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# Liver nitrosation and inflammation in septic rats were suppressed by propofol via downregulating TLR4/NF-κB-mediated iNOS and IL-6 gene expressions

Gong-Jhe Wu[a,b], Yung-Wei Lin[c], Chi-Yuan Chuang[d], Hsiao-Chien Tsai[b,e], Ruei-Ming Chen[d,e,*]

[a] Department of Anesthesiology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan
[b] Department of Anesthesiology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] Department of Urology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[d] Comprehensive Cancer Center, Taipei Medical University, Taipei, Taiwan
[e] Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan

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ABSTRACT

*Aims:* Propofol can be applied as an anesthetic or sedative agent for septic patients. Our previous studies showed that propofol ameliorated inflammation- and nitrosative stress-induced cellular insults. This study further evaluated effects of propofol on cecal ligation and puncture (CLP)-induced septic insults to rats and its possible mechanisms.

*Main methods:* Wistar rats were administered with CLP and effects of propofol on CLP-induced liver dysfunction and rat death were evaluated. Levels of hepatic or systemic nitrogen oxides (NOx) and interleukin (IL)-6 were quantified. Sequentially, inducible nitric oxide synthase (iNOS) and IL-6 gene expressions, toll-like receptor 4 (TLR4) protein levels, and nuclear factor (NF)-κB translocation were determined.

*Key findings:* Subjecting rats to CLP led to body weight loss, liver weight gain, and death. Administration of propofol lessened CLP-induced augmentations of serum and hepatic nitrosative stress and IL-6 levels. Additionally, propofol suppressed CLP-induced enhancements in levels of hepatic iNOS protein. Furthermore, the CLP-induced iNOS and IL-6 mRNA expressions in the liver were inhibited following propofol administration. Sequentially, subjecting rats to CLP enhanced hepatic TLR4 protein levels and NF-κB translocation to nuclei, but propofol inhibited these augmentations.

*Significance:* Consequently, exposure to propofol protected against CLP-induced liver dysfunction and increased the survival rates of the animals. This study shows that propofol can protect rats against septic insults through suppression of systemic and hepatic nitrosative and inflammatory stress due to inhibition of TLR4/NF-κB-mediated iNOS and IL-6 mRNA and protein expressions.

## 1. Introduction

Propofol, an intravenous anesthetic agent, is widely used to induce and maintain anesthesia during surgery [23,31]. In intensive care units (ICUs), propofol is also commonly used to manage critically ill patients who require sedation and analgesia for comfort and cooperation [15]. Propofol possesses wide applications for anesthesia and sedation because it has the advantages of rapid onset, short action duration, and speedy elimination [16]. In addition, our previous study showed suppressive effects of propofol on downregulating inflammatory cytokine gene expressions and reactive oxygen species (ROS) production in macrophages stimulated by Gram-negative bacteria [5,8,19]. In addition to anti-inflammatory and antioxidative benefits, other studies done in our lab demonstrated that therapeutic concentrations of propofol can also protect immune cells and cerebrovascular endothelial cells from nitrosative stress-induced apoptotic insults [4,7,9]. One possible mechanism illuminating how propofol can lessen nitrosative stress-induced injury is its ability to transcriptionally inhibit inducible nitric oxide synthase (iNOS) gene expression in lipopolysaccharide-activated macrophages [9,12]. Nitrosative stress participates in sepsis-induced apoptotic insults by promoting lipid peroxidation, mitochondrial dysfunction, and bioenergetic failure [1]. As a result, propofol has multiple supportive effects that would be helpful for de novo therapeutic strategies for ICU patients.

Sepsis, a severe inflammatory response to infection, remains a leading cause of morbidity and mortality in ICU patients worldwide

* Corresponding author at: Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, 250 Wu-Xing St., Taipei 110, Taiwan.
*E-mail address:* rmchen@tmu.edu.tw (R.-M. Chen).

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[11]. Sepsis usually induces cell dysfunction and subsequent organ failure, ultimately leading to death. Meanwhile, a variety of molecular events contributes to cell dysfunction. Inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α) can function as pleiotropic regulators of the immune response and play crucial roles in the complex pathophysiology of sepsis [25,30,41]. Overexpression of these inflammatory cytokines may cause dysfunction of the immune system and consequent damage to multiple tissues [30]. IL-6 is a typical proinflammatory cytokine that is considered as a first-line factor driving the dynamic process of sepsis [3,30]. Production of IL-6 and other cytokines can intensify inflammatory cascades by activating macrophages to generate ROS and nitrogen oxides (NOx) that subsequently cause organ failure [10,14,37]. Hence, repressing expressions of these inflammatory cytokine genes and production of oxidative and nitrosative stress would be helpful for treating sepsis.

In the host response, the liver is a key tissue that contributes to clearing infectious agents and products during sepsis [33]. Regrettably, sepsis can induce liver dysfunction at the same time by interfering with hemodynamic circulation or triggering insults to hepatocytes. A previous study showed presence of 12% liver failure within 28 days of the onset of sepsis syndrome [28]. For this reason, liver failure is considered to be a crucial component contributing to the severity of sepsis. In response to stimulation by septic shock, hepatocytes and Kupffer cells produce large amounts of inflammatory cytokines and NOx [14,35]. Being structurally similar to α-tocopherol, propofol was reported to possess anti-inflammatory, antioxidative, and anti-nitrosative characteristics [7,13,21]. Previous studies showed protective effects of propofol in septic hamsters and rats [20,29,43]. Thus, propofol can be of great relevance to intensive care of critically ill septic patients. However, the molecular mechanisms of propofol-induced protection against sepsis-induced damage in vivo are still not well known. Therefore, in this study, we evaluated effects of propofol on cecal ligation and puncture (CLP)-induced insults and its possible mechanisms in terms of anti-nitrosative and anti-inflammatory events in the liver.

## 2. Materials and methods

### 2.1. Animals and experimental design

All procedures were performed according to the National Institutes of Health *Guidelines for the Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Taipei Medical University, Taipei, Taiwan (Approval number: WAN-LAC-103-003). Male Wistar rats weighing 200–250 g were purchased from Animal Center of the College of Medicine, National Taiwan University (Taipei, Taiwan). Before our experiments began, rats were allowed to acclimatize for at least 1 week in animal quarters with air conditioning and an automatically controlled photoperiod of 12 h of light daily. Animals were allowed free access to rodent laboratory chow (Purina Mills, St. Louis, MO, USA).

Sepsis was induced using a CLP model as described previously [36]. Propofol, purchased from Aldrich (Milwaukee, WI, USA), is fat-soluble. In the clinic, propofol is mixed in intralipid, an emulsion of soy bean oil, egg phospholipids, and glycerin. In this study, propofol was freshly dissolved in intralipid. Animals were randomly divided into 1) sham-, 2) CLP-, 3) CLP + intralipid-, and 4) CLP + propofol-treated groups. Briefly, Wistar rats were anesthetized with an intraperitoneal (IP) injection of a mixture of 10 mg/kg xylazine and 100 mg/kg ketamine hydrochloride. A 2.0-cm incision midline laparotomy was made along midline of the abdomen, and the cecum was exposed and ligated distal of the ileocecal valve. The cecum was punctured twice in the midline with an 18-gauge needle. Sham-operated animals were subjected to a laparotomy without CLP. In the CLP + propofol-treated group, animals were IP-injected with propofol at a single dose of 60 mg/kg body weight after CLP. In comparison, the same volume of intralipid was IP-administered to rats after CLP in CLP + intralipid-treated animals. These rats remained on a heating pad until they awoke. The animals were allowed free access to food and water after induction of sepsis and drug treatment. The survival rates and body weights of these rats were determined.

### 2.2. Quantifications of serum and liver NOx

Levels of serum and liver NOx were quantified by measuring production of nitrite and nitrate using the Griess reaction method as described previously [39]. After CLP, rats were IP-injected with propofol at a single dose of 60 mg/kg body weight. Afterwards, animals were sacrificed, and blood samples and livers were collected. The serum fraction was prepared following centrifugation. Livers were homogenized, and the supernatants were prepared after centrifugation at 9000*g*. The serum and supernatant fractions were reacted with nitrate reductase. Following a reaction of the supernatant with sulfanilamide and N-1-napthylethylenediamine, a colorimetric azo compound was formed and quantified using an Anthos 2010 microplate photometer (Anthos Labtec Instruments, Lagerhausstrasse, Wals/Salzburg, Austria).

### 2.3. Assays of IL-6 levels

Levels of IL-6 were quantified using an enzyme-linked immunosorbent assays (ELISA) as described previously [41. Briefly, animals were sacrificed, and blood samples were collected. Following centrifugation at 3000 rpm for 20 min, serum fractions were obtained and immediately stored at − 20 °C until analysis. Levels of serum IL-6 were quantified following standard protocols of ELISA kits purchased from R&D Systems (Minneapolis, MN, USA).

### 2.4. Immunoblot analyses of iNOS, toll-like receptor (TLR)4, and β-actin

Protein analyses were carried out according a previously described method [7]. Briefly, animals were sacrificed, and their livers were collected. After being washed, livers were homogenized in ice-cold 1.15% potassium chloride. The 9000 g supernatant of liver homogenates was prepared as described previously [6]. Protein concentrations were quantified using a bicinchonic acid protein assay kit (Pierce, Rockford, IL, USA). Proteins (50 μg/well) were subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to nitrocellulose membranes. After blocking, iNOS was immunodetected using a mouse monoclonal antibody against mouse iNOS protein (Transduction Laboratories, Lexington, KY, USA). TLR4 was immunodetected using a goat polyclonal antibody against rat TLR4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Amounts of β-actin protein were analyzed using a mouse monoclonal antibody against rat β-actin (Sigma, St. Louis, MO, USA) as a internal standard. These protein bands were quantified using a digital imaging system (UVtec, Cambridge, UK).

### 2.5. Real-time polymerase chain reaction (PCR) assay

Messenger (m)RNA from livers was prepared for real-time PCR analyses as described previously [41]. Briefly, animals were sacrificed, and their livers were collected for total RNA preparation using an acid guanidinium thiocyanate-phenol-chloroform method. These respective oligonucleotide sequences of upstream and downstream primers for mRNA analyses were 5′-TTGGACAATGGACTGGTTG-3′ and 3′-GTGAC TGGTAGGTGAGATG-5′ for iNOS mRNA and 5′-TATGGAGAAGATTTG GCACC-3′ and 3′-ATGAGACACACCTAACCACC-5′ for β-actin mRNA. A real-time PCR analysis was carried out using iQSYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and the MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad).