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RESEARCH ARTICLE

Effect on antioxidant defense system in contra-lateral kidney after unilateral renal vessel occlusion in rats

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Manuscript Info *Abstract*

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Aim: T0 evaluate the effect of unilateral renal reperfusion injury following ischemia on contralateral kidney either in presence or absence of free radical scavengers.
Materials & method: Seven Wistar Albino rats in each group underwent unilateral renal ischemia of left renal vessels using occluder for 60 minutes of ischemia followed by 10 & 90 minutes respectively of reperfusion & the biochemical parameters such as MDA, SOD, GSH &ceruloplasmin levels were measured in the contralateral as well as occluded kidney either in the presence or absence of free radical scavengers .
Result: A significant increase in MDA & a decrease in GSH, SOD &ceruloplasmin levels in the tissues was observed in the occluded kidney. However, the levels of the above mentioned parameters in the contra- lateral renal tissue did not show any significant effect following 10 & 90 minutes of reperfusion.
Conclusion: Therefore, the results of present study showed that 60 minutes of renal ischemia followed by reperfusion for 10 & 90 minutes respectively had no significant effect on the antioxidant defense system of the contra-lateral kidney.

Introduction

Acute renal failure, a common clinical condition affecting the kidneys, accounts for nearly 50% of the morality despite prompt therapeutic interventions (Yuds et al., 1998). One of the major causes of it is renal ischemia/reperfusion, occurring in around 5% of the patients. During renal failure, often there is a period of hypotension and low blood flow to the kidney, followed by reperfusion (Yuds et al., 1998). It has been suggested that renal ischemia followed by reperfusion of the tissue enhances injury known as reperfusion injury. Several mechanisms have been postulated about reperfusion injury such as, production of oxygen metabolites and xanthine oxidase-mediated free radicals (McCord JM, 1985, Paller MS, 1992), etc., During ischemia, increased hypoxanthine and xanthine are also produced as a result of ATP degradation (Paller MS et al., 1984), and xanthine dehydrogenase is converted into xanthine oxidase (McCord JM &Fridovich I, 1970, Roy RS &Mc Cord JM, 1983). With the re-introduction of molecular oxygen upon reperfusion, purines are metabolized by xanthine oxidase, leading to the production of superoxide radicals (McCord JM, 1985, Roy RS & McCord JM, 1983). The released toxic oxygen metabolites such as superoxide radicals (O•□), hydroxyl radicals (OH•□) and singlet

oxygen has been thought to be involved in the injury process. The supportive evidence is based upon the observation that following renal artery occlusion, the free radical scavengers have been shown to offer some degree of protection against free radical-mediated renal re-perfusion injury in animal models of acute renal failure. A study conducted revealed that there were functional abnormalities associated with unilateral post-ischemic acute renal failure in the rats (Finn WT et al., 1987). Another work done on renal artery reported that the occlusion of the same produced incomplete ischemia and functional severity which is depended upon the site of occlusion (Ferwana OS & Pirie SC, 1987). An imbalance between energy supply and requirement contributes to cellular dysfunction (Paller MS, 1988). Though many reports are available in the literature regarding the effect of unilateral renal artery occlusion on contra-lateral kidney on different parameters but literature lacks with respect to the present study which is aimed to find out whether uni-lateral renal vessels occlusion could lead any imbalance in the contra- lateral kidney tissue anti-oxidant defense system. Studies conducted on isolated organs have shown that tissue functions are better preserved if antioxidants are included in the re-oxygenated medium provided the period of ischemia does not itself cause irreversible damage (McCord JM, 1985, Simpson PJ &Lucchesl BR, 1987).

Therefore, the present study was designed to find out the effect of unilateral renal reperfusion injury following ischemia on contralateral kidney either in the presence or absence of free radical scavengers.

Material Methods:

Inbred Wistar rats of either sex, weighing (200-300) gm were used in the present study. Animals were housed, (4-5) rats per cage and fed ad-lib. On the day of the experiment, 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 identified and dissected, free of the surrounding fat and tissues. Left renal artery and vein were occluded by micro vessel occluder. Renal ischemia was confirmed by inspection of the renal vessel. The vessel occluder was removed at different time intervals for reperfusion of the kidney and the onset of reperfusion was confirmed by observing the development of reactive hyperemia. Rats, which failed to develop reactive hyperemia, were excluded from the study. The abdominal viscera were covered with gauze soaked in normal saline (0.9% sodium chloride) to keep the tissues moist. Following completion of ischemia and reperfusion, the kidneys were removed and kept in cold phosphate buffered saline (PBS, 0.9%). The re-perfused kidney and normal kidney (i.e., contra- lateral kidney) were blotted dry 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 to obtain the supernatant.

Group I (n=7): the animals in this group served as control (normal control). The control values for renal tissue lipid peroxidation (MDA), glutathione (GSH), superoxide dismutase (SOD), ceruloplasmin and serum urea were measured.

Group II (n=7): the left renal artery was identified and dissected free of the surrounding fat and tissues. All experimental procedures were similar to that of Gr.I except for the induction of ischemia for 60 minutes followed by 10 minutes duration of reperfusion.

Group II a: the animals were pre-treated with Vitamin E (100mg/kg/bw) for 30 days. On 31 stday, the animals underwent 60 minutes of ischemia followed by 10 minutes of reperfusion.

Group II b: prior to ischemia reperfusion the animals were pre- treated with vitamin C (20mg/kg/bw) for 30 days . 0n 31st day, 60 minutes of ischemia was produced followed by 10 minutes of reperfusion.

Group II c: the animals were pre-treated with vitamin E αtocopherol + vitamin C in combination, for 30 days. On 31st day, the animals underwent ischemia for 60 minutes followed by 10 minutes reperfusion.

Group III (n=7): the left renal artery was identified and dissected free of the surrounding fat and tissues. The animals underwent 60 minutes of ischemia followed by 90 minutes of reperfusion

Group III a: the animals were pre-treated with Vitamin E (100mg/kg/bw) for 30 days. On 31st day, the animals underwent 60 minutes of ischemia followed by 90 minutes of reperfusion.

Group III b: prior to ischemia reperfusion the animals were pre- treated with vitamin C (20mg/kg/bw) for 30 days. 0n 31st day 60 minutes of ischemia was produced followed by 90 minutes of reperfusion.

Group III c: In this group animals were pre-treated with vitamin E (α tocopherol) + vitamin C in combination for 30 days. On 31st day the animals underwent ischemia for 60 minutes followed by 90 minutes reperfusion.

Estimation of MDA: MDA was estimated by the method described by Kartha& Krishna-Murthy (Kartha& Krishna-Murthy, 1978).TBA reactive material was expressed in terms of nano-moles of MDA/gm of wet tissue.

Estimation of GSH: glutathione was estimated standard protocol (Beutler E et al., 1963) and glutathione content was expressed in µg/gm protein.

Estimation of Superoxide dismutase: superoxide dismutase was estimated by technique explained by Fridovich (Beauchamp C &Fridovich, 1971). The activity for the homogenate was expressed as unit mg/protein.

Estimation of ceruloplasmin: tissue ceruloplasmin content of the samples was determined by Henry et al. method (Henry RJ et al., 1960). The content was expressed as mg/protein.

Protein Estimation: protein content of the tissue samples was determined by Lowry et al. method (Lowry OH et al., 1951).

Statistical analysis: All data are expressed as mean ± SD. Data was analyzed by using non-parametric (Kruskal-Wallis) test followed by multiplecomparison test**.**

Result:

The parameters such as MDA, GSH, SOD and ceruloplasmin levels were estimated under different experimental conditions in the contra - lateral renal tissue. The results obtained are summarized in Table I, II & III.

Table I: Effect of pre-treatment with α- tocopherol and vitamin C prior to the induction of ischemia for 60 minutes followed by reperfusion for 10 minutes & 90 minutes on tissue levels of MDA, GSH, SOD and ceruloplasmin. (values are expressed as mean ± SD, n=sample size)

Groups	Number of animals	MDA (nmol/gmwet tissue)	GSH µgm/gmprotein	SOD (unit/mg protein)	Ceruloplasmin (mg/gm protein)
I	7	4.35±1.59	6.74±0.453	13.74±0.62	13.5±1.87
II	7	57.30±7.318*	2.8±0.564*	5.55±0.54*	7.45±1.00*
II a	7	25.73±3.1*	3.6±0.24 *	7.96±0.46*	9.96±0.63*
II b	7	28.73±2.60*	3.2±0.30*	7.40±0.56*	10.01±0.49*
IIc	7	32.21±8.00*	3.9±0.46 8*	9.04±0.20*	10.68±0.96*
III	7	54.6 ±6.26	1.59±0.360	3.46± 0.42	4.12±0.77
IIIa	7	29.068± 2.5*	3.02±. 29*	6.55±0.369*	7.041± 0.437*
IIIb	7	31.7± 2.58*	2.127±0.495 *	6.06±0.32*	6.841±0.368*
IIIc		23.49± 4.86*	3.98±. 431*	6.788± 0.524 *	7.24± 0.88*

Gr. I versus II, II a, II b, II c, III, III a, III b & III c - significant, ***** $p \leq 0.0001$

Table II: Effect of 60 minutes of left renal artery ischemia followed by re-perfusion for 10 minutes on, tissue levels of MDA, GSH, SOD &ceruloplasmin on the contralateral kidney (values are expressed as Mean±SD, n=sample size)

Groups	Number of animals used	MDA (nmol/ gm wet tissue)	GSH (μg/gm protein)	SOD (unit/mg protein)	Ceruloplasmin gm/mg protein)
I	7	6.06±1.941	6.10±0.79	12.025± 0.80	11.77± .616
II	**7**	5.704±1.66 NS	6. 84±0.47 NS	11.72±0.12 NS	11.67±1.66 NS
II a	**7**	6.46± 5.71 NS	6.65±0.53 NS	12.31±0.659 NS	12.19+-0.97 NS
II b	7	4.90±1.75 NS	6.48± 0.84 NS	12.32±0.67 NS	11.67±1.655 NS
II c	**7**	4.23±0.94 NS	6.22±0.321 NS	11.69± 0.62 NS	12.04+-0.936 NS

Gr. I versus II, II a, II b & II c;NS- Not significant

TABLE III: Effect of pre-treatment with α-tocopherol& vitamin C prior to the induction of left renal artery ischemia for 60 minutes followed by re-perfusion for 90 minutes on tissue levels of MDA, GSH, SOD &ceruloplasmin on the contra-lateral kidney (values are expressed as Mean±SD, n=sample size)

Experimental groups		MDA (nmol/gm wet tissue)	GSH (μgm/gmprotein)	SOD (unit/mg protein)	Ceruloplasmin mg/gm protein)
I	7	6.06±1.941	6.10±0.79	12.025± 0.80	11.77± .616
III	7	5.95± 0.86	6.08± 0.47	12.73± 0.98	11.02 ± 0.77
III a	7	5.02± 1.53 NS	6.45±0.36 NS	11.87± 0.12 NS	11.63 ±1.06 NS
III b	7	5.30± 0.60 NS	6.20± 0.27 NS	12.73± 0.98 NS	11.0 ± 1.02 NS
III c	7	5.45± 2.23 NS	6.59± 1.05 NS	12.87± 0.18 NS	12.57±1.20 NS

Gr. I versus III, III a, III b & IV c; NS- Not significant

These results show that 60 minutes of renal ischemia followed by reperfusion of 10 & 90 minutes respectively had no significant effect on the antioxidant defense system of the contra- lateral kidney in the presence or absence of free radical scavengers.

Discussion:

The very existence of an individual depends on homeostatic balance between the various organ systems. Therefore, maintenance of cellular homeostasis is rather significant in terms of survival. Under normal physiological conditions, stressors can arise from internal as well as external sources (Crapo JD & Tierney DF, 1974, Fridovich I, 1978, Griffiths

HR et al., 1988). To safeguard against these stressors, the cell have evolved their own protective measures known as cellular defense systems. Such mechanisms involve mobilization of various cellular constituents and the functional integration of specific defense components (Davies KJA, 1986, Davies KJA, 1988). These components dampen the deleterious effects of external stressors and thus preserve optimum cellular activity by minimizing the disturbances that occur in cellular 'internal milieu' (Frei B et al., 1990, Heffner JE & Rapine JE, 1989). A major internal threat to cellular homoeostasis arises from the reactive oxygen species (ROS) formed as a result of normal physiological and metabolic processes that are essential for life. Antioxidant defense systems are self-sustained protective components of the cell. They protect cellular homeostasis from oxidative disruption by free radicals and other reactive molecules that are formed during oxygen metabolism (Fridovich I, 1978, Halliwell B et al., 1992, Harris ED, 1992).Involvement of oxygen free radicals have been suggested as one of the causative factors for a variety of disorders that affect the internal organs including heart, liver, lungs, brain and kidney. The most common causes for ARF are renal ischemia and/or nephrotoxins (Joannidis M et al., 1989). Ischemia causes renal functional impairment through a myriad of processes including renal vasoconstriction, tubular obstruction, tubular back leakage of glomerular filtrate and a decline in glomerular permeability (Thurau K et al., 1985, Kreisberg JI et al., 1983, Mason J, 1986).The precise mechanism underlying these changes is yet to be elucidated. The involvement of several factors has been implicated in the process of cellular injury that follows ischemia. Such factors include a decrease in high-energy phosphate, an increase in concentration of free intracellular calcium ions, loss of cellular synthetic function and the generation of membrane toxins (Leaf AJ et al., 1983). Renal ischemia results in a rapid decrease in the level of tissue ATP (Hemsda&Brosnan JT, 1970) & a rise in the ATP degradation products such as adenosine, inosine and hypoxanthine (Osswald H et al., 1977, W L Thomas RA et al., 1978, Fridovich I, 1970). The loss of adenosine from the cell by degradation of ATP during ischemia is believed to be the result of enzymatic cleavage of adenine nucleotides and the process of depletion is found to be persistent even after the re-establishment of blood flow. The cellular effects of accumulation of hypoxanthine during renal ischemia may be attributed to the generation of highly reactive oxygen free radicals that are formed during its enzymatic conversion to xanthine (Fridovich I, 1978). Such end-products of these reactions as $O_2^{\bullet}$, H_2O_2 and $OH^{\bullet}$ are known to cause cellular injury through lipid peroxidation of mitochondrial, lysosomal and plasma membranes that could induce alterations in shape and function of these membranes (Rellogg EW &Fridovich I, 1975). Experimental evidence suggest that oxygen free radicals play a role in the cellular injury following hypoxic insult. Many studies have confirmed the above findings after postmortem analysis of tissues such as brain, liver, heart and intestine obtained from rats that were subjected to ischemia. The effects of oxygen free radicals mediated injury following hypoxic insult have been found in organs like brain, liver, heart and intestine of experimental model (Flamm ES et al., 1978, Guarnieri C et al., 1980). Irrespective of kidney being an important organ, studies on the cellular and biochemical effects of free radicals in mammalian kidney are scant and the results of some pertinent studies remain debatable.Many tissues contain powerful endogenous scavengers that give protection against free radical damage and these include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbic acid & α tocopherol (Demopoulos HB et al., 1980). In the last few decades, experimental animals have been extensively studied over with an aim to design new modalities of intervention to limit the extent of renal tubular damage. Most commonly employed methods to study the effects of ischemia and reperfusion in experimental animals are bilateral occlusion of renal vessels, unilateral occlusion of renal vessels with or without contra-lateral nephrectomy (Paller MS, 1992, Zager RA etal., 1992, Gamelin LM & ,Zager RA, 1988, Greene EL &Paller MS, 1991, Schumer M et al.,1992, Jablonski P et al., 1983).

In the present study, the animals were subjected to unilateral occlusion before reperfusion of the kidney with contra- lateral kidney left intact in order to observe the effects of occlusion on contra-lateral kidney. Experimental evidence suggests that renal function can be severely impaired by injuries following short and prolonged periods of complete ischemia (Schumer M et al.,1992). When the contralateral kidney is removed prior to the ischemia, re-flow of blood to the post-ischemic kidney is more complete (Finn WF, 1987). In our study, the effect of unilateral occlusion on antioxidant defense system of the contra lateral kidney showed no significant variation IN the levels of MDA, GSH, SOD &ceruloplasmin levels. Itmight be due to the fact that, the contra-lateral kidney didn't undergo ischemia /reperfusion to produce an imbalance in the normal homeostasis through reactive oxygen species (ROS) & the bloodsupply to the contra-lateral kidney was also intact. So, the results of the present study suggest that though the ischemic kidney

showed an alteration in the biochemical parameters such as MDA, GSH, SOD &ceruloplasmin levels, no significant changes were observed in the above parameters in the contra-lateral kidney.

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