Acta Histochemica xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Original article

Effects of visfatin on the apoptosis of intestinal mucosal cells in immunological stressed rats

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ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 8 November 2016
Accepted 8 November 2016
Available online xxx

Keywords:
Visfatin
LPS induced
Intestine mucosal
Apoptosis
Caspase-3

ABSTRACT

This study was undertaken to determine if visfatin is involved in the inflammation or apoptosis introduced by LPS in rats. Forty 8-week old Wistar rats were divided into four groups (n = 10 in each group) and injected with saline, visfatin, LPS and visfatin+LPS co-stimulated via caudal vein. The duodenum, jejunum and ileum were harvested from all the rats. Compared to the saline treated group, visfatin significantly increased the number of TUNEL-positive apoptotic cells and the expression of caspase-3 protein in intestinal mucosa. Similarly, ELISA and western blot analysis also showed the up-regulation of pro-caspase-3 and cleaved caspase-3 expression in the visfatin group compared to the control group. In contrast to LPS, visfatin down-regulated the expression of cleaved-caspase-3 in the visfatin+LPS co-stimulated group, resulting in a significant decrease in apoptosis in intestinal mucosal cells. We observed more pro-caspase-3 positive cells in the visfatin+LPS co-stimulated group. The results indicate that, in the presence of LPS, visfatin plays an important role in the regulation of cell apoptosis and inflammation.

1. Introduction

Visfatin, a type of fat cell cytokine discovered in 2005, is expressed in visceral fat, and has a structure the same as the pre-B cell clone enhancement factor (PBEF) (Fukuhara et al., 2005; Lan et al., 2015; Samal et al., 1994). PBEF is expressed in neutrophils and fibroblasts, and could profoundly delay the rate of apoptosis (Jia et al., 2004). As a novel cytokine, PBEF acts on early B-lineage precursor cells and can be induced by pokeweed mitogen and superinduced by cycloheximide (Lim et al., 2008; Wu et al., 2015). Visfatin/PBEF was originally described as a cytokine-like protein which is able to regulate B cell development, apoptosis, and glucose metabolism. Visfatin has an inhibitory effect on the apoptosis of myocardial cells, endothelial cells (Borradaile and Pickering, 2009; Ucak et al., 2015), lymphocytes (Rongvaux et al., 2008), hepatocytes (Dahl et al., 2010) and other kind of cells. Moreover, it has antitumor activity (Khan et al., 2006) and can stimulate the proliferation of vascular smooth muscle cells (Wang et al., 2008). The hypothesis that visfatin could promote cell survival in macrophages subjected to endoplasmic reticulum (ER) stress (Li et al., 2008) has been confirmed by evidence that visfatin reduced caspase-3 activation (Abarikwu and Farombi, 2015; Sabry et al., 2015; Xiao et al., 2013). Furthermore, it has been further found that visfatin significantly suppresses cell apoptosis induced by palmitate by a mechanism that may be involved in up-regulation of Bcl2 and down-regulation of cytochrome C and caspase-3 (Cheng et al., 2011; Li et al., 2015). Visfatin can confer to immune system cells the ability to survive during stresses such as inflammation (Rongvaux et al., 2008). Caspase-3 is the most important member of the caspase family and is responsible for many biochemical mechanisms of apoptosis, leading to the cleavage of nuclear and cytosolic material, chromatin condensation and fragmentation of DNA into apoptotic bodies (Salvesen, 1997). The detection of activated caspase-3 is specific for identifying apoptotic cells in tissue.

The intestinal mucosa serves as the body's first line of defense and provides a resistant barrier against antigens. During infection, the integrity of the intestinal mucosa is significantly affected by apoptosis and necrosis leading to an impairment of the intestinal mucosal barrier. The intestinal mucosal immune system plays an important role in maintaining the homeostasis of the body by ensuring its immune response to pathogens and enabling the body to keep a dynamic balance between the internal and external environments (Didierlaurent et al., 2002; Shaykhiev and Bals, 2007), which are the important indicators of intestinal digestion and absorption function (Lin et al., 2014). A better understand-

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http://dx.doi.org/10.1016/j.acthis.2016.11.002
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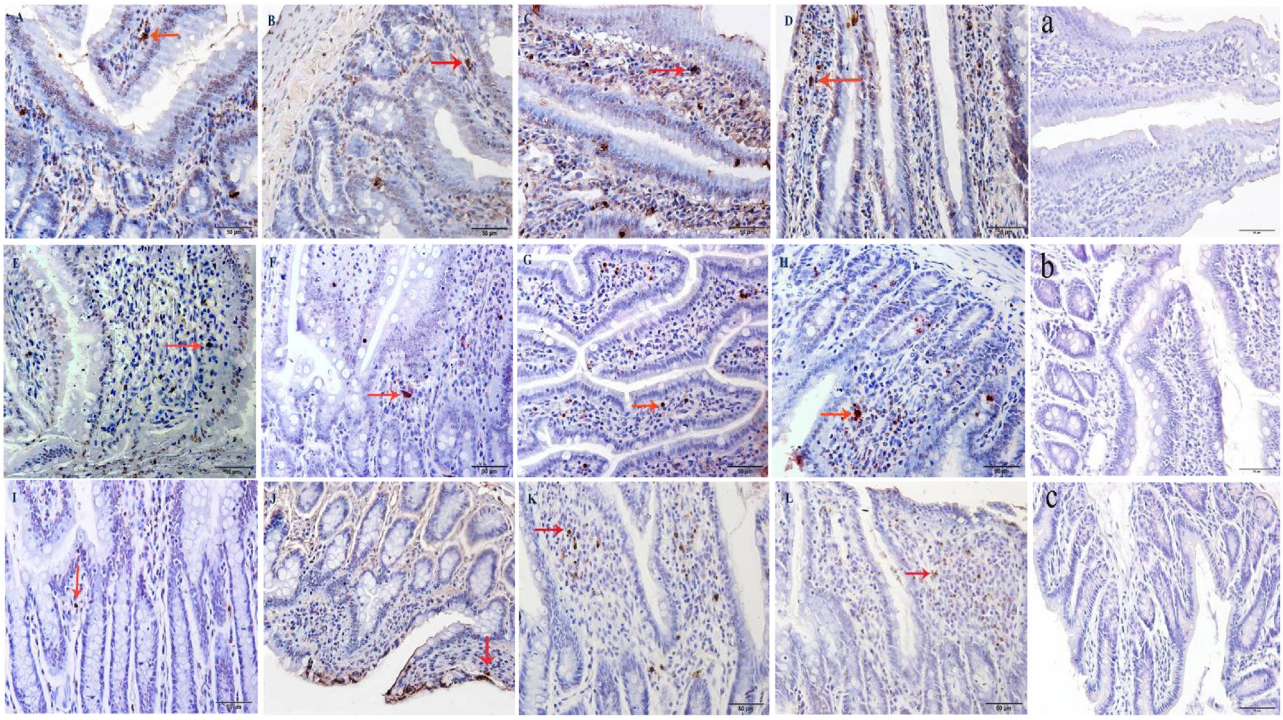

Fig. 1. Expression of apoptotic cells in the small intestinal mucosa in different treatment groups(TUNEL, 10 × 40): The expression pattern of TUNEL-positive apoptotic cells (red arrows) in duodenum (A) Control group, (B) Visfatin group, (C) LPS group, (D) Visfatin+LPS co-stimulated group; in jejunum (E) Control group, (F) Visfatin group, (G) LPS group, (H) Visfatin+LPS co-stimulated group; and in ileum (I) Control group, (J) Visfatin group, (K) LPS group, (L) Visfatin+LPS co-stimulated group. a, b and c means negative controls in duodenum, in jejunum and in ileum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ing of the mechanism of intestinal mucosal immune response is important for the prevention of intestinal diseases. Currently, the underlying mechanism of the intestinal mucosal immune system has attracted much attention among researchers, but the mechanism by which cytokines regulate the immunity has not been well characterized. Therefore, there are important practical reasons for further in-depth studies of the regulation mechanisms of intestinal mucosal cell apoptosis as they relate to the treatment of some gastrointestinal diseases. Visfatin, a peptide, can effectively regulate apoptosis of various cells, but the mechanism for regulation of intestinal mucosa cell apoptosis is not firmly established. In the present study, we describe the regulation of visfatin on intestinal mucosa cell apoptosis in LPS-induced rats.

2. Materials and methods

2.1. Reagents

Rat visfatin was purchased from Adipo Bioscience. LPS (O111:B4) was obtained from Sigma (St Louis, MO, USA). Caspase-3 expressions were quantified using a rat double-antibody sandwich indirect ELISA kit (Boster, China). The antibodies for GAPDH, CAP3 (immunohistochemistry), were obtained from Beijing Biosynthesis Biotechnology (Beijing, China). The caspase-3 polyclonal anti-body (western blot) was purchased from Proteintech (Chicago, IL, USA). The In Situ Cell Apoptosis Detection Kit I was purchased from Boster (Wuhan, China).

2.2. Animals and tissue collection

The animal experiments were approved by the appropriate local ethics committee of Huazhong Agricultural University. Eight-week old SPF Wistar rats, 200 ± 20 g, were purchased from the Hubei Provincial Center for Disease Control and Prevention. Forty rats were divided into four groups randomly (n = 10 in each group). All rats were individually housed in cages at 23–25 °C and maintained on a 12 h light/dark cycle. Water and food were provided ad libitum. The control group received one injection of isotonic saline (0.5 ml, i.v.) for seven consecutive days. The visfatin group received one injection of visfatin (50 g/kg bw, i.v.) for seven consecutive days. The LPS group received one injection of LPS (2.5 mg/kg bw, i.v.) all at once. The visfatin+LPS co-stimulated group received visfatin (50 μg/kg bw, i.v.) for seven consecutive days following a single-dose injection of LPS via the tail vein (2.5 mg/kg bw). Six hours after the last treatment, all rats were anesthetized with an i.v. injection of 1% pentobarbital (25 mg/kg bw). The duodenum, jejunum and ileum were removed. A portion of each organ was frozen in liquid nitrogen, while the remainder was fixed in 4% buffered paraformaldehyde solution.

2.3. Immunohistochemistry analysis

Tissues were fixed in 4% buffered neutral formalin solution. After the routine alcohol–xylol process, the tissue was sectioned at 4–5 um. Primary antibodies of polyclonal pro-caspase-3 (Bioss, China) and Histostain Plus Kit was used (ZSGB) as per manufacturer's instructions. Immunohistochemical staining was evaluated using a high-powered light microscope (BX51; Olympus, Tokyo, Japan).

2.4. TUNEL assay

The TUNEL assay was performed using the In Situ Cell Apoptosis Detection Kit I, POD (Boster). Sections were deparaffinized and rehydrated. Then endogenous peroxidase was blocked in 3% hydrogen peroxide and digested in Proteinase K for 15 min at 37 °C. Following the manufacturer's instructions, the slides sections were incubated with the prediluted anti-digoxin antibody, followed with

Please cite this article in press as: Zhou, Y., et al., Effects of visfatin on the apoptosis of intestinal mucosal cells in immunological stressed rats. Acta Histochemica (2016), http://dx.doi.org/10.1016/j.acthis.2016.11.002