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# Effect of ukrain on ischemia/reperfusion-induced kidney injury in rats

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ARTICLE INFO

*Article history:*
Received 9 September 2015
Received in revised form
6 December 2015
Accepted 22 December 2015
Available online 30 December 2015

*Keywords:*
Ukrain
Ischemia–reperfusion
Kidney
NGAL
iNOS
Bax

ABSTRACT

*Background:* The aim of this study was to investigate the potential protective effect of ukrain on an experimental kidney injury model induced by ischemia and reperfusion (IR) in rats.
*Material and methods:* A total of 24 male Sprague–Dawley rats were equally and randomly separated into three groups as follows: group-1: controls (C; only laparotomy); group 2: renal ischemia–reperfusion (IR; occlusion of the renal artery for 30 min and 2 h of reperfusion); and group 3: ukrain treatment and IR applied group (U + IR; occlusion of the renal artery for 30 min and 2 h of reperfusion; ukrain was intraperitoneally administered 1 h before the IR process).
*Results:* Serum total oxidant status (TOS) and total antioxidant status (TAS) levels were measured. The oxidative stress index was determined by calculating the TOS/TAS ratio. TAS serum levels significantly increased, and TOS serum levels also prominently decreased in U + IR group, when compared with the IR group ($P < 0.001$). Mean NGAL level was remarkably higher in IR group, when compared with the U + IR group ($P < 0.001$). Caspase-3 messenger RNA (mRNA) expression level increased in IR and decreased in U + IR group ($P < 0.001$). Bcl-xL serum and mRNA expression levels increased in the U + IR group ($P < 0.001$). In addition, serum iNOS and mRNA expression levels increased in IR group and decreased in U + IR group ($P < 0.001$).
*Conclusions:* Data established from the present study suggest that ukrain may exhibit protective effect against IR-induced kidney injury and that antioxidant activity primarily modulates this effect.



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http://dx.doi.org/10.1016/j.jss.2015.12.037

## Introduction

Acute kidney injury induced by ischemia and reperfusion (IR) has been regarded as a common contributor of acute and chronic renal failure, which is caused by various medical and surgical factors [1,2]. In some previous studies, it has been reported that renal IR process led to some deleterious outcomes, which in turn has prompted the present dogma of limiting renal ischemia time between 20 and 30 min during surgical interventions [3]. Small renal masses constitute 48%–66% of all diagnosed kidney-related tumors and 38% of excised renal tumors [4]. Nearly 65,000 new kidney cancer cases are reported in the United States annually, of which approximately 45,000 are amenable to nephron-sparing surgery [5]. Beside this, only 10% of the patients are eligible for nephron-sparing surgery to avoid IR-mediated kidney damage concerns [6,7]. Some non-clamping methods have been introduced in recent past to prevent IR-induced renal injury; however, these methods were associated with higher morbidity and mortality rates [8,9]. Desire to prevent the complications may give rise to a tendency toward an increased demand for partial nephrectomy. There are plenty of agents used to elongate the mean IR duration without complication. An antineoplastic agent called as "ukrain" has been evaluated for its potential clinical efficacy in patients with pancreatic, colorectal, bladder, and breast cancers. Ukrain includes three chelidonine alkaloids combined with triaziridide. It has been reported that ukrain exhibited cytotoxic and antiproliferative effects on Ewing, melanoma, lymphoma, and glioblastoma cell cultures *in vitro* [10–13]. Ukrain exhibits these effects via antiapoptotic and cytotoxic pathways [14]. In this study, we aimed to investigate whether ukrain has protective effect against renal tissue injury induced by IR via evaluating biochemical, histopathologic and genetic (real-time polymerase chain reaction, [PCR]) parameters.

## Materials and methods

### Animals

This study was approved by the Local Ethical Committee of Dumlupinar University. A total of 24 male Sprague–Dawley rats weighing between 250 and 300 g were obtained from the Experimental Research Unit of Dumlupinar University (Kutahya, Turkey). Rats were randomly and equally divided into three experimental groups as follows: (1) control group (C; n: 8); (2) renal IR group (IR; n: 8); and (3) ukrain plus IR group (U + IR; n: 8). Group 2 was exposed to kidney IR after clamping procedure, and group 3 was subjected to intraperitoneal injection of ukrain (7 mg/kg in a volume of 0.5 mL/100 g) 1 h before renal IR process. Whole animals were kept under standard laboratory conditions with controlled temperature and humidity.

### Experimental protocol and renal IR model

Rats were starved for 18 h and were allowed to access drinking water until 20–30 min before the initiation of the experiment. Rats were anesthetized with intraperitoneal injection of ketamine/xylazine HCl at 75 mg/kg/10 mg/kg of dose. A heating mat was used to keep the body temperature at 36.1°C. Animals were placed in supine position and were fixed on dissection tray. The abdominal region was shaved, cleaned with povidone–iodine 10% antiseptic solution, and 2–3 cm of abdominal midline incision was applied. This procedure was sufficient to keep the animals under anesthesia until the end of the experiment. In renal IR injury model, renal artery was occluded with a nontraumatic microvascular clamp for 30 min. At the end of this period, the clamp was removed, and kidneys were reperfused for 120 min. During the IR process, operation area was covered with a warm moist dressing to prevent hypothermia. Ukrain flacons were donated by Nowicky Pharmaceuticals (Margaretenstrasse 7, 1040 Vienna, Austria). Flacons were consistently protected from light and stored at 7°C until use.

### Biochemical analysis

Blood samples were collected in polypropylene tubes, centrifuged at 1500 × g for 10 min at 4°C, and serum samples were separated. Sera were stored at −80°C until analysis. Renal tissue was mixed with cold working solution (50 mM of phosphate buffer, pH 7.40) and homogenized with a mechanic homogenizer (Analytik Jena SpeedMill PLUS, Germany). The mixtures were then centrifuged at 10,000 × g for 15 min at 4°C, and the supernatants were preserved for biochemical analysis by storing on ice. Tissue neutrophil gelatinase–associated lipocalin (NGAL) concentrations were measured in renal tissue homogenates via using commercial enzyme-linked immunosorbent assay (ELISA) kits (Cayman Inc, Ann Arbor, MI) on a microplate reader (BMG Labtech Spectrostar Nano, GmbH, Ortenberg, Germany). Tissue protein concentrations were measured based on the Bradford method and serum urea, creatinine concentrations were measured on Beckman Coulter AU680 analyzer [15]. Renal tissue total antioxidant status (TAS) and total oxidant status (TOS) levels were measured on Beckman Coulter AU680 analyzer (Beckman Coulter, Miami, FL) using commercial reagents (Rel Assay Diagnostic, Gaziantep, Turkey). TAS and TOS were determined using a novel automated measurement method developed by Erel [16,17]. The results of TAS and TOS were expressed in terms of respectively micromolar Trolox equivalent per milligram tissue protein (mmol Trolox Eq/mg protein) and micromolar hydrogen peroxide equivalent per milligram tissue protein (μmol H2O2 Eq/mg protein).

### Histopathological analysis

Kidney tissue samples were removed and fixed in 10% neutral buffered formalin solution. Samples were then undergone routine tissue processing steps, embedded in paraffin blocks, and were cut into sections at 5-μm thickness via using a microtome. After deparaffinization, tissue sections were stained with hematoxylin–eosin for routine histopathologic observation. An optic microscope (Olympus BX-51, Tokyo, Japan) was used for evaluation. Semiquantitative evaluation method was used to describe and quantify the degree of IR-mediated injury.