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The NF-κB inhibitor celastrol attenuates acute hepatic dysfunction induced by cecal ligation and puncture in rats

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ABSTRACT

Acute hepatic dysfunction associating sepsis is mediated mainly by toll-like receptor-4 (TLR-4)/nuclear factor kappa-B (NF-κB) inflammatory pathway. This study explores potential hepatoprotective effect of the NF-κB inhibitor celastrol in cecal ligation and puncture (CLP) model in rats.

Protective effect of celastrol (1 mg/kg, i.p., 1 h before CLP) was illustrated after 24 h by preventing CLP-induced hepatic histopathological changes and elevation in serum hepatic biomarkers [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB) and gamma aminotransferase (γ-GT)] without affecting mortality. Celastrol anti-inflammatory effect was illustrated by inhibiting increased serum and hepatic mRNA expression of interleukin-6 (IL-6) without affecting IL-10 elevation. Furthermore, celastrol inhibited CLP-induced elevations in hepatic mRNA expression of nuclear factor inhibitory protein kappa-B alpha (NFκBia), TLR-4, 5-lipoxygenase (5-LOX) and prevented NF-κB/p65 nuclear translocation and activation.

In conclusion, celastrol prevented CLP-induced acute hepatic dysfunction through its anti-inflammatory effect by attenuating NF-κB activation, TLR-4 and 5-LOX expression with subsequent reduction in pro-inflammatory IL-6.

1. Introduction

Sepsis is a serious clinical syndrome that initiates over-response of host defense, leading to septic shock and multi-organ dysfunction syndrome (MODS), which is a major cause of mortality in intensive care units (Bime et al., 2016). Sepsis-associated hepatic dysfunction is expanded among septic patients (Tsai et al., 2015). There is a traditional consideration that sepsis-associated hepatic dysfunction is a late incident. However, hepatic dysfunction is presented recently in several studies as an early incident in sepsis (Groger et al., 2016).

To investigate sepsis and associated multi-organ dysfunction, cecal ligation and puncture (CLP) model is used as an experimentally-induced polymicrobial sepsis that mimics the human sepsis condition (Cuenca et al., 2010).

Inflammation is basic in many acute inflammatory conditions including sepsis (Gorbunov et al., 2013). Previous studies showed that sepsis is a severe systemic inflammation and resulted in inflammatory and immune responses represented in the activation of the toll-like receptor-4 (TLR-4)/nuclear factor kappa-B (NF-κB) pathway (Fan et al., 2016) and the exaggerated production of pro-inflammatory cytokines such as: interleukin-1 beta (IL-1β), IL-6 and tumor necrosis factor-alpha (TNF-α) (Gerin et al., 2016). In addition, activation of NF-κB results in increased gene expression and biosynthesis of pro-inflammatory mediators in sepsis (Ang et al., 2011). Accordingly, sepsis research concentrated on expanding anti-inflammatory strategies. Unfortunately, many treatments that may produce modification in systemic inflammation haven't achieved the expected success to reduce mortality rate in clinical trials (Gavins et al., 2012).

Consequently, pharmacologically active compounds extracted from natural products have been identified to innovate anti-inflammatory therapeutic strategies based on their molecular

Abbreviations: CLP, Cecal ligation and puncture; NFκBia, nuclear factor inhibitory protein kappa-B alpha; TLR-4, toll-like receptor-4; 5-LOX, 5-lipoxygenase.

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targets which are mostly centered on the inhibition of NF-κB signaling (Salminen et al., 2010).

Celastrol (also known as tripterine) is a major bioactive component extracted from celastrus and thunder of God Vine (Li et al., 2013a). Previous research has revealed its anti-oxidant, anti-inflammatory, anti-angiogenic, anti-cancer, neuroprotective, immunosuppressive (Li et al., 2012) and heat shock response (HSR) activation properties (Ma et al., 2015). Celastrol has been shown to exert an anti-inflammatory effect by inhibiting NF-κB activity and NO production, by modulating pro-inflammatory cytokines and cyclooxygenase (Kim et al., 2009b; Li et al., 2012). A previous study confirmed these findings and showed that celastrol treatment could delay the progression of diabetic liver disease in rats via reduction of hepatocyte NFκB/p65 expression which was rarely seen in the nuclei, leading to inhibition of TLR-4/NF-κB signaling cascade pathways (Han et al., 2016).

Celastrol acts through multiple mechanisms including activation of heat shock factor-1 (HSF-1) resulting in HSR, which leads to elevation in heat shock proteins (HSPs) expression. Therefore, this mechanism maintains cellular protein homeostasis by which celastrol was able to offer protection against hepatic dysfunction (Sharma et al., 2015).

Based on the potential role of celastrol in inflammation, this study examined the possible protective effect of celastrol in CLP-induced acute hepatic dysfunction in rats.

2. Materials and methods

2.1. Drugs and chemicals

The (9β,13α,14β,20α)-3-Hydroxy-9,13-dimethyl-2-oxo-24,25,26-trinoroleana-1(10),3,5,7-tetraen-29-oic acid (celastrol) was purchased from Tocris Bioscience (Minneapolis, MN, USA) and was dissolved in 1.5% ethyl alcohol in distilled water. Thiopental sodium (Anapental) was obtained from Sigmatec pharmaceutical industries (6 October City, Egypt). Formalin, sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), absolute ethanol, isopropyl alcohol (20%) and chloroform were purchased from El-Nasr Chemicals Company (Abou-Zaabal, Cairo, Egypt). Bovine serum albumin (BSA) and sodium azide were purchased from Sigma Aldrich chemical Co. (St.Louis, MO, USA) and was dissolved in distilled water.

2.2. Experimental animals

Male Sprague Dawley rats, aging 6–8 weeks were obtained from "Delta University for science and technology breeding animal house", Gamasa, Egypt. All preformed procedures complied with the ethical standards of the Research Ethics Committee, Faculty of Pharmacy, Mansoura University, Egypt.

2.3. Experimental protocol

The animals were randomly allocated into four groups each composed of (6–12) rats. **Group (1):** Sham group; **Group (2):** Celastrol group: Rats receiving Celastrol (1 mg/kg, i.p.) (Yang et al., 2014) 60 min before sham operation; **Group (3):** CLP group and **Group (4):** Celastrol + CLP group: Rats receiving Celastrol (1 mg/kg, i.p.) 60 min before CLP.

Celastrol dose in this study was selected based on our initial experiments using celastrol doses of 0.2, 0.5 and 1 mg/kg, i.p. The 1 mg/kg was optimum since the other 2 doses didn't provide a significant protection against CLP-induced changes in mortality and hepatic biomarkers. Additionally, previous studies confirmed the protective effect of this dose (1 mg/kg) in rat models (Cascao et al., 2012; Li et al., 2013b).

2.4. Induction of CLP

Rats were anesthetized with thiopental (40 mg/kg, i.p.) (Ferro et al., 2007). Under aseptic conditions, a small midline incision was made through the skin and peritoneum to expose the cecum. The cecal appendage was ligated with 4/0 surgical silk thread at the midway between distal pole and the cecal base without causing bowel occlusion. Consecutively, double punctures were performed with an 18 gauge needle and a small amount of stool was expelled out. Finally, cecum was repositioned and skin closure was performed by using the 4/0 silk threads. Rats underwent resuscitation by subcutaneous injection of 1 ml pre-warmed 0.9% saline solution (Walker et al., 2011). Control groups were sham-operated by exposing the ceca without ligation or puncture.

Twenty four hours later, the rate of mortality was calculated, then rats were anesthetized and blood was collected to separate the serum for assaying hepatic and inflammatory biomarkers. Subsequently, liver was separated for histopathological examination and assessment of mRNA expression.

2.5. Determination of serum alanine aminotranseferase (ALT), serum aspartate aminotransferase (AST), total bilirubin (TB) and gamma glutamyl tranferase (γ-GT) activities

Activities of ALT and AST were determined using a commercial kit (ELITech Clinical Systems SAS, Sées, France). Their activities were measured by a kinetic method at wavelength 340 nm and expressed as a unit per liter (U/L) (Bergmeyer et al., 1986a,b)

Level of TB was determined using a commercial kit (BioMed diagnostics, Badr City, Egypt). The TB level was measured at 578 nm and expressed as milligram per deciliter (mg/dL) (Garber, 1981).

The activity of γ-GT was determined using a commercial kit (Spinreact S.A, Girona, Spain). The γ-GT activity was measured by a kinetic method at wavelength 405 nm and expressed as U/L (Persijn and van der Slik, 1976).

2.6. Histopathological examination

At the end of the experiment, part of the right upper hepatic lobes were quickly dissected out and fixed in 10% buffered formalin. The fixed tissues were embedded in paraffin and sectioned (5 μm thick). Each section was stained with hematoxylin and eosin (H&E). The analyses were assessed with a microscope (Leica Imaging Systems, Cambridge, UK).

2.7. Determination of serum IL-6 and IL-10 levels

Levels of IL-6 and IL-10 levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, Vienna, Austria) according to manufacturer instructions. Serum IL-6 and IL-10 levels were measured at 450 nm and the concentration was calculated from a standard curve and expressed as pg/ml. The standard concentration ranges are (2000-31.3 pg/ml) and (1000-15.6 pg/ml) for IL-6 and IL-10 respectively.

2.8. Quantitative RT-PCR

The right posterior hepatic lobe was isolated, weighed and preserved in RNA Later (Qiagen, Netherlands, Germany) (50–100 mg tissue/1 ml RNA later). RNA was extracted from liver by Trizol reagent (Invitrogen, Massachusetts, USA). Then, 1 μg from each sample was reverse transcribed into complementary DNA (cDNA)