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Protective effects of curcumin supplementation on intestinal ischemia reperfusion injury

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ABSTRACT

The aim of this study was to investigate the effects curcumin on inflammation and oxidative stress markers in the intestinal ischemia reperfusion (IIR) injury induced rats. Rats were divided into four groups: sham (S), intestinal IR (IIR), curcumin plus sham (CS), and curcumin plus intestinal IR (CIIR). Curcumin was given 200 mg kg^{-1} for 20 days. IIR was produced by 45 min of intestinal ischemia followed by a 120 min of reperfusion. Although interleukin-6 levels tended to increase in IIR group tumor necrosis factor-α levels were not different. Intestinal myeloperoxidase activity in CS group was lower than IIR group. In intestine and heart tissues, malondialdehyde levels in CS and CIIR groups were lower than S and IIR groups. Superoxide dismutase activity in CIIR group was higher than IIR group in intestine and lung tissues. Curcumin has a protective role against ischemia reperfusion injury.

Introduction

Intestinal ischemia–reperfusion (IR) is a common situation for many diseases such as acute mesenteric ischemia, small bowel transplantation, abdominal aortic aneurysm, hemorrhagic, traumatic or septic shock, and even severe burns (Mallick et al. 2004). The intestine is one of the most susceptible organs to ischemia. Tissue damage is occurred not only by ischemia but also by reperfusion (Mallick et al. 2004; Pierro and Eaton 2004). The damage is dramatically augmented by a large number of events, such as formation of reactive oxygen and nitrogen species, release of iron storage, damage of the microvasculature of IR organs, inflammatory cytokines, complement activation, and neutrophil infiltration at the injury site (Carden and Granger 2000). Intestinal IR may also induce multiple organ dysfunction or remote organ injury (Pierro and Eaton 2004).

Although intestinal IR induced remote organ injury induce damage to any distant organ, the onset of the injury is generally occurred in the lungs (Carden and Granger 2000; Ishii et al. 2000; Vinardi et al. 2003). In addition to the lungs, intestinal IR induced remote organ injury was demonstrated in many vital organs such as heart (Horton and White 1991, 1993), liver (Williams et al. 2001; Pierro and Eaton 2004) and kidney (Aldemir et al. 2002). It has been reported that reactive oxygen species and inflammatory leukocytes play role in progression of intestinal IR induced remote organ injury (Granger and Korthuis 1995).

The importance of antioxidant treatment in cell protection against intestinal IR injury has been demonstrated for many years and many substances have been proposed for this purpose. Curcumin is an orange-yellow component of turmeric (*Curcuma longa*) and derived from the rhizome of the plant. Many pharmacological properties of curcumin such as antiinflammatory, antimicrobial, antiviral, antifungal, antioxidant, chemosensitizing, radiosensitizing, and wound healing activities have been demonstrated (Jagetia and Aggarwal 2007). It has been also demonstrated that curcumin has protective role in many experimental IR injury models such as cardiac (Fiorillo et al. 2008), kidney (Bayrak et al. 2008), hepatic (Shen et al. 2007) and cerebral (Shukla et al. 2008). In the present study, we hypothesized that curcumin may have a protective role in intestinal IR induced remote organ injury due to its antiinflammatory and antioxidant effects. To the best of our knowledge, only two studies (Karatepe et al. 2009; Yucel et al. 2011) have been investigated the effect of curcumin supplementation in intestinal IR. Additionally in a recent study, Guzel et al. (2012) have been demonstrated that short term curcumin supplementation (3 days) has protective effect on intestinal IR induced acute lung injury. However, no study to date has investigated the effect of long term curcumin supplementation on intestinal IR induced remote organ injury such as lungs and heart. Therefore in this study we aimed to evaluate the antiinflammatory and antioxidant effects of curcumin in experimental intestinal IR model by assessing the both proinflamatory cytokine levels in blood samples and oxidative stress and antioxidant markers in intestine, lung and heart tissues.

Methods

Animals

Forty male Wistar rats (aged 16 weeks) weighing between 300 and 350 g were used in the study. All animals were kept in

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polycarbonate cages and maintained on a 12:12-h light/dark cycle in a temperature (22 ± 2 °C) and humidity (50%) controlled room. Rats were given standard rat chow and tap water *ad libitum*. All surgical procedures and care were approved by the Animal Ethics Committee of Experimental Medicine Research and Application Center of Selçuk University. All surgical procedures performed in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institute of Health.

Experimental design and surgical procedures

Rats were randomly divided into four groups as follows:

I) Sham (S) (*n*: 10): Animals were exposed to laparotomy without clamping the superior mesenteric artery (SMA).
II) Intestinal IR (IIR) (*n*: 10): Rats were exposed to laparotomy with occlusion of the SMA for 45 min, followed by 120 min of reperfusion period.
III) Curcumin plus sham (CS) (*n*: 10): Curcumin was given orally for 20 days. Thereafter animals were exposed to laparotomy without clamping the SMA.
IV) Curcumin plus intestinal IR (CIIR) (*n*: 10): Curcumin was given orally for 20 days. Thereafter animals were exposed to laparotomy with occlusion of the SMA for 45 min, followed by 120 min of reperfusion period.

The curcumin supplemented groups received curcumin (C1386, Sigma Chemical Co., St. Louis, MO, USA) *via* oral gavage at doses of 200 mg kg body weight^{-1} per day dissolved in corn oil (C8267, Sigma Chemical Co., St. Louis, MO, USA) for 20 days before the operation. In the previous studies (Ireson et al. 2001; Mahmmoud 2005) it has been reported that naturally occurring ratio for curcuminoids is ≈5% bisdesmethoxycurcumin, ≈15% desmethoxycurcumin, and ≈80% curcumin and the effectiveness of this product and dosage has been demonstrated.

Before surgical procedures animals were fasted for 12 h with free access to water. The rats were anesthetized by intramuscular injection of ketamine hydrochloride (50 mg kg body weight^{-1}) and xylazine (10 mg kg body weight^{-1}). The abdominal region shaved and cleaned with antiseptic solution. Using sterile technique, the abdomen was opened with a middle incision. Intestines were exteriorized and the SMA was dissected. After identifying the SMA, the small intestine was subjected to the ischemia by occluding the SMA with a nontraumatic microvascular clamp. Adequate occlusion was confirmed by pallor and the absence of pulsation in the mesenteric vessels of the small intestine. The clamp was removed 45 min later and reperfusion occurred for 120 min. Reperfusion was confirmed by the restoration of pulsation and color prior to closing incision. During the reperfusion phase the abdominal cavity was closed with sutures. Rats in the sham group were submitted to the abdominal incision but not the intestinal IR. A heat lamp was used to maintain the body temperature.

At the end of reperfusion period blood samples were taken by cardiac puncture while animals still anesthetized. Rats were sacrificed by cervical dislocation and tissue samples were obtained from the small intestine −5 cm proximal to the ileocecal region-, lung and heart tissues. Tissue specimens were washed with ice-cold saline and then stored at −80 °C until the time of biochemical analysis. Blood samples were centrifuged at 1500 × *g* for 10 min and the serum samples were separated and stored at −80 °C.

Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels were analyzed in serum samples. Myeloperoxidase (MPO) activity was analyzed in intestinal tissue for determination of ischemia. Malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities were analyzed in intestine, heart and lung tissues.

Biochemical analysis

Tissue samples were homogenized in 10 volumes of ice-cold Tris–HCl buffer (50 mmol L^{-1}, pH 7.4) using a homogenizer (Wise Mix HG-15; Daihan Scientific, Seoul, Korea) after cutting the organs into small pieces. Levels of MDA and MPO and SOD activities were measured in this homogenate. Also some of the homogenate was centrifuged and its supernatant was separated. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture (5/3, volume per volume [v/v]). After centrifugation at 5000 × *g* for 30 min, the upper layer (the ethanol phase) was used in the protein assays.

Serum IL-6 and TNF-α levels were detected by enzyme-linked immunosorbent assay (ELISA). The relationship between optical density and cytokine concentrations was defined using the standard curve according to the manufacturer's instructions (Bender Med systems Diagnostics, Vienna, Austria). IL-6 and TNF-α levels were expressed as pg mL^{-1}.

For quantitative assessment of neutrophil infiltration MPO activity was measured in the intestinal tissues using a rat MPO ELISA kit according to the manufacturer's instructions (Hycult Biotechnology, Uden, the Netherlands). The absorbance was measured at 450 nm. The MPO activities were expressed as ng mg protein^{-1}.

Lipid peroxidation in the tissues was assessed by the thiobarbituric acid reactive substance (TBARS) method using a commercially available kit according to the manufacturer's recommendations and malonaldehyde as a standard (10009055, Cayman Chemical, Ann Arbor, MI). The principle of the method is based on the reaction between MDA and TBA at 100 °C and low pH. Absorbance was measured at 530 nm. MDA levels were expressed as nmol g wet tissue^{-1}.

Total SOD activity (Cu/Zn, Mn and FeSOD) was determined using a commercialized chemical SOD assay kit (706002, Cayman Chemical, Ann Arbor, MI). The kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. The reactions were initiated by adding xanthine oxidase, by incubating 20 min at room temperature, and then by reading the absorbance at 450 nm. One unit of SOD activity was defined as the amount of enzyme needed to inhibit 50% dismutation of the superoxide radical. SOD activities were expressed as U mg protein^{-1}.

Protein content of the tissues was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Statistical analysis

All statistical analyses were performed using SPSS for Windows, 15.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted after checking for normality using the Shapiro–Wilk test and homogeneity of variance using the Levene test. The result of Shapiro–Wilk test demonstrated that all variables normally distributed. Post hoc Tukey's HSD test was applied for homogeneous variances. If variances were not homogeneous, the Tamhane test was used. P values < 0.05 were regarded as statistically significant.

Results and discussion

Serum IL-6 and TNF-α levels are demonstrated in Table 1. Although IL-6 levels tended to increase in IIR group, neither intestinal IR nor curcumin pretreatment did affect IL-6 and TNF-α levels in blood samples ($p > 0.05$). Effects of intestinal IR and curcumin supplementation on intestinal MPO activity are demonstrated in Fig. 1 (304.53 ± 89.42, 363.20 ± 67.74, 253.98 ± 52.46, 316.97 ± 106.37 in S, IIR, CS and CIIR groups, respectively). Intestinal MPO activity was lower in CS group compared to the IIR group