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Short communication

The sensitivity of adipose tissue visfatin mRNA expression to lipopolysaccharide-induced endotoxemia is increased by ovariectomy in female rats

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ABSTRACT

Visfatin plays an important role in inflammatory and metabolic conditions. In this study, the effects of septic stress on the serum, white-adipose-tissue (WAT), and liver visfatin levels of male and female rats were examined. Both gonadally intact (sham) and ovariectomized (OVX) female rats were used in order to evaluate the effects of the gonadal hormonal milieu on visfatin responses. Under the saline-injected conditions, the serum visfatin levels and the hepatic, subcutaneous, and visceral WAT visfatin mRNA levels of the OVX and sham rats did not differ. The serum visfatin levels and the subcutaneous, visceral WAT, and hepatic visfatin mRNA levels of both male and female rats were increased by the injection of a septic dose (5 mg/kg) of LPS. At 6 h after the injection of LPS, the WAT visfatin mRNA levels of the OVX rats were higher than those of the sham rats, whereas the serum visfatin levels and hepatic visfatin mRNA levels of the two groups did not differ. In the cultured visceral WAT, visfatin antagonist (FK-866) attenuated the LPS-induced up-regulations of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α). The pathophysiological roles of visfatin under septic conditions remain to be clarified. In addition, the precise mechanisms responsible for the increased WAT visfatin expression seen after ovariectomy and the effects of such changes should also be clarified.

1. Introduction

Visfatin, which is also known as pre-B cell colony-enhancing factor or nicotinamide phosphoribosyltransferase, plays an important role in inflammatory and metabolic conditions [1]. Visfatin is predominantly expressed in visceral fat, the liver, and leukocytes in both humans and rodents, and it plays roles as a cytokine-like factor in a variety of metabolic and immune responses [1–3]. In adipose tissue and the liver, visfatin has pro-inflammatory effects in various chronic diseases, such as type 2 diabetes, chronic hepatitis, and cardiovascular disease [1,4]. Although it has been reported that visfatin has insulin-mimetic effects that are mediated via its binding to the insulin receptor, this result has not been reproduced in other studies [3]. Visfatin also plays roles in acute immune responses by modulating neutrophil apoptosis and inflammatory cytokine expression. In humans, serum visfatin levels are increased under septic conditions, and increased visfatin expression is associated with delayed neutrophil apoptosis [5]. In addition, the central injection of visfatin induces anorectic and febrile responses by enhancing the expression levels of hypothalamic pro-inflammatory cytokines [6], indicating that visfatin might be related to the development of such symptoms in under acute inflammatory conditions. It has been reported that visfatin promotes lung damage and myocardial impairment under acute inflammatory conditions and that the inhibition of visfatin attenuates these disorders [14,15]. In addition, it has been shown that visfatin participates in inflammatory responses in the spleen [16]. However, few studies have comprehensively evaluated the in vivo response of visfatin to septic stress. Visfatin expression might also be affected by the gonadal steroid milieu, although this is disputed. Zhou et al. showed that visfatin mRNA expression was increased by estrogen in mouse 3T3-L1 cells [7], but no such changes were observed in another study [8,17]. Similarly, adipose visfatin mRNA expression was found to be increased in pregnant humans and rats, both of which exhibit high serum estrogen levels [2,9]. On the other hand, as far as we know, the effects of ovariectomy on visfatin levels under basal and immune stress conditions have not been evaluated.

In this study, the effects of septic stress on visfatin levels were examined in male and female rats. Specifically, the serum visfatin concentration, and hepatic, subcutaneous, and visceral white adipose tissue

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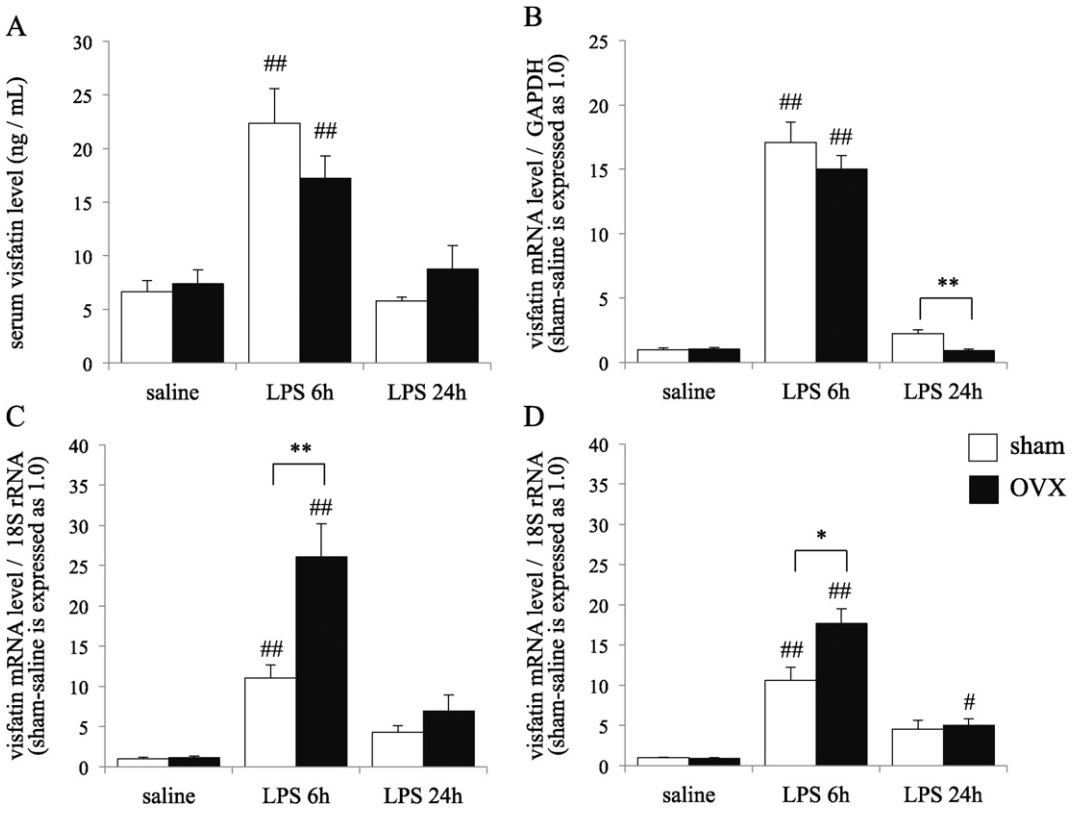

Fig. 1. Serum visfatin levels (A) and hepatic (B), subcutaneous white adipose tissue (C) and visceral white adipose tissue (D) visfatin mRNA expression levels seen under the saline-injected and LPS (5 mg/kg)-injected conditions ($n = 5$–10) in sham-operated (□) and ovariectomized (OVX) female rats (■). mRNA levels are expressed as ratios relative to the mRNA levels observed in the saline-injected sham group. Data are presented as mean ± SEM values. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. each other; $\#P < 0.05$, $\#\#P < 0.01$ vs. the value obtained for the corresponding group under the saline-injected conditions.

(WAT) visfatin mRNA expression were measured under non-stress and LPS-induced septic conditions. As the gonadal steroid milieu might also affect visfatin responses, both gonadally intact and ovariectomized rats were used in this study.

2. Materials and methods

Sprague-Dawley rats (Charles River Japan, Tokyo, Japan), which were housed in a room under controlled conditions, were used in this study. All animal experiments were conducted in accordance with the ethical standards of the animal care and use committee of the University of Tokushima. All surgical procedures were carried out under anesthesia with sodium pentobarbital. At 10 weeks of age, female rats underwent a sham operation (sham group) or bilateral ovariectomy (ovariectomized (OVX) group). After being allowed to recover for 6 or 7 weeks, the female rats were used for the study. In a separate experiment, gonadally intact male rats were used at 14 weeks of age.

The female rats in the sham and OVX groups were sub-divided into saline-injected and LPS (0111:B4; Sigma, St. Louis, MO, USA) (5 mg/kg)-injected groups. The rats in these groups were intraperitoneally injected with saline or LPS dissolved in saline. The injected volume of LPS did not exceed 0.2 mL. In the LPS-injected groups, tissue samples were collected at 6 h and 24 h after the injection. The females were divided into 6 groups, the sham-saline ($n = 10$), sham-6 h LPS ($n = 8$), sham-24 h LPS ($n = 5$), OVX-saline ($n = 10$), OVX-6 h LPS ($n = 8$), and OVX-24 h LPS ($n = 6$) groups. At each time point, the selected rats were deeply anesthetized, and their blood was collected from the left cardiac ventricle. Then, the rats were perfused with phosphate buffered saline, and their visceral (parametrial) and subcutaneous (inguinal) WAT was collected. The male rats were also sub-divided into saline-injected ($n = 6$) and LPS-injected groups ($n = 7$). At 6 h after the injection, blood was collected from the males using the same procedure as was employed in the females. Serum visfatin levels, and hypothalamic and WAT visfatin mRNA levels were measured.

To evaluate the roles of visfatin under LPS-induced inflammatory condition, explant culture were prepared by collecting perigonadal WAT from OVX rats as reported in previously. WAT was rinsed in PBS and dissected into 60–100 mg fragment. Each piece was transferred into 12-well plate filled with 2 mL medium (DMEN/F-12 (1:1) with L-glutamine and 15 mM HEPES, antibiotics, and 10% fetal bovine serum), and incubated at 37 °C, 90% humidity, and 5% CO2 for 24 h. Thereafter the medium was removed and replaced by 2 mL fresh medium (control), medium with 500 ng/mL LPS (LPS), medium with 500 ng/mL LPS and visfatin antagonist FK-866 (LPS + FK-866 10 nM, LPS + FK-866,100 nM, or LPS + FK-866,500 nM) (Funakoshi, Tokyo, Japan) and incubated for 24 h. The mRNA expression levels of IL-1β, IL-6, and TNF-α were quantified in cultured WAT. These doses of FK-866 were used in previous reports [20–22].

The rats' serum visfatin levels were measured using enzyme-linked immunosorbent assays (BioVendor Inc., Brno, Czech Republic). Total RNA was isolated and cDNA was synthesized as described previously [10]. Real-time PCR analysis was performed using the StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and SYBR green in order to quantify the relative mRNA expression levels of visfatin. All visfatin expression levels were normalized to the mRNA expression level of 18S rRNA or GAPDH. The following forward and reverse primers were used: visfatin (forward: 5′-AGC GGC AGA GCA CAG TAC CAT A-3′, reverse: 5′-CCA CAG ACA CAG GCA CTG ATG A-3′); IL-1β (forward: 5′-GCT GTG GCA GCT ACC TAT GTC TTG-3′, reverse: 5′-AGG TCG TCA TCA TCC CAC GAG-3′); IL-6 (forward: 5′-TCC TAC CCC AAC TTC CAA TGC TC-3′, reverse: 5′-TTG GAT GGT CTT GGT CCT TAG CC-3′); TNF-α (forward: 5′ - AGC CCT GGT ATG AGC CCA TGT A-