Biomedicine & Pharmacotherapy 83 (2016) 160–166

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Investigation of the effect of safranal and crocin pre-treatment on hepatic injury induced by infrarenal aortic occlusion

Ziya Taner Ozkececi[a,*], Yucel Gonul[b], Yasemin Yuksel[c], Afra Karavelioglu[d], Kamil Tunay[e], Yusuf Gulsari[b], Onder Cartilli[b], Omer Hazman[f], Ahmet Bal[a]

[a] Department of General Surgery, Faculty of Medicine, Afyon Kocatepe University, Afyon, 03200, Turkey
[b] Department of Anatomy, Faculty of Medicine, Afyon Kocatepe University, Afyon, 03200, Turkey
[c] Department of Histology and Embryology, Zekai Tahir Burak Women's Health and Research Hospital, Ankara, 06260, Turkey
[d] Department of Pediatric Surgery, Faculty of Medicine, Afyon Kocatepe University, Afyon, 03200, Turkey
[e] Department of Emergency Medicine, Faculty of Medicine, Afyon Kocatepe University, Afyon, 03200, Turkey
[f] Department of Chemistry, Biochemistry Division, Faculty of Science and Arts, Afyon Kocatepe University, Afyonkarahisar 03200, Turkey

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 8 May 2016
Accepted 14 June 2016

Keywords:
Ischemia-reperfusion
Liver injury
Apoptosis
Bax
Blc-2
Caspase-3

ABSTRACT

Ischemia-reperfusion (IR) injury of the liver is an unresolved problem that occurs during certain surgical approaches, including hepatic, cardiac and aortic operations. In this study we aimed to investigate whether crocin and safranal had protective effects on liver IR injury induced in an infrarenal aortic clamping (IRAC) model. Male Wistar-Albino rats (n = 32) were divided into four groups with 8 animals each as follows: Sham, IR, IR + crocin, and IR + safranal. The infrarenal aorta (IRA) was clamped for 60 min for the ischemic period and allowed to reperfuse for 120 min. Blood and tissue samples were collected for biochemical, histological and immunohistological analysis. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found to be significantly higher in the IR group than the sham group (respectively; $p = 0.015$, $p < 0.001$). There were significant differences between the IR group and the IR + crocin group or the IR + safranal group in AST levels (respectively; $p = 0.02$, $p < 0.001$). ALT showed a significant decrease in the IR + crocin group compared to the IR group ($p < 0.05$). We also observed histopathological changes among the groups. Bax and Caspase-3 expression in the IR group was remarkably higher than in the other groups. Caspase-3 and Bax expression in the IR + crocin and the IR + safranal groups were significantly lower than in the IR group. Nevertheless, there were no significant differences in BCL2 expression among the groups. IRAC is a cause of IR injury in the liver. This study showed that crocin and safranal have protective effects on IR induced liver injury.

1. Introduction

Remote organ injury caused by infrarenal aortic clamping (IRAC), which can be encountered during surgical interventions related to the abdominal aorta, is a complex process affecting the lower extremities as well as remote organs such as the heart, liver, kidney, and lungs [1–3]. Reperfusion of acutely ischemic abdominal organs causes local and distant organ damage and may contribute to high morbidity and mortality [4].

Several factors have been suggested to play a role in the pathophysiology of ischemia-reperfusion (IR) injury, such as free oxygen radicals, polymorphonuclear leukocytes, the complement system and endothelial cells. These are cellular and humoral mechanisms that have complex relationships with each other. While the amount of oxygen, the decrease in energy production and synthesis of the antioxidant enzymes, toxic substances, ions such as Ca and Na, pro-inflammatory cytokines and leukocyte adhesion molecules increase during ischemia. This makes cells relatively prone to damage during reperfusion. When blood flow resumes to ischemic tissue, cells are exposed to large amounts of oxygen, which is a source of radical oxygen species in the previously damaged tissue. However, internal antioxidant mechanisms are insufficient during IR injury. This is why many drugs have been used to reduce the effects of hepatic IR injury [5–8]. Nevertheless, studies on this subject are as yet insufficient to reach a definitive result.

* Corresponding author at: Department of General Surgery, Faculty of Medicine, Afyon Kocatepe University, Ali Cetinkaya Campus, Izmir Road 8. Km, 03200 Afyonkarahisar, Turkey.
E-mail address: tanerozkececi@gmail.com (Z.T. Ozkececi).

http://dx.doi.org/10.1016/j.biopha.2016.06.027
0753-3322/

Bcl-2 is protective against both apoptotic cell death and multiple cellular events. It was reported that apoptosis can be prevented by a decreased ratio of Bax/Bcl-2 [9]. Intracellular Bcl-2 to Bax ratio is of paramount importance and may give a clue to whether a cell would progress to apoptosis. If Bax is greater, then the cell begins to progress towards apoptosis. However, apoptosis will be inhibited when Bcl-2 is greater [2]. Various substrates like Bax, Bak and Caspase trigger the apoptotic process; however Bcl-2, Bcl-xL or Mcl-1 family proteins generally act as antiapoptotic mediators, which may cause a delay or inhibition in apoptotic enzyme release. Moreover, overexpression of antiapoptotic proteins provide a survival benefit in cells treated with chemotherapeutic agents [10].

As shown in rat models, liver [8,11] and kidney [2] remote organ injury mediated IRAC can be attenuated using different protective agents. Our primary goal is to minimize damage after IRAC. Therefore, we investigated crocin and safranal as strong protective agents of liver IR induced by IRAC.

Crocus sativus L., cultivated in Southwest Asia, Spain, France, Italy, Turkey and Iran, is a flowering plant used in traditional medicine. Saffron, derived from the flower of *Crocus sativus* L., contains at least 150 chemicals. Crocin and safranal, which are the main components of saffron, are known to have anti-inflammatory, anticonvulsant, antitussive, antioxidant, anxiolytic and antidepressant effects [12]. In our previous studies, we showed that crocin has anti-inflammatory, antiapoptotic, antioxidant and protective effects on acute kidney injury following IRAC [2] and brain injury following four vessels oclusion [13].

As far as we know, the effects of crocin and safranal on liver IR injury mediated by IRAC have not been studied thus far. In this study we therefore aimed to investigate whether crocin and safranal had protective and anti-apoptotic effects on IRAC induced liver IR injury using hematoxylin and eosin staining and Caspase-3, Bax, and Bcl-2 immunohistochemical staining.

2. Material and methods

Study protocols and experimental procedures were approved by the Ethics and Animal Welfare Committee of Afyon Kocatepe University and the experiments were performed according to 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 (NIH issue no. 85–23, 1985 revised) prepared by The National Institutes of Health.

2.1. Animals

Thirty two male Wistar-Albino rats, weighing 250–300 g, were included in the study. During the experimental procedure, the animals were kept under standard laboratory conditions including 24–26 °C room temperature, 50–60% humidity, 12 h of light and night cycle and were allowed access to food and water ad libitum. The animals were fasted 12 h before the experiment.

2.2. Study groups

The 32 rats were randomly divided into four groups comprising 8 rats in each group as follows:

Group I (Sham, n = 8): Except for aorta clamping, procedures for laparotomy and dissection of the infrarenal aorta (IRA) were performed the same as in the other groups for equivalent operational time and stress. After closing the abdominal incision, the animals were kept under observation for 3 h to simulate the IR interval in the other groups. No drugs were administered to the rats.

Group II (IR, n = 8): After laparotomy, the IRA was dissected carefully and clamped to create ischemia for 60 min and the clamp was removed to apply reperfusion for two hours.

Group III (IR + Crocin, n = 8): 100 mg/kg crocin (Sigma-Aldrich Chemical Co., St Louis, USA) was injected intraperitoneally 30 min before laparotomy [14]. The IRA was clamped for 60 min to create IR, achieved by opening the clamp for two hours.

Group IV (IR + Safranal, n = 8): 100 mg/kg safranal (Sigma-Aldrich Chemical Co., St Louis, USA) [15] was administered intraperitoneally 30 min before the surgical procedure. After applying 60 min of ischemia by blocking IRA blood flow, reperfusion was supplied by opening the aortic clamp for two hours.

2.3. Surgical intervention

Animals were fasted for 12 h before the surgical procedure. After intramuscular injection of 5 mg/kg Xylazine (Rompun, Bayer, Turkey) for premedication, 40 mg/kg ketamine (Ketalar, Parke-Davis, Eczacibasi, Turkey) was used for anesthesia. Rats were fixed to the operating table from four extremities to prevent movement. A median laparotomy was performed after shaving, washing with povidone-iodine solution and covering the abdomen under sterile conditions. The intestines were covered with warm, wet gas compresses to avoid heat and fluid loss after deviating to the right. The abdominal aorta was explored with retroperitoneal dissection and turned 1 cm below the renal vein and 1 cm above the iliac bifurcation. After administering 150 U/kg heparin intraperitoneally and waiting 5 min for anticoagulation, the IRA was obstructed for 60 min using atraumatic microvascular clamps (Vascu-Statt II, midi straight 1001-532; Scanlan Int., St. Paul, MN, USA). Disappearance of distal aortic pulsation verified occlusion of the IRA and determination of convincing pulsation in the femoral region with Doppler ultrasonography (BT-200 Vascular Doppler HI-dop, 7th Fl., A Bldg., Woolim Lions Valley 5-cha, 302, Korea) confirmed reperfusion after removing the clamps. Before removing the clamps for reperfusion, 1 mg/kg intraperitoneal protamine sulphate was given to the rats to neutralize the effect of heparin. Following hemostasis, the compresses covering the intestines were removed and the intestines were placed into the abdomen. Fluid resuscitation was performed intraperitoneally with 10 cc of saline and the abdominal incision was closed with a sterile dressing. Rats were sacrificed to collect tissue/blood samples after reperfusion for 120 min. Crocin and safranal were prepared just before application to the rats. Crocin was dissolved in physiological saline at a dose of 100 mg/kg. Safranal was emulsified in physiological saline at a dose of 100 mg/kg before being administered to the experimental animals intraperitoneally. While 100 mg/kg crocin was injected intraperitoneally in group III, 100 mg/kg safranal was administered intraperitoneally in group IV 30 min before laparotomy. No drugs were administered in groups I and II.

2.4. Collection of blood and tissue samples

Blood samples (3 ml) were taken from the vena cava inferior for biochemical analysis and centrifuged. Serum samples obtained in this manner were preserved at −70 °C until biochemical analysis. In addition, the right lobe of the liver was removed for histopathological and immunohistochemical assays.

2.5. Biochemical analysis

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed in an autoanalyser (Cobas 6000, Roche, Switzerland) as indicators of liver ischemia.