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A Study on the Protective Effect of Single Dose of N-2- Mercaptopropionyl Glycine Administration Prior to Renal Reperfusion on Wistar Rats

[1]Vinodini N.A., [2]Tripathi Y., [3]C.V. Raghuveer, [4]Asha Kamath, [5]C. Ramaswam

[1]Department of Physiology Kastruba Medical College Mangalore (Unit of Manipal university Manipal Karnataka India)
[2]Tripathi Y Present address: P.O.Box No:64832, Ghariyan, Libya
[3]Department of pathology Malaka Manipal College Manipal Karnataka India
[4]Department of community Medicine Kasturba medical College Manipal Karnataka India
[5]Department of physiology Saveetha Medical College Chennai India

Abstract: N-2-mercaptopropionylglycine (NMG) is a powerful antioxidant which inhibits synthesize of superoxide radicals. It has been tested as a preventive agent against metabolic and structural damage induced during ischemia/reperfusion (I/R) process. To validate the efficacy of NMG on renal tissue this work was devised. During the experiment, the wistar strain rats were divided into five groups; the group I as normal control, the group II & III as I/R controls, and the group IV & V as I/R treated groups. The I/R control and I/R treated groups were subjected to ischemia and reperfusion either for 10 minutes (Gr.II & IV) or 90 min. (Gr.III & V). The I/R treated groups in addition received N-2- mercaptopropionylglycine (50mg/kg bw) intravenously prior to the onset of reperfusion. The results suggest that 60 minutes of ischemia followed by 10 (Gr.II) and 90 minutes (Gr.III) of reperfusion increased lipid peroxidation (MDA), and decreased glutathione (GSH), & Superoxide dismutase levels in the renal tissues compared to normal control. However, the GSH and SOD levels were higher and the lipid peroxidation level was lower in I/R treated groups than I/R controls. Thus, the present study confirms the protective effect of N-2- mercaptopropionylglycine on the reperfusion injury and also extends that protective effect on renal tissue.

Key words: ischemia, reperfusion, NMG, MDA, GSH, SOD.

INTRODUCTION

Renal ischemia initiates a complex and interrelated sequence of events, resulting in injury and death of the renal cells (Dong Kyun Rah etal ,2007). Reperfusion, although essential for the survival of the ischemic tissue, causes additional damage (termed as reperfusion injury (Lieberthal W and Levine JS, 1996). Together, I/R of the kidney contribute to the renal dysfunction and associated with ischemic acute renal failure (Gueler, F etal, 2004). Interruption of blood circulation produces biochemical and structural cell disintegration that evolve to organelle disorganization with subsequent cellular death (Souza AP etal 1991; & Lefer AM, Lefer DJ, 1993). The oxidative-reduction activity, mainly mediated by newly formed highly reactive chemical radicals, is one of the processes responsible for this condition (Del Maestro RF 1980; & Gao W 1991).

When ischemic tissue is reperfused, high concentrations of xanthine-oxidase will use the incoming oxygen to produce hydro peroxide and subsequently liberate great amounts of aggressive free radicals (Souza AP etal 1991). The dissociation of oxygenated water by metallic ions such as iron and copper stimulates the production of free radical hydroxyls and is highly harmful to the reperfused tissue causing rupture of cellular and sub-cellular structures (Del Maestro RF 1980; Belzer FO, Southard JH.1988). The anti oxidative effect of N_2-mercaptopropionylglycine (N_2 -MPG), that partially prevents the conversion of xanthine-dehydrogenase into xanthine-oxidase, was previously demonstrated by the preservation of miocutaneous graft after 12 hours of normothermic ischemia (Emilio Elias Abdo et al 2003). The tissue protective effect of N_2-MPG was also demonstrated in the acute pancreatitis model, where low blood flow and cellular damage, resembles the ischemia/reperfusion process (Lefer AM, Lefer DJ 1993). But, not a single report was available about protective role of N2-NMG on renal tissue in limiting free radical-induced tissue reperfusion damage. Therefore, the present study was designed to find out the importance of the protective effect of N-2- mercaptopropionyl glycine on renal tissue I/R.

Corresponding Author: Dr Vinodini NA Associate professor Department of physiology center for Basic Sciences Kasturba Medical College Bejai Mangalore Karnataka 575004 Mob : 919448214870

MATERIAL AND METHODS

Animals:

Inbred wistar rats (WI) weighing about 200-300gm were used in this study. The rats were obtained and maintained in the Animal house of the department of physiology center for Basic Sciences Kasturba Medical College Mangalore. All experimental protocols were approved by the ethical committee of Manipal University Manipal, Karnataka. During the experimental periods, the rats were kept in an individual cage and food & water were given ad lib.

Grouping Protocol:

Group I: (n=7; Normal control). The animals in this group served as control.

Group II& III: (n=7 each; I/R control) In this; the rats underwent ischemia for 60minutes followed by 10 minutes (II) & 90 minutes (III) of reperfusion respectively.

Group IV & V: (n=7each; I/R treated group) The rats underwent 60 minutes of ischemia and were treated with a loading dose of NMG (50mg/kg/bwt) prior to the commencement of reperfusion for 10 minutes (IV) & 90 minutes (V) respectively.

Procedure for Ischemia/reperfusion:

The rats were anesthetized intraperitoneally with pentabarbitone sodium (40mg/kg/bw) under strict aseptic conditions. The abdomen was opened by left flank incision. Left renal artery and vein were occluded by micro vessel occluder. The animals were subjected to renal ischemia for 60 minutes. Renal ischemia was confirmed by inspection of the renal vessel. At the end of ischemic period the vessel occluder was removed for reperfusion of the kidney for 10 minutes (II & IV) or 90 minutes (III & V). The Gr.IV & Gr.V also received a loading dose of NMG (50/kg/bwt) prior to the onset of reperfusion. The abdominal viscera were covered with gauze soaked in normal saline (0.9% sodium chloride) to keep the tissues moist.

Procedure for Harvesting the Tissue:

Following completion of ischemia and reperfusion, the kidneys were removed and kept in cold phosphate buffered saline (PBS, 0.9%). The reperfused kidney was blotted, dried and minced. The minced tissues were transferred to a glass homogenizer containing 10 ml of cold PBS (PH 7.4) and were centrifuged at 3000 rpm for 30 minutes. The supernatant obtained was used for the biochemical estimation such as MDA, GSH and SOD.

Estimation of MDA:

MDA was estimated by the method described by Kartha & KrishnaMurthy (1978). The development of pink color was measured at 535nm by using a Spectronic D-20 Spectrometer. TBA- reactive material was expressed in terms of nano moles of malondialdehyde (MDA)/gm of wet tissue.

Estimation of GSH:

GSH was estimated standard protocol (Beutler E, etal, 1963). The OD of the blank test tube with all the reagents except the samples and the test tube with all the reagents with the sample were measured at 412 nm by using a Spectron D-20 spectrometer. The glutathione content of tissue was expressed as GSH in µg /gm of tissue protein.

Estimation of SOD:

SOD was estimated by the technique explained by Fridovich(1971).The reduction of NBT by $O_2^{\cdot -}$ was measured at 560 nm using a Spectron D-20 Spectrophotometer. The activity was expressed as unit/ mg protein

Statistical Analysis:

The data were expressed as means ±SD from 7 animals per group. The differences between the groups were compared for statistical significance by the student t test with the level of significance set at $P<0.05$.

Results:

The MDA level, the measure of lipid peroxidation increases significantly in both I/R control groups (Gp II & III) ($p<0.0001$) when compared with normal control (Gp I). However, administration of N-2-

mercaptopropionyl glycine (NMG) prior to reperfusion decreased the MDA level. As a result, I/R treated groups (Gp. IV & V) showed a decrease in MDA level with both I/R controls (P<0001 for all). (Fig: 1).

The I/R control groups II & III showed a decrease in GSH and SOD level in comparison with the normal control, group I (p<0.0001). But the GSH & SOD level of these I/R control groups (II & III) were significantly more than the I/R treated groups of IV & V (P< 0.0001 for all) (Fig: 2 & Fig;3).

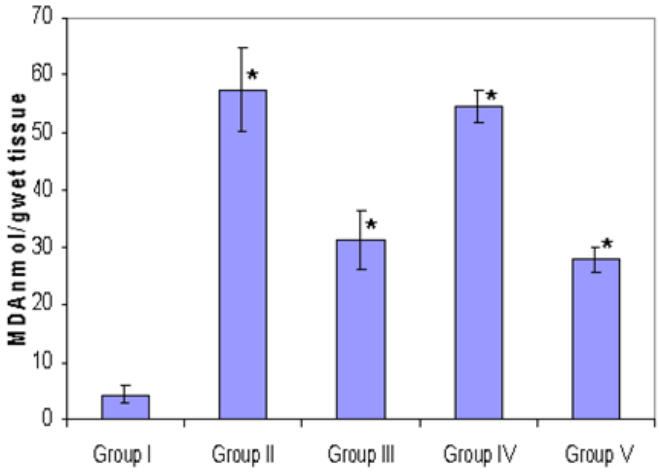

Fig. 1: Effect of NMG administration prior to reperfusion on tissue level of MDA following renal ischemia Gr. I vs Gr. II, Gr. III, Gr. IV and Gr. V n-sample size=7 p<0.0001.

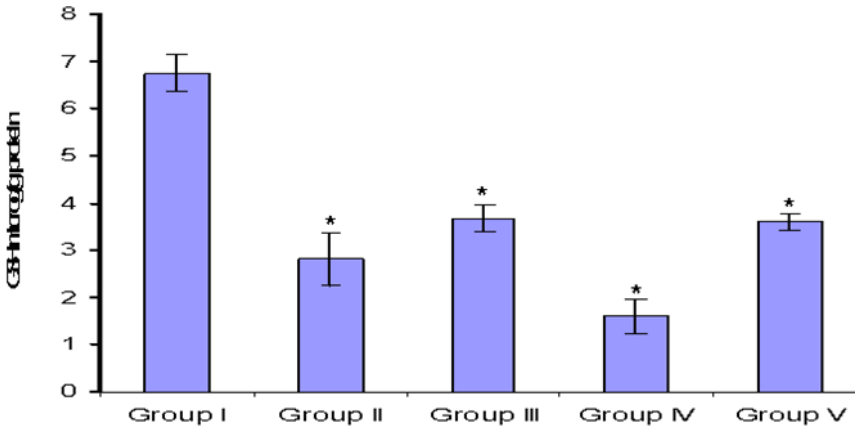

Fig. 2: Effect of NMG administration prior to reperfusion on tissue GSH level following renal ischemia Gr. I vs Gr. II, Gr. III, Gr. IV and Gr. V n-sample size=7 p<0.0001.

Discussion:

Oxygen free radicals are usually produced by the aerobic metabolism of living cells. An overproduction of these radicals has been reported in ischemic/reperfused organs, leading to a high degree of tissue damage (Epstein FH 1985, Granger DN et al 1989, Kobelt F, et al 1994 Vento AE etal 1999). Prevention of these deleterious effects, by the administration of antioxidative substances has been attempted in organ transplantation and surgical emergencies (.Horwitz LD et.al 1994. Ihnken K et al, 1995, Vento AE etal 1999).

After a significant period of ischemia, blood recirculation produces large amounts of super oxide residues derived from the abnormal activity of the newly formed xanthine-oxidase and is responsible for tissue lesion (Del Maestro RF etal 1980 ,Widman SC et a 1994). The N_2-MPG prevents the formation of xanthine-oxidase (Emilio Elias Abdo 1988) and thereby reduces the reperfusion injury by decreasing the production of oxidative free radicals. The result of the present study which shows, a decrease in the level of tissue peroxidation and increase in the level of GSH & SOD, after the administration of N_2-MPG prior to reperfusion following ischemia also in agreement with the above statement. As the protective effect of N_2-MPG in this study is more evident with group received reperfusion of 10 minutes than the group received 90 minutes reperfusion indicates that the protective action of N_2-MPG is short lived.

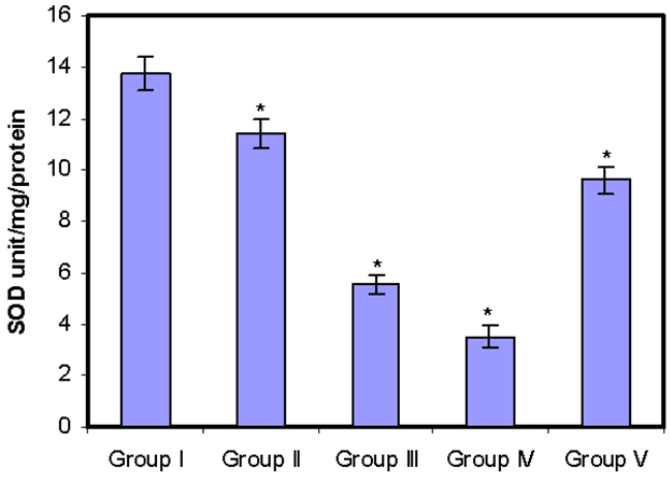

Fig. 3: Effect of NMG abministration prior to reperfusion on tissue SOD level following renal ischemia Gr. I vs Gr. II, Gr. III, Gr. IV and Gr. V n-sample size=7 $p<0.0001$.

Thus, the results of this study support the present notion that N_2-MPG is potent in decreasing the renal injury mediated due to oxidative stress which is responsible for tissue destruction. This study also suggest for to get better protective effect against reperfusion injury at least in renal tissue, along with a single dose of prior administration, repeated shots of N_2-MPG at certain intervals during reperfusion may be needed; a new finding has to be investigated further.

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