

The lipoxin A₄ agonist BML-111 attenuates acute hepatic dysfunction induced by cecal ligation and puncture in rats

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Abstract Sepsis is a systemic inflammatory response associating severe infection leading to multi-organ failure, such as hepatic dysfunction. This study investigates the possible hepatoprotective effect of the lipoxin A₄ agonist (BML-111) in cecal ligation and puncture (CLP) model in rats. Pretreatment with BML-111 (1 mg/kg, i.p., 1 h before CLP) protected against CLP-induced mortality after 24 h. BML-111 prevented marked inflammatory cells in liver tissues and decreased elevation in serum hepatic biomarkers [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), gamma-glutamyl transferase (γ-GT)] induced by CLP. Additionally, BML-111 attenuated elevated serum level of interleukin-6 (IL-6) and downregulated hepatic IL-6 mRNA expression. Meanwhile, BML-111 further increased serum IL-10 and upregulated hepatic IL-10 mRNA expression, while it downregulated hepatic mRNA expression of nuclear factor inhibitory protein kappa-B alpha (NFκB α), toll-like receptor-4 (TLR-4), and 5-lipoxygenase (5-LOX). Moreover, BML-111 prevented NF-κB/p65 nuclear translocation and activation. In conclusion, BML-111 attenuated CLP-induced acute hepatic dysfunction through its anti-inflammatory effect by decreasing NF-κB activity, TLR-4,

and 5-LOX expression with subsequent decrease in pro-inflammatory IL-6 and elevation in anti-inflammatory IL-10.

Keywords LXA₄ · BML-111 · Cecal ligation and puncture · Hepatic dysfunction · NF-κB · IL-6 · IL-10

Abbreviations

CLP Cecal ligation and puncture

NFκB α Nuclear factor inhibitory protein kappa-B alpha

Introduction

Sepsis is a fatal condition that causes multi-organ failure and shock. It activates host immune, inflammatory, and coagulation responses, leading to tissue injury, hypoxia, and organ dysfunction (Takahashi et al. 2013).

Incidence of sepsis-associated hepatic dysfunction among sepsis patients ranges from 34 to 46% (Tsai et al. 2015) and their mortality rates range from 54 to 68% (Yan et al. 2014). Traditionally, sepsis-associated hepatic dysfunction is viewed as a late event. However, recent studies have revealed hepatic dysfunction as an early event in sepsis (Yan et al. 2014).

To study sepsis and associated multi-organ dysfunction, the surgical model, cecal ligation and puncture (CLP), is considered to be the gold standard for the experimental induction of polymicrobial sepsis. This is a realistic model resembling the human condition and is clinically relevant (Brooks et al. 2007).

Since inflammation plays a vital role in sepsis, thus, sepsis research focused mainly on the development of anti-inflammatory regimens (Nullens et al. 2016). However, in clinical trials, several therapies that possibly affect systemic inflammation have widely failed to reduce incidence of mortality in cases with severe sepsis (Gavins et al. 2012).

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Therefore, new understanding of the pathophysiological mechanisms is needed to develop novel therapeutic strategies.

Lipoxins, particularly lipoxin A₄ (LXA₄), exhibit anti-inflammatory and proresolution effects, including its capability to reduce vascular permeability, to impair migration of new neutrophils into sites of injury and to promote infiltration of monocytes into the site of inflammation. LXA₄, the arachidonic acid metabolite, is biosynthesized by two main routes, and both are influenced by lipoxygenases (LOX) (Sordi et al. 2013).

Since LXA₄ is rapidly inactivated, potent analogs have been created, including the LXA₄ agonist BML-111. BML-111 exhibits protective effects in many experimental inflammatory conditions, such as ischemic stroke and acute lung injury in rat models and acute pancreatitis-associated lung injury in mice (Wang et al. 2014a). However, its role in sepsis-induced inflammation and hepatic dysfunction is not clear.

Therefore, in this study, we aimed to investigate the possible protective role of BML-111 in acute hepatic dysfunction induced by CLP surgical model in rats.

Materials and methods

Drugs and chemicals

5(S), 6(R), 7-Trihydroxyheptanoic acid methyl ester (BML-111), bovine serum albumin (BSA), and sodium azide were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and were dissolved in distilled water. Thiopental sodium (Anapental) was purchased from Sigmatec Pharmaceutical Industries (6 October City, Egypt). Absolute ethanol, chloroform, isopropyl alcohol (20%), disodium hydrogen phosphate (Na₂HPO₄), sodium dihydrogen phosphate (NaH₂PO₄), and formalin were obtained from El-Nasr Chemicals Company (Abou-Zaabal, Cairo, Egypt).

Experimental animals

Male Sprague-Dawley rats, aging 7–8 weeks and weighing 160–200 g, were purchased from Delta University for Science and Technology Breeding Animal House, Gamasa, Egypt. All procedures performed were in accordance with the ethical standards of the Research Ethics Committee, Faculty of Pharmacy, Mansoura University, Egypt.

Experimental protocol

The animals were randomly divided into four groups each consisting of 6–12 rats: group 1, sham group; group 2, BML group—rats receiving BML-111 (1 mg/kg, i.p.) 60 min before sham operation; group 3, CLP group; and group 4, BML + CLP group—rats receiving BML-111 (1 mg/kg, i.p.) 60 min before CLP.

CLP procedure

Rats were anesthetized with Anapental (40 mg/kg, i.p.) (Ferro et al. 2007). A small midline incision was made through the skin and extended till it reached the peritoneal cavity to expose the cecum. The cecum was ligated exactly between the distal pole and base of the cecum with 4/0 surgical silk. Then, the cecum was punctured two times using an 18-gauge needle. A small amount of fecal matter was squeezed out of the perforated cecum. The cecum was relocated back into the abdominal cavity, and the skin was further closed in double layers with 4/0 silk sutures. Pre-warmed 0.9% saline solution (1 mL/100 g; s.c.) was injected to replace fluid loss during surgery (Walker et al. 2011). In sham-operated controls, ceca were exposed without ligation or puncture (Walker et al. 2011).

After 24 h, mortality rate was calculated, then rats were anesthetized and blood was collected to obtain serum for assessment of hepatic and inflammatory biomarkers. In addition, the liver was excised immediately for histopathological examination and measurement of mRNA expression.

Determination of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), total bilirubin (TB), and gamma-glutamyl transferase (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 a wavelength of 340 nm and expressed as units 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 milligrams 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 a wavelength of 405 nm and expressed as units per liter (Persijn and van der Slik 1976).

Histopathological examination

At the end of the experiment, portions of the right upper hepatic lobes were rapidly dissected out and fixed in 10% buffered formalin for 24 h, embedded in paraffin, sectioned (5 μm thick), stained with hematoxylin and eosin (H&E), and were then assessed microscopically for the presence of inflammation using (Leica Imaging Systems, Cambridge, UK).

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's 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 picograms per milliliter.

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 the liver by Trizol reagent (Invitrogen, MA, USA). Then, 1 μ g from each sample was reverse transcribed into complementary DNA (cDNA) using GoScript Reverse Transcription System (Promega Corporation, Madison, USA) according to the manufacturer's procedures. Quantitative RT-PCR was performed with a thermocycler Rotor Gene Q (Qiagen), using HOT FIREPol EvaGreen qPCR mix plus kit (Solis BioDyne, Tartu, Estonia).

The mRNA levels of IL-6 (NM_012589), IL-10 (NM_012854.2), inhibitory protein kappa-B alpha (NF κ B α) (NM_001105720.2), toll-like receptor-4 (TLR-4) (NM_019178.1), and 5-lipoxygenase (5-LOX) (NM_012822.1) were measured and normalized relative to 18S ribosomal RNA (Rn18S) (NR_046237.1) in the same sample. IL-6, IL-10, NF κ B α , TLR-4, 5-LOX, and Rn18S primers were as follows:

IL-6 (sense, AGCCAGAGTCATTCAGAGCAA; anti-sense, TGGAAGTTGGGGTAGGAAGG, amplicon size = 98)

IL-10 (sense, CACTTCCCAGTCAGCCAGA; anti-sense, GTCAGCAGTATGTTGTCCAGC, amplicon size = 107)

NF κ B α (sense, GTGACTTTGGGTGCTGATGT; anti-sense, ACACTTCAACAGGAGCGAGA, amplicon size = 111)

TLR-4 (sense, TGTTCCCTTCCTGCCTGAGA; anti-sense, GGTTCTGGTTGAATAAGGGATG, amplicon size = 129)

5-LOX (sense, CAAGATTGTTCCCATCGCCA; anti-sense, GCCAGTCGTATTTGAGTCCG, amplicon size = 89)

Rn18S (sense, AGTTGGTGGAGCGATTTGTC; anti-sense, GAACGCCACTTGTCCTCTA, amplicon size = 121)

The results were expressed as an *n*-fold change of the relative expression levels of target genes from control group using $\Delta\Delta C_t$ method.

Immunohistochemistry for NF- κ B/p65

Paraffin-embedded liver tissue sections were used for the immunohistochemical study of NF- κ B/p65 expression. After de-

paraffinization, the slides were rehydrated and the antigen was retrieved. Unspecific binding sites were blocked by using 0.2% BSA and 15 mM sodium azide in 10 mM phosphate-buffered saline (PBS), pH 7.6, for 1 h at RT before incubation with the primary antibody. Tissue sections were incubated overnight at 4 °C with rabbit anti-NF- κ B/p65 polyclonal antibody (Thermo Fisher Scientific, Fremont, CA, USA) as a primary antibody (Vazquez et al. 2015). After washing with PBS (3–5 min), the sections were incubated with secondary antibody (Genemed Biotechnologies, South San Francisco, USA) for 2 h at RT, then washed with PBS. All antibodies were used in a dilution of 1:100. Immunocompleted slides were visualized according to the manufacturer's protocol and then counterstained with H&E and washed again with PBS. Negative control sections were performed by omission of the primary antibody from the procedure. Finally, observation and imaging of immunolocalization were carried out using a light microscope (Leica).

Statistical analysis

Data are expressed as mean \pm standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparisons post hoc test. Additionally, Kruskal-Wallis test followed by Dunn's post hoc test were used in non-parametric measurements (mortality rate). The level of significance was set at $p < 0.05$. Statistical analysis and graphing were performed with GraphPad Prism v 5.02 (GraphPad Software Inc., San Diego, CA, USA).

Results

Effect of BML-111 on CLP-induced mortality in rats

Mortality was significantly ($p < 0.05$, $n = 12$) increased in CLP group by 50% when compared to sham group (Table 1). Pre-injection of rats with BML-111 produced a significant decrease in CLP-induced mortality rate to 8.3%. Additionally, BML-111 had no significant effect on mortality in sham group (Table 1).

Table 1 Effect of BML-111 on CLP-induced mortality in rats

	Sham	BML-111	CLP	BML-111 + CLP
Mortality rate	0	0	50*	8.3 [#]

Data are expressed as the mean of mortality percentage of 4 independent experiments with a total of 12 rats. BML-111 (1 mg/kg, i.p.) was given 1 h before induction of CLP, and after 24 h, its effect on mortality was calculated

*, [#] $p < 0.05$, significantly different from sham and CLP groups, respectively, using Kruskal-Wallis test followed by Dunn's post hoc test

Effect of BML-111 on CLP-induced elevation in hepatic biomarkers in rats

CLP caused a significant ($p < 0.05$, $n = 6$) increase in ALT, AST, TB, and γ -GT by 2.3-, 1.8-, 2.7-, and 4.5-fold, respectively, when compared to sham group (Fig. 1). Pre-injection of BML-111 before CLP caused a significant decrease in CLP-induced elevation of ALT, AST, TB, and γ -GT. Moreover, BML-111 had no significant effect on serum hepatic biomarkers in sham group (Fig. 1).

Effect of BML-111 on CLP-induced changes in histopathological examination of liver tissues of rats

Rats in sham and BML-111 groups revealed normal liver architecture (Fig. 2a, b). CLP caused moderate inflammatory cell infiltration, hepatocellular necrosis, and central vein dilation and congestion (Fig. 2c). The administration of BML-111 prior to CLP attenuated the histopathological changes induced by CLP (Fig. 2d).

Effect of BML-111 on CLP-induced changes in serum level and hepatic mRNA expression of IL-6 and IL-10 in rats

CLP caused a significant ($p < 0.05$, $n = 6$) increase in serum IL-6 and IL-10 by 9.4- and 4.5-fold, respectively, when compared to sham group (Fig. 3a, b).

Pre-injection of BML-111 before CLP caused a significant decrease in CLP-induced elevation in serum IL-6 compared to CLP group (Fig. 3a). Conversely, BML-111 produced a

significant increase in CLP-induced elevation in serum IL-10 compared to sham and CLP groups (Fig. 3b).

Similarly, the hepatic mRNA expressions of IL-6 and IL-10 were increased in CLP group by 6.7- and 4.7-fold, respectively, compared to sham group (Fig. 3c, d). Pretreatment with BML-111 before CLP significantly downregulated hepatic mRNA expression of IL-6 compared to CLP group, while it upregulated hepatic mRNA expression of IL-10 significantly compared to sham and CLP groups (Fig. 3c, d). The BML-111 had no significant effect on serum IL-6, IL-10, and their hepatic mRNA expression in sham group.

Effect of BML-111 on CLP-induced changes in hepatic mRNA expression of NF κ B α , TLR4, and 5-LOX in rats

CLP upregulated hepatic mRNA expression of NF κ B α , TLR-4, and 5-LOX significantly by 2.7-, 5.8-, and 3.3-fold, respectively, compared to sham group (Fig. 4). Pretreatment with BML-111 before CLP downregulated hepatic mRNA expression of NF κ B α , TLR-4, and 5-LOX significantly compared to CLP group. Additionally, BML-111 had no significant effect on hepatic mRNA expression of NF κ B α , TLR-4, and 5-LOX in sham group (Fig. 4).

Effect of BML-111 on CLP-induced changes in immunohistochemical expression of NF- κ B/p65 in liver tissues of rats

Hepatic cells obtained from the sham and BML-111 groups revealed marked cytoplasmic staining (Fig. 5a, b) showing

Fig. 1 Effect of BML-111 on CLP-induced changes in serum hepatic biomarkers. BML-111 (1 mg/kg, i.p.) was given 1 h before induction of CLP, and after 24 h, serum was collected to measure ALT (a), AST (b), total bilirubin (c), and γ -GT (d). Data are expressed as mean \pm SD, $n = 6$. *, # $p < 0.05$, significantly different from sham and CLP groups, respectively, using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. ALT alanine transaminase, AST aspartate transaminase, γ -GT gamma-glutamyl transferase

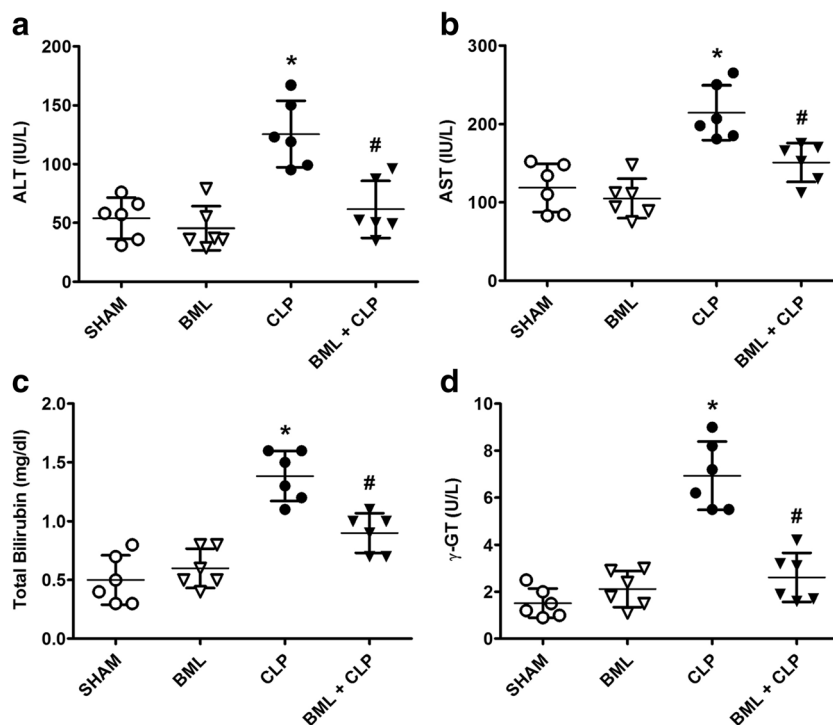
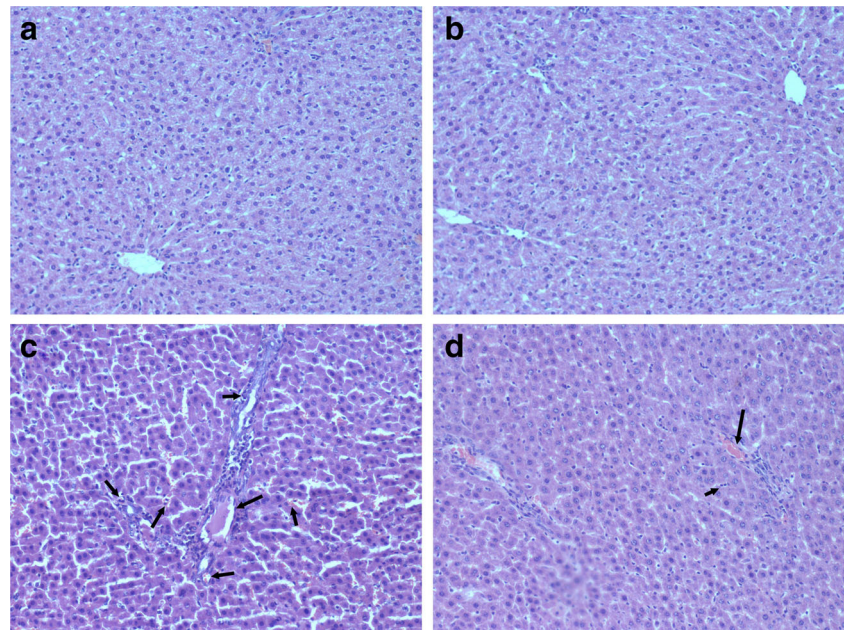


Fig. 2 Effect of BML-111 on CLP-induced changes in histopathological examination of liver. BML-111 (1 mg/kg, i.p.) was given 1 h before induction of CLP, and after 24 h, liver tissues were collected for histopathological examination using H&E stain ($\times 200$). Rats in sham (a) and BML-111 + sham (b) groups revealed normal liver architecture. Portal liver tissue of CLP group (c) showed moderate infiltration of mononuclear inflammatory cells (arrows), hepatocellular necrosis, and central vein dilation and congestion. BML-111 + CLP group (d) showed mild mononuclear cells infiltrate both portal (long arrow) and parenchymal (short arrow)

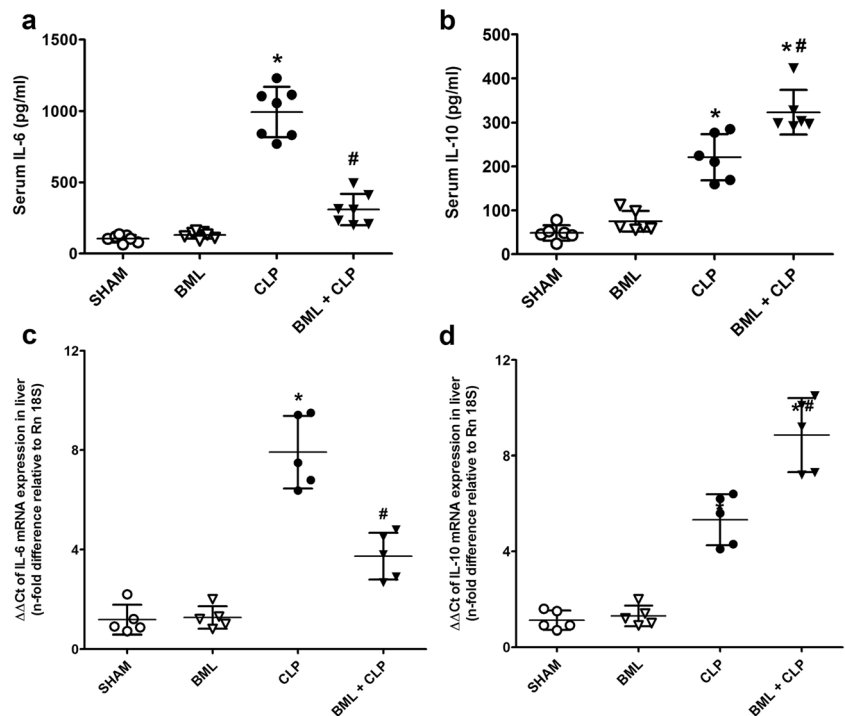


that p65 was limited to the cytoplasm, indicating the presence of NF- κ B in its inactive form. In liver tissue collected from CLP rats, there was a substantial increase in immunostaining for p65 in the nuclei of hepatic cells (Fig. 5c), indicating translocation of NF- κ B to the nucleus. In rats injected with BML-111 prior to CLP, p65 was limited to the cytoplasm in all hepatic cells (Fig. 5d), indicating that BML-111 suppressed translocation of NF- κ B into the nucleus.

Discussion

In this study, the lipoxin A₄ analog BML-111, was able to protect against CLP-induced acute hepatic dysfunction. Sepsis is commonly associated with hepatic dysfunction (Wang et al. 2015). Various human (Groger et al. 2016; Tsai et al. 2015) and animal models (Bu and Yu 2015; Dangi et al. 2016) confirmed the association between sepsis and hepatic dysfunction.

Fig. 3 Effect of BML-111 on CLP-induced changes in serum level and hepatic mRNA expression of IL-6 and IL-10. BML-111 (1 mg/kg, i.p.) was given 1 h before induction of CLP and after 24 h serum was collected to measure IL-6 (a) and IL-10 (b). Additionally, liver samples were collected to measure mRNA expression of IL-6 (c) and IL-10 (d). Data are expressed as mean \pm SD, $n = 6$. *, # $p < 0.05$, significantly different from sham and CLP groups, respectively, using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test



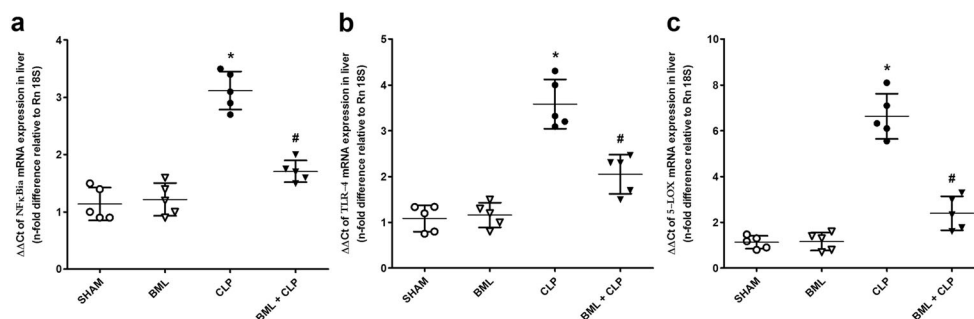


Fig. 4 Effect of BML-111 on CLP-induced changes in hepatic mRNA expression of NF κ B, TLR-4, and 5-LOX. BML-111 (1 mg/kg, i.p.) was given 1 h before induction of CLP, and after 24 h, liver samples were collected to measure mRNA expression of NF κ B (a), TLR-4 (b), and 5-LOX (c). Data are expressed as mean \pm SD, $n = 6$. *, # $p < 0.05$,

A previous study showed that CLP-induced hepatic dysfunction is associated with elevation in serum ALT, AST, TB, and γ -GT activities (Bu and Yu 2015). Our work showed that pretreatment with BML-111 protected against CLP-induced hepatic dysfunction through decreasing hepatic biomarkers (ALT, AST, TB, and γ -GT).

Moreover, a marked liver injury is characterized by the deterioration of histopathology (Wang et al. 2015). Our results showed that CLP caused a marked inflammation in the liver tissue of rats with significant granulocyte infiltration and disruption of cellular morphology which confirms the presence of liver dysfunction. In our study, BML-111 was able to improve survival and restore the histologic architecture of liver

of CLP rats. These results confirm that BML-111 resulted in a marked protection against CLP-induced hepatic dysfunction. In CLP-induced hepatic dysfunction, inflammation plays a major role (Li et al. 2015). A previous study showed that CLP-induced hepatic dysfunction is associated with elevation in TLR-4, NF- κ B, IL-6, and IL-10 (Deng et al. 2013).

TLR-4 is expressed on most cell types in the liver, including Kupffer cells, endothelial cells, and hepatocytes (Rivera et al. 2010). NF- κ B is a transcription factor that is triggered by TLR-4 activation during inflammation (Kim et al. 2016). NF- κ B is responsible for activation of an inflammatory cascade leading to stimulation of the expression of pro-inflammatory cytokines involved in sepsis, such as IL-6, in

significantly different from sham and CLP groups, respectively, using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. 5-LOX 5-lipoxygenase, NF κ B nuclear factor inhibitory protein kappa-B alpha, TLR-4 toll-like receptor-4

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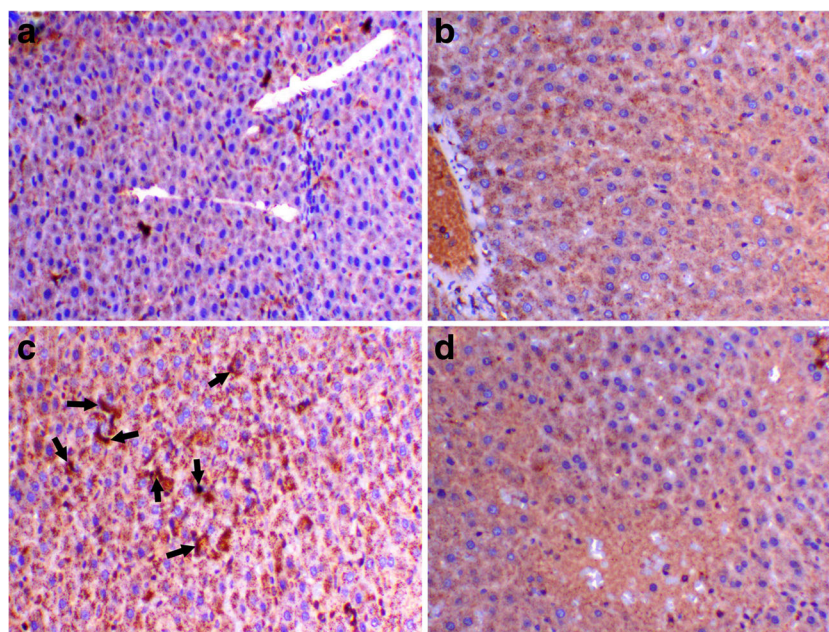


Fig. 5 Effect of BML-111 on CLP-induced hepatic changes in immunohistochemical expression of NF- κ B p65. BML-111 (1 mg/kg, i.p.) was given 1 h before induction of CLP. After 24 h, liver tissues were collected for immunohistochemistry (IHC) staining using H&E stain ($\times 200$). Liver tissue of sham (a) and BML-111 + sham (b) groups showed diffuse cytoplasmic staining for NF- κ B/p65 in hepatocytes mostly within the

centrolobular areas. Liver tissue of CLP group (c) showed marked intranuclear expression and translocation for NF- κ B/p65 within hepatocytes (arrows). Liver tissue of BML-111 + CLP group (d) showing cytoplasmic staining for NF- κ B/p65 and marked intracytoplasmic expression of NF- κ B within hepatocytes

addition to anti-inflammatory cytokines, such as IL-10 (Deng et al. 2013; Murakami et al. 2011).

The p65 subunit of NF- κ B is a key transcriptional factor responsible for regulating most of NF- κ B's transcriptional activity (Fan et al. 2011). NF- κ B/p65 heterodimer is present in the cytoplasm in inactive form. Activation of NF- κ B/p65 allows nuclear translocation of NF- κ B transcription factor and increases the expression of many pro-inflammatory cytokines (Shafik et al. 2015).

Previous studies showed an increased TLR-4 and NF- κ B expression after CLP induction in rat and mice models (Petronilho et al. 2012; Wu et al. 2015b). Another study reported that NF- κ B transcriptional activity was increased secondary to overexpression of NF- κ B/p65 (Wang et al. 2013). Therefore, we examined the effect of CLP on mRNA expression of TLR-4 and NF κ Bia as a main regulator of NF- κ B activation (Harvey et al. 2013) in addition to the immunohistochemical expression of NF- κ B/p65 and mRNA expression of the inflammatory cytokines IL-6 and IL-10.

Our results showed that CLP increased hepatic mRNA expression of TLR-4, NF κ Bia, and nuclear translocation of NF- κ B/p65. In addition, CLP increased serum level and hepatic mRNA expression of IL-6 and IL-10. Meanwhile, pretreatment with BML-111 decreased CLP-induced increase in mRNA expression of TLR-4, NF κ Bia, and NF- κ B/p65 nuclear translocation and activation. Moreover, BML-111 prevented CLP-induced elevation in serum level and mRNA expression of IL-6. Conversely, BML-111 further increased CLP-induced elevation in serum and mRNA expression of the anti-inflammatory cytokine IL-10.

These results are in accordance with previous findings that alterations in the CLP-induced elevated expression of TLR-4 may be one of the molecular mechanisms underlying the protective effect of BML-111 in rat model (Liu et al. 2015). Furthermore, BML-111 was shown to possess potent anti-inflammatory activities as it can effectively suppress NF- κ B activation (Kong et al. 2015). This protective role was strongly associated with reduced expression and production of pro-inflammatory cytokine IL-6 and increased production and expression of anti-inflammatory IL-10 protein level (Wang et al. 2014b). However, Walker et al. (2011) showed that LXA₄ pretreatment before CLP in rats reduced IL-10 expression compared to CLP-operated rats 48 h after surgery (Walker et al. 2011). This variation from our results may be due to time variability of examination after CLP surgery. It was previously reported that examination of the anti-inflammatory effect of LXA₄ and its analog BML-111 24 h after surgery is mediated by the release of IL-10 via increasing its production and mRNA expression (Souza et al. 2007; Wang et al. 2014a).

In addition, 5-LOX has been shown to increase in sepsis/CLP. 5-LOX products derived from arachidonic acid are pathogenic mediators in endotoxemia and are responsible for inflammatory reactions (Chen 2011).

Therefore, we examined the effect of CLP on hepatic mRNA expression of 5-LOX. In the present study, CLP increased hepatic mRNA expression of 5-LOX. Administration of BML-111 decreased CLP-induced elevation in mRNA expression of 5-LOX. This is in accordance with previous findings that BML-111 effectively inhibited 5-LOX enzyme produced after sepsis induction by bacterial inoculation in mice model (Sordi et al. 2013). Measurement of protein levels of 5-LOX and TLR-4 would further confirm our results and requires further investigation.

In addition, previous studies reported that LXA₄ and its analog BML-111 increased neutrophil bacterial clearing function and reduced blood bacteria load in CLP and bacterial inoculation sepsis models, indicating an antibacterial effect of BML-111 (Sordi et al. 2013; Wu et al. 2015a). Although we did not measure this antibacterial effect of BML-111 in our study, however, this antibacterial effect cannot be excluded as a mechanism of the hepatoprotective effect of BML-111 in this study.

In conclusion, this study showed that BML-111 has a protective effect against CLP-induced acute hepatic dysfunction in rats through its anti-inflammatory effect. This effect may be mediated by decreasing the hepatic mRNA expression of TLR-4, 5-LOX, and NF κ Bia in addition to suppressing NF- κ B/p65 nuclear translocation and activation with subsequent increase in the hepatic mRNA expression and serum level of IL-10 and the decrease in the hepatic mRNA expression and serum level of IL-6. Therefore, these findings provide a potential beneficial effect of BML-111 in the prevention of acute sepsis-associated hepatic dysfunction.

Compliance with ethical standards

Ethical approval All procedures performed were in accordance with the ethical standards of the Research Ethics Committee, Faculty of Pharmacy, Mansoura University, Egypt.

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