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Expression and Changes of Visfatin in the Intestinal Mucosal of LPS-Induced Piglets

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Abstract: The purpose of this study was to elucidate the function of visfatin in the mucosal immune barrier of weaned piglets by observing histological changes in the small intestine and visfatin expression in LPS-induced piglets. The mucosal structure of the small intestine was damaged. The duodenum, jejunum, ileum and the intestinal villous were atrophied and shorter. Epithelial lymphocytes and lamina propria lymphoid tissue was reduced and aggregates of lymphoid nodules were increased. Visfatin-positive products were expressed in the small intestinal mucosa and mainly distributed in lamina propria lymphoid tissue. After LPS administration, visfatin-positive cells were reduced in the epithelial lymphocytes, lamina propria and intestinal gland but increased in aggregates lymphoids. This study suggested that an LPS-induced immune stress model could induce structural changes of piglet small intestine and cause changes of intestinal mucosal immune barrier function. In addition, the distribution and quantity of visfatin-positive cells were altered, suggesting visfatin is involved in the humoral immune responses of piglets.

Key words: Visfatin, LPS, small intestinal mucosa, expression changes, tissue

INTRODUCTION

Visfatin is an adipocytokine that promotes the synthesis and accumulation of adipose tissue, regulates lipid and glucose metabolism and is involved in immune and inflammatory responses. Following its discovery, study of the relationship between diabetes and obesity, adipose tissue and inflammation and its role and mechanism of action have increased. It is widely expressed in bone marrow stromal cells, activated lymphocytes, macrophages, liver, uterus, pancreas, muscle tissues, embryolemma and visceral adipose tissue (Ognjanovic and Bryant-Greenwood, 2002; Samal *et al*., 1994).

Currently, visfatin research is mainly focused on acute and chronic inflammatory disorders, obesity and metabolic diseases (Huang *et al*., 2011; Eker *et al*., 2010; Bao *et al*., 2009; Zhu *et al*., 2008; Stephens and Vidal-Puig, 2006). In a preliminary study, researchers found that visfatin is expressed in the immune organs of mice and chickens, visfatin have significant roles in regulating the level of serum IFN-γ and the structure of immune organ. Although, visfatin might be involved in immune responses, its role in the piglet intestinal mucosa immune barrier has not been previously reported. Researchers used an induced stress model *in vivo*, to study the structural changes of the small intestinal mucosa in immune-stressed piglets and the expression level of visfatin protein, to clarify its role in piglet mucosal immune barrier and provide a new direction for the treatment of metabolic and autoimmune diseases.

MATERIALS AND METHODS

Ethics statement: Animal experiment was fully compliant with the guidelines of Hubei Municipality on the Review of Welfare and Ethics of Laboratory Animals approved by the Tenth People's Congress Standing Committee of Hubei Province.

Sample collection and processing: Ten, 3 weeks old healthy white weaned piglets were purchased from a breeding pig farm at Huazhong Agricultural University and were randomly assigned to two groups (n = 5). Piglets received treatment intraperitoneally as follows: saline group, piglets received 0.9% NaCl solution; Lipopolysaccharide (LPS) group, piglets received LPS (O55:B5) (100 μg kg^{-1}). All animals were sacrificed at 6 h while under deep anesthesia using pentobarbital (20 mg kg^{-1} body weight by intravenous injection). Spleen tissue specimens were quickly removed and were fixed with 4% paraformaldehyde and 0.1% phosphate buffer, embedded in paraffin and sectioned to a thickness of 4 μm. Sections were stained with Hematoxylin and Eosin (HE) and by immunohistochemistry. Images were captured by Olympus microscope (Olympus, Philippines).

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The immunohistochemical staining (SABC) was performed after the intact tissue morphology has been determined by HE staining. Following immunohistochemistry, the cytoplasm of positive cells was brownish yellow. Researchers determined the strength of positivity based on the chromogenic staining as follows (Ji *et al*., 2005): '+++' dark brown, strongly positive; '++' medium coloring, brown, positive; '+' light color, light yellow, weakly positive. The staining in control group sections was negative '-'.

Statistical analysis: Results are presented as the mean±SE. To make comparisons between two groups an unpaired Student's t-test was used. The $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Structural changes in the small intestinal mucosa of LPS-induced piglets: After administration of LPS to piglets, the structure of the small intestine was damaged as assessed by microscopic observation. Although, the duodenum, jejunum, ileum and intestinal villi were damaged and the intestinal villi were shortened (Fig. 1a and d), this was not significantly different from the control group ($p>0.05$). The intestinal crypt depth was significantly ($p<0.01$) increased in the propria (Fig. 1a and d). Changes in goblet cell numbers in mucosal epithelium were not obvious but the intraepithelial lymphocytes and lymphoid tissue near the bottom of the mucosal epithelium was reduced (Fig. 1e and f). Aggregated lymphoid nodules were increased in the ileum but cells in the lymph nodes were sparse (Fig. 1g and h). Specific changes of the intestinal villi and intestinal crypts are shown in Table 1.

Table 1: The intestinal mucosal morphology of weanling piglets after LPS challenge

Items	Control group	LPS group	p-values
Villus (height μm^{-1})			
Duodenum	303.16	251.64	0.2300
Jejunum	278.98	221.85	0.0886
Ileum	214.22	221.30	0.8584
Crypt (depth μm^{-1})			
Duodenum	159.04**	281.81**	0.0023
Jejunum	84.03**	213.64**	<0.0001
Ileum	128.32**	264.00**	0.0014

Numbers represent the mean value of villus height or crypt depth. *p<0.05, **p<0.01

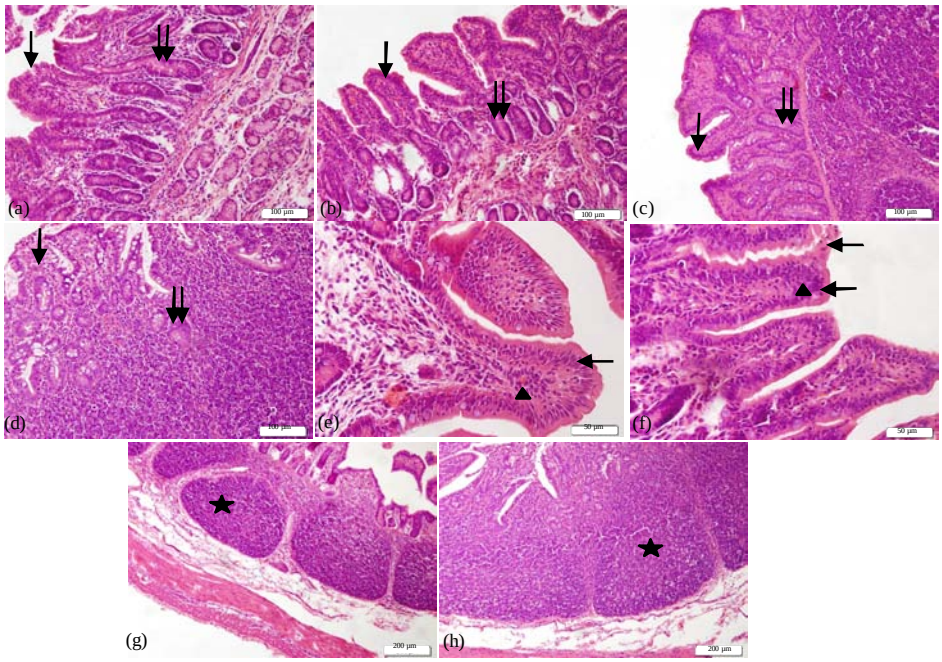

Fig. 1: The changes of histological structure of piglet small intestinal mucosa in LPS group and in control group. a) The duodenum in LPS group; b) in control group; c) the ileum in LPS group; d) in control group showing intestinal villus (single arrow) and intestinal crypt (double arrow); x200. e) The duodenum in LPS group; f) in control group showing lymphocytes between epithelium (arrow) and lymphoid tissue in lamina propria (triangle); x400. g) The ileum in LPS group; h) in control group showing lymphoid aggregate (pentagon); x100 and HE staining

Expression and changes of visfatin-positive cells in LPs-induced piglet intestinal mucosa: Immunohistochemical analysis demonstrated that visfatin-positive cells were round or oval and the color of positive products ranged from yellow to brown with most positive products located in the cytoplasm. Some nuclei had a light yellow color. In the control group, researchers found that visfatin-positive cells were expressed in the mucosa of duodenum, jejunum and ileum. Visfatin-positive cells were mostly expressed in the lamina propria where the immunopositive products were strongly positive (+++) as well as in cells of the small intestine (++). However, visfatin was rarely expressed in intraepithelial lymphocytes. In addition, visfatin-positive cells were also expressed in the duodenal gland and aggregates of lymphoid nodules (Fig. 2h) and were strongly positive. The quantity of positive cells gradually increased from the duodenum, jejunum to the ileum and positive cells were significantly increased in the ileum of the small intestinal glands (Fig. 2b, d and f). After administration of LPS, visfatin-positive cells formed a diverse population and were observed as round, oval, spindle or irregularly shaped. Visfatin-positive cells were reduced in the epithelial lymphocytes, lamina propria and intestinal gland (Fig. 2a, c and e) but were increased in the aggregates lymphoids (Fig. 2g).

The gut is the largest digestion and absorption organ and also functions as a mechanical and immune barrier of the intestinal mucosa, composed of organized lymphoid tissues and scattered lymphocytes in the intestinal wall including intestinal mucosa aggregates of lymphoids, isolated lymphoid nodules, diffuse T and B lymphocytes, plasma cells, mast cells and epithelial lymphocytes (Blikslager *et al*., 2007).

The small intestine is the main site of nutrient absorption and transport, the villi are the main structures that provide absorption and the intestinal glands have a secretory capacity. LPS is a characteristic component of the cell wall of Gram-positive bacteria and is absorbed by the gastrointestinal system into the circulatory system, causing intestinal morphogenesis changes (Wang *et al*., 2010). The good condition of the small intestine is the physiological basis of nutrient digestion and absorption and the normal growth of animals. Villus height and intestinal crypt depth reflects the intestinal condition.

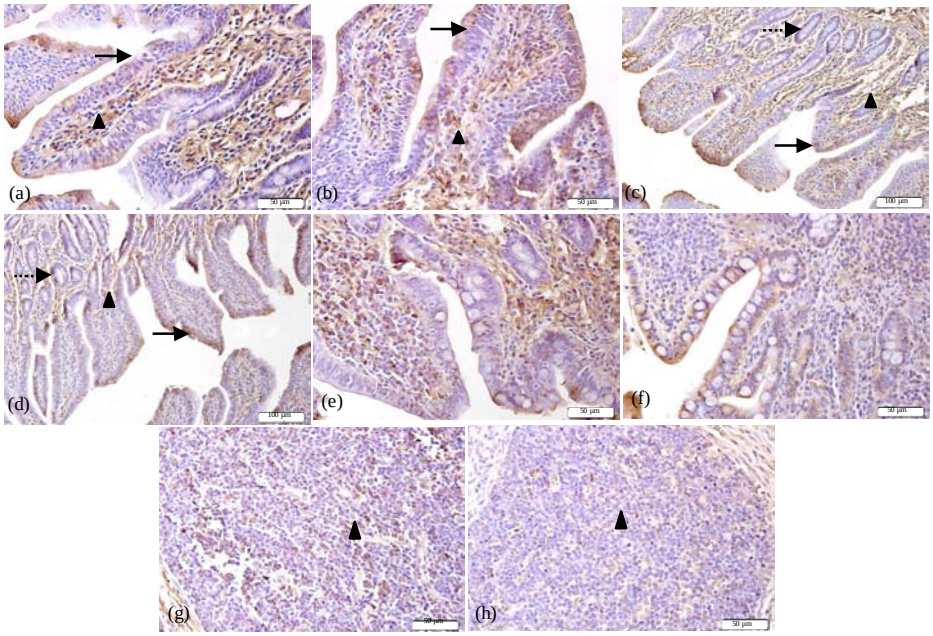

Fig. 2: Visfatin-positive cell distribution in piglet small intestinal mucosa in LPS group and in control group. Immunohistochemical staining of small intestine with anti-visfatin antibody. a) Visfatin-positive cells in the mucosal epithelium of duodenum and lamina propria in LPS group and b) in control group; x400. c) Visfatin-positive cells in the epithelium (solid arrow), lamina propria (triangle) and small intestine gland (dotted arrow) in jejunal mucosa in LPS group and d) in control group; x200. e) Visfatin-positive cells in the epithelium (solid arrow), lamina propria (triangle) and small intestine gland (dotted arrow) in ileum mucosa in LPS group; f) in control group; x400. g) Visfatin positive cells in ileum lymphoid aggregates in LPS group and h) in control group x400

Villus height is positively correlated with the quantity of cells but only mature villus cells are involved in nutrient absorption. Crypt depth may reflect the rate of cell generation and shallow crypts suggest increased maturation rate and enhanced secretory functions (Ke *et al*., 2007). Researchers observed that the intestinal mucosa villi were atrophied and shortened in the duodenum, jejunum and ileum in LPS group. There was an increase in small intestinal crypt depth that promoted small intestinal structural damage and shortened villus, reduced numbers of mature cells, poor nutrient absorption capacity and decreased absorption. Increased intestinal crypt depth indicates a reduction in cells maturation rate and secretion function. In addition, small intestinal epithelium lymphocytes and mucosal epithelial lymphoid tissue were decreased in LPS group which due to inducible Nitric Oxide Synthase (iNOS) overexpressed in the intestine after LPS administration and iNOS can damage cell respiration functions and accelerate the apoptosis of lymphocytes in the small intestine (Hoffman, 2000). Aggregates lymphoid nodes in intestinal mucosa are part of the mucosal immune system which mainly distributed in ileum. The main function is to capture antigens and induce an immune response. In this study, aggregates of lymphoid nodes were increased in the ileum after processing with LPS suggesting that the immune response was enhanced while the structure of lymph nodes was sparse and contained reduced numbers of cells. Thus, immune stress might induce immune suppression leading to the increased apoptosis of lymphocytes.

The intestinal mucosa consists of mucosa epithelium, lamina propria and mucosa muscle layer. The mucosal immune system includes gut-associated lymphoid tissue, cells and molecular components. Gut-associated lymphoid tissue is divided into organizational lymphoid tissues and scattered lymphocytes throughout the entire intestinal wall. It includes Peyer's patches, isolated intestinal mucosa lymphoid nodules, intraepithelial lymphocytes, dispersed lymphocytes in intestinal mucosa, macrophages, plasma cells, mast cells, lysozyme and antibacterial peptides. According to its distribution and position in the intestinal mucosa, visfatin-positive products were mainly distributed in the subepithelial lymphoid tissue of the lamina propria and in intraepithelial lymphocytes. The intestinal lamina propria lymphoid tissue, surface epithelium and intraepithelial lymphocytes form the first line of defense against pathogens, suggesting that visfatin participate in intestinal mucosal immune responses.

Aggregates of lymphoid nodules in the ileum are the main source of B lymphocytes. After LPS administration, the quantity of lymphoid nodules increased. The number of visfatin positive cells was increased and visfatin-positive products were enhanced, suggesting that B lymphocytes are the main source of visfatin protein. After processing with LPS, the expression of visfatin was induced in activated B lymphocytes. According to the variation law of the expression of positive products, visfatin may participate in the initial stages of adaptive humoral immune responses during immune-stressed conditions.

It is a coincidence that after stimulation with antigen, lymphoid tissues changed as the emergence and proliferation of pyronine lymphocytes enhanced the immune responses (Thorbecke *et al*., 1962; Ward *et al*., 1959). Pyronine lymphocytes are lymphocytes or plasma cells stimulated by antigen.

CONCLUSION

In the current research, researchers found that visfatin-positive products were reduced in intestinal mucosal epithelial lymphocytes and the lamina propria lymphoid tissue which may be due to the LPS-mediated inflammatory response increasing apoptosis. Activation by LPS can cause high expression of related cytokines or acute phase reactants and cause apoptosis but the exact mechanism needs further research.

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