risk*	*Zone of infarction in zone at risk*
I	MDA	2.8±0.32	7.4±1.22*	17.82±1.56**
(Saline reperfused n = 8)	GSH	3.94±0.86	1.12±0.18*	0.25±1.0**
II	SOD	6.75±1.3	2.1±1.0*	0.52±0.35**
(MPG treated n = 8)	MDA	1.4±0.24	6.64±1.13*	11.76±1.7**
	GSH	4.52±0.50	1.41±0.24*	0.9±0.04***
	SOD	7.6±1.40	3.45±1.75*	1.32±0.14***

Values are mean ± SEM
*P<0.001 as compared to non-ischemic zone, ***P<0.001 as compared to zone at risk in Gr. I
***P<0.001 as compared to zone of infarction in Gr. I
MDA : Malondialdehyde (n mol/g tissue), GSH : Glutathione (µg/g tissue protein)
SOD : Superoxide dismutase (units/mg tissue protein)

DISCUSSION

Endogenous antioxidants like superoxide dismutase, glutathione and catalase play an important role against free radical attack (5, 6). Ischemia depletes antioxidant enzymes and makes ischemic myocardium vulnerable to free radical damage at the time of reperfusion (2, 23). Results of our study show that infusion of MPG at the onset of reperfusion prevented the incidence of ventricular fibrillation. Further, MPG treatment was effective in preventing a significant amount of ischemic tissue from becoming necrotic whether calculated as infarction in area at risk or as percentage of total ventricular weight.

It has been reported that changes in myocardium are detectable by light microscopy only after 120–140 min of ischemia (24, 25). Coronary artery occlusion of 15–20 minutes followed by reperfusion halts myocardial cell necrosis and 40 minutes of ischemia results in focal necrosis (25). Studies examining the effect of reperfusion have reported reduction in infarct size provided that reperfusion was maintained upto 6 h (9–11, 26). In other studies where reperfusion period varied from 1 to 4 days, no reduction in infarct size could be demonstrated (7, 8, 12). Choice of 90 min ischemia and 4 h reperfusion was based on earlier studies (10, 11). We could not find any evidence of necrosis either by light microscopy or by gross examination of myocardium after 90 min of ischemia (10). It was in agreement with other studies (24, 25). We have earlier shown that reperfusion in 90 min ischemic myocardium results in severe edema, myocardial cell separation, hemorrahage and contraction band necrosis (10, 11). In our study, MPG was infused at the time of reperfusion and not during ischemia. Some of the studies have reported reduction in infarct size by using antioxidants during the course of ischemia (4, 9). Infusion of antioxidant before reperfusion may slow down the development of ischemic injury. Therefore, it may not be possible to assess whether observed cardioprotection following reperfusion was due to reduction in reperfusion injury or was secondary to anti-ischemic effects of antioxidant (27). In our study, animals receive MPG at the time of reperfusion to

show that observed cardioprotection was not due to anti-ischemic effects of MPG. It has been reported that OFR burst peaks at 2 minutes after reperfusion and continues upto 3 h following reperfusion in dogs subjected to 15 minutes of coronary artery occlusion (28). LVEDP, which represents pre-load was maximum after 90 min of ischemia and remained unchanged during reperfusion in saline treated animals. A significant improvement in LVEDP was noted in MPG treated animals. It shows that MPG can be effective in improving left ventricular functions. LVSP remained unchanged in both the groups following ischemia and reperfusion. It was probably due to localized cardiac muscle damage in set up of experimentally induced myocardial infarction. Oxygen free radicals peroxidise polyunsaturated membrane lipids and damage their structure and functions (29). Oxygen free radical damage can be monitored by measuring Malondialdehyde (MDA) (30). In the present study, higher levels of MDA, lower levels of GSH and SOD in necrotic zone of myocardium in saline treated control animals suggest the involvement of OFR in reperfusion injury. MPG contains reduced sulfhydryl group (9). It has been suggested that reaction between MPG and OFR form toxic disulfide which can terminate free radical reaction and MPG can scavenge both superoxide and hydroxyl radicals (9). Therefore, the observed cardioprotection in our study might have been due to free radical scavenging properties of MPG.

In conclusion, the present study shows that MPG can reduce infarct size and improve left ventricular performance in dogs subjected to 90 min of ischemia and 4 h of reperfusion.

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