EFFECT OF ANTIOXIDANTS (VITAMIN C) ON TISSUE CERULOPLASMIN FOLLOWING RENAL ISCHEMIA REPERFUSION IN WISTAR RATS

Vinodini NA*[1], Tripathi Y[2], Raghuveer C V[3], Ranade AV[4], Asha Kamath[5], Sheila R Pai[1]
[1*]Department of Physiology Kastruba Medical College Mangalore Karnataka India (A Unit of Manipal University Manipal)
[2]Department of Physiology, Santhosh Medical College, Maliwara, Nehru Nagar II, Ghaziabad 201001, Uttar Pradesh
[3]Department of Pathology, Srinivas Medical College, Mukka po, Surathkal Mangalore Karnataka, India
[4]Department of Anatomy, Gulf Medical College, PO Box 4184, Ajman, United Arab Emirates
[5]Department of community Medicine Kasturba medical College Manipal Karnataka India (A Unit of Manipal University, Manipal)
E-mail of Corresponding Author: Vinodini1967@gmail.com

Abstract
Oxidant injury has been implicated in the pathogenesis of inflammatory, metabolic and toxic insults in ischemic –reperfusion injury. Oxidant injury is initiated by free radicals and reactive oxygen molecules .However when the cells are exposed to oxidant stress several different antioxidant defense systems operate to prevent or limit the injury. Ceruloplasmin one among such antioxidants; a powerful free radical scavenger.
Aim: To study the effect of vitamin C on tissue ceruloplasmin level following renal reperfusion.
Materials and Methods: *Wistar albino* rats were divided into Group I, II & III the Gr. II the experimental groups) were subjected to ischemia for 60 minutes followed by 24 hrs of reperfusion. The Gr.III was pre- treated with vitamin C (20mg/kg.bw) for 30 days followed by 60 minute ischemia & 24hrs of reperfusion. After the experimental procedure was over; the kidneys were removed and homogenized. The homogenized tissue was used for biochemical estimation of lipid peroxidation & ceruloplasmin.
Result: A significant in the levels of tissue lipid peroxidation (MDA) and increase in tissue ceruloplasmin level was observed in Group II compared to those in Group I (normal control) However, the pre- treated group (Group III) showed an increase in the levels of ceruloplasmin and a decrease in lipid peroxidation in comparison to group II.
Conclusion: The results of the present study suggest that administration of vitamin C prior to renal ischemia reperfusion protect the renal tissue from the free radical induced reperfusion injury.
Keywords: renal ischemia reperfusion; MDA; ceruloplasmin; Vitamin C

1. Introduction

Oxidant injury has been implicated in the pathogenesis of inflammatory, metabolic and toxic insults, in ischemic-reperfusion injury[1]. Oxidant injury is initiated by free radicals and reactive oxygen molecules which are generated by activated neutrophils, monocytes, and mesangial cells, during normal and abnormal metabolic processes. When cells and organs are exposed to oxidant stress, several different antioxidant defense mechanisms operate to prevent or limit oxidant injury[1] oxidant injury results from the shift in the oxidant/antioxidant balance[1].
Renal ischemia-reperfusion injury constitutes the most common pathogenic factor for acute renal failure and is the main contributor to renal dysfunction[2]. Many studies have demonstrated that reactive oxygen species play an important role in ischemic acute renal failure,[3-6]. Several in vivo animal studies document a role of reactive oxygen molecules in causing tissue injury[7-10] Ascorbic acid has been used to protect against corneal damage from free radicals in rabbits [11]. In addition, it has also been used to improve the renal hemodynamics and decrease oxidative stress, inflammation and fibrosis in the ischemic kidney of pigs [12]. While previous studies have shown the role of vitamin E in reducing injury, the role of vitamin C has been less extensively studied.
The Ceruloplasmin is a copper based glycoprotein with a molecular weight of

132,000 that can bind to six or seven copper ions under physiological conditions and mediates free radical metabolism in the extracellular compartment ions[13] Previous studies have shown that the level of lipid peroxidation and scavenging enzymes such as glutathione & SOD levels were altered following renal reperfusion.[14,15] However not many studies have been conducted to co relate the role of vitamin C on ceruloplasmin level following renal reperfusion. Therefore, the present study was designed to investigate the efficacy of ascorbic acid as a free radical oxygen scavenger on ceruloplasmin level following renal reperfusion

2. Materials &Methods :

Inbred *Wistar* rats weighing (200-300) gm were used in the present study. Animals were housed;
(4-5) rats per cage and fed adlib. All experimental protocols were approved by the ethical committee of Manipal university [formerly known as Manipal Academy of Higher Education (MAHE)], Manipal, Karnataka, India. For the experimental purpose, the animals were divided into the following groups;

Group I: Normal control.

Group II. Experimental control (The animal underwent 60 minutes ischemia followed by 24hrs of reperfusion.

Group III: Pre- treatment with Vitamin C for 30 days followed by 60 min ischemia & 24 hrs reperfusion.

2.1 Experimental procedure: On the day of the experiment the rats were anesthetized intraperitoneally with pentabarbitone sodium (40/mg/kg/bw) under strict aseptic conditions. The abdomen was opened by left flank incision. Left renal artery and vein were identified and dissected free from the surrounding fat and tissues. Animals were pre-treated with vitamin C for 30 days. On 31st day animals were anesthetized and renal ischemia was produced for 60 min followed by 24 hrs reperfusion. After 24 hrs of reperfusion the kidneys were removed and kept in cold phosphate buffered saline (PBS, 0.9%). The reperfused kidney was 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. The supernatant obtained were used to estimate the biochemical parameters such as MDA & ceruloplasmin.

2.2 .1 Estimation of MDA: MDA was estimated by the method described by Kartha& KrishnaMurthy[16]. Thiobabutric acid reactive material was expressed in terms of nano moles of MDA /gm wet tissue.

2.1.2 Protein Estimation: Protein content of the tissue samples was determined by Lowry et al method [17]

2.1.3 Ceruloplasmin Estimation: ceruloplasmin content of the tissue samples was determined by Henry etal method The content was expressed as mg/protein[18].

2.1.4 Statistical analysis: All data are expressed as mean ± SD. Data were analyzed by using non-parametric (Kruskal- Wallis) test followed by multiple comparison test with $p< 0.05$ considered as being significant.

3. Results:

Renal ischemia of 60 minutes followed by 24 hrs.of reperfusion showed a significant increase in tissue lipid peroxidation level (MDA, 56.47 ± 5.4 Table .1) as compared to the normal control (4.37± 1.59P < 0.0001) ; whereas the level of ceruloplasmin showed a decrease 4.533±0.681 :.13.5± 1.87). Treatment with vitamin C showed a reverse effect (ie the tissue MDA level was significantly reduced (31.40± 3.79: 56.47±5.46)and ceruloplasmin level showed a significant increase (7.015± 0.522 compared to experimental control. 4.533± 0.68 Table .1)

4. Discussion:

The results of our study showed that the renal tissue ceruloplasmin concentration decreased following renal ischemia reperfusion like other antioxidant such as reduced glutathione & superoxide dismutase. The Ceruloplasmin (Cp) is a copper based glyco protein with a molecular weight of 132,000 that can bind to six or seven copper ions under physiological conditions and mediates free radical metabolism in the extracellular compartment ions[13].Goldstein et al[19].have suggested that ceruloplasmin is capable of scavenging superoxide radicals and its free radical scavenging activity was confirmed by Gutteridge[20]. However, its ability to fight oxidative damage is found to be weaker than superoxide dismutase. Thus, the interaction of

ceruloplasmin with free radical might be one of the reasons that its level decreased following induction of ischemia and reperfusion. Ceruloplasmin (Cp) is a ferroxidase that oxidizes toxic ferrous iron to its nontoxic ferric form [21]

Previous studies have shown that the levels of Cp are likely to increase during injuries because CP is an acute phaseprotein & further investigators suggest that antioxidant property of Cp is likely to be crucial during CNS injury such as ischemia or mechanical trauma[22]. Ceruloplasmin prevents free copper ions from catalyzing oxidative damage. The ferroxidase activity of ceruloplasmin (oxidation of ferrous iron) facilitates iron loading onto its transport protein, transferrin, and may prevent free ferrous ions (Fe2+) from participating in harmful free radical generating reactions.[21]Ceruloplasmin is an effective antioxidant for a variety of radicals and has a potent peroxidase property to decompose hydrogen peroxide in the presence of reduced glutathione. It inhibits the peroxidase activity of myeloperoxidase in a concentration-dependent manner and shows selective binding to myeloperoxidase *in vitro*[22], [23] However, upon pre-treatment with vitamin C the level of Cp significantly increased compared to the experimental control. Previous studies on laboratory animals and young humans reported that with high dose of vitamin C intake decreased the levels of serum ceruloplasmin.[24,25] Findings of this study is not in agreement with the previous study ; which may be due to the difference in the dosage levels. In the present study the dosage used was 20mg/kg b.w. It plays an important role in antioxidant protection against organic and inorganic oxygen radicals from iron and ascorbate. Since not many studies have examined the tissue ceruloplasmin following renal reperfusion injury; further studies may be required to elucidate the exact role of ceruloplasmin in renal ischemia followed by reperfusion.

References:

1. Sharon P. Andreoli Reactive oxygen molecules, oxidant injury and renal disease. Pediatr Nephrology 5: 733-742 2001
2. Yu DS, Char DL, Chang SY, Ma CP.Pathogenesis of ischemia reperfusion injury of the kidney after transient renal arterial clamping in rats. J Formos Med Assoc 1998; 97: 606-613.
3. Greene EL, Paller MS. Oxygen free radicals in acute renal failure.Mineral Electrolyte Metab 1991; 17: 124-132.
4. Yoshida N, Granger DN, Anderson DC, Rothlein R, Kvietys PR. Anoxia / reoxygenation induced neutrophil adherence to cultured endothelial cells. Am J Physiol 1992; 262: H1891-H1898.
5. Linas SL, Whittenburg D, Parsons PE, Repine JE. Mild renal ischemia activates primed neutrophils to cause acute renal failure. Kidney Int 1992; 92 : 610-66.
6. Baud L, Ardaillou R. Involvement of reactive oxygen species in kidney damage.Br Med Bullet 1993; 49: 621-629.
7. Baud L and Ardaillou R. Reactive oxygen species production and role in the kidney. Am J Physiol 1986; 251: F765-F776
8. Shah SV. Role of reactive oxygen metabolites in the experimental glomerular disease. Kidney Int 1989; 35:1093-1106
9. Baud L, Fouqueray B, Phillippe C and ArdaillouR.Reactive oxygen species as glomerular autacoids. Am J SocNephrol 1992; 2:S 132-S 138.
10. Paller MS, Neumann TV. Reactive oxygen species and rat renal epithelial cells during hypoxia and reoxygenation. Kidney Int 1991; 40 : 1041-1049.
11. AR, Rodriguez-Porcel M, Herrmann J, Krier JD, Zhu X,Lerman A, Lerman LO. Beneficial effects of antioxidant vitaminson the stenotic kidney. *Hypertension* 2003;42:605–12.
12. Nagel E, Meyer zu Vilsendorf A, Bartels M, Pichlmayr R.Antioxidative vitamins in prevention of ischemia/reperfusion injury.*Int J Vitam Nutr Res* 1997;67:298–306
13. Macintyre G, Gutfreund KS, Martin WR, Camicioli R, Cox DW. Value of an enzymatic assay for the determination of serum ceruloplasmin.*J Lab Clin Med.* 2004;144:294–301.[PubMed]
14. Baker GC, Coory RJ, Autor AP. Oxygen free radical induced damage in kidneys subjected to warm ischemia and reperfusion. Ann surg 1985; 202: 628-641.
15. Linas SL, Dennis, Whittenburg D, Repine JE. O_2 metabolites cause reperfusion injury after short but not prolonged renal ischemia. Am J Physiol 1987;253:F685-F691

16. Kartha VNR, Krishnamurthy S. Factors effecting in vitro lipid peroxidation in rat homogenate.Ind J PhysiolPharmacol.1978; 22: 44-52.
17. Lowry OH, Roscbrought NJ, Faro AI, Randal RJ. Protein measurement with folin phenol reagent. J BiolChem 1951; 193:265-275
18. Henry RJ, Chiamori N, Segalore N. ProcSocExpBiol Med 1960; 104:620-
19. Goldstein IM, Kaplan HB, Edcison HS, Weissmann.Ceruloplasmin a scavenger of superoxide anion radicals.J BiolChem 1979; 254:4040-4045.
20. Gutteridge JMC and Halliwell B. The antioxidants proteins of exctracellular fluid In; Cellular antioxidant defense mechanisms. Edited by Chowok, Boca Raton Fl CRC, 1988; 1-23
21. Bharatkumar N. Patel, Robert J. Dunn, Suh Young Jeong, Qinzhang Zhu, Jean-Pierre Julien, andSamuel DavidCeruloplasminregulates iron levels in the CNS and Prevents FreeRadical Injury.The Journal of Neuroscience, August 1, 2002, *22*(15):6578–6586
22. Giurgea N, Constantinescu MI, Stanciu R, Suciu S, Muresan A. Ceruloplasmin – acute-phase reactant or endogenous antioxidant? The case of cardiovascular disease.*Med SciMonit.*2005;11:RA48–51.[pubmed)
23. Dumoulin, M.-J., et al., "Comparative antioxidant and cardioprotective effects of ceruloplasmin, superoxide dismutase and albumin." *Arzneim.Forsch.*, 1996 :**v.** 46:9,: 855-861
24. Finley EB, Cerklewski FL Influence of ascorbic acid supplementation on copper status in young adult men**.**.Am J ClinNutr. 1983 Apr;37(4):553-6.
25. Robert A, Jacobs James H, Skala , Stanely T, omaayeAndjudith R, TurnlAoe ND Effect of Varying Ascorbic Acid Intakes on Copper absorption and Ceruloplasmin Levels of Young Men J. /Yuir. 1987; 117:2109-2115.

TABLE 1: Effect of pre-treatment with vitamin C prior to the induction of 60 minutes of renal ischemia followed by 24 minutes of reperfusion on tissue levels of MDA &ceruloplasmin

Groups	Animals	MDA nmol/gm wet tissue	Ceruloplasmin gm/mg protein
Group I	7	4.37± 1.59	13.5± 1.87
Group II	7	56.47±5.40*	4.533±0.681*
Group III	7	31.40± 3.79*	7.015± 0.522

(values are expressed as mean ±SD. n=7).

(Gr. I versus Gr II &Gr.III. * P<0.0001).