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Involvement of progesterone receptors in ascorbic acid–mediated protection against ischemia-reperfusion–induced acute kidney injury

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ABSTRACT

Background: Ascorbic acid (AA) is an established antioxidant and has been used for treatment of various disorders. Recent reports suggest that administration of AA increases the level of steroids such as progesterone in the body. The present study investigated the protective role of progesterone against ischemia-reperfusion–induced acute kidney injury (AKI) and possible involvement of progesterone receptors in AA-mediated renoprotection in rats.

Materials and methods: The male rats were subjected to bilateral renal ischemia for 40 min followed by reperfusion for 24 h to induce AKI. The rats were treated with progesterone (5 and 10 mg/kg, intraperitoneally) and AA (500 mg/kg, intraperitoneally for 1, 2, and 5 d) before AKI. In separate groups, mifepristone, the progesterone receptor antagonist was administered to rats before progesterone (10 mg/kg) and AA treatment (5 d). Various parameters including creatinine clearance, serum urea, uric acid, potassium level, fractional excretion of sodium, lactate dehydrogenase, and microproteinuria were used to assess kidney injury. Moreover, renal tissues were subjected to quantification of oxidative stress and evaluation of histopathologic changes.

Results: The exogenous administration of progesterone afforded protection against AKI in a dose-dependent manner that was abolished by mifepristone. The administration of AA for 1, 2, and 5 d induced significant increase in serum progesterone levels and afforded protection against AKI. The antioxidant and renoprotective effect of AA was abolished by prior treatment with mifepristone.

Conclusions: It is concluded that exogenous administration of progesterone exerts significant antioxidant and renoprotective effect. Moreover, the progesterone receptors find their explicit involvement in AA-mediated renoprotection against ischemia-reperfusion –induced AKI in rats.

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1. Introduction

Acute kidney injury (AKI) shares one of the major reasons of morbidity and mortality in hospitalized patients involving medical and surgical procedures [1]. The AKI is characterized by abrupt decline in glomerular filtration rate that leads to the accumulation of nitrogenous and other biochemical wastes in blood [2]. Ischemia-reperfusion injury (IRI) is one of the common reasons for renal dysfunction observed in various clinical situations involving kidney transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm, and ureteral obstruction [3]. The IRI is well documented to be associated with altered mitochondrial oxidative phosphorylation, activation of various enzymes, such as protein kinases, phosphatases, proteases, and lipases along with significant oxidative stress that results in loss of organ function [4,5]. Moreover, IRI leads to the activation of various inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin 1, 6, and 18 along with the secretion of chemokines such as monocyte chemoattractant protein-1 [6,7]. Progesterone is a hormone responsible for maintaining reproductive functions, such as ovulation and implantation in females and androgen biosynthesis in males [8]. Apart from its role in reproductive physiology, the role of progesterone is explored as neuroprotective agent against brain injury and stroke [9,10]. Moreover, progesterone suppresses various inflammatory cytokines, including TNF-α and interleukin-1, 6, and 18, increases various antiapoptotic proteins, such as Bcl-2, and reduces lipid peroxidation along with increase in reduced glutathione level and antioxidant enzymes including catalase (CAT) [11–13].

Many reports indicate that administration of antioxidants provide benefit against wide range of stress stimuli in renal tissues [14,15]. Ascorbic acid (AA) is widely accepted as an antioxidant and is an essential nutrient required for various metabolic reactions. The active part of AA is ascorbate ion that acts as an electron donating entity and is involved in biosynthesis of steroids, collagen, and peptide hormones [16,17]. Moreover, AA increases the activity of endogenous antioxidant defense including superoxide dismutase, reduced glutathione (GSH), and CAT [18]. The AA is well documented to be cardioprotective, hepatoprotective, anticonvulsant, as well as renoprotective through its antioxidant and free radical ions scavenging activity [18–21]. Various studies suggest that AA is involved in the biosynthesis of progesterone in females [21,22]. The role of exogenous administration of progesterone in renal IRI and the possible involvement of progesterone receptors in AA-mediated biological response has not been explored so far. The present study investigated the role of progesterone against ischemia-reperfusion–induced AKI in male rats. Moreover, possible involvement of progesterone receptors in AA-mediated renoprotection has been explored.

2. Materials and methods

The present study was carried out in accordance with the guidelines framed by committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Male Wistar rats weighing 200–250 g were used in the present study. They were maintained on standard chow and water *ad libitum* and were exposed to 12-h light and dark cycle. Two-day acclimatization period was given to rats in metabolic cages before surgical procedure.

The AKI was induced by using bilateral ischemia-reperfusion model in rats. The rats were anesthetized with ketamine (50 mg/kg, intraperitoneally [i.p.]) and xylazine (10 mg/kg, i.p.). The anesthetized rats were placed on surgical platform in dorsal position, and rectal temperature was maintained at 37°C throughout the experimental procedure. Both kidneys were exposed through flank incisions, and renal pedicles were occluded using bulldog clamp for 40 min. The clamps were then removed to start reperfusion for next 24 h. The surgical site was sealed by continuous sutures in two layers. In sham group, the animals were exposed to similar surgical procedure except for occlusion of renal pedicles. The animals were returned to their metabolic cages for urine collection.

After 24 h, the rats were anesthetized using ketamine (50 mg/kg, i.p.). The blood samples were collected using retro-orbital puncture, and rats were killed by cervical dislocation. The serum isolated from blood was used for estimation of creatinine, urea, uric acid, sodium, potassium level, and lactate dehydrogenase (LDH) activity. Moreover, the creatinine, sodium, and protein content in urine were estimated. The kidneys were removed and washed with saline. A part of renal tissue was preserved for histopathologic studies, the small portion was used for estimation of superoxide anion generation (SAG), and the rest of the tissue was minced and homogenized (10% wt/vol) in 1.17% potassium chloride solution using teflon homogenizer. The contents were centrifuged at 800*g* for 20 min. The pellet obtained was used for estimation of myeloperoxidase (MPO) activity, whereas the clear supernatant was used to estimate lipid peroxides in terms of thiobarbituric acid reactive substances (TBARS), reduced GSH levels, and CAT activity.

2.1. Estimation of serum progesterone level

The serum progesterone level was estimated by liquid phase radioimmunoassay using progesterone antisera raised in the Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana using procedure of Ghuman *et al.* [23].

2.2. Estimation of creatinine clearance

The serum and urine creatinine level was assayed by alkaline picrate method using creatinine assay kit (Span Diagnostics Ltd, Surat, India). The creatinine clearance (CrCl) was calculated using the formula: [CrCl = urine creatinine × urine flow rate/plasma creatinine]. The results were expressed as milliliters per minute per kilogram of rat weight.

2.3. Estimation of serum urea, uric acid, and potassium level

The urea, uric acid, and potassium level was assayed using commercially available kit (Span Diagnostics Ltd and Crest