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Comparative Study of Montelukast with Dexamethasone for Possible Protection against Endotoxic Effect of Lipopolysaccharide in Male Mice

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Abstract

Endotoxic septic shock is a serious clinical syndrome caused mostly by gram-negative bacterial infections; It refers to infected patients with high bloodstream endotoxin levels and activity with high rate of cardiovascular, pulmonary, hepatic, and renal failure. The endotoxin in the bacterial cell wall can evoke neutrophil monocytes and stimulate secretion of inflammatory mediators like cytokines, nitric oxide, histamine, and bradykinin, causing local and systemic inflammation, severe vasodilation, endothelial dysfunction, increased capillary permeability, and disseminated intravascular coagulation. Mice were utilized as experimental animals for research by induction of endotoxemia/septic-like condition that closely simulates septic shock in humans by administration of lipopolysaccharide isolated from a particular bacterial strain at an appropriate dose. This study aimed to determine if male mice may be protected from lipopolysaccharide-induced endotoxicity by using montelukast compared with dexamethasone. Twenty-eight Albino mice were randomly assigned to four groups (seven per group): Group I (control) mice were given 0.25-0.5 ml of distilled water by oral gavage once daily for a week and euthanized on the 8th day. Group II mice received 10 mg/kg intraperitoneal lipopolysaccharide and were euthanized on the second day. Group III mice received oral gavage dexamethasone 2.5 mg/kg once daily for a week. Two hours following the final dosage, lipopolysaccharide was given and euthanized on the eighth day. Group IV mice received oral gavage montelukast 20 mg/kg once daily for a week. Two hours after the last dose, lipopolysaccharide was given and euthanized on the eighth day. Results show that pretreatment with both drugs lowers serum tumor necrosis factor-alpha, interleukin-6, receptor activator nuclear factor kappa B, and osteoprotegerin levels, as well as cathepsin-G and neutrophil elastase gene expression. In conclusion, montelukast may have a promising protective effect from lipopolysaccharide-induced endotoxicity and may be an option as a protective drug from inflammatory consequences in human.

Keywords: Endotoxic Septic Shock, Inflammatory Mediators, Lipopolysaccharide, Montelukast.

Introduction

The bone helps the body move, provides strength and rigidity, protects soft tissues, and it is constantly changed because of resorption and generation [(1)]. Receptor activator nuclear factor kappa B ligand (RANKL) modulation by osteocytes is one of many mechanisms that govern this complex process. Although RANKL is usually linked to the cell membrane, it can be split into sRANKL[(2)]. RANKL binds to osteoclasts and stimulates receptor activator nuclear factor kappa B (RANK) to start osteoclastogenesis[(2)]. Normally, osteocytes help osteoblasts manufacture osteoprotegerin (OPG), a soluble decoy receptor that binds RANKL and blocks its interaction with osteoclast precursors' RANK protein[(3)].

Bone marrow; the spongy gelatinous tissue presents in the hollow of bone , harbors immune cells like neutrophils. Besides; the spleen and lung also retain neutrophils [(4)]. In addition to its long-standing role in neutrophil clearance, the spleen possesses a large resident neutrophil population in its red pulp that aids in infection response. Both mature, motile Ly6Ghigh neutrophils and immature, static Ly6Gint neutrophils live in the mouse spleen in a steady state. According to spleen intravital microscopy, these neutrophil populations, as well as red pulp and marginal zone macrophages, contribute to the clearance of *Streptococcus pneumoniae* [(5)].Lipopolysaccharide (LPS) , or endotoxin , can

induce sepsis and septic shock. It strongly activates the inflammatory cascade. Endotoxic septic shock (ESS) is characterized by elevated endotoxins and a history of hepatic dysfunction, acute renal damage, and endothelial dysfunction [6]. ESS is more over 50% of patients, making it a terrible condition [7]. Sepsis kills about 11 million people worldwide [8], with septic shock killing over 40% of patients before they leave the hospital [9].Multiple variables interact to cause sepsis, including host susceptibility (e.g., age, environment, genetics) and pathogen burden, pathogenicity, and pathogen-associated molecular patterns (PAMPs)[10]. The action of endotoxin is mediated by a cascade of proteins that includes Toll-like receptor 4 (TLR4), myeloid differentiation factor 2 (MD-2), and the cluster of differentiation 14 (CD14), with the latter also requiring lipopolysaccharide-binding protein (LBP). There are three main ways that endotoxin can be recognized: (i) the TLR4-MD-2 receptor pathway, which detects it outside of cells; (ii) the caspase 4/5 mechanism, which detects it inside cells (for example, following infection by bacteria inside cells); and (iii) complement, which binds to endotoxin in the blood[11]. Figure 1.Even low amounts of endotoxin have significant impacts on human organs and systems[12].LPS causes the following actions: TNF-α and IL-6 production leads to the release of prostaglandins and leukotrienes. A cascade of histamine release begins with complement system activation (C3a, C3b, and C5a). This stimulation causes neutrophil chemotaxis and accumulation. Direct endothelial cell stimulation releases nitric oxide (NO) and numerous reactive oxygen species (ROS). Activation of factor XII and coagulation cascade. Plasmin activation produces bleeding and fibrinolysis. Kinin activation releases vasoactive peptides such as bradykinins. Results include inflammation, intravascular coagulation, bleeding, tissue hypoperfusion, hypotension, and septic shock[13]. Figure 1.Endotoxin activity assay (EAA) uses whole blood and a monoclonal antibody against lipid A. Ag-Ab complex binding to blood sample endotoxin causes neutrophils attraction to the complex and release of ROS which can be measured by a luminometer. EAA measures endotoxin activity relatively[14]. When endotoxin activity is 0.60 or greater, mortality rises [15]. Endotoxin-specific septic shock is defined by high endotoxin activity, but endotoxin-targeting medications may not help patients with small organ failure burdens (e.g., SOFA < 7) who have a low mortality risk[16]. However, fluid resuscitation and blood culture before empirical intravenous antibiotic administration are crucial for treatment success [17].Anakinra (an IL-1 receptor antagonist) and eculizumab (a monoclonal antibody to C5) may be unsafe for sepsis patients if the infection is still active [7]. Endotoxin-neutralizing proteins, Lipid A (E5564) analogs, and alkaline phosphatase have failed to target the compound [18,19]. In extracorporeal circulation, adsorptive medical devices can remove considerable levels of endotoxin from the bloodstream. This may help people with elevated endotoxin activity assay levels[7]. Using dexamethasone before or after endotoxin injection can reduce fever and enhance plasma and cerebrospinal fluid interleukin IL-6, prostaglandins PGE2, and PGF2-α concentrations [20]. Synthetic glucocorticoid dexamethasone is widely utilized in clinical settings due to its anti-inflammatory and immunosuppressive properties. When nuclear factor kappa B (NF-κB) is suppressed, several cytokines and chemokines cannot be transcribed, disrupting LPS signal transduction [21].Montelukast, an oral leukotriene receptor antagonist with anti-inflammatory and antioxidant properties, binds with high affinity and selectivity to the cysteinyl leukotriene type 1 receptor and blocks the physiological actions of LTC4, LTD4, and LTE4 at the receptor, aiding asthma or allergic rhinitis[22]. NF- κB inhibition at high dosages of montelukast regulates IL-6, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) production[23].

The study was designed to determine the possible protective effect of montelukast in comparison with dexamethasone on mice bone and spleen utilizing measurement of the following parameters: (1) Serum TNF-α and IL-6 by ELISA. (2) Serum RANK and OPG by ELISA. (3) Cathepsin-G (CTG) and neutrophil elastase (NE or ELANE) gene expression in mice spleen utilizing real-time polymerase chain reaction.

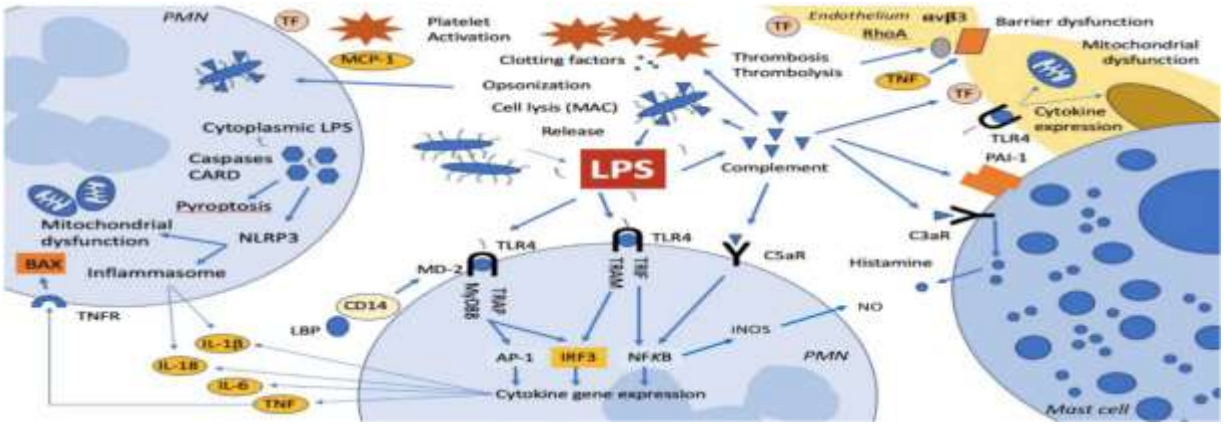


Figure 1. Mechanisms of endotoxic septic shock. Endotoxin is released from bacteria during proliferation/cell death, and LBP is the receiver of LPS. TLR4/MD-2/CD14 complex in PMN is the primary LPS receptor that results in cytokine release. Intracellular LPS sensed through caspases 4,5 activation leading to inflammasome resulting in mitochondrial dysfunction. LPS activates the complement system, directly activating NF-kB in PMN, histamine release from mast cells, and the clotting cascade[7].

Materials and Methods

Chemicals and kits

Lipopolysaccharide endotoxin *E.coli* serotype O55:B5 was purchased from Sigma Aldrich-Germany. Dexamethasone raw powder obtained from SDI-Iraq.Montelukast raw powder obtained from Pioneer-Iraq. Phosphate buffer saline liquid PH=7.4 from Elabscience® USA. Di-ethyl-ether Scharlau-Spain was given from the medicinal store of Baghdad Pharmacy College.

The interleukin-6 sandwich ELISA kit and the osteoprotegerin sandwich ELISA kit were purchased from Elabscience® USA.Tumor necrosis factor-α sandwich ELISA kit purchased from Cloud-Clone Crop®-USA. RANK (TNF superfamily member 11A) sandwich ELISA kit purchased from Fine Test®-Korea.TransZol Up Plus® RNA Kit, EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix, PerfectStart® Green qPCR SuperMix(Taq DNA polymerase, SYBER Green1, dNTPs, PCR enhancer and stabilizer) all purchased from Trans Gen biotech – China. Granulocyte elastase primer and Cathepsin G primer were purchased from Macrogen, South Korea. β -Actin primer as (reference gene) purchased from Alpha ADN, SENC- Montreal. The primer pairs reference gene nucleotide sequence was as follows: β-Actin; Forward (F) and Reverse (R) from (5'→3') CCGCGGGAGACAAGCTT and GGAATGGAAGAAGGGCTTGATC. For CTG; F and R from (5'→3') is AGTCCAGAAGGGCTGAGTGCTT and GCACTGTGATGAGTTGCTGGGT. For ELANE, F and R from (5'→3') are CAGGAACTTCGTCATGTCAGCAG and AGCCATTCTCGAAGATCCGCTG.

Experimental animals

Twenty-eight albino male mice weighing (30-45 gm) were obtained from the animal house of the College of Pharmacy/University of Baghdad. Mice were kept in cages at standard humidity, temperature (25°C), and light/dark cycle with free access to diets (commercial pellets) and water. Mice were divided randomly into four groups, each group containing seven mice (n=7 per group); each group received the following: (1) Control group: Mice received distilled water 0.25-0.5 ml by oral gavage once daily for seven days and on the 8th day euthanized. (2) LPS-model group: Mice received a single dose intraperitoneal injection (IP) of LPS (10mg/kg) and then euthanized the next day after LPS administration[24]. (3) DEX-LPS group: Mice received dexamethasone (2.5 mg/kg) by oral gavage once daily for seven days, and after two hours, all were given LPS (10mg/kg) IP. On the 8th day, euthanized[25]. (4) MONT-LPS group: Mice received montelukast (20 mg/kg) by oral gavage daily for 7 days, and after two hours, all were given LPS (10mg/kg) IP. On the 8th day, euthanized [25].

Drugs preparation

Lipopolysaccharide presented as 10 mg vial lyophilized powder, dissolved in a sterile distilled water, 9 mg/3 ml stock solution then the working solution was prepared by calculating the total desired dose for each group (simple ratio and proportion) then make the second dilution using an equivalent amount of sterile distilled water and

shaking by vortex before each dose. (LPS 1mg out of 10 mg was used for the pilot study test).Dexamethasone raw material off-white powder was dissolved in sterile distilled water, stock solution was prepared by dissolving 0.6 mg/1 ml in a borosilicate glass using a small spatula for grinding and stirring particles until a homogenized, off-white suspension was obtained. Then diluted by its equivalent volume of distilled water to prepare the working solution. Shaking by vortex before each dose.Montelukast raw material white powder was dissolved in sterile distilled water, stock solution was prepared by dissolving 3 mg/0.5 ml in a borosilicate glass using a small spatula for grinding and stirring particles until a homogenized, clear solution was obtained. Then diluted by its equivalent volume of distilled water to prepare the working solution. Simple stirring before each dose.

Serum separation

After mice anesthesia in a glass jar using di-ethyl-ether and collection of blood, the sample was left for clotting for 30 minutes at room temperature. Then, centrifuged in a cold centrifuge at 4000 rpm for 15 minutes and 4°C. A micropipette withdrew serum, then was transferred into labeled Eppendorf tubes and stored at -20 °C until the serum biomarkers assessment.

Preparation of spleen for quantitative-real time PCR

After euthanizing and dissection, the spleen was excised for each animal, cleansed from residual blood by placing in ice-cold phosphate buffer saline PBS solution PH=7.4 in a small petri dish, then dried on filter paper, and cut the required weight for analysis about 50 mg and placed in 700µL Genezol® reagent in 2ml Eppendorf tubes, stored at -20°C.

Enzyme-Linked Immunosorbent Assay

Tests for TNF-α, IL-6, RANK, and OPG can be conducted using ELISA kits. Briefly, after incubating the sample, standards, and blank on the pre-coated antibody plate for 30-60 min at 37°C, biotinylated-specific antibody, enzyme conjugate, chromogenic substrate, and stop solutions were added sequentially. A microplate reader measured 450 nm optical density.

RNA extraction, reverse transcriptase-PCR, and quantitative real-time PCR reactions

Transzol Up Plus® RNA Kit isolates cell and tissue total RNA. After lysing with Transzol Up and adding an RNA Extraction Agent like chloroform, the solution separates into an upper colorless aqueous phase (carrying RNA), an interphase hazy layer (containing DNA), and a lower pink organic phase. A silica-based spin column binds RNA in water. Add ethanol to bind all RNA. After multiple centrifugations, add a clean buffer and wash buffer solutions. Next, an opened centrifuge will extract all ethanol from RNA during drying. After several minutes, add RNAse-free water to the filter column center and centrifuge. The RNA will be at the bottom of the tubes. Then, use RNAse-free water drops to zero the nanodrop reader, install RNA, and read the data. The RNA sample's absorbance 260/280 ratio of 1.99–2.06 indicated purity. An elute of pure RNA was kept at -20 °C.Unique genomic DNA remover and EasyScript® First-Strand cDNA Synthesis SuperMix remove genomic DNA and synthesize cDNA with reverse transcriptase. After calculating and preparing the required volumes of random primers, oligo dT, and gDNA remover, place them in RNAse-free tubes with RNA samples, spin for seconds, and place them in a thermocycler to activate reverse transcriptase enzyme at 42°C and inhibit it at 85°C for a few seconds.As in the supplied leaflet, CTG and NE primers were reconstituted with nuclease-free water. A workable solution for the needed volume was made by combining each primer's forward and reverse with nuclease-free water. Add the necessary amounts of master mix, primers, nuclease-free water, and cDNA to the reaction mixture, run in the Rotor-Gene Q device, and read the results. To make up to a billion exact copies of the DNA strand, denaturation, annealing, and extension were repeated 30-45 times at varied temperatures. The ultimate product is two identical DNA strands. To quantify fold change, each gene was compared to the reference gene using the 2-ΔΔct technique.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 25 was used to examine data. To determine if sample data were normally distributed, all measurable parameters were tested. Results were given as mean ± standard error analyzed using one-way ANOVA and post hoc Tukey multiple comparisons for normally distributed values. In a skewed distribution, data was presented as median ± interquartile range, and the Kruskal-Wallis test and post-hoc one-way ANOVA (k samples) were used for multiple pairwise comparisons. Group

differences were judged significant when the P-value was < 0.05.

Results

Effects on serum TNF-α and IL-6.

The serum levels of TNF-α and IL-6 were significantly elevated (p<0.05) in group II that received intraperitoneal (IP) LPS. In both groups, III (Dexamethasone DEX-LPS) and IV (Montelukast MONT-LPS), The serum levels of TNF-α and IL-6 were significantly lower (p<0.05) when compared with the levels in the LPS-model group. Table 1, Figure 2 (a &b).

Effects on serum RANK and OPG.

Administration of LPS by (IP) caused a significant elevation (p<0.05) in the level of serum RANK and OPG in (group II) compared with their levels in the control group (group I). In groups III (DEX-LPS) and IV (MONT-LPS), The levels of serum RANK and OPG showed a significant reduction (p<0.05) compared with their levels in the LPS-model group. Table 2, Figure 2 (c &d).

Effect on Cathepsin G (CTG) and Neutrophil elastase (ELANE) genes expression in spleen.

Administration of LPS by (IP) caused a significant elevation (p<0.05) in the gene expression of CTG compared with the control group. In groups III (DEX-LPS) and IV (MONT-LPS), a significant reduction (p<0.05) in gene expression of CTG when compared with the LPS-model group. Table 3, figure 2 (e). Statistical analysis revealed that P= 0.097 (p>0.05) regarding the ELANE gene. So, despite the downregulation, there was no significant difference among groups. Table 3, Figure 2 (f).

Table 1. Effect on serum biomarkers TNF-α and IL-6 among groups.

Groups/ Parameter	Serum TNF-α levels (pg/ml)	Serum IL-6 levels (pg/ml)
Control	282.022 ± 8.760	109.486 ± 1.374
LPS-model	884.964 ± 8.613*	342.311 ± 1.262*
DEX-LPS	170.906 ± 4.972#	218.984 ± 0.791#
MONT-LPS	253.055 ± 8.559~+	152.709 ± 2.922~+

The values are reported as mean ± SEM.(*) indicates a significant difference from the control group.(**#, ~**) indicates a significant difference from the LPS-model group.(+) indicates a significant difference from DEX-LPS and an advantage if present. No significant change between groups(**o**).

Table 2. Effect on serum RANK and OPG among groups.

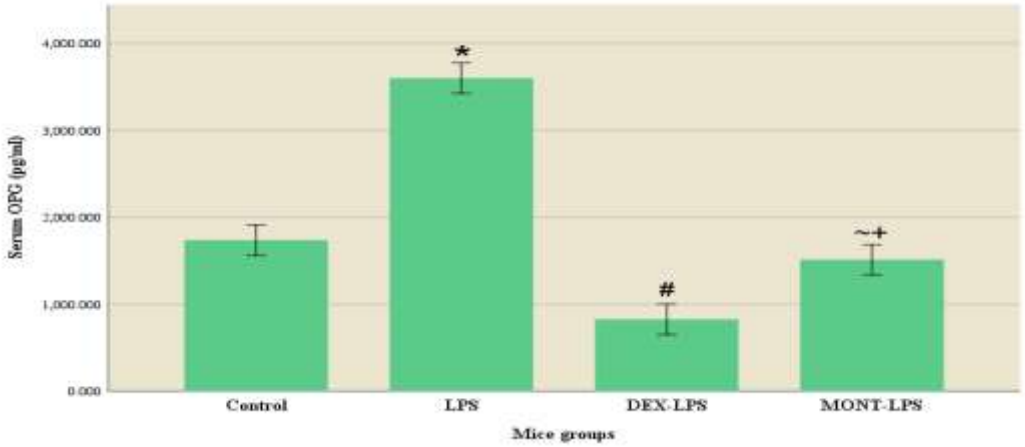
Group/ Parameter	Serum RANK levels	Serum OPG levels
Control	641.119 ± 166.753	1737.040 ± 79.149
LPS-model	1852.033 ± 81.101*	3599.093 ± 42.556*
DEX-LPS	530.935 ± 139.102#	828.648 ± 29.254#
MONT-LPS	516.877 ± 175.931~	1513.215 ± 111.768~+

Results are shown as median ± IQR for serum RANK and mean ± SEM for serum OPG.(*) indicates a significant difference from the control group.(**#, ~**) indicates a significant difference from the LPS-model group.(+) indicates a significant difference from DEX-LPS and an advantage if present. No significant change between groups(**o**).

Table 3. Effect on Cathepsin-G (CTG) and Neutrophil elastase (ELANE) gene expression relative to β-Actin in spleen among groups.

Group/ Parameter	CTG-β-Actin fold change	ELANE-β-Actin fold change
Control	0.74 ± 0.15	0.94 ± 0.19○
LPS-model	2.19 ± 0.76*	0.57 ± 0.61○
DEX-LPS	0.25 ± 0.03#	0.42 ± 0.87○
MONT-LPS	0.67 ± 0.07~+	0.41 ± 0.48○

Values presented as median ± IQR.(*) indicate a significant difference from the control group.(**#, ~**) indicates a significant difference from the LPS-model group.(+) indicates a significant difference from DEX-LPS and an advantage if present. No significant change between groups(**o**).



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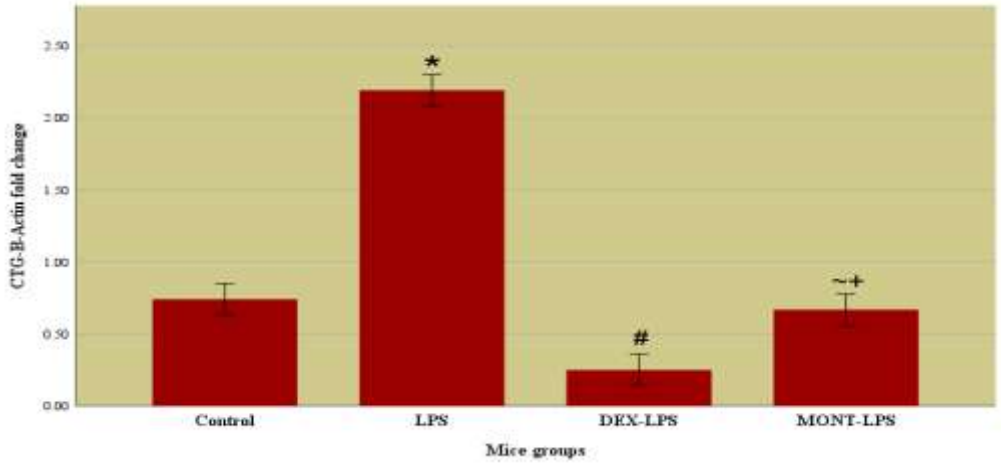

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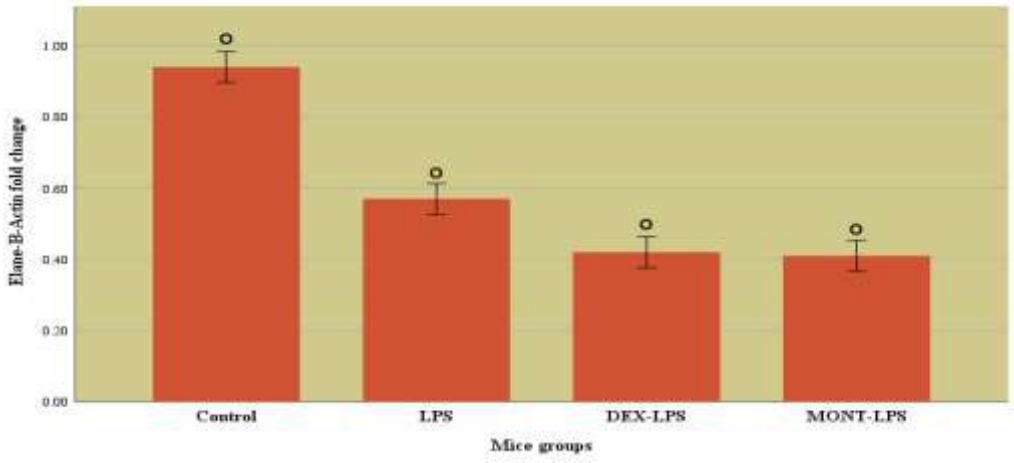

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Figure (2) form (a-f): Parameters affected in each group;(a)Serum TNF-α.(b)Serum IL-6.(c)Serum RANK. (d)Serum OPG.(e)CTG-B-Actin fold change.(f)ELANE-B-Actin fold change. (*) denotes the significant elevation ($P<0.05$) of the LPS group in comparison with the control group. (**#, ~**) denotes the significant reduction ($P<0.05$) of DEX-LPS and MONT-LPS groups in comparison with the LPS group. (**+**) denotes the significant difference in comparison to the DEX-LPS group and also notifies the advantage if present. (**O**) refers to no significant difference among groups.

Discussion

Lipopolysaccharide induces a strong immune and inflammatory response in certain doses, so it is widely used as an experimental tool in animals to simulate infection in humans. *Kang et al (2018)* found that montelukast potently inhibits RANKL-induced osteoclast production and bone loss in vivo [26]. *Sherwin et al (2012)* concluded in a systematic review that using low-dose corticosteroids for septic shock may lead to faster recovery [27]. Early administration of dexamethasone to mice that had been challenged with LPS resulted in a dose-dependent improvement in survival, with the higher dose resulting in a higher survival rate [28].This study found that a single dosage of LPS (IP) significantly increased proinflammatory cytokines such as TNF-α and IL-6 (Table 1, figure 2 a&b). Toll-like receptors-4 (TLR-4) belong to tyrosine kinase receptors and are present in neutrophils, monocytes, and macrophages rapidly activated producing innate immune response [29,30]. These receptors transduce intracellular signals via myeloid differentiation-88 (MYD-88) dependent and independent pathways [31,32]. The net result is the activation and translocation of NF-κB, AP-1, and IRF-3 to the nucleus and transcription of many proteins, including proinflammatory cytokines [33].Tumor necrosis factor-α, a master proinflammatory cytokine, contributes to the pathogenesis of many mouse diseases like cholestasis, rheumatoid arthritis, and benign prostatic hyperplasia [34,35,56]. Mice pretreated with 2.5 mg/kg dexamethasone orally daily for a week showed a significant decrease in serum TNF-α levels, even below the control group. Corticosteroids prevent LPS-induced TNF-α release. This opposition or antagonism is noncompetitive. Increased stimulatory drug doses cannot abolish the optimal inhibitory corticosteroid effect[36]. Dexamethasone completely blocked TNF production after a short exposure, even when given to cells at the same time as endotoxin. Dexamethasone also inhibited endotoxin-induced TNF in human monocytes. However, dexamethasone inhibition was optimal after 48 hours of cell pre-incubation[37]. Montelukast significantly reduced TNF-α levels in the MONT-LPS group compared to the LPS group (P=0.00, p<0.05). Additionally, MONT-LPS did not significantly reduce TNF-α compared to the control group (P=0.07, p>0.05). Montelukast has a more beneficial effect than dexamethasone , reducing TNF-α release to near-normal values without suppression in this experiment (Table 1, figure 2 a&b). Similarly, both medicines significantly lower serum IL-6 levels compared to the LPS-model group (P=0.000, p<0.05). Statistical analysis revealed a significant P=0.00 (p<0.05) and strong positive linear correlation (Pearson correlation 0.822) between TNF-α and IL-6, indicating the ability of TNF-α to stimulate and release interleukin-6. Other sources of IL-6 stimulation and secretion, such as LPS and IL-6 itself, can also amplify its release through a positive feedback loop[38].Montelukast can reduce IL-6 by reducing eosinophil activity, secretions, and survival, as well as by inhibiting the activity of NF-κB [39]. It acts on LTC_4 and LTD_4 receptors as a leukotriene antagonist, these are G-protein coupled receptors of the $G_{q/11}$ subtype and can act as P_2Y_{12} antagonist and PPARγ agonist (intracellular receptors) [40]. Studies have demonstrated that PPARγ agonists have anti-inflammatory effects by inhibiting NFκB, particularly in macrophages[40]. Activating phospholipase C (PLC) via leukotrienes through $G_{q/11}$ subtype LTC4 and LTD4 receptors generates protein kinase C (PKC) from downstream signaling molecules. PKC phosphorylates and activates downstream signaling molecules, including MAP Kinase. The response may be affected by trans-activation or trans-repression of signaling pathways. The MAP Kinase cascade is one of the tyrosine kinase receptor pathways (mitogenic pathway) and this is the intersection point since LPS can activate this pathway. Montelukast, a PPARγ agonist, suppresses NF-κB, resulting in an anti-inflammatory response after LPS exposure in mice[40]Previous studies indicate that proinflammatory cytokines, including IL-1β, IL-6, and TNF-α promote osteoclastogenesis via RANKL upregulation resulting in bone resorption[41,42,45]. Anti-inflammatory drugs may prevent bone fractures [43]. While glucocorticoids like dexamethasone may have some positive effects by their anti-inflammatory effect, they also increase the risk of bone resorption since they enhance RANKL expression and decrease OPG expression [44].This study found raised blood RANK and IL-6 levels, which increased bone resorption risk. However, LPS treatment raised blood OPG levels too, as reported in a previous study [46]. The soluble decoy receptor OPG competes with RANK for RANKL attachment.

As they are receptors for the same ligand, RANKL [47], OPG may have a higher affinity than RANK[48]. Data indicates montelukast maintains greater OPG levels than dexamethasone with a significant difference (P=0.00, p<0.05), despite no significant difference in RANK decline between DEX and MONT groups (P=0.559, p>0.05).OPG levels were not significantly different between montelukast and control groups (P=0.163, p>0.05), suggesting that montelukast maintains OPG levels closer to the control group (Table 2, figure 2 c&d).Neutrophil serine proteases (NSPs) like CTG and NE help maintain the inflammatory response's delicate balance between tissue protection and destruction. They damage tissue and modulate inflammation when their concentrations exceed their inhibitors [49]. Through proteolytic regulation, NSPs can regulate cell surface chemokines, cytokines, growth factors, and receptors. Bloodstream proteases stimulate lymphocytes and break down adhesion and apoptotic molecules. They control inflammation-related immune responses by preserving pro- and anti-inflammatory characteristics.Neutrophil elastase (NE), a contradictory feature of innate immunity, serves as both a host protection and tissue-damaging agent. In response to TLR-4 agonists like LPS, macrophages generate cytokines that NE can cleave. This can weaken the host's immune system. NE activity breaks opsonins and phagocytic receptors, preventing neutrophils and macrophages from killing and clearing microbes[50]. NE degrades monocyte CD14, a major LPS receptor[51]. Moreover, Even at high NE concentrations, *P. aeruginosa* cells were viable and morphologically intact, unlike *Escherichia. Coli*, which was killed by even very low NE concentrations[52].The LPS-model group showed a significant increase in CTG (P=0.038, p<0.05) but not in NE (P= 0.097 p>0.05), consistent with a previous study[53] that found NE levels decreased after 6 hours of LPS administration, while in this study, samples were collected the day (24 hours) after single dose LPS. Neutrophils have at least four types of proteases; therefore, NE's function may be obscured or redundant[55]. Low or deficient NE or CTG levels do not change neutrophil recruitment to inflammation, oxidative assault, or phagocytic activity in mice[54].NE overactivation during early inflammation may subvert the innate immune response, which might worsen the prognosis[55]. So, the site, severity, and length of the inflammatory response may determine the coordinated, progressive development and release of these NSPs.By interfering with neutrophil CTG expression, montelukast and dexamethasone can lower CTG expression below the LPS-model group. Dexamethasone's immune-suppressant effects significantly reduced CTG expression levels (P=0.000, p<0.05) compared to the LPS-model group, control group, and MONT-LPS group (P=0.038, p<0.05). Montelukast nearly restored normal CTG expression levels that LPS dramatically elevated, as there was no significant difference between MONT-LPS and the control group (P=0.537, p>0.05)

Limitations and Recommendations

Blood samples were difficult to obtain at different times (for instance after three, six, and twelve hours of LPS injection) to make a valuable comparison in blood levels of these NSPs. Additional groups can be added for further work using other protective drugs (like atorvastatin) with montelukast or giving treatment after LPS injection (like hydrocortisone injection). Studying the montelukast effect on other dangerous mediators in sepsis; for instance, high mobility group box-1 (HMGB-1). It is unclear, however, using observational and quantitative methods, how much time these mice need to recover from the negative effects of a single LPS IP injection in terms of symptoms using a score table.

Conclusion

Firstly, administration of Montelukast before LPS can effectively alleviate the acute inflammatory process as evidenced by TNF-α and IL-6 serum levels. Secondly, regarding its diverse anti-inflammatory action; Montelukast exerts an anti-resorptive effect on bone as guided by serum RANK and OPG levels. Lastly, montelukast has an immunomodulator action as proved by the normalization of CTG-gene expression in the spleen; Besides, it causes a non-substantial downregulation of ELANE-gene expression.

Acknowledgment

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Conflicts of Interest

None

Funding

None

Ethics Statements

The Scientific and Ethical Committee of the College of Pharmacy, University of Baghdad, approved the present study.

Author Contribution

Study conception and design: Dr Ahmed Hamed Jwaid. Data collection: M.Sc. Rami Isam Kamal. Analysis and interpretation of results: M.Sc. Rami Isam Kamal and Dr Ahmed Hamed Jwaid. Draft manuscript preparation: M.Sc. Rami Isam Kamal.

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دراسة مقارنة بين دواء المونتيلوكاست مع الديكساميثازون في الوقاية المحتملة من التأثير السمي لمادة عديد السكاريد الدهني في ذكور الفئران

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الخلاصة

الصدمة الانتانية التسممية هي متلازمة سريرية خطيرة سببها غالبا عدوى ببكتريا سالبة الغرام، ويشير الى المرضى المصابين بمستوى ونشاط عاليين للمادة السمية الداخلية في مجرى الدم، بالاضافة الى حدوث عالي لفشل الاعضاء ومن ضمنهم فشل الجهاز القلبي الوعائي، الرئوي، الكبدي، والكلوي. المادة السمية الداخلية هي جزء من مكونات الجدار الخلوي البكتيري تستطيع اجتذاب العديد من الخلايا المناعية مثل الكريات العدلة والوحيدة لكي تفرز المواد الوسطية الالتهابية مثل السايتوكين، احادي اكسيد النتريك، الهستامين، البراديكنين فيحصل تفاعل التهابي موضعي وفي عامة الجسم وبالنتيجة سيحصل توسع شديد للاوعية الدموية، خلل وظيفي في الخلايا المبطنة للاوعية الدموية ، زيادة نفاذية الاوعية الشعرية الدموية، انتشار التخثرات داخل الاوعية الدموية. من الممكن الاستفادة من الفئران في البحوث التجريبية بتحفيز حالة التسمم او الانتان في الدم التي تحاكي حالة الصدمة الانتانية في الانسان عن طريق اعطاء مادة عديد السكاريد الدهني المعزولة من بعض السلالات البكتيرية وبالجرعة المناسبة. هذه الدراسة تفحصت الحماية المحتملة لدوائي المونتيلوكسات والديكساميثازون من التأثيرات التسممية الداخلية المحفزة بمادة عديد السكاريد الدهني في ذكور الفئران. ثمانية وعشرون فأر تم تقسيمهم عشوائيا الى اربعة مجموعات بواقع سبعة لكل مجموعة وذلك كالتالي: المجموعة الاولى تم اعطائها الماء المقطر ٠,٢٥-٠,٥ مل بواسطة انبوب الزق الفموي مرة يوميا و لمدة اسبوع، ثم التضحية بها في اليوم الثامن. المجموعة الثانية تم اعطائها جرعة منفردة من مادة عديد السكاريد الدهني ١٠ ملغ لكل كغم زرقا في البيريتون، ثم التضحية بها في اليوم الثاني. المجموعة الثالثة تم اعطائها الديكساميثازون ٢,٥ ملغ لكل كغم بواسطة انبوب الزق الفموي مرة يوميا ولمدة اسبوع ، وبعد ساعتين من الجرعة الاخيرة اعطيت مادة عديد السكاريد الدهني، ثم التضحية بهم في اليوم الثامن. المجموعة الرابعة تم اعطائها المونتيلوكاست ٢٠ ملغ لكل كغم بواسطة انبوب الزق الفموي مرة يوميا ولمدة اسبوع ، وبعد ساعتين من الجرعة الاخيرة اعطيت مادة عديد السكاريد الدهني، ثم التضحية بهم في اليوم الثامن . كشفت النتائج ان العلاج المسبق بكلا الدوائين قلل مستويات مواد عامل تنخرالورم، انترليوكين-٦، مفعل مستلمات العامل النووي كابا- بي، ومستلمات الاوستيوبروتيجرين، كذلك تقليل مستويات التعبير الجيني لكل من جينات الكاثيبسين جي وايلاستيزالكريات العدلة. كاستنتاج؛ هناك تأثير وقائي واعد لدواء المونتيلوكاست من التأثيرات التسممية الداخلية لمادة عديد السكاريد الدهني، وربما يكون خيارا كدواء وقائي من المضاعفات الالتهابية في هذه الحالات في البشر.

الكلمات المفتاحية: الصدمة الانتانية التسممية، الوسائط الالتهابية، عديد السكاريد الدهني ،المونتيلوكاست.