Original Article

Preventive Effects of NSAIDs on Lung Tissue Oxidative Damage in an Animal Sepsis Model

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Abstract

Background: Sepsis is a heterogeneous syndrome caused by a dysregulated host response to infection. Inflammatory cascades have a critical role in sepsis and can potentially be suppressed by anti-inflammatory compounds. Therefore, this study was focused on the antiseptic effects of non-steroidal anti-inflammatory drugs (NSAIDs) on lung injuries based on cecal ligation and puncture (CLP) surgery.

Materials and Methods: Male Wistar rats were divided into 6 groups (n=60) as follows: Control, laparotomy (LAP), CLP, and three treatment groups including indomethacin, celecoxib, and aspirin (2 mg/Kg b.w) taken orally every 12 hours for 2 days. The rats were sacrificed after 48 hours, and the lung tissue was subjected to determine lipid peroxidation (LP), myeloperoxidase (MPO), glutathione enzyme (GSH)) and inflammatory genes expression (cyclooxygenase-2 (COX-2), CD177, and MPO).

Results: Cecal ligation and puncture caused lung injury by changes in antioxidant enzymes and genes expression ($P<0.05$). Treatments with indomethacin, celecoxib, and aspirin as anti-inflammatory compounds significantly improved antioxidant enzymes by reducing LP and MPO levels and genes expression and increasing the level of GSH ($P<0.05$).

Conclusion: Sepsis caused oxidative damage in the lung tissue, and NSAIDs effectively prevented and improved these injuries.

Keywords: Sepsis, Inflammation, Antioxidant Activity, Genes Expression, Cecal Ligation, and Puncture Surgery

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are available as over-the-counter drugs, and they are mostly used to relieve pain or inflammatory conditions (1). NSAIDs inhibit cyclooxygenase (COX), preventing the production of prostaglandins from arachidonic acid. They are divided into two major categories based on COX selectivity, including non-selective NSAIDs, which block COX isoforms, and COX-2-selective inhibitors (2, 3). COX-1 is present in most body tissues and is involved in synthesizing prostaglandins, and thromboxane A2 and COX-2 are expressed in response to injury or inflammation (4).

Indomethacin is one of the NSAIDs with antipyretic, analgesic, and anti-inflammatory activity. It is a non-selective inhibitor of cyclooxygenase 1 and 2 (3). Celecoxib as COX-2 specific inhibitor is another NSAID (5). Acetyl-salicylic acid (aspirin) is also a

non-selective inhibitor of cyclooxygenase enzyme that is subject to significant first-pass metabolism (6, 7).

NSAIDs have beneficial effects in numerous experimental models and are considered a potentially life-saving treatment for sepsis (8, 9). Sepsis is defined as life-threatening acute organ dysfunction secondary to an infection that affects more people each year. The initial host response in sepsis includes activating proinflammatory and anti-inflammatory innate immune pathways. The severity of immune suppression and organ dysfunction after sepsis treatment is influenced by host response, pathogen characteristics, and the quality of early sepsis treatment (10, 11).

NSAIDs were used frequently for septic patients, and long-term use may lead to severe side effects (3). Therefore, we examined the effects of some non-steroidal anti-inflammatory drugs on lung tissue in septic rats.

Methods

Animals

Male Wistar rats (200-250 gr) (Pasteur Institute, Tehran, Iran) were housed under standard conditions with normal diet and sterile water *ad libitum* on a 12 h light-dark cycle (20 -25 °C, humidity 50 ± 5%). Animal experimentation was performed according to ethical committee and the general ethical principles of the Declaration of Helsinki (World Medical Association 2001) (Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964) and local institutional animal care (approved the rat protocol from Pasteur Institute, Iran) and ethical guidelines (approved the rat protocol from Payame Noor University on October 19-20, 2018, Iran) (etic code was100245/45/1). All rats were randomly divided into 6 groups (n=10): the control group (without any surgery and treatment), laparotomy group (LAP) (without sepsis induction and treatment), cecal ligation, and puncture surgery group (CLP) (sepsis induction without any treatment), three different treatment groups (sepsis induction with CLP and treatment); A) indomethacin, B) celecoxib and C) aspirin at a dose of 2 mg/kg b.w orally in 12h intervals after CLP induction (all drugs diluted in distilled water). The dose of drugs was determined according to Rubin et al. (12). In the LAP group, all procedure was done as same as the CLP group, but the cecum was handled without ligation and puncture. In CLP induced groups, rats were anesthetized using xylazine and ketamine, then 1.5 cm of the cecal tip was ligated with 4-0 silk and punctured twice using an 18-gauge needle LAP group, the cecum was isolated but without ligated and punctured (13). After 48h of surgery, rats were killed, and lung tissue was collected for further analysis.

Antioxidant assay

Table 1: Primer oligonucleotide sequencing used in this study.

Primers	Sequence (5′→3′)	Product Length (bp)
COX2 forward	ACCTCTGCGATGCTCTTC	188
COX2 reverse	AGGAATCTCGGCGTAGTAC	
CD177 forward	ATACCAGTGCTGACCCTTCTG	145
CD177 reverse	CCTCGCAGGTTTTCTCACC	
MPO forward	GCGATAGGTTTTGGTGGGAG	165
MPO reverse	AGCTCACAAAGTCTCGGGG	
GAPDH forward	TGCCAGCCTCGTCTCATAG	197
GAPDH reverse	ACTGTGCCGTTGAACTTGC	

The lipid peroxidation was determined by the measurement of Malondialdehyde (MDA) according to the MDA assay kit (Teb Pazhouhan Razi (TPR), Tehran, Iran) at 530-540 nm. Results were stated as nmol/mg of protein. Myeloperoxidase (MPO) activity was evaluated by myeloperoxidase assay kit (NampoxTM) at 450 nm. The MPO activity was expressed as µmoles/min/ml or mg protein.

Glutathione enzyme (GSH) activity was determined by reduced glutathione (GSH) assay kit (Navand Salamat, Urmia, Iran) at 414 nm. The GSH content was expressed as nmol/ mg protein.

Gene expression

Total RNA was isolated from the lung tissues using the RNA total kit (GeneAll, Korea). The quantity of extracted RNA was measured by Nanodrop2000 (Thermo Scientific NanoDrop™). Then, complementary DNA was transcripted from the same amount of total extracted RNA by the PrimeScript ™ RT reagent kit (Takara Bio Inc, Japan) and oligo dt primers (Takara Bio Inc, Japan). Real-time PCR was performed using SYBR® Green Master Mix (Amplicon) and was conducted on a real-time PCR system (Rotor-Gene Q-QIAGEN). For analysis, the cDNA samples were run in triplicate. The typical thermal profile used was at 95°C for 15 min, followed by 40 cycles of 95°C for 30 s, 56-61°C for 30 s and 72°C for 30 s. The formula 2–ΔΔCt calculated the fold changes in selected genes. The primer sets are used as follows (Table 1).

Statistical analysis

Data were presented as means±SD, and significant

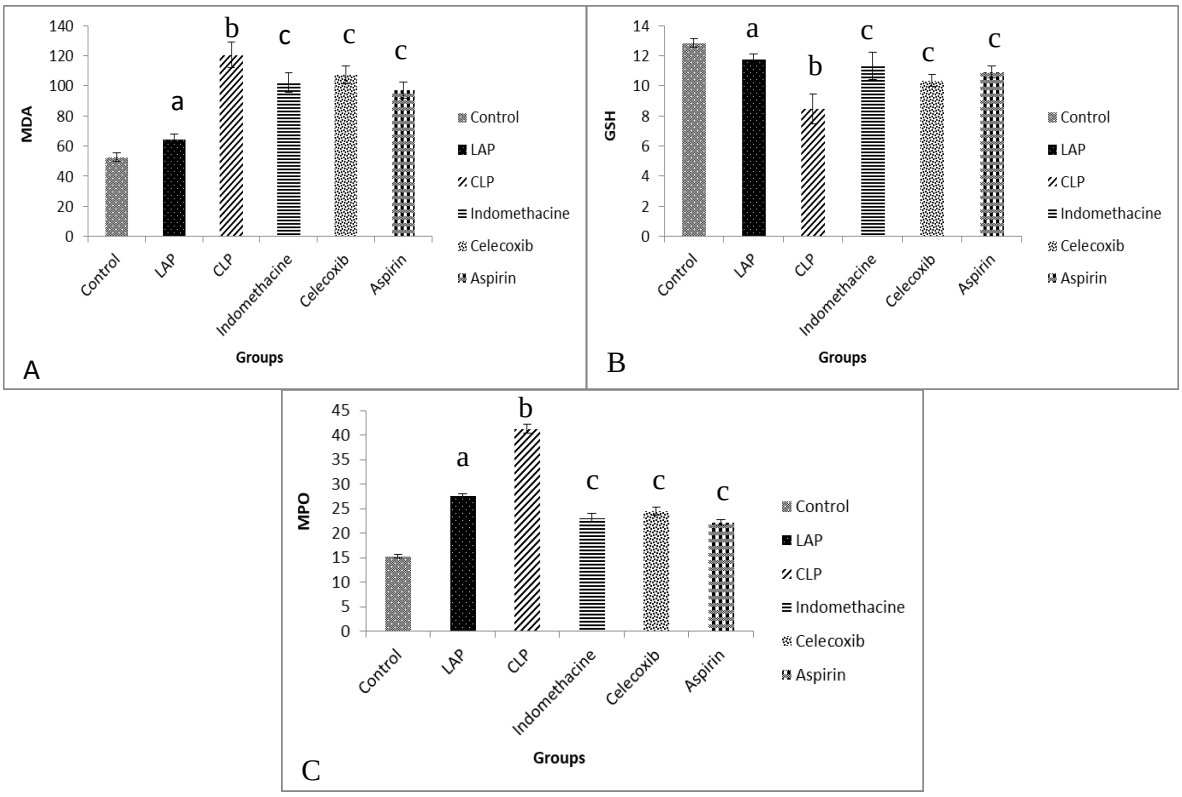

Figure 1. The effect of NSAIDs on antioxidant parameters in septic rats (A: MDA, B: GSH, and C: MPO)
[a] P <0.05 is considered significantly between control and LAP groups.
[b] P <0.05 is considered significantly between LAP and CLP groups.
[c] P < 0.05 is considered significantly between the CLP group and treatment groups.
Values represent the Mean ± SD of each group.

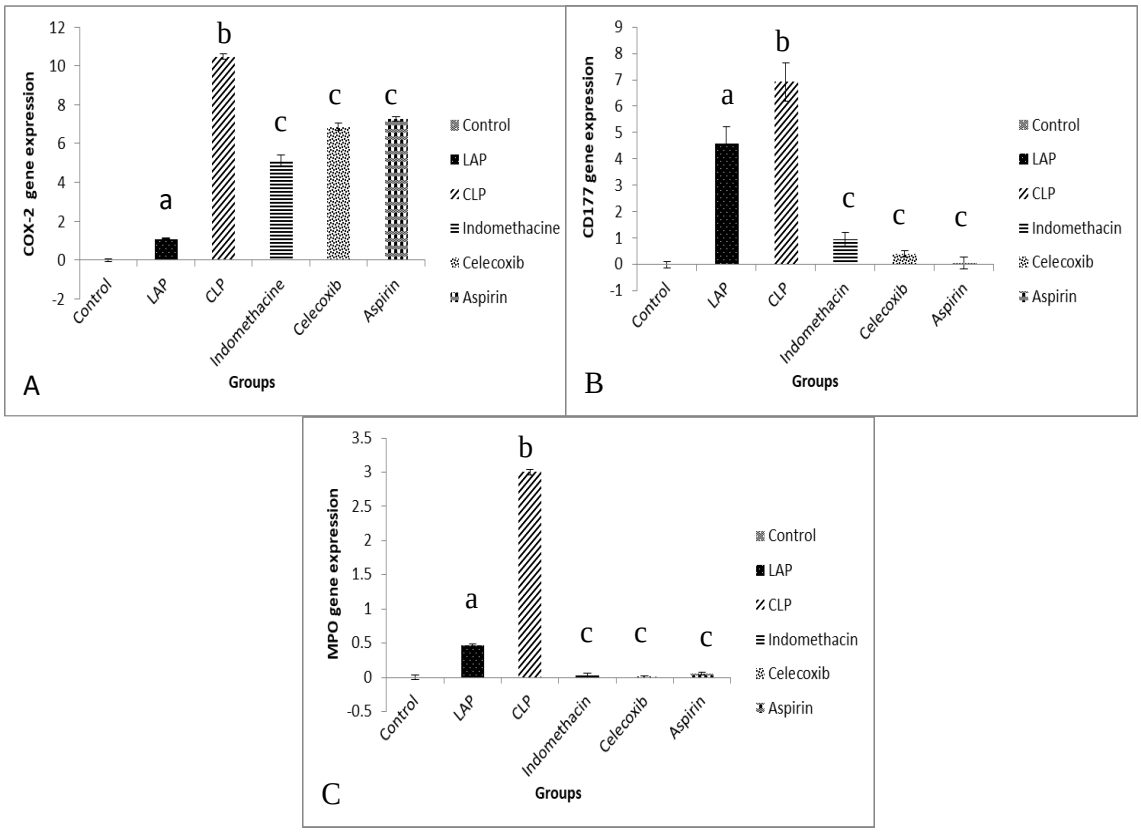

Figure 2. The effect of NSAIDs on genes expression in septic rats (A: COX-2, B: CD177, and C: MPO)
[a] P < 0.05 is considered significantly between control and LAP groups.
[b] P < 0.05 is considered significantly between the LAP and CLP groups.
[c] P < 0.05 is considered significantly between the CLP group and treatment groups.
Values represent the Mean ± SD of each group.

levels were defined as P<0.05. The results were subjected to one-way ANOVA followed by LSD using SPSS (version 15.0) software.

Results

Determination of the antioxidant activity of NSAIDs

Figure 1 showed that CLP increased the concentration of MDA and MPO activity in rats compared to the control and LAP groups (P<0.05). Also, the CLP group significantly showed a decrease in GSH content compared to the control and LAP groups. In contrast, treatment with selected NSAIDs at the dose of 2 mg/kg b.w after 48h significantly decreased MDA concentration and MPO level as well as increasing of GSH compared to the CLP group (P<0.05) (Figure 1).

Determination effect of NSAIDs on genes expression levels

Results have revealed that CLP significantly increased the mRNA expression levels of COX-2, CD177, and MPO genes compared to the control and LAP groups (P<0.05). The results showed that indomethacin, celecoxib, and aspirin treatment decreased the genes

expression levels in lung tissue of septic rats compared to the CLP group (Figure 2).

Discussion

Sepsis is a complex pathophysiological event involving systemic inflammatory response syndrome, multiple organ dysfunction syndromes, and tissue damage such as acute lung injury (ALI), acute respiratory distress syndrome (ARDS), and even death (14).

Lung injury during sepsis is characterized by the accumulation of a large number of neutrophils in the lungs, increased generation of reactive oxygen species (ROS), and increased production of proinflammatory cytokines (14). Many studies focused to clear sepsis physiopathology and develop new treatment strategies (15). Some studies are still actively being performed on both clinics and in laboratory animals for the determination of potential drug candidates (16-18).

Among different animal models, CLP is a reliable animal model of human sepsis that is mostly preferred, for it mimics the clinical situation of bowel perforation and bacterial infection in humans (19). It is a hallmark of the progression and characteristics of human sepsis and is one of the most commonly used methods to induce experimental sepsis in laboratory animals compared to other animal models for sepsis research.

Moreover, the CLP model as a mechanism to inform novel therapeutics can elucidate the immune and inflammatory pathways that can be tested in human trials (20). Our results showed that CLP could injure the lung by affecting genes expression and oxidative stress/antioxidant status. Results of the present study indicated that sepsis could increase levels of LP and MPO with decreasing GSH levels (Figure 1).

In sepsis, oxidative stress plays an important role by affecting cellular integrity with a mechanism of ROS reacting with the unsaturated fatty acid of cellular and subcellular membranes (21, 22). Miri et al. (23) demonstrated CLP-induced lung injury by changing oxidative enzymes in a previous study. Also, COX-2, CD177, and MPO expression increased in the CLP group compared with the control group (Figure 2). The present study's results concur with the reports of the following studies (13, 23), supporting the idea that sepsis promoted lung inflammation. COX-2 plays a role in inflammatory responses, initiating oxidative tissue damage. Elevated levels of COX-2 could be provoked by inflammation (24, 25). Also, MPO is one of the formations of ROS and oxidation of biological substances released in a high percentage by activated neutrophils for antibacterial activities and causes the increase of degranulated neutrophils in severe sepsis (26). On the other hand, CD177 is a neutrophil-specific molecule involved in severe antibody-dependent infections. Therefore, the measurement of CD177 can be a useful diagnostic tool for distinguishing some infectious diseases (27).

Moreover, our data suggested that indomethacin, celecoxib, and aspirin could protect the lung by restoring the ideal inflammatory and antioxidant parameters (Figures 1 and 2). One study expresses that aspirin can manipulate the processes in sepsis (7), and COX-2 is sensitive to low doses of aspirin (28). A study by Houshmand et al. (29) revealed that pioglitazone combined with low doses of indomethacin can be used to control inflammation in patients with diabetes.

Also, one study showed that mortality in septic mice treated with indomethacin strongly related to the concentration of indomethacin, i.e., the higher the concentration, the shorter the survival period (30). The findings of Solomon et al. (31) confirmed that celecoxib at moderate dosages could be safe among other NSAIDs in patients with prior cardiovascular events. Since many septic patients use NSAID drugs, our results have considerable public health implications. Results demonstrate that although NSAIDs at proper dosage are useful to cure sepsis, they are not enough. Thus, further studies are required to confirm this finding in sepsis.

Conclusion

This study indicated the effectiveness of the NSAIDs drugs in modulating the oxidative stress injury parameters and genes expression as an inflammatory factor disturbed in septic rats after CLP surgery.

Acknowledgment

None.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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