Effects of Allopurinol and Apocynin on Renal Ischemia-Reperfusion Injury in Rats

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ABSTRACT

Background. This study evaluated the effects of allopurinol (ALP), a xanthine oxidase inhibitor, and apocynin (APC), a NADPH oxidase inhibitor, administered alone or together, on kidney damage caused by renal ischemia-reperfusion (IR) in rats.

Methods. Thirty rats were randomly assigned to 5 groups. Group 1 was a sham group. Group 2 was the renal IR control group (30-min ischemia followed by 24-h reperfusion). In groups 3 and 4, ALP or APC, respectively, was administered 1 h before the ischemia. In group 5, ALP and APC were co-administered. Blood urea nitrogen (BUN) and serum creatinine (Cr), renal tissue malondialdehyde (MDA) and superoxide dismutase (SOD), and histological changes were evaluated.

Results. A significant increase in BUN and Cr level, and histological damage was seen in the IR control group, indicating renal injury. Elevated MDA and decreased SOD levels in the IR control group demonstrated that renal damage occurred through oxidative stress. Pretreatment with ALP or APC alone or together prevented IR-induced renal damage. However, there was no significant difference between treatment with a single drug and co-administration of ALP and APC.

Conclusions. The use of ALP and/or APC before ischemia may be beneficial to ameliorate renal IR injury.

RENAL ischemia-reperfusion (IR) injury, the main causative factor for acute kidney injury, occurs in a variety of clinical conditions, including renal transplant, partial nephrectomy, shock, and sepsis [1–3]. Cell and tissue damage during the ischemic period is paradoxically aggravated further when blood flow is restored, called reperfusion. Although many mechanisms have been implicated in renal IR injury, reactive oxygen species (ROS) are a critical mediator of reperfusion injury [4,5]. Excessive production of ROS promotes renal IR injury by affecting the function of cellular DNA, proteins, and lipids [6,7]. During IR injury, ROS are generated by numerous sources, including xanthine oxidase (XO), NADPH oxidase (NOX), and the mitochondrial respiration chain [8]. Neutralization of ROS by endogenous antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), or catalase reduces the toxic effects [9]. Furthermore, to ameliorate renal injury induced by IR, several therapeutic strategies to inhibit XO and/or NOX have been investigated experimentally [10–12].

Many studies have demonstrated that allopurinol (ALP), an XO inhibitor, protects against renal IR injury [13]. During the ischemic period, adenosine triphosphate is degraded to hypoxanthine and xanthine. Xanthine dehydrogenase (XDH) is also converted to XO, which is

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related to the generation of ROS [6]. Furthermore, apocynin (APC, 4-hydroxy-3-methoxy-acetophenone) has been used as an antioxidant to prevent the formation of ROS in a number of studies. The action mechanism for APC relates to the inhibition of NOX, a critical enzyme that catalyzes the electron reduction of molecular oxygen to promote superoxide anion production [8,14]. Stimuli by various cytokines and vasoactive mediators result in enhancement of the NOX in the endothelium, which in turn generates excessive ROS [8].

ALP and APC inhibit ROS production through different mechanisms. Thus, it was proposed that combined administration of these inhibitors may be more effective than individual treatment. We therefore conducted this study to investigate the renoprotective effect of ALP and APC during renal IR injury individually and in combination.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (275–325 g) were used in this study (Central Lab Animal Inc, Seoul, Korea). All animal procedures were approved by the Kyungpook National University Institutional Animal Care and Use Committee.

Experimental Protocol

Rats were pretreated with either ALP (50 mg/kg, i.p.) or APC (20 mg/kg, i.p.) 1 h before the renal IR procedure. Under ketamine (60 mg/kg, i.p.) and xylene (10 mg/kg, i.p.) anesthesia, a right flank incision was made and right nephrectomy was performed. A left flank incision was then made, and the renal artery and vein were clamped to induce ischemia with the use of a nontraumatic vascular clamp. After 30 min of ischemia, the clamp was removed to allow reperfusion, and the skin was sutured. At 24 h after reperfusion, blood samples were obtained from the heart and organs were harvested. The serum was separated through centrifugation at 3000 rpm for 15 min. The left kidney was divided into longitudinal sections. One section was processed for histopathological examination and the other section was placed in liquid nitrogen and stored at −80°C for the malondialdehyde (MDA) and SOD analysis.

Rats (n = 30) were randomly divided into 1 of 5 groups for the experiments. Group 1 was the sham group, in which the rats underwent a sham operation without the renal IR procedure and received normal saline (sham); group 2 was the renal IR control group, in which the rats underwent the renal IR procedure and received normal saline (IR control); group 3 was the ALP treatment group, in which the rats received an ALP i.p. injection 1 h before the renal IR procedure (ALP); group 4 was the APC treatment group, in which the rats received an APC i.p. injection 1 h before the renal IR procedure (APC); and group 5 was the ALP and APC treatment group, in which the rats received ALP and APC i.p. injection 1 h before the renal IR procedure (ALP + APC).

Biochemical Analysis

Blood urea nitrogen (BUN) and creatinine (Cr) levels were used to assess renal function. Serum levels of BUN and Cr were analyzed with the use of a colorimetric assay, following the manufacturer's protocols (Asan Pharmacy, Seoul, Korea). The renal MDA levels were determined spectrophotometrically with the use of thiobarbituric acid reactive substances [15]. The absorbance of the reaction mixture was measured at 535 nm. The MDA level was expressed as nmol/g tissue, according to a standard curve. The SOD activity assay was performed with the use of the pyrogallol autoxidation method [16]. Tris-HCl and pentetic acid buffer were used as a reaction medium, and the decrease in pyrogallol absorbance was monitored at 420 nm spectrophotometrically. SOD activity was evaluated as the amount of enzyme that reduced color change by 50% and calculated as U/mg protein.

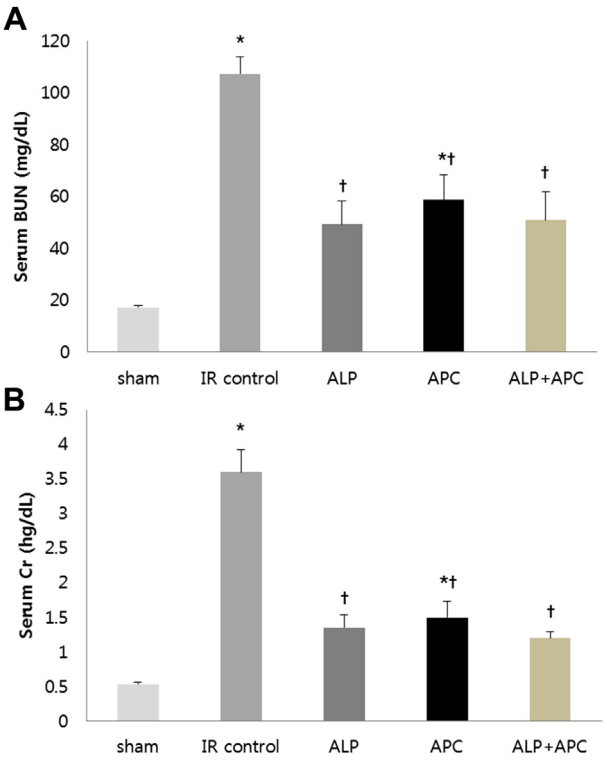

Fig 1. Serum BUN (**A**) and Cr (**B**) levels in the experimental groups. Compared with the sham group, the IR control group showed significant increases in serum BUN and Cr levels. Administration of ALP or, APC alone, and ALP + APC significantly reduced the IR-induced increase in BUN and Cr levels. There were no significant differences among the 3 drug treatment groups. *$P < .05$ versus the sham group; †$P < .05$ versus the IR control group. Results are expressed as mean ± SEM (n = 6 rats/group).

Histopathological Analysis

Kidney specimens were fixed immediately in 10% phosphate-buffered formalin, embedded in paraffin, cut in 4-μm sections, and stained with periodic acid Schiff (PAS). The sections were evaluated and scored for tubular cell form, lumen dilatation, and cast formation. Results were analyzed and graded by a scale from 0 to 3 (0, normal; 1, minimal; 2, moderate; 3, severe) [17]. The sections were evaluated by use of light microscopy at ×200 magnification.

Statistical Analysis

Data analysis was performed with the use of statistical software (SPSS, version 18.0 for Windows; SPSS, Chicago, Ill, United States). All data are expressed as mean ± SEM. The 1-way analysis of variance (ANOVA), followed by Bonferroni test for post hoc