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Investigation of the effect of crocin pretreatment on renal injury induced by infrarenal aortic occlusion

Fahri Adali, MD,[a,*] *Yucel Gonul, PhD,*[b] *Mustafa Aldemir, MD,*[a] *Omer Hazman, PhD,*[c] *Ahmet Ahsen, MD,*[d] *Mehmet Fatih Bozkurt, PhD,*[e] *Ozlem Gulec Sen, MD,*[a] *Ibrahim Keles, MD,*[f] *and Hikmet Keles, PhD*[e]

[a] *Department of Cardiovascular Surgery, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[b] *Department of Anatomy, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[c] *Biochemistry Division, Department of Biochemistry, Faculty of Science and Arts, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[d] *Department of Internal Medicine, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[e] *Department of Pathology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[f] *Department of Urology, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*

ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form
10 February 2016
Accepted 10 March 2016
Available online 19 March 2016

Keywords:
Crocin
Infrarenal aortic occlusion
Renal injury
Apoptosis
Ischemia–reperfusion

ABSTRACT

Objective: Ischemia and reperfusion (IR) injury is an important complication of abdominal aortic surgery, and it mainly affects the lower extremities and remote organs. In the present study, we aimed to investigate the possible protective effect of crocin in IR-mediated kidney damage.
Materials and methods: A total of 24 adult male Wistar–Albino rats were equally and randomly separated into three groups as follows: sham laparotomy, IR, and IR + crocin. Infrarenal aortic occlusion and reperfusion was applied for 1 and 2 h, respectively. Tissue samples were removed and collected. Biochemical and histopathologic analyses were performed.
Results: Urea, blood urea nitrogen, creatinine, renal tissue tumor necrosis factor α, interleukin (IL)-6, IL-18, interferon gamma, IL-1β, total oxidant status, and oxidative stress index levels were significantly higher in IR group, when compared with other groups. These improvements were also demonstrated with some parameters including total score of histopathologic damage, Tunel, Bax, and Caspase-3 expression levels, and these parameters were prominently higher in the IR group, when compared with the other groups. Nevertheless, Bcl2 expression degree was prominently lower in the IR group than those in the other two groups.
Conclusions: Data established from the present study suggest that crocin can preclude renal damage in infrarenal aortic occlusion models.

Introduction

Transient occlusion of the abdominal aorta (AA) at infrarenal level may lead to ischemia and reperfusion (IR)–induced tissue injury not only in lower limbs but also in various remote organs such as kidneys, lungs, and heart.[1] Remote organ dysfunction after AA surgery is an important complication, which is closely related to postoperative mortality and

* *Corresponding author*. Department of Cardiovascular Surgery, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar 03200, Turkey. Tel.: +90 505 6370016; fax: +90 272 246 3300.
E-mail address: drfadali@yahoo.com (F. Adali).
0022-4804/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2016.03.022

morbidity. IR-induced acute kidney injury (AKI) is a frequently seen condition with incidental risk between 15% and 22%. AKI may lead to a subsequent acute renal failure condition, which can eventually enforce the patients to take mandatory dialysis treatment.[2,3] Revascularization process after prolonged ischemia of the lower limbs may cause renal failure, and this clinical condition is called as "myonephropathic–metabolic syndrome".[4] Postulated underlying mechanisms such as hypoxia, inflammatory responses, and free radical damage are probably multifactorial, and these are associated with each other in IR-induced organ damage.[5] Reactive oxygen species (ROS) and massive release of systemic inflammatory mediators involve in the pathophysiology of IR-mediated injury. Endogenous antioxidants that act against ROS during the reperfusion period carry out a crucial functionality to reduce this damage.[6]

Crocin is a carotenoid compound, which is mainly found in saffron, crocus, and gardenia plants. It is a water-soluble diester formed from disaccharide gentiobiose and the dicarboxylic acid crocetin. Saffron has been widely used in treatment of many diseases including depression and cancer in the past, and it has three basic metabolites: crocin, crocetin, and safranal.[7,8] Crocin (crocetin glycoside) is the main chemical component responsible for the yellow color of saffron. Antioxidant, hypolipidemic, neuroprotective, and analgesic effects of crocin have been reported in previous studies.[9]

In the present study, we aimed to investigate whether crocin has anti-inflammatory, antiapoptotic, antioxidant, and protective effects on IR-induced AKI after infrarenal aortic occlusion (IAO) via using biochemical, histopathologic, and immunohistochemical methods.

Materials and methods

Animals

A total of 24 male Wistar–Albino rats weighing between 250 and 300 g (mean 270 g) were included in this study. Rats were kept under standard laboratory conditions with 24°C-26°C temperature, 50%-60% humidity, and 12 h of light and dark cycle, which comply with the Principles and Guidelines for Experimental Animals issued by The National Health and Medical Research Council and Guide for the Care and Use of Laboratory Animals (NİH issue no. 85-23, 1985 revised) prepared by The National Institutes of Health. Animals were fed with standard rat chow and regular water. Rats were starved for 12 h before the experiment. The protocol and experimental method of this study were approved by the Ethical and Animal Welfare Committee of Afyon Kocatepe University.

Study design

Animals were equally and randomly separated into three groups as follows:

Group I (sham): Laparotomy and dissection of the infrarenal AA were carried out with the identical intervention time and stress of the other groups, but, the aorta was not clamped. The abdominal incision was closed, and the animals were followed up for 3 h to stimulate the IR interval in the other groups.

Group II (IR): After laparotomy and dissection of the infrarenal AA, ischemia was induced by clamping infrarenal AA for 60 min, and reperfusion was applied by removing the clamp for 2 h.

Group III (IR + crocin): In this group, crocin (100 mg/kg b.w.)[10] was intraperitoneally (i.p) given 30 min before the laparotomy. Ischemia was induced by clamping of the infrarenal AA for 60 min, and then, reperfusion was performed for 2 h.

Anesthesia and surgical procedure

Rats were starved for 12 h before the operation. Xylazine (Rompun; Bayer, Turkey) was intramuscularly injected at 5 mg/kg dose as premedication, and animals were anesthetized with ketamine (Ketalar; Parke-Davis, Eczacibasi, Turkey) at 40 mg/kg dose. After anesthesia, rats were fixed on the operation table to avoid instabilization. The abdomen was covered under sterile conditions and incised through median laparotomy after shaving and washing with povidone–iodine solution. The intestines were gently deviated to right, taken out, and wrapped with the warm wet compresses to minimize the hypothermia and dehydration. The retroperitoneum was accessed with dissection, and the AA was explored. After turning the AA through 1 cm inferior to the renal vein and 1 cm superior to the iliac bifurcation, anticoagulation was performed with 150 U/kg i.v. heparin. After 5 min of heparin application, the infrarenal AA was occluded for 60 min using atraumatic microvascular clamps (Vascu-Statt II, Midi Straight 1001-532; Scanlan Int, St. Paul, MN). Aortic ischemia was confirmed with the loss of the distal aortic pulsation, and reperfusion was confirmed by the observation of satisfactory pulsation in the femoral region with Doppler ultrasonography (BT-200 Vascular Doppler HI-dop; Korea) after removing the clamps.

All the subjects were injected with 1 mg/kg i.v. protamine sulfate in the beginning of the reperfusion process to neutralize the effect of heparin. After bleeding control, the intestines were inserted into the abdomen. After the intraperitoneal administration of 10-mL saline solution, the abdomen was closed with sterile drapes. After 120 min of reperfusion, rats were killed, and tissue/blood samples were collected. Unlike other groups, group III was intraperitoneally injected with crocin (Sigma-Aldrich Chemical Co, St Louis) at 100 mg/kg b.w. dose 30 min before the laparotomy.

Kidney tissue and blood samples

Blood samples at 3-mL volume were collected from the inferior vena cava for biochemical analysis. The samples were centrifuged, and sera were obtained. Serum samples were preserved at −70°C until biochemical analysis. In addition, the right kidneys were removed for histopathologic and immunohistochemical evaluation, whereas the left kidneys were taken to analyze oxidative stress parameters and cytokines.

Biochemical analysis

Serum levels of creatinine, urea, and blood urea nitrogen were measured in an autoanalyzer (Cobas 6000; Roche,