JOURNAL OF SURGICAL RESEARCH 197 (2015) 412–418

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Vasopressin inhibits endotoxin binding in activated macrophages

Ya-Ying Chang, MD,[a,b] *Chen-Hsien Yang, MD,*[a,b] *Shih-Ching Wang, MD,*[a,b] *Ming-Chang Kao, MD,*[a,b] *Pei-Shan Tsai, PhD,*[c] *and Chun-Jen Huang, MD, PhD*[a,b,*]

[a] *Department of Anesthesiology, Taipei Tzu Chi Hospital, Taipei, Taiwan*
[b] *School of Medicine, Tzu Chi University, Hualien, Taiwan*
[c] *Graduate Institute of Nursing, College of Nursing, Taipei Medical University, Taipei, Taiwan*

ARTICLE INFO

Article history:
Received 13 December 2014
Received in revised form
14 March 2015
Accepted 14 April 2015
Available online 18 April 2015

Keywords:
Vasopressin
CD14
Nuclear factor-κB
Lipopolysaccharide

ABSTRACT

Background: Vasopressin possesses potent anti-inflammatory effects. Endotoxin recognition (mediated by cluster of differentiation 14 [CD14]), endotoxin binding, and subsequent nuclear factor-κB (NF-κB) activation are essential mechanisms for initiation of the inflammatory response. We elucidated the effects of vasopressin on these essential mechanisms of inflammation with the hypothesis that vasopressin could inhibit CD14 expression, endotoxin binding, and NF-κB activation in activated macrophages.

Methods: Murine macrophage-like cell line RAW264.7 cells were stimulated with endotoxin (lipopolysaccharide [LPS]; 100 ng/mL) or LPS plus vasopressin (1000 pg/mL; designated as the LPS and the LPS + V groups, respectively). After reaction, between-group differences in inflammatory molecule concentrations and levels of NF-κB activation, endotoxin-macrophages binding, and CD14 expression were compared. Analysis of variance was performed for statistical analysis.

Results: The concentrations of chemokine macrophage inflammatory protein 2 and cytokine interleukin 6 of the LPS + V group were significantly lower than those of the LPS group ($P = 0.004$ and $P < 0.001$). The nuclear concentration of phosphorylated NF-κB p65 and cytosolic concentration of phosphorylated inhibitor-κBα of the LPS + V group were significantly lower than those of the LPS group (all $P < 0.05$). In addition, the level of endotoxin-macrophages binding of the LPS + V group was significantly lower than that of the LPS group ($P < 0.001$). The level of surface CD14 expression of the LPS + V group was also significantly lower than that of the LPS group ($P = 0.019$).

Conclusions: This study confirmed the potent anti-inflammatory effects of vasopressin. The mechanisms underlying the anti-inflammatory effects of vasopressin may involve its effects on inhibiting CD14 expression, endotoxin binding, and subsequent NF-κB activation.

* *Corresponding author*. Department of Anesthesiology, Taipei Tzu Chi Hospital, 289, Jianguo Rd, Sindian District, New Taipei City 231, Taiwan. Tel.: +886 2 66289779x2639; fax: +886 2 66289009.
E-mail address: huangcj1112@gmail.com (C.-J. Huang).
0022-4804/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2015.04.042

1. Introduction

Vasopressin, a nanopeptide synthesized in paraventricular and supraoptic nuclei in the hypothalamus, is well known for its physiological characteristics of vasoconstriction, antidiuresis, and behavioral regulation [1–3]. As circulating level of endogenous vasopressin is low in septic shock [3,4], clinical guidelines have suggested exogenous vasopressin replacement as part of the therapies against severe sepsis [5]. On top of that, exogenous vasopressin replacement has been shown to remedy inflammation-induced damages, as vasopressin could decrease sepsis-induced pulmonary inflammation [6] and preserve renal and mesenteric blood flow in septic animals [7,8]. Our previous study also confirmed that vasopressin could inhibit upregulation of inflammatory molecules in endotoxin-stimulated murine macrophages [9].

Although the anti-inflammatory effect of vasopressin is confirmed, the underlying mechanisms remain to be elucidated. Expression of inflammatory molecules is tightly regulated by the upstream transcription factor nuclear factor-κB (NF-κB) [10]. Moreover, cellular recognition of endotoxin and subsequent endotoxin binding is essential for endotoxin-induced activation of NF-κB [11,12]. In line with this concept, it is likely that vasopressin may act through inhibiting endotoxin recognition, endotoxin binding, and/or mitigating NF-κB activation to exert its effect on inhibiting upregulation of inflammatory molecules. Of note, endotoxin recognition is tightly regulated by the cell surface pattern recognition receptor cluster of differentiation 14 (CD14) [12,13]. Downregulation of membrane-bound CD14 expression can decrease endotoxin-macrophages binding and subsequently inhibit macrophages activation [14–17]. To elucidate further, we thus conducted this study with a widely used endotoxin-activated murine macrophages cell model [18,19]. Our hypothesis was that vasopressin could inhibit CD14 expression, endotoxin binding, and NF-κB activation in activated murine macrophages.

2. Materials and methods

2.1. Cell culture and cell simulation protocols

We used an immortalized murine macrophage-like cell line RAW264.7 cells to facilitate investigation. RAW264.7 cells were grown in Dulbecco modified Eagle medium (Life Technologies, Grand Island, NY), supplemented with 10% fetal bovine serum and 1% penicillin and/or streptomycin (Life Technologies), and incubated in a humidified chamber at 37°C in a mixture of 95% air and 5% CO_2. Cells under passage 20 were used for experiments. For cell simulation, confluent RAW264.7 cells were treated with gram (−) endotoxin (lipopolysaccharide, LPS, 100 ng/mL, *Escherichia coli* Serotype 0127:B8 endotoxin; Sigma–Aldrich, St. Louis, MO), according to previous reports [18,19].

2.2. Experimental protocol

Confluent RAW264.7 cells were randomized to receive phosphate-buffered saline (PBS; Life Technologies), vasopressin (1000 pg/mL; Life Technologies), LPS or LPS plus vasopressin, and designated as the PBS, the V, the LPS, and the LPS + V groups, respectively. The dosage of vasopressin was determined according to our previous report [9]. Vasopressin was administered immediately after LPS.

2.3. Enzyme-linked immunosorbent assay for inflammatory molecules

We assayed the concentrations of chemokine macrophage inflammatory protein 2 (MIP-2) and cytokine interleukin 6 (IL-6) to confirm the anti-inflammatory effects of vasopressin. In brief, six different cell cultures from each group (i.e., $n = 6$ in each group) were harvested at 24 h after reaction with LPS or at comparable time points in groups without LPS. The collected cultures media were then analyzed for the concentrations of MIP-2 and IL-6 using enzyme-linked immunosorbent assay. Commercially available enzyme-linked immunosorbent assay kits of MIP-2 (R&D Systems, Inc, Minneapolis, MN) and IL-6 (Pierce Biotechnology, Inc, Rockford, IL) were used, and the procedures were performed according to the manufacturer's instructions.

2.4. Immunoblotting assay for NF-κB

NF-κB activation begins with phosphorylation of inhibitor-κB (I-κB) followed by subsequent NF-κB nuclear translocation [20,21]. This study performed immunoblotting assay to measure the protein concentrations of phosphorylated I-κB in cytosol and phosphorylated NF-κB in nucleus, as we have previously reported [20,21]. To facilitate investigation, cell cultures were harvested at 0, 15, 30, 45, and 60 min after reaction with LPS or at comparable time points in groups without LPS. For each time point, six different cell cultures from each group were harvested (i.e., $n = 6$ in each group). Preparations of nuclear and cytosolic extracts were performed, according to our previous report [20]. Immunoblotting assay was then conducted. In brief, the proteins were separated by gel electrophoresis and then transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). For nuclear extracts, the nitrocellulose membranes were incubated overnight at 4°C with anti–phosphorylated NF-κB p65 antibody (p-NF-κB p65 [Ser536], 1:500 dilution; Cell Signaling Technology, Inc, Danvers, MA) or anti–histone H3 antibody (internal standard, 1:500 dilution; Cell Signaling Technology) to facilitate assaying NF-κB nuclear translocation. For cytosolic extracts, the nitrocellulose membranes were incubated overnight at 4°C with anti–phosphorylated I-κB antibody (p-I-κBα [Ser32], 1:1000 dilution; Cell Signaling Technology) or anti-actin antibody (internal standard; 1:5000 dilution; Millipore Corporation, Burlington, MA) to facilitate assaying I-κB phosphorylation. Horseradish peroxidase–conjugated anti–mouse IgG antibody (Amersham Pharmacia Biotech, Inc, Piscataway, NJ) was used as the secondary antibody. Bound antibody was detected by chemiluminescence (ECL plus kit; Amersham). The protein band densities were quantified using densitometric technology (Scion Image for Windows; Scion Corp, Frederick, MD).