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Explicit role of peroxisome proliferator–activated receptor gamma in gallic acid–mediated protection against ischemia-reperfusion–induced acute kidney injury in rats

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ABSTRACT

Background: Gallic acid is a polyphenolic compound and is reported to be renoprotective because of its antioxidant activity in various preclinical studies. Gallic acid has been reported to activate peroxisome proliferator–activated receptor gamma (PPAR-γ) *in vitro*. However, the relevance of the interplay between gallic acid and PPAR-γ in various pathologic conditions is yet to be established *in vivo*. The present study investigated the protective role of gallic acid against ischemia-reperfusion–induced acute kidney injury (AKI) and the possible involvement of PPAR-γ in gallic acid–mediated renoprotection.

Materials and methods: The AKI was induced in rats through bilateral clamping of renal arteries for 40 min followed by reperfusion for 24 h. The AKI was assessed by the quantification of creatinine clearance, blood urea nitrogen, uric acid, potassium level, fractional excretion of sodium, and urinary microproteins. The oxidative stress in renal tissues was quantified in terms of myeloperoxidase activity, thiobarbituric acid reactive substances, superoxide anion generation, and reduced glutathione level. The histopathologic changes in renal tissues were assessed by hemotoxylin and eosin staining. The rats were administered gallic acid (50, 100, and 200 mg/kg) orally for 7 d before subjecting them to AKI.

Results: The renal ischemia–reperfusion induced significant changes in plasma, urinary, and tissue parameters. The administration of gallic acid at three dose levels offered a significant protection against renal ischemia-reperfusion–induced AKI. The prior treatment with PPAR-γ antagonist, bisphenol A diglycidyl ether, significantly abolished the renoprotective effect of gallic acid that confirms the involvement of PPAR-γ in gallic acid–mediated renoprotection.

Conclusions: It is concluded that the activation of PPAR-γ significantly contributes toward gallic acid–mediated protection against ischemia-reperfusion–induced AKI.

1. Introduction

Acute kidney injury (AKI) is defined as an abrupt loss of kidney function characterized by the accumulation of nitrogenous and other biochemical wastes and an imbalance of electrolytes in body fluids. AKI is a serious medical problem that occurs in about 4%–20% patients admitted to the hospital and its incidence is growing almost 10% annually [1]. The

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ischemia–reperfusion injury (IRI) is one of the established reasons to induce renal dysfunction and is observed in various clinical conditions including kidney transplantation, partial nephrectomy, renal artery angioplasty, and ureteral obstruction [2]. The IRI accounts for 30% of total cases of delayed graft dysfunction after renal transplantation [3].

The IRI involves dysfunction of both endothelial and tubular cells in kidney. It leads to mitochondrial dysfunction, depletion of adenosine triphosphate (ATP), and guanosine triphosphate resulting in necrosis and apoptosis, respectively [4,5]. Moreover, the ATP depletion accounts for the dysfunction of ATP-dependent membrane ion pumps with a consequent rise in both mitochondrial and cytosolic calcium levels [6,7]. This rise in the calcium level activates phospholipase A_2 and the consequent production of arachidonic acid metabolites [8]. The IRI results in the release of proinflammatory cytokines, such as interleukin (IL)-1, 6, 8 and tumor necrosis factor α (TNF-α), and the increased expression of monocyte chemoattractant protein 1 in renal tissues [9–11]. Moreover, reactive oxygen species (ROS) generated during IRI activate profibrotic factors, such as transforming growth factor β, nuclear factor κB, and cascade of reactions, resulting in death of renal tissues [12,13].

The peroxisome proliferator–activated receptor-γ (PPAR-γ) is a nuclear receptor involved in fat storage and glucose metabolism. The PPAR-γ is being explored for their antioxidant and anti-inflammatory activities against IRI of various organs including heart, lungs, brain, sciatic nerve, liver, and gut [14–19]. The protection offered by PPAR-γ agonists in IRI has been attributed to their inhibitory role against the overexpression of inducible nitric oxide synthase, monocyte chemoattractant protein 1, intracellular cell adhesion molecule-1, IL-1, and TNF-α [20].

The naturally occurring polyphenols are known to possess antioxidant activity. Gallic acid is a polyphenolic compound present in the number of plants and is reported to protect against myocardial, gastric, and hepatic damage [21–23]. The role of gallic acid in few models of renal dysfunction except IRI has been explored [24–26]. The protection offered by gallic acid in various models of organ damage is attributed to its antioxidant potential. Gallic acid has been reported to activate the expression of PPAR-γ *in vitro* [27]. However, the relevance of the interplay between gallic acid and PPAR-γ in various pathologic conditions is yet to be established *in vivo*. The present study has been designed to explore the role of gallic acid against IRI-induced AKI. Moreover, the possible involvement of PPAR-γ in gallic acid–mediated renoprotection has been investigated.

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, Ministry of Environment and Forests, Government of India. Male Wistar albino 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 cycles. The rats were allowed to acclimatize in metabolic cages for 24 h before subjecting them to surgical treatment.

The animals were anesthetized with ketamine (50 mg/kg, intraperitoneally [i.p.]) and xylazine (10 mg/kg, i.p.) and placed on a surgical platform in a dorsal position. Both kidneys were exposed through flank incisions and renal pedicles were occluded using bulldog clamps for 40 min. The clamps were then removed to start reperfusion for next 24 h. The surgical site was sealed with continuous sutures in two layers. In the sham group, animals were exposed to the similar surgical procedure except occluding 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 sacrificed by cervical dislocation. Plasma isolated from the blood was used for the estimation of creatinine, blood urea nitrogen (BUN), uric acid, sodium, and potassium levels. Moreover, the creatinine, sodium, and microproteins in urine were estimated. The kidneys were removed and washed with saline. A part of renal tissue was preserved in neutral-buffered formalin for histopathologic studies, a small portion was used for the estimation of superoxide anion generation (SAG) and the rest of tissue was minced and homogenized (10% wt/vol) in 1.17% potassium chloride solution using a Teflon homogenizer. The contents were centrifuged at 800*g* for 20 min. The pellet obtained was used for the estimation of myeloperoxidase (MPO) activity, whereas the clear supernatant was used to estimate lipid peroxides in terms of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) levels.

2.1. Estimation of creatinine clearance

The estimation of creatinine in plasma and urine samples was done by an alkaline picrate method using a commercially available kit (Angstrom Biotech Pvt Ltd, Vadodara, India). The creatinine clearance (CrCl) was calculated using the formula (CrCl = urine creatinine × urine volume/plasma creatinine × 24 × 60 × animal weight) and expressed as milliliters per minute per kilogram of rat weight.

2.2. Estimation of BUN and uric acid

The BUN and uric acid level was estimated in plasma using a commercially available kit (Angstrom Biotech Pvt Ltd). The value of BUN was expressed as milligrams per deciliter of plasma.

2.3. Estimation of plasma potassium level

The potassium level was estimated in the plasma sample using a commercially available kit (Crest Biosystems, Goa, India). The potassium level was expressed as millimoles per liter of plasma.

2.4. Estimation of fractional excretion of sodium

The sodium level was estimated in the plasma and urine samples using a commercially available kit (Crest Biosystems). The fractional excretion of sodium (Fe_{Na}) was calculated by using the formula (Fe_{Na} = plasma sodium × urine creatinine × 100/urine sodium × plasma creatinine) and expressed as percentage change.