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Lactoferrin and Metoprolol Supplementation Increase Mouse Survival in an Experimental LPS-Induced Sepsis Model

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Abstract

Sepsis, a result of a hyperreaction of the immune system to acute infection, has been recognized as a significant healthcare challenge due to the considerable associated morbidity and mortality. In this study, the antisepsis effects of bovine lactoferrin (bLF) and metoprolol were evaluated in a lipopolysaccharide (LPS)-induced sepsis model in mice. Eighty mice were divided into two equal groups and received LPS or PBS inoculation. Prior to LPS/PBS inoculation, each group was further divided into four equal groups to receive saline solution (SS), metoprolol, bLF, or bLF + metoprolol. Mouse survival was monitored at 0, 3, 24, 48 and 96 h after LPS/PBS inoculation, and blood was collected for inflammatory cytokine and lactate measurements. Morphological and structural changes in vital organs, such as the heart, kidney, and liver, were recorded. The survival rates of septic mice treated with metoprolol, bLF, and bLF + metoprolol significantly improved compared to those of mice treated with SS. The levels of inflammatory cytokines and lactate decreased in the bLF and bLF + metoprolol groups, and tissue injury was diminished in all groups compared with septic LPS-SS mice. This study shows an antisepsis effect of bLF against LPS-induced sepsis in mice, an effect that is boosted in combination with metoprolol, indicating a novel option to prevent or treat sepsis.

Keywords Lactoferrin · Metoprolol · Lipopolysaccharide · Sepsis · Mice

Introduction

Sepsis is a serious medical condition that involves a systemic inflammatory response against microbial infections (Rello et al. 2017). This condition represents one of the leading causes of death among hospitalized patients, causing the deaths of 250,000 individuals in the USA and 30–40% of annual mortality worldwide (Camacho-Gonzalez et al. 2013; Hajj et al. 2018). Microbial pathogens that can cause sepsis include fungi, viruses, and bacteria, in particular Gram-negative bacteria (Bizzarro et al. 2005). Moreover, specific bacterial structures, such as lipopolysaccharides (LPS) and lipoteichoic acid, found in Gram-negative and Gram-positive bacteria, respectively, are often reported to cause sepsis by dysregulating immune system responses by high production of pro-inflammatory cytokine (Liu et al. 2017). Cytokines plays a central role in sepsis process, they mediate the response of infection but in sepsis there an overreaction, and TNF, IL-6, IL-1, IL-8 are associated with systemic inflammatory response syndrome (SIRS), they participate in activating endothelial cells to stimulate the synthesis of chemokines to attract immune system cells, secretion of procoagulant factors and in endothelial cell dysfunction/necrosis to increase vascular permeability and nitric oxide

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production (Kellum et al. 2007). On the other hand, anti-inflammatory cytokine as IL-10, they are secreted to try to block the action of pro-inflammatory cytokine and modulate the overreaction response (Chousterman et al. 2017). The pathogenesis of sepsis, in addition to immune system dysfunction and inflammatory response, involves oxidative stress, endothelial dysfunction, and coagulative disorders (Zhou et al. 2019). As consequence of it, some biomarkers will be elevated in blood, one of the most important is lactate, the high blood levels of lactate in sepsis are associated with higher risk of mortality (Toffaletti 1991), in fact the blood lactate levels are the most widely utilized biomarker that indicate injury and organ dysfunction in sepsis (Billeter et al. 2009). The early detection of biomarkers related of sepsis are very important to choose the correct treatment to patients, including those involving antibiotics, steroids, resveratrol, and beta blockers (β-blockers), such as propranolol and metoprolol (van Loon et al. 2018; Zhou et al. 2019).

Metoprolol has been shown to attenuate the effects of sepsis through immune-modulating effects, regulation of the cardiovascular system, coagulopathy, and attenuation of the hypermetabolic state (van Loon et al. 2018).

Despite the availability of multiple strategies to treat this pathology, patient deaths from sepsis remain high. The challenge of developing more effective therapeutic methods is complicated by the roles the host immune system plays in the progression of local infection and eventual systemic dissemination. Compounds that modulate oxidative stress and inflammation and regulate host immune responses may improve patient outcomes. Lactoferrin (LF), a nonheme and cationic glycoprotein with high affinity to iron, represents a promising strategy for the development of new antisepsis therapies (Zagulski et al. 1989). LF is naturally produced by the mammary gland and secondary granules of neutrophils and has been classified as a multifunctional protein presenting, among other functions, antibacterial, antitumor and antioxidant activities (Baggiolini et al. 1970; Masson and Heremans 1971). Moreover, it has been suggested that LF can exert immunomodulatory activity by the regulation of both innate and adaptive immune responses with the activation of natural killer cells, neutrophils, lymphocytes and immune cell recruitment and the modulation of cytokine/chemokine production (Fischer et al. 2006; Kuhara et al. 2006; Wakabayashi et al. 2006). All these LF functions rely on not only its capacity to sequester iron but also its property to interact with the molecular and cellular components of both the host and pathogens, including LPS and its receptor (Elass-Rochard et al. 1995). Zagulski et al. reported that LF treatment protected mice receiving a lethal dose of *Escherichia coli* in an experimental infection model (Zagulski et al. 1989). Guillén et al. investigated the response to *Staphylococcus aureus* infection in transgenic mice carrying a functional human LF gene, with transgenic mice having better bacterial clearance than their congenic littermates, which was further associated with trends of reduced incidence rates of septicemia and mortality (Guillen et al. 2002).

To further elucidate the immunomodulatory activity of LF and the relationship with sepsis prevention, we evaluated the antisepsis effect of bLF and the beta blocker metoprolol in an LPS-induced sepsis mouse model by determining the mouse survival rate, the levels of lactate and cytokines, and the structural changes in the heart, kidney, and liver.

Materials and Methods

Mouse Studies

Studies were approved by the Research Committee of the School of Medicine, Autonomous University of Sinaloa, Mexico (SE-2018-037). All mice were 6- to 8-week-old females weighing 20–25 g and had a Balb/*CAnN Hsd* background. Prior to treatment intervention, they were housed in standard cages in a temperature- and light-controlled room and fed ad libitum.

Mouse Treatments and Sample Collection

Eighty mice were randomly divided into two groups of 40 mice: one group was inoculated with lipopolysaccharide (LPS) from *E. coli* O111:B4 (Sigma, Aldrich, St. Louis, Missouri, United States) to induce sepsis, and the other group was inoculated with phosphate-buffered saline (PBS). Prior to LPS/PBS inoculation, both groups were divided into four groups of 10 mice to receive one of four treatments: intragastric saline solution, intraperitoneal metoprolol (Hikma Pharmaceuticals, London, UK, 2.5 mg/kg), intragastric bLF saturated at 20% iron (Nutriscience, Connecticut, USA, 1.95 mg/kg), or intragastric bLF + intraperitoneal metoprolol. Treatments were applied every 12 h for a total of 48 h. The inoculated group then received intraperitoneal LPS (20 mg/kg) and non-inoculated intraperitoneal PBS solution (Fig. 1). The mouse survival rate was monitored at 0, 3, 24, 48, 72 and 96 h after LPS/PBS inoculation, and blood was collected from the tail cut for lactate and cytokine measurements. Mice were sacrificed by cervical dislocation, and macroscopic analysis of damage in organs was made, them the heart, liver, and kidney were collected for histological analysis.

Cytokine Measurements

Blood samples were taken at 0, 3, 24, 48, and 96 h after LPS/PBS inoculation and centrifuged at 1500 rpm for 10 min to

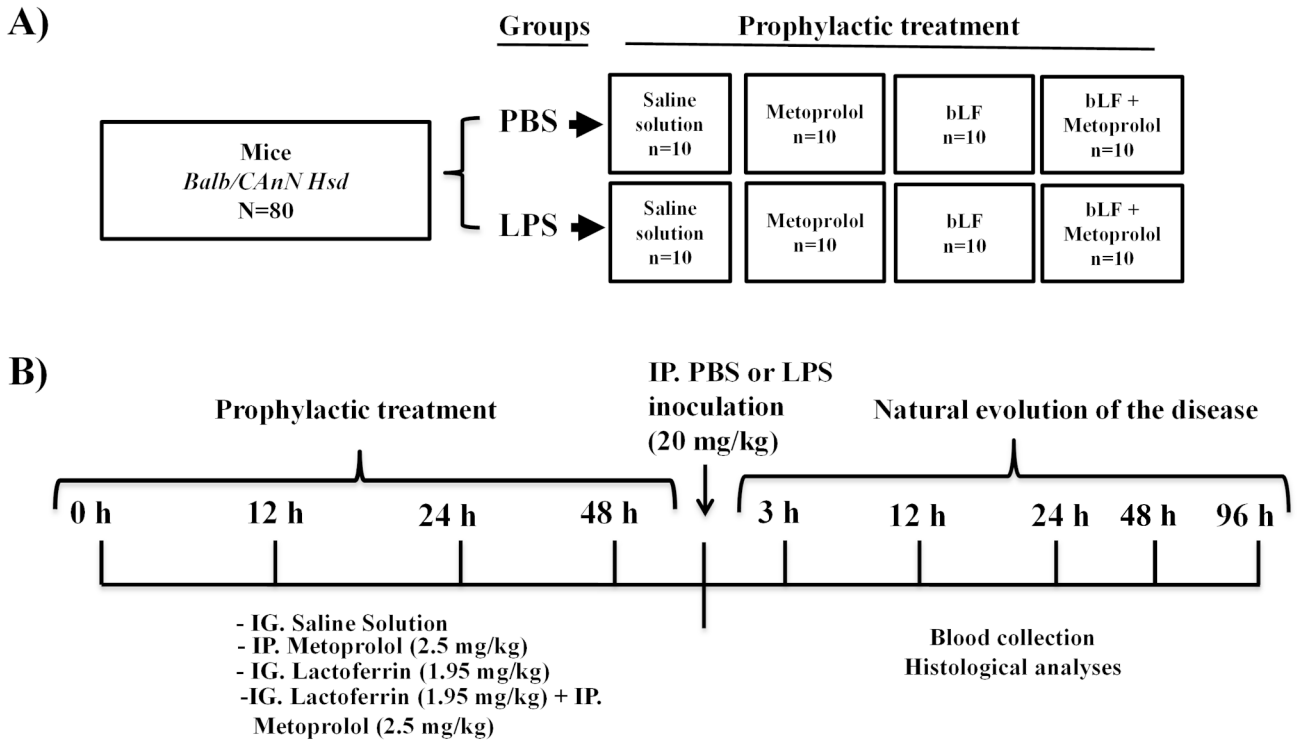

Fig. 1 Experimental design. (A) Eighty mice were randomly divided into two equal groups to receive PBS or LPS, both groups were divided into four groups and pretreated with saline solution, metoprolol, bLF, or bLF + metoprolol. (B) Treatments were applied every 12 h for a total of 48 h. Then mice were inoculated with intraperitoneal LPS or PBS, the mouse survival rate was monitored at 0, 3, 24, 48 and 96 h after LPS/PBS inoculation, blood was collected for lactate and cytokine measurements. Mice were sacrificed and the heart, liver, and kidney were collected for histological analysis. LPS, lipopolysaccharide; PBS, phosphate-buffered saline; bLF, bovine lactoferrin; Ip, intraperitoneal; Ig, intragastric.

obtain serum. Serum was used to measure pro- and anti-inflammatory cytokines, including interleukin (IL) 2, 4, 6, 10, 17, tumor necrosis factor (TNF), and interferon γ (INF γ), by flow cytometry (BD Accuri C6, New Jersey, USA) using the CBA mouse Th1/Th2/Th17 cytokine kit (BD Bioscience, New Jersey, USA) following the manufacturer's instructions.

Lactate Measurement

Serum lactate was determined using a lactate assay kit II (Sigma-Aldrich, MAK065) according to the manufacturer's protocol. Serum was first deproteinized with a 10-kDa cut-off spin filter to remove lactate dehydrogenase. The lactate reaction mixture, consisting of 50 µl of lactate reagent and 50 µl of filtered serum, was incubated at room temperature in the dark for 30 min. Subsequently, absorbance was measured at 450 nm (A_{450}) using a 96-well microplate reader (Multiskan™ FC Microplate Photometer, ThermoFisher Scientific, Helsinki, Finland), and the results were calculated using a lactate standard plot through linear regression and expressed as mmol of lactate per liter of the sample (mmol/L).

Histological Analysis

Liver, kidney, and heart tissues were collected from mice at 24 h post-LPS/PBS inoculation and fixed in neutral-buffered formalin. Fixed tissues were embedded in paraffin and sectioned at 8-µm thickness for hematoxylin-eosin staining. Samples were sent to pathology services of Civil Hospital of Culiacan for it analyze. Structural changes such as centrilobular necrosis, inflammatory infiltrate hemorrhagic congestion in the liver; hypertrophy, myocarditis, and muscle cell damage in the heart; and tubular necrosis and glomerular injury in the kidney of stained sections were examined by a histologist to determine organ damage. Damage was classified as follows: – null; + low; ++ moderate; and +++ high.

Statistical Methods

Statistical significance ($p \leq 0.05$) was determined by a non-parametric Kruskal-Wallis test. Kaplan-Meier method was used to determine the survival rate of mice. All data were analyzed using SigmaPlot version 12.0 (Sytastac Software Inc. CA, USA).

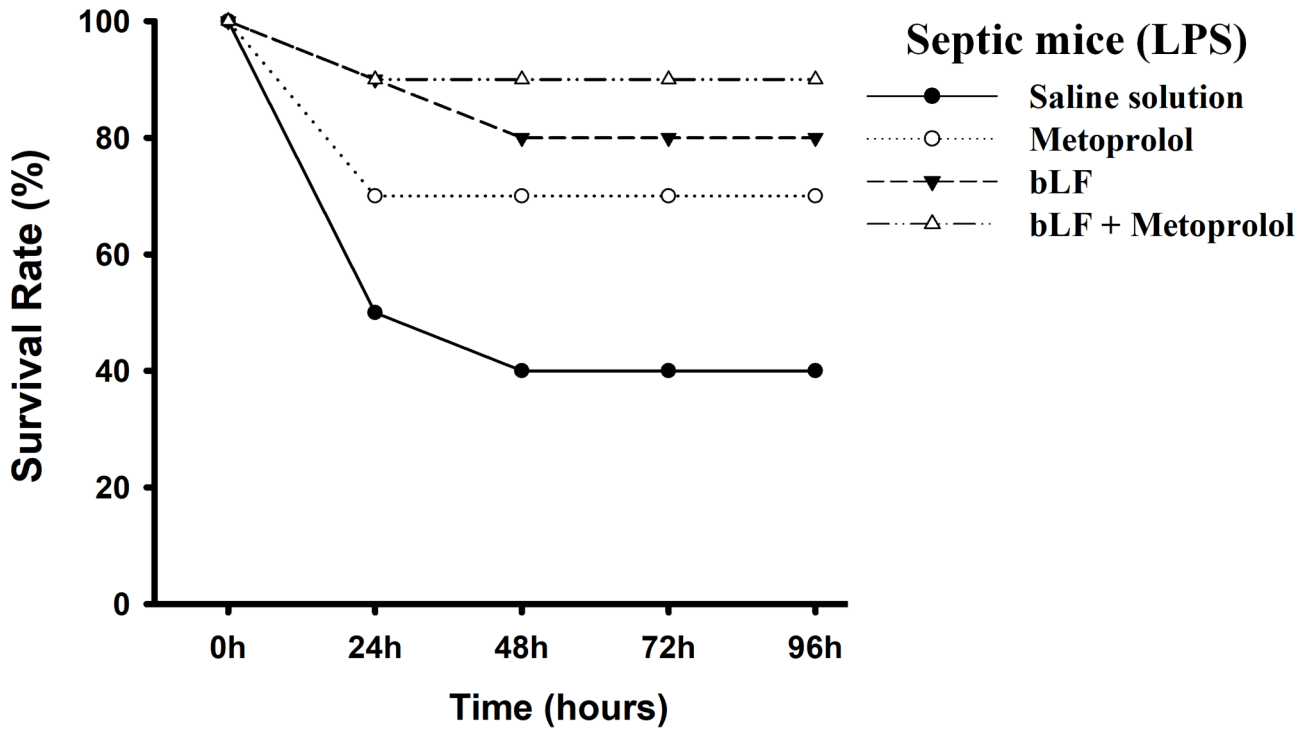

Fig. 2 Survival rates at 0, 24, 48, 72, and 96 h post-LPS/PBS inoculation of mice pretreated with saline solution, metoprolol, bLF, or bLF + metoprolol. All non-septic mice showed 100% survival. Septic mice treated with saline solution exhibited 40% decreased survival at 48 h after LPS inoculation. Septic mice pretreated with metoprolol, bLF, or bLF + metoprolol exhibited 70%, 80%, and 90% survival at 48 h post-LPS inoculation. Survival rate was estimated by Kaplan-Meier method LPS, lipopolysaccharide; PBS, phosphate-buffered saline; bLF, bovine lactoferrin.

Results

Bovine Lactoferrin And Metoprolol Increase The Mouse Survival Rate in LPS-Inoculated Mice

To determine the antisepsis effect of bLF and metoprolol, the mouse survival rate was first analyzed in septic (LPS-inoculated mice) and non-septic mouse (PBS-inoculated mice) mice treated with saline solution, metoprolol, bLF, or bLF + metoprolol. Saline/septic mice exhibited a marked decrease in the survival rate, reaching levels of 50% survival at 24 h of LPS inoculation. The metoprolol/septic, bLF/septic, and bLF + metoprolol/septic mice had higher survival rates than the saline/septic mice, with survival rates of 70% with metoprolol and 90% with bLF and bLF + metoprolol. From 48 to 96 h, the bLF + metoprolol/septic mice continued to have the highest survival rate (90%). All non-septic mice had 100% survival independent of the treatment (Fig. 2). By the Kaplan-Meier method, the survival of metoprolol + bLF/septic mice had was higher compared to bLF/septic, metoprolol/septic, and saline solution/septic mice.

Modulation of Inflammatory Cytokine Production by Bovine Lactoferrin and Metoprolol in LPS/PBS-Inoculated Mice

To evaluate the effect of bLF and metoprolol on reducing the inflammatory response caused by sepsis, the levels of proinflammatory (IL-2, IL-6, IL-17, TNF, and INF-γ) and anti-inflammatory (IL-4 and IL-10) cytokines were determined at 0, 3, 24, 48, and 96 h after LPS/PBS inoculation (Fig. 3). Serum levels of pro- and anti-inflammatory cytokines increased in saline solution/septic mice 3 h (IL-6, IL-10, and TNF), and 24 h (IL-17 and IFN-γ) after LPS inoculation, the highest levels of cytokines are shown below (pg/ml): 35,977.32, 2.34, 847.01, 302.81, and 99.43 for IL-6, IL-17, TNF, INF-γ and IL-10, respectively. After 24 h, most of cytokines dropped significantly in septic groups were treated, in comparison with saline solution/septic mice. bLF + metoprolol/ septic mice decreased significantly ($p \leq 0.05$) the levels of IL-6 (16.65 vs. 10,838.30 pg/ml), IL-10 (0.0001 vs. 39.83 pg/ml), TNF (25.92 vs. 258.02 pg/ml) and IFN-γ (0.36 vs. 302.81 pg/ml), in comparison with saline solution/septic mice, (Fig. 3). In bLF/septic mice significantly reduction was observed after 24 h of IL-6 (232.48 vs. 10,838.30 pg/ml), IL-10 (9.33 vs. 39.83 pg/ml), and IFN-γ (1.22 vs. 302.81 pg/ml) in comparison with saline

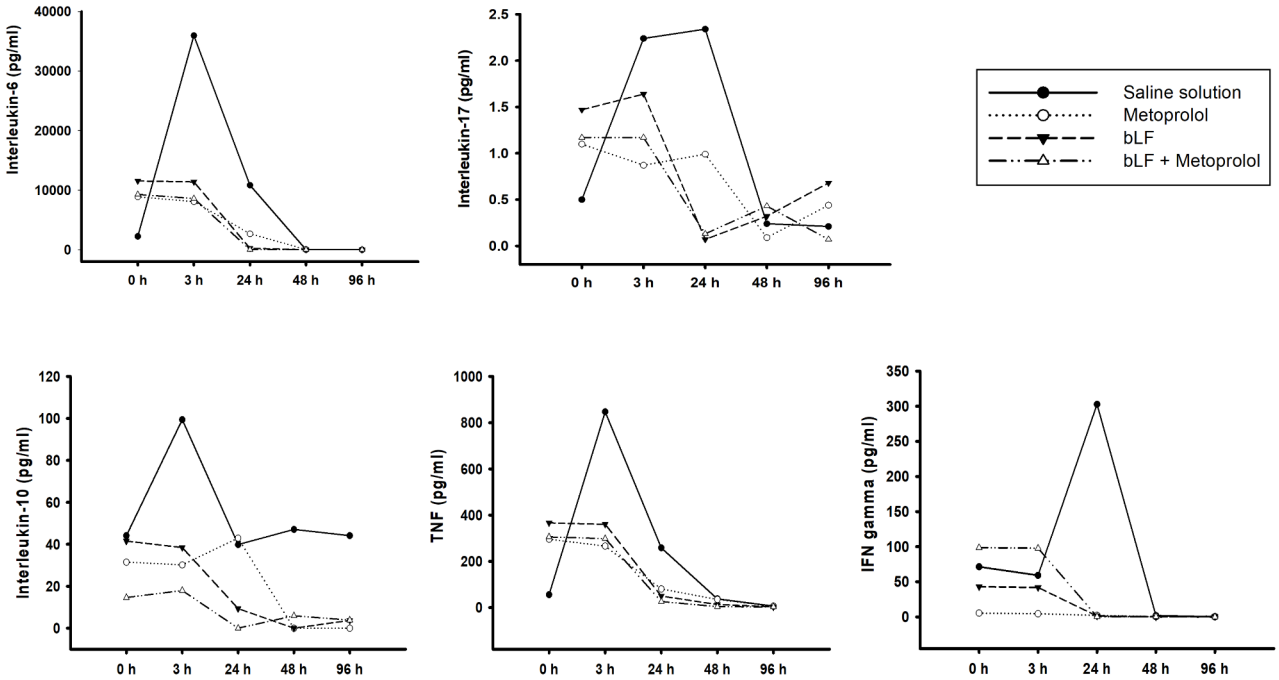

Fig. 3 Regulation of serum IL-6, IL-17, IL-10, TNF, and IFNγ at 0, 3, 24, 48, and 96 h post-LPS inoculation in mice pretreated with saline solution, metoprolol, bLF, or bLF + metoprolol. All three selected pretreatments resulted in reductions in IL-6, IL-17, IL-10, TNF, and IFNγ expression compared with saline solution LPS-septic mice. IL-2 and IL-4 were found below the limit of detection in all LPS-septic mice. PBS-inoculated mice presented no alterations in cytokine levels. Differences between the saline solution group and the metoprolol, bLF, and bLF + metoprolol groups were analyzed at 24 h post-LPS inoculation using the nonparametric Kruskal-Wallis test. $p \leq 0.05$ was considered statistically significant (significantly different values are shown in the main text).

solution/septic mice. On the other hand, metoprolol/septic mice only decreased the IFN-γ (2.13 vs. 302.81 pg/ml). Additionally, after 24 h on ward, no significant difference among groups was detected in terms of anti and pro inflammatory cytokines in comparison with saline solution/septic mice, (Fig. 3). Regarding IL-2 and IL-4, there were no significant changes between mice and all non-septic mice presented no alterations in cytokine levels (data not shown).

Bovine Lactoferrin and Metoprolol Decrease Lactate Levels in LPS-Inoculated Mice

To further evaluate the antisepsis potential of bLF and metoprolol, lactate serum levels were determined at 3, 24, 48, and 96 h after LPS inoculation (Fig. 4). Serum lactate levels increased 3 h after LPS inoculation in saline solution/septic mice, with a value of 9.6 mmol/L. Septic mice treated with metoprolol, bLF and bLF + metoprolol showed lower levels (8.2, 5.6 and 5.5 mmol/L at 3 h after LPS inoculation, respectively), although the differences were not significant, in comparison saline solution/septic mice. From 3 to 96 h, no significant differences were found in lactate levels among all the mice.

Protective Effect of bLF and Metoprolol on Sepsis-Induced Damage in Heart, Liver, and Kidney Tissue

The organ-protective effect of bLF and metoprolol against sepsis were first evaluated based on morphological changes (Fig. 5). Septic mice treated with saline solution presented inflammation in both the intestine and liver and necrosis in the spleen, while treated with bLF and metoprolol presented inflammation only in the intestine. No visible damage in bLF + metoprolol/Septic mice was detected among different organs, presenting similar size, color, and morphology to the PBS/non-septic groups (Fig. 5). Organ damage was then examined based on structural changes, and the results are shown in Table 1. saline solution/septic mice had marked organ damage presenting high centrilobular necrosis, high inflammatory infiltrate, and high hemorrhagic congestion in the liver; high hypertrophy, high myocarditis, and high muscle cell damage in the heart; and moderate tubular necrosis and moderate glomerular injury in the kidney. The metoprolol/septic and bLF/septic mice exhibited signs of reversal of organ damage caused by sepsis, ranging overall from null to moderate damage. bLF + metoprolol/septic mice showed the highest protective effect, exhibiting low centrilobular

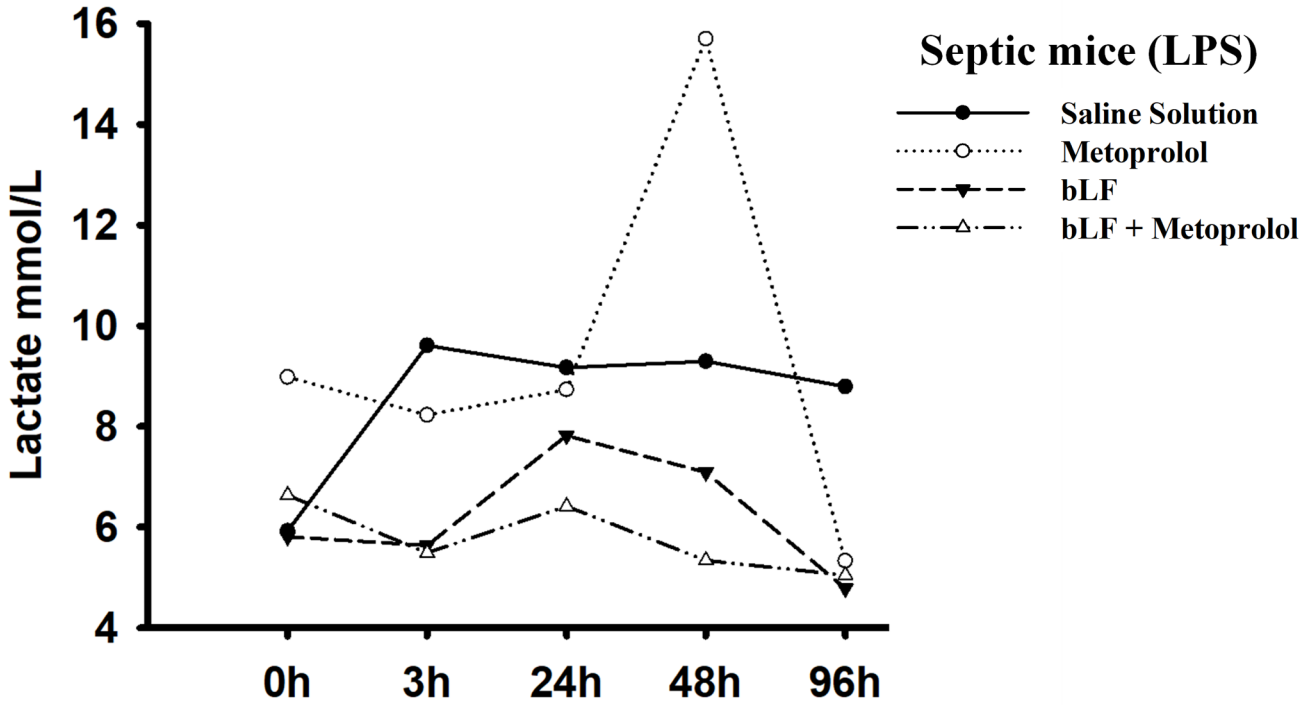

Fig. 4 Serum lactate levels at 0, 3, 24, 48, and 96 h post-LPS inoculation of mice pretreated with saline solution, metoprolol, bLF, or bLF + metoprolol. At 3 h post-LPS inoculation, metoprolol, bLF and bLF + metoprolol showed overall lower lactate levels, although not statistically significant, than saline solution. Differences between the saline solution group and the metoprolol, bLF, and bLF + metoprolol groups were analyzed at 3 h post-LPS inoculation using the nonparametric Kruskal-Wallis test. $p \leq 0.05$ was considered statistically significant.

necrosis, low inflammatory infiltrate, and low hemorrhagic congestion in the liver; low hypertrophy, low myocarditis, and null muscle cell damage in the heart; and low tubular necrosis and null glomerular injury in the kidney, similar to the non-septic mice.

Discussion

Despite significant scientific and clinical efforts, the number of patients diagnosed with sepsis is increasing worldwide (Paoli et al. 2018). Therefore, the development of novel therapies is a paramount concern for the scientific community. The present study shows that bLF and bLF in combination with metoprolol decrease the levels of proinflammatory cytokines (after 24 h), lactate, and organ damage in a mouse sepsis model. The immunomodulatory activity of bLF contributed to the organ-protective effects exhibited by both the bLF and bLF + metoprolol groups, as was observed in the morphologies of the liver, kidney, and heart tissues. Moreover, bLF treatment increased the mouse survival rate, an effect that was boosted when the mice were treated in combination with metoprolol.

Sepsis and severe sepsis management remain major challenges for healthcare systems, with an estimation of more than 6.3 million deaths annually in developing countries (de Souza et al. 2017). In this context, the search for novel and effective therapies to face the challenges that sepsis poses is of the utmost importance. In 1995, Dhainaut et al. studied the immunomodulatory activity of a "humanized" monoclonal antibody against human antitumor necrosis factor-alpha (TNF-alpha) in patients with septic shock. They reported a decrease in TNF-alpha levels after 30 min of antibody inoculation, although mortality was found to be similar to the placebo group (Dhainaut et al. 1995; Chen et al. 2019) demonstrated that oral administration of *Lactobacillus rhamnosus* reversed sepsis-induced microbiota dysbiosis in an experimental model of sepsis (Chen et al. 2019).

In this study, using an LPS-induced mouse model of sepsis, it was shown that the administration of bLF increased the survival rate in comparison to saline solution alone (Fig. 2). The preventative effect of bLF on sepsis has been suggested previously in different models. Akin et al. (2014) determined increased sepsis prevention rates in premature children taking LF (200 mg/day) in a placebo-controlled trial, reporting 4.4 and 17.3 sepsis events/1000 patients per day for LF and placebo, respectively (Akin et al. 2014).

Some of the antisepsis mechanisms known for LF are bactericidal and bacteriostatic effects. Mosquito et al. (2010) evaluated the bactericidal effect of bLF in a sepsis mouse

Intervention					
PBS	+	-	-	-	-
LPS	-	+	+	+	+
Pre-treatment					
Saline solution	+	+	-	-	-
Metoprolol	-	-	+	-	+
bLF	-	-	-	+	+

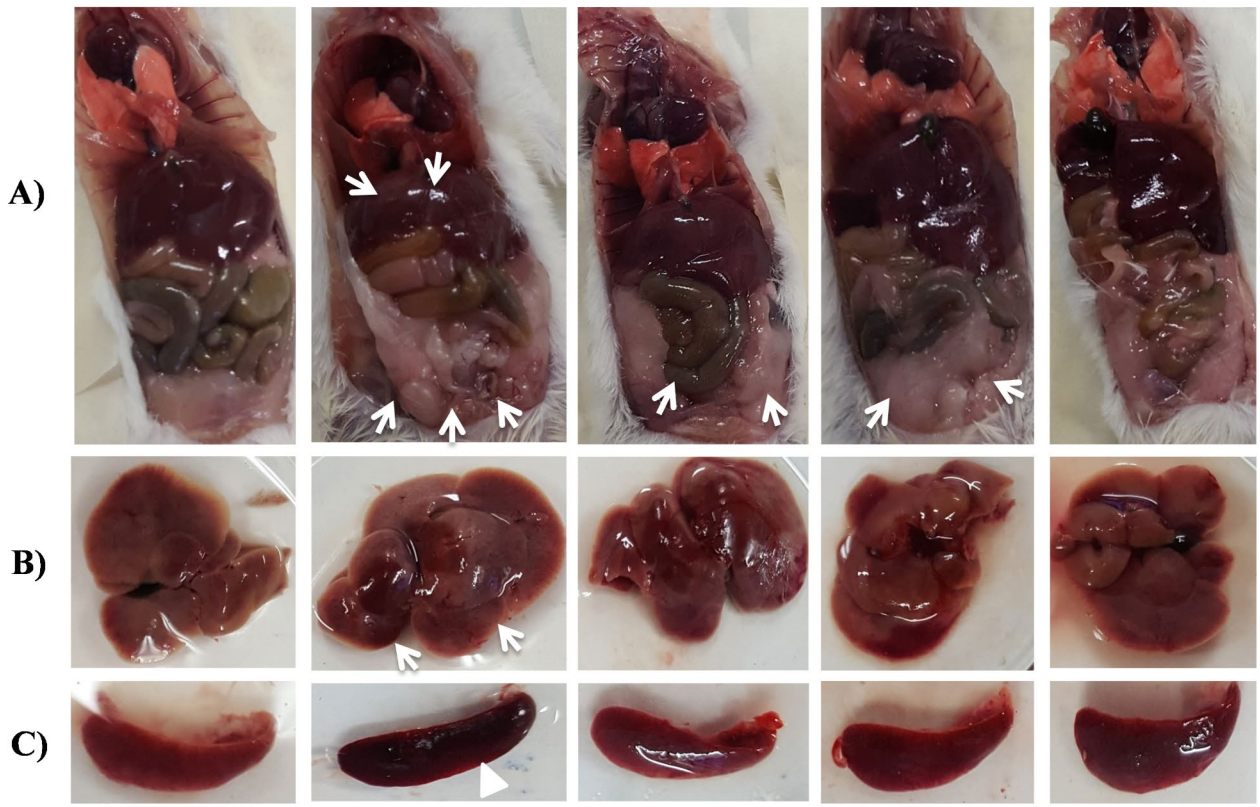

Fig. 5 Macroscopic changes in organ tissues involved in the sepsis process in analyzed mice. From left to right: mice inoculated with PBS and pretreated with saline solution; mice inoculated with LPS and pretreated with saline solution; metoprolol; bLF; and bLF + metoprolol. At 24 h post-LPS/PBS inoculation, mice were opened, and the size, color and morphology of organs were analyzed in the (A) thoracic and abdominal cavities, (B) liver, and (C) spleen. Indication of inflammation: white arrow, Indication of necrosis: white arrowhead. LPS, lipopolysaccharide; PBS, phosphate-buffered saline; bLF, bovine lactoferrin.

model inoculated with *Salmonella enterica* Typhimurim, which resulted in a lower mortality rate than the control (Mosquito et al. 2010). Similarly, the bactericidal effect of bLF was previously reported by our group in an *E. coli* O157:H7-infected mouse model. bLF produced a marked decrease in bacterial density and a higher mouse survival rate than the control (Flores-Villasenor et al. 2012). Using LPS in this study negates the possibility of LF-derived bacteriostatic and bactericidal effects.

Moreover, LF has also shown significant immunomodulatory activity (Uchida et al. 1994; van de Graaf et al. 1991). During sepsis, LPS is bound on the surface of macrophages to cluster of differentiation 14 (CD14) which facilities the transfer of LPS to complex toll-like receptor 4 (TLR4)/myeloid differentiation 2 (MD-2) protein, the complex recognizes the LPS, which triggering a signal cascade into de macrophages to its activation, as result of it, starts the production of proinflammatory cytokines (Wright et al. 1990). In this study, bLF and the combination of bLF + metoprolol decreased the production of IL-6, IL-10, IL-17, TNF, and INFγ (Fig. 3). Recent studies suggest that at least some of the immunomodulatory effects of LF rely on its capacity to form complexes with LPS blocking LPS-TLR4 or CD14 interaction and thereby inhibiting macrophage activation and the production of cytokines (Kim et al. 2012; Puddu et al. 2009). The results of the present study are in line with those reported by Kruzel et al. 2002, in which LF decreased IL-6 and TNFα levels in the serum of LPS-treated

Table 1 Histological analysis of liver, heart, and kidney at 24 h post-PBS/LPS inoculation of mice pretreated with saline solution, metoprolol, bLF, and bLF + metoprolol.

Group	Treatment	Liver			Heart			Kidney	
		Centrilobular necrosis	Inflammatory infiltrate	Hemorrhagic congestion	Hypertrophy	Myocarditis	Muscle cell damage	Tubular necrosis	Glomerular injury
PBS	Saline solution	+	+	+	+	-	-	-	-
	Metoprolol	+	+	-	+	-	-	-	-
	bLF	-	-	-	+	-	-	-	-
	bLF + Metoprolol	-	-	-	+	-	-	-	-
LPS	Saline solution	+++	+++	+++	+++	+++	+++	++	++
	Metoprolol	+	+	+	+	++	-	+	-
	bLF	++	+	+	++	+	+	+	-
	bLF + Metoprolol	+	+	+	+	+	-	+	-

Abbreviations: PBS: phosphate-buffered saline; LPS: lipopolysaccharide. Damage level: – null; + low; ++ moderate; and +++ high

mice (Kruzel et al. 2002). Moreover, in lactoferrin knockout mice infected with *Staphylococcus mutans*, intravenous LF administration abrogated the overproduction of the proinflammatory cytokines INFγ, TNFα, IL-1 and IL-6 (Velusamy et al. 2014a).

Here, for the first time, the antisepsis effect of the combination bLF + metoprolol was determined. This combination presented the highest immunomodulatory activity and the best protective effect of the selected treatments, with the highest mouse survival rate and the lowest production of inflammatory cytokines. Although the underlying mechanism by which β-blockers such as metoprolol decrease cytokine levels has not been well elucidated, this effect has been observed previously and can be partially explained by the modulatory activity of metoprolol on the adrenergic system and macrophage activity (Boost et al. 2007; Deng et al. 2004; Muthu et al. 2005; Novotny et al. 2009).

To further investigate the antisepsis effect of bLF and metoprolol, lactate serum levels were determined in all groups. Lactate is considered an important marker of tissue damage associated with inflammation and mortality in patients and has previously been shown to predict the occurrence and prognosis of sepsis (Filho et al. 2016; Nguyen et al. 2004; Scott et al. 2017). Additionally, new treatment strategies have focused on the clearance of lactate (Barzegar et al. 2016). In 2018, Xu J. et al. reported that ergosterol decreased lactate levels by more than 50% in septic rats inoculated with LPS and modulated myocardial marker enzymes altered by sepsis, such as CK and LDH (Xu et al. 2018). The bLF + metoprolol group exhibited the most marked decrease in lactate 3 h after LPS inoculation compared to bLF or metoprolol alone (Fig. 4), which could be the result of a synergistic effect on the immunomodulatory activity of bLF and metoprolol.

Finally, the protective effect of lactoferrin and metoprolol against sepsis was directly determined in organs by measuring morphological and structural changes. Notably, treatments that showed the highest immunomodulatory activity and the lowest lactate levels (bLF and bLF + metoprolol) exhibited notably lower damage in the liver, heart, and kidney (Table 1). This organ-protective effect of LF has been previously observed in the liver and spleen of an LF knockout mouse infected with *Aggregatibacter actinomycetemcomitans* (Velusamy et al. 2014b).

The limitation of this study was that some mice had been already dead before the time points of blood collection, also then histological images were not included in the study. Both facts could provide important information.

Conclusions

In conclusion, this study shows a protective effect of bLF against LPS-induced sepsis in mice. Moreover, for the first time, the synergic immunomodulatory activity between bLF and metoprolol against sepsis, which exhibited the highest protective effect, is shown. This synergism suggests that this combination could represent a novel alternative therapy to prevent or treat sepsis.

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Author Contribution Jesús Javier Martínez-García and: Data curation, Writing-Original draft preparation, conceptualization; Adrian Canizalez-Roman: Visualization, Investigation, Methodology; Uriel A. Angulo-Zamudio and Jorge Velazquez-Roman: Formal analysis, Data curation; Héctor Flores-Villaseñor and Marco A. Valdez-Flores: Writing- Reviewing and Editing; Efren Rios-Burgueño, David Moran-Portela: Supervision, Validation and Reviewing; Nidia Leon-Sicairos: Writing-Original draft preparation, conceptualization, Project administration, Funding acquisition. All authors contributed to the writing of the final manuscript.

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest None of the authors have any proprietary interests or conflicts of interest related to this submission.

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