The effect of 5-aminosalicylic acid on renal ischemia-reperfusion injury in rats

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ABSTRACT

Objectives: Ischemia-reperfusion (IR) contributes to the development acute renal failure. Oxygen free radicals are involved in the pathophysiology of IR injury (IRI). This study was designed to investigate the effects of 5-aminosalicylic acid (5-ASA), which is known antioxidant agent, in IR-induced renal injury in rats.

Materials and Methods: Male Wistar albino rats were unilaterally nephrectomized and subjected to 45 min of renal pedicle occlusion followed by 24 h of reperfusion. 5-ASA (300 mg/kg, i.p) was administered prior to ischemia. After 24 h reperfusion, urine and blood samples were collected for the determination of creatinine (Cr) and nitric oxide (NO) levels, and renal samples were taken for the histological evaluation.

Results: Treatment with 5-ASA significantly decreased serum Cr and NO levels, also significantly increased urinary Cr level and decreased histopathological changes induced by IR.

Conclusion: Treatment with 5-ASA had a beneficial effect on renal IRI. These results may indicate that 5-ASA exerts nephroprotective effects in renal IRI.

KEYWORDS: 5-aminosalicylic acid, antioxidant, nitric oxide, renal ischemia-reperfusion injury

Introduction

Ischemia (cessation of blood flow), followed by reperfusion (re-establishment of blood supply), causes serious damage to organs. Ischemia compromises the continuous supply of oxygen required by tissues to maintain physiological function. Ischemia of kidney is a common problem during kidney transplantation, or hydronephrosis, leading to renal dysfunction and injury. Moreover, when reperfusion is established, additional renal reperfusion injury occurs. This involves the development of oxidative stress via the generation of superoxide anions (O$_2^-$). Generation of reactive oxygen species (ROS) such as hydroxyl radical (OH) and O$_2^-$ as well as reactive nitrogen species (RNS) such as nitric oxide (NO) and peroxynitrite (OONO$^-$) or the decline of antioxidant defense lead to oxidative stress, which plays a critical role in the development of renal ischemia-reperfusion injury (IRI) and ischemic acute renal failure (ARF). The interaction of O$_2^-$ with NO generates OONO$^-$ that causes cellular injury via DNA strand breakage and nitration of tyrosine residues on proteins. NO, OONO$^-$ and ROS, cause profound injury to renal cell structures, particularly those of the proximal tubular cell. A major result is ATP depletion, which contributes to renal cell dysfunction and damage. Cell death occurs via a combination of necrosis or apoptosis, depending on the level of oxidative stress. Excessive ROS generation contributes to IRI. ROS scavengers, and antioxidants that remove ROS can protect against renal IRI.

5-aminosalicylic acid (5-ASA), a prescribed drug for ulcerative colitis, is a potent antioxidant and scavenger of oxygen free radicals. 5-ASA, the anti-inflammatory drug commonly used in the treatment of inflammatory bowel diseases, has been shown to possess antioxidant properties and has been found to protect against oxidative stress and tissue damage.
considered to be of particular importance in the pathologic context of these diseases. 5-ASA, mesalamine, has superoxide and hydroxyl radical scavenger properties.\textsuperscript{10,11}

Therefore, ROS and RNS were shown to contribute to the cellular damage induced by ischemia-reperfusion. The aim of the present study was to examine the potential effects of 5-ASA on renal IRI. For this purpose, we measured the levels of NO and creatinine (Cr) and assessed histological changes in rats subjected to renal IRI.

**Materials and Methods**

**Animals**

In this study, twenty male Wistar albino rats (weighing 200–250 g) were obtained from the experimental animal research center, Medical Faculty, Iran University, Iran. The rats were housed in a temperature (21 ± 2°C) and humidity (60 ± 5%) controlled room in which a 12–12 h light-dark cycle was maintained. They had free access to standard water and food. The study was approved by the University Ethics Committee.

**Surgery and Experimental Protocol**

Under anesthesia (75 mg/kg ketamine hydrochloride and 8 mg/kg xylazine, intraperitoneal injection), right nephrectomy was performed and then, the left renal pedicle (artery and vein) was occluded by placing a microvascular clamp for 45 min to induce ischemia and then placed into metabolite cage, after 24 h reperfusion, urine samples were collected.

**The Rats were Divided into Two Groups**

- Saline + ischemia-reperfusion (IR) group (control group, \( n = 10 \))
- 5-ASA + IR group (\( n = 10 \)).

5-ASA (Sigma, St. Louis, MO, USA) was administered as a 300 mg/kg single dose, intraperitoneally prior to ischemia.\textsuperscript{12}

**Biochemical Analysis**

Urine and blood samples were obtained after 24 h of reperfusion in each group; the left kidneys were removed. The blood samples were centrifuged at approximately 4000 g for 10 min at 4°C. The Cr and NO levels in the serum and urine were measured; the Cr levels were determined to assess the renal function using the Autoanalyzer (Alcyon 300, USA).

**Measurement of Nitric Oxide Concentration**

Rats under anesthesia were sacrificed after 24 h reperfusion. Serum was obtained from the blood samples. As NO is rapidly oxidized to nitrite and nitrate in biological fluids, nitrite and nitrate concentrations in serum samples were determined as a proxy for NO. Nitrite and nitrate concentrations were measured using a commercial kit according to the manufacturer's protocol (R & D Systems). The kit (total NO and nitrite/nitrate parameter assay kit), uses a modified version of the Griess test, a colorimetric assay that measures absorbance at 540 nm. Nitrite and nitrate concentration was calculated using a standard curve and expressed in micromoles (µM) per liter.\textsuperscript{13}

**Histological Evaluation**

The renal tissues were fixed in 10% buffered formalin solution, dehydrated in ascending grades of alcohol, and embedded in paraffin. Sections of 5 µm were taken, stained with hematoxylin-eosin, and examined under a light microscope (Olympus BH-2, Tokyo, Japan) in a blinded manner by a pathologist. Renal tissues were evaluated in terms of tubular lumen dilation, tubular epithelial cell vacuolization, tubular epithelial cell degeneration, and interstitial inflammatory infiltration. Histological changes were scored on a 4-point scale: (−) none, (+) mild, (++) moderate, and (+++) severe damage.\textsuperscript{14}

**Statistical Analysis**

All data are presented as a mean ± standard deviation. Significance testing between groups was performed using one-way analysis of variance with SPSS version 19 and multiple comparison post hoc test to determine significant differences between groups. A \( P < 0.05 \) was considered statistically significant.

**Results**

The effect of 5-ASA on renal IRI was investigated in 45 min of renal ischemia followed by 24 h reperfusion. Biochemical analysis results are outlined in Tables 1 and 2, and the results of the histological evaluation are shown in Table 3.

**Effects of 5-aminosalicylic Acid on Kidney Function**

Serum Cr level in the 5-ASA + IR group was significantly lower than that in the IR group (\( P < 0.05 \)). Urinary Cr level in the 5-ASA + IR group was significantly higher than that in the IR group (\( P < 0.0001 \)).

**Effects of 5-aminosalicylic Acid on Nitric Oxide Levels**

Serum NO levels in the 5-ASA + IR group were significantly lower than that in the IR group (\( P < 0.0001 \)). Urinary NO levels in the 5-ASA + IR group were higher than that in the IR group, but the difference was not statistically significant (\( P > 0.05 \)).

**Effects of 5-aminosalicylic Acid on Renal Ischemia-Reperfusion**

In the IR group, renal injury was very obvious. There were tubular lumen dilation, vacuolization, degeneration, and mononuclear cell infiltration [Figure 1a]. 5-ASA pretreatment resulted in marked attenuation of tubular lumen dilation, tubular epithelial cell degeneration, vacuolization, and mononuclear cell infiltration induced by IR [Figure 1b]. A minimum of ten fields for each kidney slide were examined and assigned for severity of changes using scores on a scale of (−) none, (+) mild, (++) moderate, and (+++) severe damage (\( n = 7 \) for each group).

**Discussion**

Renal IR is a common result of clinical procedures such as partial nephrectomy, or transplantation. Furthermore, renal IRI is a leading cause of ARF which is associated with high mortality rates. ARF is characterized by increased vascular resistance in the kidney, a low rate of filtration through the glomeruli, and tubular necrosis. These deleterious effects have been attributed to ROS generation during renal reperfusion.\textsuperscript{15,16} The main sources of free radicals are NO synthase (NOS) and the mitochondrial electron transport chain.\textsuperscript{17,18} NO plays an important role in renal function under both normal and pathophysiological conditions. Up-regulation
of NO may be associated with the cytotoxicity resulting from oxidative stress. Based on this evidence, NO is an important contributor to the pathophysiology of ARF. Our study showed that 5-ASA significantly reduced serum NO levels, which could be protective against renal IRI. NO arises from renal IR, resulting in subsequent tissue injury. A possible mechanism for the protective effect of 5-ASA following renal IR is the reduction of NO levels. Kennedy et al. demonstrated that 5-ASA dose-dependently inhibited NO production. In addition, they concluded that 5-ASA inhibits inducible NOS expression and NO production at therapeutically relevant concentrations. Couto et al. reported that 5-ASA was shown to be a strong scavenger of NO and ONOO−; also, 5-ASA showed the best ROS and RNS scavenging effects.

Our study results indicated that 5-ASA significantly reduced serum Cr level and increased urinary Cr level in rats subjected to renal IR. Based on this, 5-ASA results in an increase of glomerular filtration rate in the kidney and improves renal function after IRI. This beneficial effect may be related to a reduction in NO levels.

In our study, histological evaluation showed that IR caused changes in tubules as shown by tubular lumen dilation, vacuolization, and degeneration. Renal IR also caused an increase in interstitial inflammatory infiltration. 5-ASA severely attenuated the histopathological changes, nearly the normal renal tissue structure was preserved by 5-ASA pretreatment. This cytoprotective effect of 5-ASA may be due to its powerful antioxidant properties.

### Table 1:

**Effect of 5-aminosalicylic acid on creatinine levels**

| Groups          | IR group (mg/dl) | 5-ASA + IR group (mg/dl) |
|-----------------|------------------|----------------------------|
| Serum creatinine | 0.81±0.35        | 0.47±0.20†                 |
| Urinary creatinine | 0.84±0.18        | 3.33±0.63†                 |

†Significantly decreased when compared with IR group, P<0.05. 5-ASA=5-aminosalicylic acid, IR=Ischemia-reperfusion

### Table 2:

**Effect of 5-aminosalicylic acid on nitric oxide levels**

| Groups          | IR group (μM) | 5-ASA + IR group (μM) |
|-----------------|---------------|-----------------------|
| SNO             | 2.16±0.17     | 1.66±0.09†            |
| UNO             | 0.44±0.16     | 0.63±0.32             |

†Significantly decreased when compared with IR group, P<0.05. SNO=Serum nitric oxide, UNO=Urinary nitric oxide, 5-ASA=5-aminosalicylic acid, IR=Ischemia-reperfusion

### Table 3:

**Tubulointerstitial changes in the kidney after 24 h reperfusion (hematoxylin and eosin stain)**

| Groups          | Tubular lumen dilation | Tubular epithelial cell vacuolization | Tubular epithelial cell degeneration | Interstitial inflammatory infiltration (mononuclear cell infiltration) |
|-----------------|------------------------|--------------------------------------|-------------------------------------|---------------------------------------------------------------------|
| IR              | +++                    | +++                                  | +++                                 | +++                                                                 |
| 5-ASA           | −                      | −                                    | −                                   | −                                                                   |

5-ASA=5-aminosalicylic acid, IR=Ischemia-reperfusion

In conclusion, the current study demonstrated that treatment with 5-ASA could prevent renal IRI in a rat model. On the other hand, findings of our study suggest that 5-ASA treatment may exert antioxidant effects by decreasing NO levels. Thus, 5-ASA may have potential as a therapeutic for various clinical conditions involving IRI. However, further studies are required to clarify the exact mechanisms mediating the effect of 5-ASA in renal IRI.

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**Conflicts of Interest**

There are no conflicts of interest.

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