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Propofol lowers serum PF4 level and partially corrects hypercoagulopathy in endotoxemic rats

Jing Tang [a,d,1], Yijuan Sun [a,1], William Ka Kei Wu [b], Tianyu Zhong [c], Yawei Liu [d], Jinfang Xiao [a], Tao Tao [a], Zhenlong Zhao [a], Miaoning Gu [a,*]

[a] Department of Anesthesia, Nanfang Hospital, Southern Medical University, 1838 Guangzhou Avenue North, Guangzhou 510515, China
[b] Institute of Digestive Diseases, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China
[c] Clinical Laboratory, Nanfang Hospital, Southern Medical University, 1838 Guangzhou Avenue North, Guangzhou 510515, China
[d] Department of Pathophysiology, School of Basic Medical Science, Southern Medical University, 1838 Guangzhou Avenue North, Guangzhou 510515, China

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ABSTRACT

Propofol, an anesthetic drug, has been shown to exhibit antioxidant and anti-inflammatory properties *in vitro* and *in vivo*. Hypercoagulopathy is a common clinical feature of sepsis, but the effects of propofol on the coagulation system in septic conditions are unclear. Using the gel-based comparative proteomic approach, together with Western blot analysis, ELISA, antithrombin III activity assay, and blood coagulation test, the effect of propofol on serum proteomic profiles in endotoxemic rats was examined. We identified that serum platelet factor-4 (PF4), an endogenous pro-coagulant, was up-regulated in LPS-challenged rats ($p<0.001$). Endotoxemia also resulted in hypercoagulopathy as evidenced by the shortening of thromboplastin time and thrombin time. Administration of propofol attenuated LPS-stimulated PF4 release and partially reversed the effect of LPS on thromboplastin time ($p=0.0012$) and thrombin time ($p=0.0072$). We demonstrated that propofol reduces serum levels of PF4 and partially corrects the hypercoagulopathy associated with endotoxemia in rats.

1. Introduction

Sepsis is a serious medical condition characterized by a whole-body inflammatory state (systemic inflammatory response syndrome or SIRS) resulting from infection and is associated with high morbidity and mortality [1]. Septic patients sometimes require mechanical ventilation and in these cases propofol may be an ideal agent for sedation [2]. Propofol is a safe and effective intravenous anesthetic, being used widely for induction and maintenance of anesthesia in surgery, and for sedating patients suffering from critical illness such as sepsis [3]. Since the report by Musacchio et al. that propofol could inhibit the production of malondialdehyde in rat liver mitochondria and microsomes as well as in rat brain synaptosomes treated with inducers of lipid peroxidation [4], there have been many studies demonstrating the anti-inflammatory effects of propofol. For instances, Jawan et al. found that propofol pre-treatment attenuates LPS-induced granulocyte-macrophage colony-stimulating factor production in cultured hepatocytes [5]. Moreover, propofol inhibited LPS-induced hypoxia-inducible factor 1 activation and suppresses glucose metabolism in macrophages [6]. Propofol has also been shown to reduce both lipid peroxidation and the fall in arterial oxygen tension during endotoxemia [7].

Despite *in vitro* evidence, the antioxidant and anti-inflammatory effects of propofol *in vivo* have been questionable [8]. For example, it had been reported that propofol exhibited no significant effects on neutrophil function and immune status in patients with severe brain injury who required long-term sedation [9]. During sepsis, a large number of host-derived mediators, including cytokines, chemokines, and products of the complement system, circulate in the bloodstream to mediate the systemic inflammatory response [10]. These mediators activate endothelial cells to promote the expression of adhesion molecules, resulting in increased platelet adhesion, leukocyte trafficking and blood hypercoagulability [10–12]. Conversely, blood clotting not only leads to fibrin deposition and platelet activation, but it also results in endothelial cell activation, which contributes to leukocyte activation [13]. We therefore speculated that propofol might affect one or more of these serum proteins to mediate its anti-inflammatory effect. Utilising the comparative proteomic approach, we sought to clarify the changes of serum protein expression in endotoxemic rats and the effect of propofol on serum protein profile.

2. Materials and methods

2.1. Rat endotoxemia model and serum sample collection

Eighteen male Sprague-Dawley (SD) rats (180–210 g) were maintained in the experimental animal center of the Southern Medical University and had free access to standard laboratory chow

* Corresponding author. Tel./fax: +86 20 61641881.
E-mail address: gumiaoning@126.com (M. Gu).
[1] J. Tang and Y. Sun contributed equally to this work.

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and water. All animal procedures were approved and conducted in accordance with the guidelines for the care and use of animals of the ethic committee of the Southern Medical University. Animals were anesthetized with urethane (1.0 $g.kg^{-1}$) intra peritoneal, and divided into control ($n=6$), LPS ($n=6$) and LPS plus propofol ($n=6$) groups. Rats in the control group received a continuous infusion of 10% Intralipid® (20 $mg.kg^{-1}\,h^{-1}$) (Sino-Sweden Pharmaceutical Corp. Ltd., China) via the femoral vein for controlling EDTA present in the lipid diluent of propofol. Rats in the LPS group were infused with Intralipid® immediately after intravenous injection of 5 $mg.kg^{-1}$ LPS (*Escherichia coli* EH100, Sigma), and rats in the LPS plus propofol group were injected with LPS (5 $mg.kg^{-1}$) followed by intravenous infusion with propofol (Astrazeneca, UK) at 20 $mg.kg^{-1}h^{-1}$. After 6 h, blood samples were collected from each rat via the carotid artery, and serum isolated was stored at −80 °C until analysis.

2.2. 2D electrophoresis

Immobiline Dry strip (pH 4–7, length 24 cm, GE Healthcare) was rehydrated with 1500 μg protein in 450 ml rehydration buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 65 mM DTT, 20 mM Trizma base, 1% IPG buffer and 0.002% bromophenol blue for 14 h at room temperature. Isoelectric focusing (IEF) was performed using the Ettan IPGphor 3 IEF System (GE Healthcare, USA) for a total of 70 kVh. The strip was then subjected to two-step equilibration in a buffer containing 6 M urea, 20% glycerol, 2% SDS and 50 mM Tris–HCl (pH 8.8) with 2% w/v DTT for the first step, and 2.5% w/v iodoacetamide for the second step. The second-dimension SDS-PAGE (12% T, $260\times200\times1.5\ mm^3$) was performed using a Ettan DALTsix Large Vertical system (Amersham, USA) according to the following procedures: 45 min at a constant power of 5 watts followed by 20 watts per gel until the bromophenol blue reached the bottom of the gel. Subsequently the gels were stained with 0.12% w/v Coomassie Brilliant Blue G250. Each group was run in triplicate to minimize run-to-run variation. The Coomassie Blue-stained protein 2D gels were scanned using an Amersham Biosciences Imagescanner and analyzed using DeCyder software package (GE Healthcare, USA).

2.3. In-gel digestion

Protein spots were excised from the gel with a scalpel, destained twice with 30 mM potassium ferricyanide and 100 mM sodium thiosulfate (1:1 v/v) and then equilibrated in 100 mM NH_4HCO_3 to pH 8.0. After dehydrating with acetonitrile (CAN) and drying in nitrogen at 37 °C for 20 min, the gel pieces were rehydrated in 10 μl trypsin solution (12.5 ng/μl in 50 mM NH_4HCO_3) at 4 °C for 30 min and incubated at 37 °C overnight. Peptides were extracted twice using 0.1% trifluoroacetic acid (TFA) in 60% CAN and dried with the RCT60 (Jouan, France).

2.4. MALDI-TOF MS identification

The peptide mixtures were solubilized with 0.1% TFA and desalted with C18 ZipTip (Millipore, USA). The peptide was then eluted by saturated a-cyano-4-hydroxy-trans-cinnamic (CHCA) solution in 0.1% TFA/60% acetonitrile as the matrix and analyzed using 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems, USA). Mass spectra were internally calibrated with angiotensin I (M_r: 1296.6853).

2.5. Protein identification and database searching

Protein identification using peptide mass fingerprinting (PMF) and peptide sequence tag (PST) was performed by the MASCOT search engine (http://www.matrixscience.com/, MatrixSicence Ltd., London, UK) against the SwissProt protein database. The errors in peptide masses were in the range of 50 ppm. One missed tryptic cleavage site per peptide was allowed during the search. Proteins matching more than four peptides and with a MASCOT score higher than 64 were considered significant ($p<0.05$). Carboamidomethylation of cysteine was selected as the fixed modification and oxidation of methionine as the variable modification. Protein identification results were filtered with GPS software.

2.6. Western blot analysis

To verify the 2D electrophoresis protein expression data, another 24 SD rats were divided into four groups control, LPS, LPS plus propofol, and propofol groups. In the propofol group, rats were infused with propofol at 10 $mg.kg^{-1}h^{-1}$, and the other three groups were treated the same as above. The amount of PF4 in serum was determined by Western blot. Equal amounts of total protein (50 μg) were loaded and run on 12% PAGE gels with 5% stacking gels and transferred onto polyvinylidene difluoride membranes (PVDF) (Amersham Pharmacia Biotech, Piscataway, NJ). The membrane was

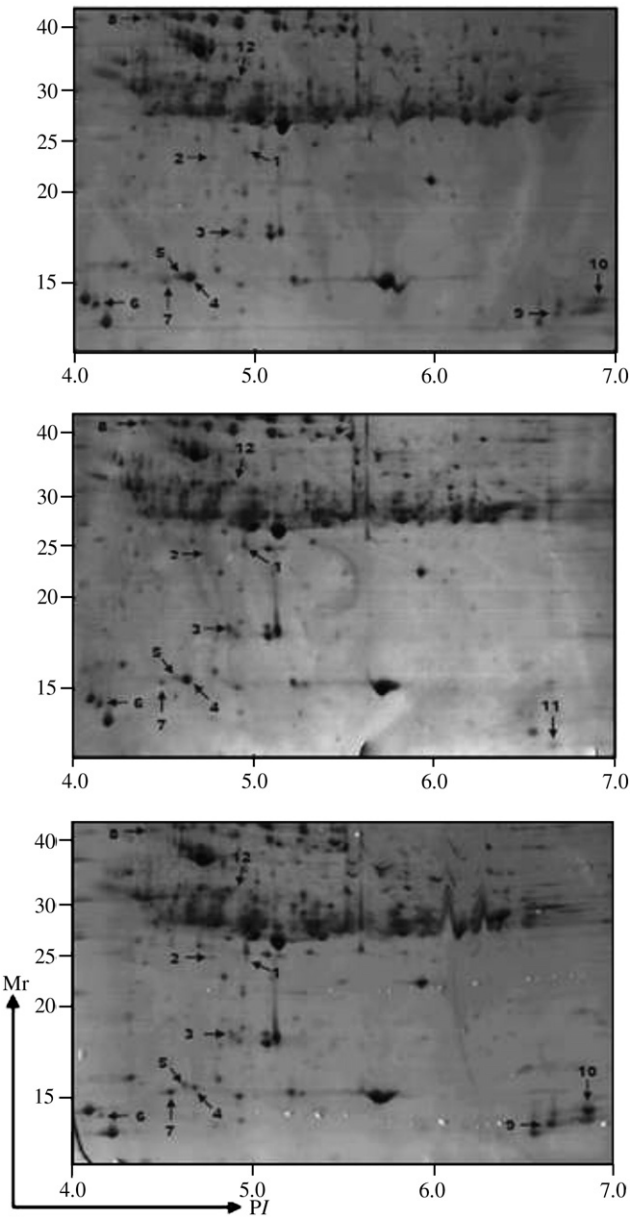

Fig. 1. Representative 2D gels. Control group (upper), LPS plus Intralipid group (middle) and LPS plus propofol group (lower). All the 12 differentially expressed proteins were identifed by MALDI-TOF MS.