Biomedicine & Pharmacotherapy 86 (2017) 583–589

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Original article

# Celecoxib administration reduced mortality, mesenteric hypoperfusion, aortic dysfunction and multiple organ injury in septic rats

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ARTICLE INFO

*Article history:*
Received 12 October 2016
Received in revised form 23 November 2016
Accepted 24 November 2016

*Keywords:*
Celecoxib
Sepsis
Survival and multiple organ damage
Vascular hyporeactivity
Mesenteric arterial blood flow

ABSTRACT

*Background:* The cyclooxygenase (COX)-2 overexpression is associated with vascular injury and multiple organ failure in sepsis. However, constitutive COX-1 and basal COX-2 expressions have physiological effects. We aimed to investigate the effects of partial and selective COX-2 inhibition without affecting constitutive COX-1 and basal COX-2 activities by celecoxib on mesenteric artery blood flow (MABF), vascular reactivity, oxidative and inflammatory injuries, and survival in septic rats accomplished by cecal ligation and puncture (CLP).
*Methods:* Wistar rats were allocated into Sham, CLP, Sham + celecoxib, CLP + celecoxib subgroups. 2 h after Sham and CLP operations, celecoxib (0.5 mg/kg) or vehicle (saline; 1 mL/kg) was administered orally to rats. 18 h after drug administrations, MABF and responses of isolated aortic rings to phenylephrine were measured. Tissue samples were obtained for biochemical and histopathological examinations. Furthermore, survival rate was monitored throughout 96 h.
*Results:* Celecoxib ameliorated mesenteric hypoperfusion and partially improved aortic dysfunction induced by CLP. Survival rate was%0 at 49th h in CLP group, but in CLP + celecoxib group it was 42.8% at the end of 96 h. Serum AST, ALT, LDH, BUN, Cr and inflammatory cytokine (tumor necrosis factor-alpha, interleukin-1 beta and interleukin-6) levels were increased in CLP group that were prevented by celecoxib. The decreases in liver and spleen glutathione levels and the increases in liver, lung, spleen and kidney malondialdehyde levels in CLP group were blocked by celecoxib. The histopathological protective effects of celecoxib on organ injury due to CLP were also observed.
*Conclusions:* Celecoxib has protective effects on sepsis due to its preservative effects on mesenteric perfusion, aortic function and its anti-inflammatory and antioxidative effects.


## 1. Introduction

Sepsis, the body's systemic and life-threatening response to an infection, severe sepsis (sepsis with acute organ dysfunction), and septic shock (severe sepsis with hypotension despite adequate fluid resuscitation) still have very high mortality rates of 30%, 50%, and 80%, respectively, despite all intensive treatment strategies [1,2]. Septic shock is a serious circulatory failure associated with systemic vasodilation, vasoplegia, hyporesponsiveness to vasoconstrictors [3] and hypoperfusion of vital organs that lead to multiple organ dysfunction [4]. Respectively, diminished blood flow to the mesenteric vascular bed, increased vascular resistance and impaired responsiveness to exogenously applied vasoconstrictor agents are related to hypoperfusion and multiple organ failure. The translocation of intestinal bacteria from the intestinal lumen to the systemic circulation following mesenteric ischaemia was shown to contribute to the high mortality rate in sepsis-related syndromes both in animals and in humans [5].

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http://dx.doi.org/10.1016/j.biopha.2016.11.102
0753-3322/

The prostaglandins (PGs) and thromboxane $A_2$ ($TXA_2$) are collectively termed prostanoids and formed from arachidonic acid which is a major component of cellular membrane phospholipids. Two isoforms of Cyclo-oxygenase (COX), COX-1 and COX-2, catalyze the rate-limiting step of the prostanoid biosynthesis [6,7]. Under physiological conditions COX-1 is constitutively expressed in most tissues, whereas COX-2 is expressed at low levels in only a few tissues such as brain and kidney. The prostanoids are normally produced at low levels that regulate the homeostasis, gastrointestinal epithelial cytoprotection, intestinal barrier maintenance, mucosal secretion, smooth muscle function and vascular tonus [8]. The expression of COX-2, an inducible enzyme, is dramatically and readily increased in certain pathophysiological conditions such as inflammation, organ damage and malignant transformation that leads to the production of deleteriously large amounts of prostanoids [9,10]. The COX-2 overexpression is associated with increased vascular and intestinal permeability, systemic dissemination of the microorganisms, massive and uncontrolled secretion of inflammatory cytokines and free radicals, development of multisystem hypoperfusion, multiple organ failure and eventual death in sepsis [11]. Partial and selective COX-2 inhibition without affecting the constitutive COX-1 and basal COX-2 expressions might be a good potential therapeutic target for sepsis. Celecoxib, a selective COX-2 inhibitor, provides partial inhibition of COX-2 when it is administered to the rodents by oral gavage (*o.g.*) at a dose of 0.5 mg/kg [10]. In the light of these previous findings, we aimed to investigate the possible beneficial effects of celecoxib in sepsis induced by cecal ligation and puncture (CLP) with particular attention to the decreases in survival, mesenteric blood flow and vasopressor response of the aortic muscle, histopathological and biochemical injury in target organs such as liver, lung, kidney and spleen.

## 2. Materials and methods

Fifty-one female Wistar albino rats (weighing 250–300 g) were purchased from the animal shelter of Selcuk University Experimental Research Center, Konya, Turkey. The animals were housed in a temperature and humidity controlled room (21 ± 2 °C and 30–70%, respectively) under a 12-h light/12-h dark illumination sequence with *ad libitum* access to tap water (drinking bottle) and standard pellet dairy chow. All experimental procedures were performed according to The Recommendations from the Declaration of Helsinki together with The Guiding Principles in the Care and Use of Laboratory Animals. Before the commencement of any intervention, this study was approved by the Animal Care Committee of Selcuk University Experimental Research Center (Approval Number: 414757552015/39).

### 2.1. Experimental protocols

Animals were allocated into four groups: the 1 st group was sham operated and saline administrated (Sham group); the 2nd group was CLP operated and saline administrated (CLP group); the 3rd group was sham operated and celecoxib treated (0.5 mg/kg, o.g., Sham + celecoxib group); the 4th group was CLP operated and celecoxib treated (0,5 mg/kg, o.g., CLP + celecoxib group). Rats were administered with celecoxib (0.5 mg/kg/day, o.g) or its solvent (nonpyrogenic sterile saline; 0.9% NaCl, w/v) two hours after Sham and CLP operations [10,12]. The dose of celecoxib was choosen on the basis of a previous report. According to this report, low dose celecoxib (0.5 mg/kg) administration inhibits the COX-2 overexpression selectively and partially [10]. All rats were fasted overnight before the operations, but were allowed ad libitum access to drinking water.

For survival assessment, 26 rats were observed at 6-h intervals until 96 h after the Sham and CLP operations. Another 25 rats were used for mesenteric artery blood flow (MABF) and isometric measurements, histopathological and biochemical analyses. In these 25 rats, MABF was measured with a Doppler ultrasound flowmeter twenty hours after the Sham and CLP operations. Twenty hours after CLP operation represents the late phase of polymicrobial sepsis [13]. At the end of MABF measurement, blood samples were collected via cardiac puncture and transferred to Eppendorf tubes. After 15–20 min the blood samples were centrifuged for 5 min at 4000 × *g* and the serum sample aliquots were stored at −80 °C until the assays were performed. After the collection of the blood samples, aorta was removed quickly for isometric measurements and, the samples of liver, spleen, kidney and lung tissues were removed for biochemical and histopathological analysis. The tissue pieces of liver, spleen, kidney and lung were transferred to Eppendorf tubes and were stored at −80 °C until the time of malondialdehyde (MDA) and total glutathione (GSH) analyses. Furthermore, the other tissue pieces were harvested accordingly and fixed in 10% neutral buffered formaldehyde solution for histopathologic analyses.

### 2.2. Model of polymicrobial sepsis

Polymicrobial sepsis was induced in rats using the CLP method as described previously [12,13]. In brief, the rats were intraperitoneally anesthetized with sodium pentobarbital (60 mg/kg), a 2-cm ventral midline incision was then performed and the cecum was carefully exposed. The cecum was tightly ligated with a 3.0-silk suture just below the ileocecal valve and punctured twice with an 18-gauge needle. A small amount of feces were extruded from the perforation sites via gentle squeeze of the cecum. The cecum was returned to its normal intra-abdominal position and the laparotomy site was then closed in two layers using atraumatic 3-0 silk sutures. After the operations, normal saline (3 mL/100 g, s.c.) were injected immediately to the nape of the neck of rats for fluid resuscitation. For Sham procedure all of the same steps were applied, outside ligation and puncture of the cecum.

### 2.3. Mesenteric artery blood flow measurement

The animals were anesthetized with sodium pentobarbital and a ventral midline incision was then performed again 20 h after the Sham and CLP operations. The mesenteric artery was carefully exposed and blood flow was measured by a Doppler ultrasound flowmeter. The Doppler flow probe was hitched to a Transonic T206 Small Animal Flowmeter System (Transonic Inc., Ithaca, N.Y., USA) which provides absolute blood flow readings (mL/min), and located around the common mesenteric artery trunk. The obtained signals from the flowmeter were also recorded on a computer using a MP35 Biopac data recording system (Goleta, Calif., USA) while the animals were stabilized for 15 min before recording MABF values [14].

### 2.4. Isometric measurements

The changes in alpha-receptor mediated vasoconstriction during sepsis were evaluated on isolated rat aortic rings via cumulative administration of phenylephrine, an $\alpha_1$-receptor agonist, into the organ bath. After the thoracotomy, thoracic part of aorta was removed and put into the cold Krebs Henseleit (K-H) solution (as mM; sodium chloride 118, sodium bicarbonate 25, potassium chloride 4.7, calcium chloride 2, monopotassium dihydrogen phosphate 1.2 magnesium sulfate 1.2, and glucose 10) that was gassed with carbogen (95% $O_2$/5% $CO_2$). The isolated aorta was carefully cleaned of the fat and connective tissues and