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Mangiferin protects against intestinal ischemia/reperfusion-induced liver injury: Involvement of PPAR-γ, GSK-3β and Wnt/β-catenin pathway

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ABSTRACT

Aim: Mangiferin (MF), a xanthonoid from *Mangifera indica*, possesses anti-inflammatory, immunomodulatory, and potent antioxidant effects; however, its protective effect against mesenteric ischemia/reperfusion (I/R)-induced liver injury has not been fully clarified. The study was designed to assess the possible mechanism of action of MF against mesenteric I/R model.

Main methods: Male Wister rats were treated with MF (20 mg/kg, i.p) or the vehicle for 3 days before I/R, which was induced by clamping the superior mesenteric artery for 30 min followed by declamping for 60 min.

Key findings: The mechanistic studies revealed that MF protected the 2 organs studied, *viz.,* liver and intestine partly *via* increasing the content of β-catenin and PPAR-γ along with decreasing that of GSK-3β and the phosphorylated NF-κB-p65. MF antioxidant effect was evidenced by increasing contents of total antioxidant capacity and GST, besides normalizing that of MDA. Regarding the anti-inflammatory effect, MF reduced IL-1β and IL-6, effects that were mirrored on the tissue content of MPO. Moreover, MF possessed anti-apoptotic character evidenced by elevating Bcl-2 content and reducing that of caspase-3. In the serum, intestinal I/R increased the activity of ALT, AST, and creatine kinase.

Significance: The intimated protective mechanisms of MF against mesenteric I/R are mediated, partially, by modulation of oxidative stress, inflammation, and apoptosis possibly *via* the involvement of Wnt/β-catenin/NF-κβ/ PPAR-γ signaling pathways.

1. Introduction

Intestinal ischemia/reperfusion (I/R) is seen in a variety of clinical syndromes, such as vasculopathies, coagulopathies, mechanical obstruction (Levine and Jacobson, 1995), trauma or hemorrhagic shock (Chandler et al., 1997).

In I/R injury, progressive systemic inflammatory response syndrome often ensues due to imbalance between free radical (FR) generation and endogenous antioxidant mechanisms leading to the development of I/R-induced organ injury (Fink, 2003). Moreover, after reperfusion, lymphocytes accumulate other organs resulting in neutrophil infiltration and parenchymal damage (Deitch et al., 2006). Gut hypoxemia itself, in the absence of blood loss or tissue injury, induces pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and IL-6 (Scannell et al., 1993; Ertel et al., 1995) contributing to systemic inflammatory response syndrome (SIRS).

Cytokines and TNF-α production induce the expression of adhesion molecules on vascular endothelial cells and stimulate neutrophil-attracting chemokines release (Jaeschke et al., 1990), with the consequent neutrophils infiltration. IL-1β induces TNF-α and IL-6 release mainly by stimulating hepatic cells (Dinarello, 1996). Nuclear factor kappa B (NF-κB) family regulates all these inflammatory cytokines and plays an important role in mesenteric ischemia (Yao et al., 2009). This injurious cascade entails also the liver, which is the first remote organ to be injured by intestinal I/R because of the washout of these inflammatory mediators from the reperfused intestine (Clavien et al., 1996; Horie et al., 1997b).

Apart from its role in inflammation, IL-6 released in the small intestine induces apoptosis (Wu et al., 2003), which is a major contributor for I/R-induced destruction in small intestinal epithelial cells (Schaller and Graf, 2004). Following ischemia, the pro-apoptotic proteins, Bax and Bad, are activated promoting the release of cytochrome c (Abe et al., 2004; Dluzniewska et al., 2005) that imitates a downstream signal ending with the executioner protein caspase-3 (Chiang et al., 2007).

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The Wnt/β-catenin is one of the signaling pathways implicated in intestinal I/R insult (Cuzzocrea et al., 2007), which can further be modulated by glycogen synthase kinase (GSK)-3β (Woodgett, 2001). The latter controls many cellular events, like apoptosis, proliferation, differentiation, and oxidative stress (Thompson and Monga, 2007) and affects the activity of NF-κB (Ali et al., 2001).

The nuclear factor, peroxisome proliferator-activated receptor-γ (PPAR-γ), is involved in the regulation of intestinal inflammation (Su et al., 1999) by providing a protective effect against both local and remote organs injury after intestinal I/R *via* inactivating NF-κB in intestinal tissue (Nakajima et al., 2001).

Mangiferin (MF), a naturally occurring glucosyl xanthone, possesses miscellaneous pharmacological activities including, antioxidant (Sellamuthu et al., 2013), antitumor (Rajendran et al., 2008), immunomodulatory (Makare et al., 2001; Leiro et al., 2004), antiviral, and antimicrobial (Yoosook et al., 2000) effects. Furthermore, it displays anti-apoptotic and anti-inflammatory activities, as verified in different animal models (Marquez et al., 2012; Gong et al., 2013). Despite its vast pharmacological effects described, the potential hepatoprotective effect of MF against intestinal I/R insult has not been evaluated, which is the goal of the current work, besides studying some of the possible mechanism(s) of action.

2. Material and methods

2.1. Animals

Adult male Wister albino rats (250–300 g), obtained from the animal house of National Research Center (Giza, Egypt), were used in this study. Rats were housed at the facility of Faculty of Pharmacy, October 6 University, for one week prior to the experiment. Animals were housed under controlled conditions (temperature of 22 ± 2 °C, humidity 60–70%, and 12 h light/dark cycles) and were allowed a free access to tap water and pelleted standard rat chow diet. The animal experiments described later comply with the recommendations of the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health. The study protocol was approved by the guidelines of the Research Ethical Committee of the Faculty of Pharmacy, Cairo University, Cairo, Egypt (Permit Number: PT 240). All surgery was performed under thiopental anesthesia, and all efforts were made to minimize animal suffering.

2.2. Induction of mesenteric ischemia/reperfusion (I/R) injury

Ischemia/reperfusion (I/R) was performed according to Murata et al. (2006). Rats under thiopental anesthesia (25 mg/kg, i.p) (Totsuka et al., 1998) were placed supine, where a midline laparotomy was performed. The superior mesenteric artery (SMA) was identified and was occluded temporarily with a microvascular bulldog clamp for 30 min; afterwards reperfusion was allowed for 1 h by gently declamping the artery. The laparotomy was closed with two small clamps immediately after the termination of the 30 min of ischemia.

2.3. Experimental design

Twenty male Wistar albino rats were randomly allocated into three groups; in the first group (n=6), rats received saline and the SMA was manipulated only without occlusion to serve as the sham-operated group. In the other two groups (n=7), rats were subjected to 30 min mesenteric ischemia and 1 h reperfusion. Group 2 was donated as the untreated I/R control and animals received saline only, while in group 3, rats were injected with MF (Sigma-Aldrich Chemical Company; MO, USA; 20 mg/kg; i.p) for 3 days before exposure to mesenteric I/R to serve as MF+I/R group. The MF dose was the most commonly used one in rat models of experiments (Mahmoud-awny et al., 2015).

2.4. Specimen collection / blood and tissue sampling

At the end of the reperfusion period, blood samples were collected from the aorta and were centrifuged to separate serum. The latter was used for the estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase (CK). Afterwards, the animals were euthanized and the organs (liver and intestine) were harvested, weighed, and then homogenized in ice-cold saline and kept in aliquots at −80̊ °C until the estimation of the following biomarkers.

2.5. Assessment of homogenate redox parameters

Lipid peroxidation (nmol/g tissue) was determined according to the method of Mihara and Uchiyama (1978), where the thiobarbituric acid reactive substances (TBARS), measured as malondialdehyde (MDA), was used as an index of lipid peroxides. The MDA-TBA adduct develops pink color, which was extracted by *n*-butanol and measured at two wave lengths, *viz*., 520 and 535 nm. The activity of glutathione transferase enzyme (GST) was assessed according to the method of Habig et al. (1974). GST catalyzes the reaction between reduced glutathione and the substrate CDNB (1-chloro-2,4-dinitrobenzene) to form dinitrophenyl thioether, which can be detected spectrophotometrically at 340 nm at one min interval for 3 min (nmol/min/mg protein). The protein content (mg/g tissue) was determined according to Cannon et al. (1974). Total antioxidant capacity (TAC) was assessed using the corresponding ELISA kit (Cell Biolabs Inc., CA, USA) according to the manufacturers' procedures.

2.6. Assessment of inflammatory and apoptotic markers

ELISA kits were used for the measurement of the homogenate contents of the inflammatory [IL-1β (Ray Biotech Inc., Norcross, USA), IL-6 (KOMA Biotech Inc, Seoul, Korea), MPO (Ray Biotech, Inc., Norcross, USA)], and apoptotic [B-cell leukemia/lymphoma-2 (Bcl-2, USCN Life Science Inc., Wuhan, China), caspase-3 activity (Cusabio Biotech Co., WUHAN, Hubei Province, China)] biomarkers according to the manufacturer's instructions provided.

2.7. Assessment of signaling molecules

The tissue contents of the signaling molecules were measured using the corresponding ELISA kit according to the manufacturer's instructions; GSK-3β and β-catenin (Invitrogen Corporation: Camarillo, CA), phospho-NF-κB p65 (S536) (Abcam, Cambridge, USA), PPAR-γ (Abcam, Cambridge, USA), MMP-9 (Cusabio Biotech, Wuhan, China).

2.8. Assessment of liver/intestinal injury parameters

The serum levels of ALT and AST were determined using the standard commercial biochemical assay kits (Biodiagnostic Co, Cairo, Egypt) based on the method described by Reitman and Frankel (1957). As marker of intestinal function, the activity of CK was estimated using Stanbio CK-NAC dignostic kit (San Antonio, TX, USA) according to a modification of the kinetic method of Rosalki (1967) and Szasz (1976) technique. When serum is added to the reaction mix, the CK in the sample converts ADP to ATP, which is then detected by a coupled enzymatic reaction. ATP is used to produce glucose 6-phosphate, which is consequently used by a third enzyme (glucose-6-phosphate dehydrogenase (G-6- PDH)) to produce NADPH from NADP+. NADPH is produced at a rate directly proportional to the CK activity; the increase in NADPH absorbance is examined per min at 340 nm.

2.9. Statistical analysis

Data are expressed as mean ± S.E.M of 6 animals. Statistical comparisons between means were carried out using one-way analysis