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Effects of Lacosamide in Rats with Lipopolysaccharide Induced Hepatic Pathology

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Abstract

Bacterial lipopolysaccharides (LPS) generally increase the pathogenicity of the agent. This study aimed to examine the hepatic pathology and possible prophylactic effects of lacosamide (LCM) in a LPS-induced sepsis rat model. Overall, 24 1-year-old female Wistar Albino rats were divided into three groups: Group I (control), Group II (LPS group: 5 mg/kg LPS intraperitoneally, single dose), and Group III (LCM group: 40 mg/kg LCM intraperitoneally once daily for 3 days plus 5 mg/kg LPS 30 min after the last LCM treatment). Animals were euthanized 6 hours after LPS administration. Blood and liver samples collected during necropsy were analyzed biochemically, pathologically, and immunohistochemically. LPS caused a significant increase in serum aspartate aminotransferase, alanine aminotransferase, total bilirubin, direct bilirubin, indirect bilirubin, and alkaline phosphatase levels. Histopathological analysis revealed numerous neutrophil leucocyte infiltrations, slight hemorrhages in the liver, and degenerative or necrotic changes in hepatocytes. Increased expressions of malondialdehyde, C-reactive protein, heat shock protein-70, interleukin-1β, and tumor necrosis factor-α were observed in the LPS administered group. LCM ameliorated the biochemical, histopathological, and immunohistochemical findings. The present study results revealed that LCM ameliorated the LPS-induced liver damage in the rat models as evidenced by the biochemical and pathological findings.

Keywords: immunohistochemistry, lacosamide, lipopolysaccharide (LPS), liver, pathology.

Introduction

Lipopolysaccharides (LPS) are large lipid- and polysaccharide-containing molecules found in the outer membrane of gram-negative bacteria.[1] LPS in some bacteria play a pivotal role in the pathophysiology of sepsis.[2] The intraperitoneal application of LPS is an experimental model for inducing systemic and hepatic inflammation in rodents. LPS may lead to dysfunction or failure of numerous organs, including the liver.[3,4] Acute phase proteins (APPs) are increased after LPS administration.[5] Endotoxemia is indicative of severity of the sepsis and the leading cause of death.[6]

Lacosamide (LCM) is a third-generation antiepileptic drug (AED) that was first approved in 2008 for use in adjunctive therapy for partial-onset seizures in adults. In 2014, LCM was approved by the FDA for use in monotherapy for partial-onset seizures. Unlike conventional sodium channel blockers, LCM stimulates the slow inactivation of sodium channels selectively. This action mechanism results in the stabilization of overexcitable neuronal membranes, inhibition of neuronal firing, and reduction of the long-term availability of channels without affecting the physiological function.[7] LCM does not stimulate or inhibit the cytochrome P450 enzyme. It has low protein-binding ability (< 15%) due to its numerous destruction mechanisms and

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does not interact with most clinically prescribed drugs.[7,8] The entry of calcium (Ca2+) into the cytoplasm is the most common signaling factor of cell damage in all cell types. The normal concentration of intracellular Ca2+ is typically lower than that of extracellular Ca2+.[9,10] Therefore, excessive intracellular Ca2+ may activate degradative processes and cause toxic effects in cells.[9,11] Similarly, sodium (Na+), which is the major cation in the extracellular space, can enter cells through various routes, especially during increased cell membrane permeability.[12] A significant increase in Na+ is characteristic of tissue injuries.[13,14] The use of sodium channel blockers reduces both Na+ entry and apoptotic neuronal death.[14] A well-known fact is that Na+ influx into the cell is accompanied by chloride ions (Cl−) and water that can lead to acute cell swelling and damage.[15] The inhibition of Na+−H+ exchange attenuates ischemia-induced cell death.[16,17] The primary focus of pharmaceutical research has been to discover effective therapeutic approaches that target voltage-gated Na+ channels.[18] LCM facilitates slow inactivation of sodium channels.[7,8]

Ameliorative effect of LCM on sepsis induced polyneuropathy previously reported.[19] But there is no knowledge about effect of LCM on LPS induced hepatic pathology. The objective of this study was to evaluate of the pathogenesis of sepsis and effect of LCM on LPS induced liver lesions. For this purpose, we evaluated the effects of LCM on liver via biochemical, histopathological and immunohistochemical findings in an in vivo rat model of sepsis.

Materials and Methods

Animals

The experiments were performed in accordance with the guidelines for animal research of the National Institutes of Health and were approved by the Committee on Animal Research at Burdur Mehmet Akif Ersoy University, Burdur, Turkey. Animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals in the Experimental Animal Center of Burdur Mehmet Akif Ersoy University (approval number:308).

Before the experiment health status of all rats were controlled by a veterinarian who was responsible for the animals. Overall, 24 1-year-old female Wistar Albino rats were used and divided into three groups: Group I (control) (0.1 ml/oral and i.p. saline, single dose), (n=8), Group II (LPS group: 5 mg/kg LPS intraperitoneally, single dose; (i.p. lipopolysaccharide, 500 mg flk, 048K4126, Sigma-Aldrich, USA), [20] (n=8), and Group III (LCM group: 40 mg/kg LCM intraperitoneally once daily for 3 days and 5 mg/kg LPS 30 min after the last LCM treatment; (Benvida 100 mg tb/ Adeka Farmacy, Turkey)[19], (n=8). LCM was dissolved in normal saline. Four rats weighing 350– 400 g were kept in a conventional cage, two cages used for each group, they placed in a temperature- (21–22 °C) and humidity (60 + 5%)-controlled room in which a 12:12 h light–dark cycle was maintained. All the rats were fed ad libitum a standard commercial chow diet (Korkuteli yem; Antalya, Turkey) and drinking water during the study. The rat numbers were minimalized and total numbers of the animals selected for reliable statistical analysis. Animals were euthanized 6 hours after LPS administration. During necropsy, blood and liver samples were collected for biochemical, pathological, and immunohistochemical analyses.

Biochemical Analysis

An autoanalyzer (Beckman Coulter AU680, California, USA) was used to determine the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T. bil.), direct bilirubin (D. bil.), indirect bilirubin (I. bil.), albumin (ALB), and alkaline phosphatase (ALP) levels.

Histopathological Examinations

During necropsy, the collected liver samples were fixed in 10% neutral formalin. Samples were then processed routinely using an automatic tissue processor equipment (Leica ASP300S, Wetzlar, Germany) and embedded in paraffin wax. Tissue sections of 5-µm thickness were cut using a rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany); they were then stained with hematoxylin–eosin (H&E), placed on a mounting media and coverslipped, and examined under a light microscope.[21]

Immunohistochemical Examinations

For immunohistochemical analysis, liver samples were immunostained with antibodides raised against malondialdehyde (MDA) [Anti-Malondialdehyde antibody (ab6463)], C-reactive protein [Anti-C Reactive Protein antibody - Aminoterminal end (ab65842)], heat shock protein-70 (HSP-70) [Anti-Hsp70 antibody [5A5] (ab2787)], interleukin-1β (IL-1β) [Anti-IL1 beta antibody (ab2105)], and tumor necrosis factor-α (TNF-α) [Anti-TNF alpha antibody (ab6671)] using the streptavidin–biotin technique. All primary serums and secondary antibodies were purchased from Abcam (Cambridge, UK), and all primary antibodies used were 1/100 dilution. Primary antibodies were incubated for 60 min, and immunohistochemistry was performed using biotinylated secondary antibody and streptavidin–ALP conjugate. Ready-to-use kits [EXPOSE Mouse and

Rabbit Specific HRP/DAB Detection IHC kit (ab80436)] were used as secondary antibodies and 3,3-diaminobenzidine as chromogen for 5 min. For negative controls, primary antiserum step was omitted. Histopathological and immunohistochemical examinations were performed on blinded samples. The percentage of immune-positive cells for each marker was determined by counting 100 cells in 10 different fields for every section under 40× objective magnification for all groups.[22] Statistical analyses were performed on the results obtained from the image analyzer. Morphometric analyses were performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

Statistical Analysis

One-way analysis of variance test was used to determine significant differences among the groups. The groups were compared using the non-parametric Kruskal–Wallis and Dunnett tests. Biochemical parameters that fit the normal distribution were obtained, and ANOVA, post-hoc LSD, Bonferroni, and Tukey tests were used to compare the groups. Differences among groups in the histopathological and immunohistochemical analyses were determined using the Bonferroni multiple comparison method. Calculations were made using the SPSS 20.0 program, and $p < 0.05$ was set as the level of significance.

Results

Biochemical Findings

LPS caused a significant increase in the serum AST, ALT, T. bil., D. bil., I. bil., and ALP levels. Serum ALB levels were not affected by LPS administration. The results of biochemical analysis are summarized in Table 1.

	CON	LPS	LCM	P
AST (IU/L)	153.00±10.84	588.00±322.02	169.00±41.99	Con-LPS <0.01 Con-LCM (NS) LPS-LCM (NS)
ALT (IU/L)	70.25±16.14	178.75±54.63	35.33±7.91	Con-LPS<0.01 Con-LCM (NS) LPS-LCM (NS)
T.bil (mg/dL)	0.39±0.17	0.71±0.28	0.36±0.12	Con-LPS <0.05 Con-LCM (NS) LPS-LCM <0.05
D.bil (mg/dL)	0.020±0.002	0.040±0.001	0.015±0.002	Con-LPS< 0.05 Con-LCM (NS) LPS-LCM (NS)
I.bil (mg/dL)	0.37±0.17	0.67±0.26	0.35±0.12	Con-LPS <0.05 Con-LCM (NS) LPS-LCM <0.05
ALB (g/dl)	2.50±0.39	2.55±0.37	2.61±0.19	Con-LPS (NS) Con-LCM (NS) LPS-LCM (NS)
ALP (IU/L)	183.12±140.59	205.33±145.44	45.79±18.69	Con-LPS (NS) Con-LCM (NS) LPS-LCM (NS)

Table 1: Statistical analysis results of serum biochemical analysis and oxidative stress markers.

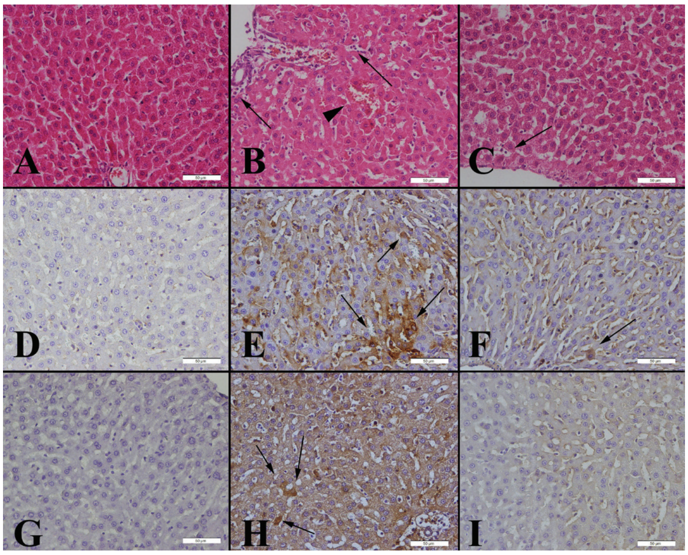
Necropsy Findings

At necropsy hyperemia at the hepatic vessel were the marked finding in LPS group. There was no pathological finding observed in CON and LCM group.

Histopathological Findings

Histopathological analysis revealed normal tissue architecture in the control group. Microscopic examination of the LPS group revealed marked hyperemia, inflammatory reaction comprised of neutrophil leucocyte, and hemorrhages in livers. Additionally, degenerations in some cells were the other common findings in this group. However, LCM treatment ameliorated the histopathological findings (Fig.1A-C).

Figure 1: Histopathological appearance of the livers. A) Normal histology in control group. H&E; B) Inflammatory reaction (arrows) and hemorrhage (arrow head) in the LPS group. H&E; C) decreased inflammatory reaction (arrow) in LCM-treated group. H&E. Bar = 50 µm. MDA immunoreaction in the livers. D) Negative immunoreaction in the control group. E) Increased immunoexpression (arrows) in hepatocytes in the LPS group. F) Slight immunoexpression (arrow) in the LCM-treated group. CRP immunoreactions in the livers. G) Negative immunoreaction in the control group. H) Increased immunoexpression (arrows) in hepatocytes in the LPS group. I) No immunoexpres-sion in the LCM-treated group. Streptavidin–biotin peroxidase. Bars = 50 µm.



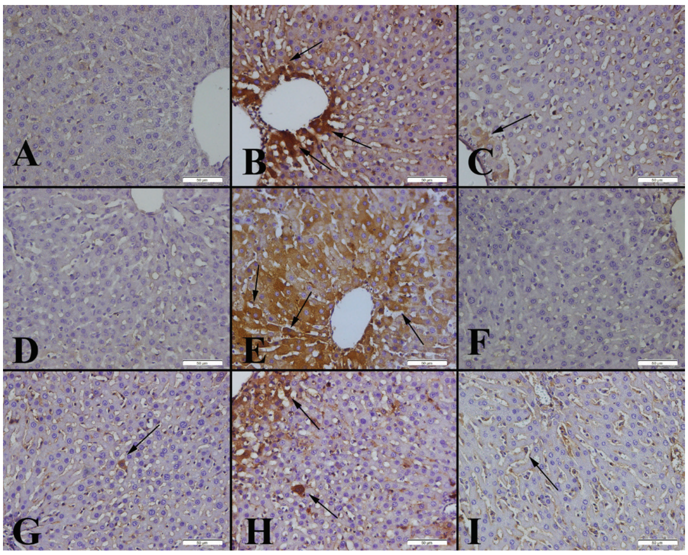
Immunohistochemical Findings

Immunohistochemical analyses revealed little or no expression of the investigated molecules in the control group. However, LPS caused an increase in the expressions of MDA, CRP, HSP-70, IL-1β, and TNF-α in hepatocytes and sinusoidal endothelial cells intracytoplasmically. The most significantly expressed markers were IL-1β, HSP-70, and TNF-α, respectively. Notably, LCM had an ameliorative effect and caused a statistically significant decrease in the expression of all markers (Figs. 1D-I, 2), (Table 2).

Markers	Control	LPS	LCM	P value
MDA	0.50±0.37	6.83±0.98	1.33±0.51	Con-LPS<0.001 Con-LCM (NS) LPS-LCM<0.001
CRP	1.25±0.36	11.83±2.15	2.07±0.36	Con-LPS<0.001 Con-LCM (NS) LPS-LCM<0.001
HSP-70	1.00±0.32	16.16±1.10	2.16±0.70	Con-LPS<0.001 Con-LCM (NS) LPS-LCM<0.001
IL1-β	0.50±0.26	27.33±1.52	2.16±0.70	Con-LPS<0.001 Con-LCM (NS) LPS-LCM<0.001
TNF-α	1.85±0.50	15.66±1.22	2.50±0.42	Con-LPS<0.001 Con-LCM (NS) LPS-LCM <0.001

Table 2: Statistical analysis of immunohistochemically positive cell numbers in the livers among the groups.

Figure 2: HSP-70 immunoreaction in the livers. A) Negative immunoreaction in the control group. B) Increased immunoexpression (arrows) in hepatocytes in the LPS group. C) Slight immunoexpression (arrows) in the LCM-treated group. IL-1β immunoreaction in the livers. D) No immunoreaction in hepatocytes in the control group. E) Increased immunoexpression (arrows) in hepatocytes in the LPS group. F) Negative immunoexpression in the LCM-treated group. TNF-α immunoreaction in the livers. G) Slight immunoreaction in hepatocytes (arrow) in the control group. H) Increased immunoexpression (arrows) in hepatocytes in the LPS group. I) Decreased immunoexpression (arrow) in the LCM-treated group. Streptavidin–biotin peroxidase. Bar = 50 μm.



Discussion

This study revealed that LPS caused liver damage in rats and that the pathogenesis was associated with increased expression of IL-1β, HSP-70, TNF-α, CRP, and MDA. LCM can be a possible drug choice in the prevention of LPS-induced liver lesions.

LD50 dose of the LPS for rats previously reported as greater than 20 mg/kg in normal rats at 24 hours.[23] The most preferred dose for single intraperitoneal injection of LPS is 5mg/kg for rats.[24–26] Dose selection were made based of the previous studies.

The induction of hepatic injury becomes evident because of the elevated levels of serum AST and ALT, which can be measured using standard clinical chemistry. Typically, when an inflammation-modifying substance needs to be tested, it is mandatory to analyze a control group simultaneously that only receives LPS and has no further intervention.[4] Bacterial LPS, also known as endotoxin or lipoglycan, is the major component of the outer surface of gram-negative bacteria.[27] Our study observed significant increases in the levels of serum ALT, AST, ALP, T. bil., D. bil., and I. bil. in the LPS group. However, LCM treatment attenuated the biochemical results.

LPS activates Toll-like receptor 4 and eventually nuclear factor-kappa B (NF-κB) mechanism followed by the release of inflammatory cytokines, such as TNF-α, IL-1α/β, IL-6, IL-12, IL-18, and GM-CSF.[4] Oxidative stress is also a well-known mechanism of LPS-induced hepatic injury which is supported by reactive oxygen species.[28] A large proportion of proinflammatory mediators comprising cytokines are responsible for metabolic changes associated with cellular injury.[29] Cytokines function as mediators of the immune and acute phase responses. TNF-α, IL-1β, and IL-6 are the major mediators of acute phase response in humans.[30] Additionally, IL-6 functions as an endogenous pyrogen that stimulates the immune system and, in conjunction with TNF-α, can stimulate the synthesis of acute phase proteins, such as CRP from hepatocytes.[31] The immunohistochemical examination of our study revealed substantially increased expression of MDA, CRP, HPS-70, IL-1β, and TNF-α. However, LCM was observed to be effective in reducing both inflammatory and oxidative damage markers. We believe that the most suitable markers to evaluate LPS-induced hepatic damage are IL-1β, HSP-70, TNF-α, CRP, and MDA.

Despite several studies have been focused on this subject, the mechanism of LPS-induced endotoxemia is still not completely clear. It has been suggested that oxygen-derived radicals are generated during endotoxic shock that induce tissue injury. Lipid peroxidation may then be initiated, inducing further tissue damage.[28-32] Lipid peroxidation with unsaturated lipids generates a wide variety of oxidation products. Notably, the primary products of lipid peroxidation are lipid hydroperoxides. The secondary and most mutagenic product formed during lipid peroxidation is MDA.[33] A majority of the toxic effects of LPS are mediated by proinflammatory cytokines, such as TNF-α, IL-1α, and IL-1β, that are produced by monocytes and macrophages.[34] TNF-α reportedly plays a central role in the pathology of LPS-induced lethality.[3] Furthermore, CRP is an acute phase reactant that is synthesized and released by various cells, including hepatocytes in response to microbial infection, tissue injury, and immunomodulatory stimuli.[35] Additionally, HSPs are a group of stress proteins that are actively synthesized when macrophages are exposed to bacterial toxins.[36] HSPs are present in almost all eukaryotic cells, including hepatocytes, and are transiently overexpressed when cells are exposed to heat shock.[37] HSP-70 is an abundant and well-conserved, stress-inducible protein that plays a vital role in the cellular stress response and enables organisms to survive multiple environmental stresses. It is constitutively expressed and is essential in the normal functioning of cells.[38] Notably, when cells are exposed to a stressor, the rapid increase in HSP-70 levels reportedly protects cells from the harmful effects of the stressor.[39] The protective effect of HSP-70 has been demonstrated in a variety of cells, tissues, and organs.[39,40] In this study, we examined LPS-induced liver toxicity and the effect of LCM on acute sepsis through biochemical, histopathological, and immunohistochemical methods. We identified that MDA, CRP, HSP-70, IL-1β, and TNF-α play important roles in LPS-induced hepatic pathology. Liver histopathology revealed that hyperemia, hemorrhage, inflammatory reaction mainly comprising neutrophil accumulation, and necrotic changes in hepatocytes in the LPS group indicated classic signs of hepatic damage. Increased immunoexpression of the markers was indicative of lipid peroxidation and acute phase reaction, which are critical factors of LPS-induced hepatic damage.

Few reports have suggested an increase in enzymes and toxicity in the liver following LCM treatment[41-43] with hepatic impairment considered to be a severe side effect of LCM.[44] However, in all these cases, LCM was administered for a longer term with multiple doses. Conversely, our results suggested that three doses of LCM can produce ameliorative effects in liver damage. Unfortunately, the mechanism of this effect is elusive. Nonetheless, the possibility of an interaction of LCM—a sodium channel inhibitor—with the epithelial sodium channels of hepatocytes via an un-known mechanism is suggested. Notably, in rat and mice, the brain and liver intestine sodium channels (BLINaC) are predominantly expressed in the brain, liver, and intestine.[45] However, the functions of these channels remain unknown.[46] Therefore, we suggest that LCM affects these channels to prevent sodium influx in hepatocytes that can cause degenerative changes and decrease inflammatory and free radical injury via an unknown mechanism.

In our study, we used only 40 mg/kg for three days followed by one dose of LPS (5 mg/kg) and obtained unexpected results because the LCM improved the liver condi-tion. Chronic nature of epilepsy necessitates lifelong use of AEDs. Generally, epilepsy cannot be controlled with a single AED in more than 30% of patients; therefore, multiple AEDs are needed.[7] New AEDs, such as levetiracetam and LCM, are more suitable options for patients with hepat-ic impairment compared with the older AEDs[46-47] because these drugs are less likely to exacerbate liver damage. Moreover, these drugs show low or no serum protein-binding capacity,[47] lower drug-to-drug interaction, and minimal toxicity risk.[5,48] LCM is metabolized by the CYP450, 2C19 system, but its metabolites are inactive. Its protein-binding capacity is lower than 15%, and approximately 40% of it is excreted in the urine. LCM is also a safe choice because of its linear pharmacokinetics.[46] Although the long-term usage of LCM may produce liver damage, we observed that three doses of LCM did not induce liver injury but surpris-ingly ameliorated serum biochemical levels of liver enzyme and improved histopathological and immunohistochemical findings. We also reported that ameliorative effects of LCM on LPS induced neuro inflammation and urogenital system damage by using related organs of these study rats recently.[49,50]

Therefore, to conclude, three doses of LCM can prevent LPS-induced acute liver damage by suppressing both inflammatory and oxidative injuries through an unknown mechanism. However, further studies are warranted to understand this mechanism entirely. However, the possibility of exacerbation of liver damage must be considered when using this drug for a longer duration.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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