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Activation of IGF-1/IGFBP-3 signaling by berberine improves intestinal mucosal barrier of rats with acute endotoxemia

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ABSTRACT

Insulin-like growth factor I (IGF-I) and binding protein 3 (IGFBP-3) play a role in the maintenance of gut mucosal barrier function. Nevertheless, IGF-I/IGFBP-3 and tight junction protein (TJP) expression in small intestinal mucosa are often impaired during endotoxemia. In this model of acute endotoxemia, the regulatory effect of berberine on IGF-I/IGFBP-3 and TJP expression in ileal mucosa was evaluated. The findings revealed systemic injection of lipopolysaccharide (LPS) suppressed mRNA and protein expression of IGF-I and IGFBP-3, but berberine ameliorated their production. LPS injection inhibited occludin and claudin-1 protein generation, and this inhibitory effect of LPS was abolished by berberine. Inhibition of IGF-I/IGFBP-3 signaling by AG1024 or siRNAs reduced berberine-induced occludin and claudin-1 production. Additionally, GW9662 was found to repress berberine-induced IGF-I/IGFBP-3 expression, indicating of a cross-link between PPARγ and IGF-I/IGFBP-3 axis.

1. Introduction

IGF-I is an insulin-like polypeptide to regulate cell proliferation, apoptosis, migration, immune and inflammation [1,2]. In gastrointestine, IGF-I not only increases enterocyte number, crypt depth, villus height and surface, but also maintains gut barrier function and prevents luminal bacteria and toxins translocation [3–5]. It is well known that the bioactivity of IGF-I is mainly modulated by IGFBPs and IGF-1/IGFBP-3 plays a key role in the maintenance of gut homeostasis [5–7]. Moreover, IGF-I not only induces IGFBP-3 gene transcription [5], but also increases TJP production [8,9]. In gastrointestine the IGF-I and IGFBP-3 abundances are moderate and predominately localized on the surface epithelium [10]. LPS has been reported to inhibit IGF-I and IGFBP-3 expression [11–13].

Peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor transcription factor, has pleiotropic effects. The model of acute endotoxemia shows LPS not only increases PPARγ expression, but also induces PPARγ phosphorylation [14,15]. The cirrhosis model reveals IGF-I/EGF attenuates hepatic ischemic/reperfusion (I/R) injury and protects hepatocyte, which is closely linked to PPARγ overexpression [16]. The insulin resistance model indicates PPARγ agonist reduces alcohol-induced insulin/IGF resistance and induces insulin/IGF-responsive gene expression [17]. In addition, PPARγ is involved in the regulation of IGF-I hormone secretion and gene expression [18,19]. Interestingly, the involvement of IGFBP-3 in modulating PPARγ pathway has been reported [20].

Berberine is a plant alkaloid mainly isolated from the roots and bark of several herbs such as coptis chinensis (goldenthread), *Berberis aquifolium* (oregon grape), *Berberis vulgaris* (barberry), *Hydrastis canadensis* (goldenseal), and berberis aristata (tree turmeric). The functions of berberine include anti- inflammation, anti-tumor, anti-diabetes and anti-atherosclerosis. Besides, berberine contributes to the maintenance of intestinal barrier function [21–23]. Berberine may activate PPARγ pathway and inhibit p38 signaling to repress COX-2 expression [14,24].COX-2 activation may suppress IGF-I expression [13]. Activation of IGF-I/IGFBPs axis by berberine-containing medicine has been reported [25,26].

2. Materials and methods

2.1. Experimental animal

Male Wistar rats (about 250–300 g) were obtained from the Laboratory Animals of Nanjing Medical University and maintained under controlled temperature (20–22 °C) and light conditions (from 07:00 to 19:00). The food and tap water were available ad libitum. The rats were used following adaptation to environment one week. All procedures conformed to the guidelines approved by the Ethics Committee for Laboratory Animals of Nanjing Medical University.

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2.2. Pretreatment with berberine, GW9662 and AG1024

Berberine hydrochloride and GW9662 were obtained from Sigma-Aldrich Corporation, USA. AG1024 was obtained from Selleck Chemicals Corporation, USA. These reagents were dissolved in 1% (*v*/v) DMSO and given at 24-h and 12-h before LPS injection. Berberine was given by gavage at the dose of 25 mg/kg (ber25) or 50 mg/kg (ber50). AG1024 or GW9662 was i.p. injected at the dose of 3 mg/kg.

2.3. Pretreatment with siRNAs

IGF-I siRNA, IGFBP-3 siRNA and negative control siRNA were provided by Shanghai GenePharma Company, China [27] and Dharmacon siDESIGN center, USA [28]. These siRNAs were dissolved in deionized water and delivered by systemic injection at the dose of 120 mg/kg/d for 6 days [15] before LPS administration. The functional assessment of siRNA transfection consisted of TJP expression.

2.4. Induction of acute endotoxemia

LPS (serotype O55:B5) was obtained from Sigma-Aldrich Corporation, USA. LPS was dissolved in sterile saline. The rat model of acute endotoxemia was induced by intraperitoneal injection of LPS at the dose of 2 mg/kg. The rats were slaughtered by decapitation 48 h after LPS injection.

2.5. Preparation of intestinal mucosal scrapings

The distal small intestine (ileum) of each rat was removed and rinsed with sterile saline. The fresh intestinal mucosa was scraped with a glass slide on ice and rapidly frozen in liquid nitrogen, and these specimens then were stored at − 80 °C refrigerator [14].

2.6. RT-PCR analysis

Total RNA was extracted from fresh small intestinal mucosal scrapings. Semi-quantitative RT-PCR was performed as previously described [14,24] to determine the mRNA abundance of IGF-I and IGFBP-3. The forward and reverse primers for IGF-I and IGFBP-3 [13] and for internal reference β-actin [14] were listed as follows (Table 1).

PCR thermal cycle (Takara Biomedicals, Japan) started with an initial denaturation of 95 °C for 5 min, and 32 cycles consisting of denaturation at 95 °C for 30 s, annealing at 60 °C for 1 min and extension at 72 °C for 30 s, followed by an extension at 72 °C for 10 min. PCR products were electrophoresed on 1.8% agarose gel in TAE buffer containing 0.1% ethidium bromide.

2.7. Western blot analysis

The ileal mucosal scrapings were mixed with lysis buffer (1% Triton X-100; 50 mmol/L Tris-HCl, pH 7.6; 150 mmol/L NaCl; and 1% protease inhibitor) and centrifuged at 12,000 r/min for 5 min at 4 °C. The supernatant was separated and collected. Total proteins were exacted with the extraction kit (KeyGEN Biotech Co., China) following the manufacturer's instructions. The protein concentration was assayed with bicinchoninic acid assay. After being separated by SDS-PAGE, the proteins were transferred to a PVDF membrane. The membrane was blocked with 5% skim milk in Tris-buffered saline containing 0.05% Tween-20, incubated with primary antibodies: IGF-I, IGFBP-3, occludin, claudin-1 or β-actin antibody overnight at 4 °C and then incubated with secondary antibody for 2 h at 37 °C. The bands were photographed and quantified with the ChemiDoc XRS system (Bio-Rad Laboratories). The intensity of β-actin band was designated as internal reference.

Table 1
The forward and reverse primers for IGF-I, IGFBP-3 and β-actin.

Gene	Primers		Product (bp)
IGF-I	Forward	5′-GCTATGGCTCCAGCATTCG-3′	62
	Reverse	5′-TCCGGAAGCAACACTCATCC-3′	
IGFBP-3	Forward	5′-GGAAAGACGACGTGCATTG-3′	78
	Reverse	5′-GCGTATTTGAGCTCCACGTT-3′	
β-actin	Forward	5′-CTACAATGAGCTGCGTGTGG-3′	527
	Reverse	5′-AAGGAAGGCTGGAAGAGTGC-3′	

2.8. Statistical analysis

Data are expressed as mean and standard deviation (SD). After the analysis of homogeneity, the data of variance homogeneity or heterogeneity was tested by One-way ANOVA or Welch analysis. The least significant difference (LSD) or Dunnett T3 test was used to determine the difference of means among different groups. $P < 0.05$ was considered statistically significant. All statistical analyses were done with the SPSS16.0 statistical software package (SPSS Inc., Chicago, USA).

3. Results

3.1. Berberine improves LPS-downregulated IGF-I and IGFBP-3 expression

As indicated in Fig. 1, endotoxin significantly inhibited IGF-I and IGFBP-3 expression, leading to a reduction in IGF-I and IGFBP-3 mRNA by 44.9% ($P < 0.001$) and 50.8% ($P < 0.001$) compared with saline-treated group. Berberine effectively ameliorated IGF-I and IGFBP-3 expression. Ber25 raised LPS-decreased IGF-I and IGFBP-3 mRNA by 43.6% ($P = 0.008$) and 40.4% ($P = 0.046$), respectively; and ber50 by 64.0% ($P < 0.001$) and 68.1% ($P = 0.001$), respectively. Similarly, ber50 raised LPS- reduced IGF-I and IGFBP-3 protein by 47.6% ($P = 0.001$) and 58.6% ($P < 0.001$), respectively (Fig. 4).

3.2. Berberine ameliorates LPS-downregulated claudin-1 and occludin expression

As shown in Fig. 2, endotoxin showed an inhibition on TJP expression. LPS significantly decreased claudin-1 and occludin protein by 39.6% ($P < 0.001$) and 33.4% ($P = 0.003$) compared with saline-treated group. This inhibitory effect of LPS was also weakened by berberine. Ber25 and ber50 raised LPS-reduced claudin-1 by 44.7% ($P = 0.006$) and 63.8% ($P < 0.001$), respectively; and raised occludin by 27.8% ($P = 0.089$) and 53.7% ($P = 0.002$), respectively.

3.3. AG1024 suppresses berberine-upregulated claudin-1 and occludin expression

AG1024 was used to test the hypothesis if berberine increases TJP expression via IGF-I signaling. As indicated in Fig. 3, AG1024 was found to inhibit TJP expression. In the LPS plus ber50 plus AG1024 group claudin-1 and occludin were significantly decreased by 54.2% ($P < 0.001$) and 35.0% ($P < 0.001$) in comparison with those in LPS plus ber50 group.

3.4. GW9662 inhibits berberine-activated IGF-1/IGFBP-3 signaling pathway

GW9662 was also applied to test the hypothesis whether berberine-activated IGF-I/IGFBP-3 axis is affected by PPARγ. As shown in Fig. 4, ber50 markedly improved LPS-decreased IGF-I and IGFBP-3 protein. Inhibition of PPARγ activation by GW9662 decreased ber50-induced IGF-I and IGFBP-3 by 22.1% ($P = 0.014$) and 21.4% ($P = 0.012$), respectively.

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