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Effects of Epidermal Growth Factor on Glutamine and Glucose Absorption by IPEC-J2 Cells Challenged by Lipopolysaccharide using the Ussing Chamber System

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XT conducted the experiment, analyzed the data, wrote the article and provided the financial support for this study. KX helped in the experimental design and participated in data analyzed.

Key words
Epidermal growth factor, Glucose, Glutamine, IPEC-J2 cells, Ussing chamber

ABSTRACT

The aim of this study was to investigate the effects of epidermal growth factor (EGF) on glutamine and glucose absorption of IPEC-J2 cells using the Ussing chamber system. Cells were treated with: (1) EGF (0 ng / mL) + LPS (0 μg / mL) (Control group), (2) EGF (100 ng / mL) + LPS (0 μg / mL) (EGF group), (3) EGF (0 ng / mL) + LPS (1 μg / mL) (LPS group), and (4) EGF (100 ng / mL) + LPS (1 μg / mL) (EGF+LPS group) for 24 h for ussing chamber assay. The transepithelial electrical resistance (TEER, Ω.cm2), glutamine and glucose absorption rate (%) were measured. The results showed that: (1) EGF significantly ($P < 0.05$) increased the TEER of TPEC-J2 cells induced by LPS; (2) EGF significantly ($P < 0.05$) increased the absorption of glucose and glutamine of IPEC-J2 cells induced by LPS. It can be speculate that EGF repaired the injured cells by reducing cell permeability and increasing the absorption of glutamine and glucose.

INTRODUCTION

Glutamine is one of the most abundant amino acid in blood, which is an important precursor in the biosynthetic process of proteins, nucleic acids, glucose, amino sugars, and the main source of energy for intestinal epithelial cells (Huang *et al*., 2007; Pochini *et al*., 2014). Glucose is the main carbon and energy source of eukaryotic cells, and glucose transport to mammalian cells is the rate-limiting step in glucose utilization (Chaudhry *et al*., 2012). The transport of glucose, glutamine and other nutrients through the intestinal mucosa from the gastrointestinal tract to the epithelial cells is crucial for the growth and development of animals. Epidermal growth factor (EGF), a small mitogenic polypeptide comprising 53 amino acid residues, has been established as a trophic factor for the epithelial cell homeostasis (Tang *et al*., 2016, 2018) and nutrients transport in the small intestine (Huang *et al*., 2007; Trapani *et al*., 2014; Wang *et al*., 2019). Although, previous studies have demonstrated that EGF had a positive effect on the absorption of glutamine (Huang *et al*., 2007) and glucose (Xu *et al*., 2015; Wang *et al*., 2019). However, the studies of the effects of EGF on absorption of glutamine and glucose under stress conditions were rarely reported.

The Ussing chamber system is consists of a circuit system, diffusion chambers, inserts, electrodes and a data collection system (He *et al*., 2013), which provides a simple, but powerful technique for measuring the transport of ions (Muscher *et al*., 2012), drugs (Sjoberg *et al*., 2013) and nutrients (Albin *et al*., 2007) across intestinal epithelial tissues. There are published studies involving in the use of Ussing chambers to evaluate the absorption of glucose in small intestine of animals (Millar *et al*., 2002; Awad *et al*., 2013). However, the studies of the effects of EGF on absorption of glutamine and glucose under stress conditions using Ussing chamber are lacking. Therefore, the aim of the present study was to investigate the effect of EGF on glutamine and glucose absorption of IPEC-J2 cells induced by lipopolysaccharide (LPS), a endotoxin usually used to establish cellular injure model, using the Ussing chamber system.

MATERIALS AND METHODS

Cell culture

Porcine intestinal epithelial cells (IPEC-J2) were kindly provided by Dr. Bie Tan (Institute of Subtropical Agriculture, Chinese Academy of Science, Changsha, China). IPEC-J2 cells were cultured in DMEM / F12

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medium (GE Healthcare life sciences, South Logan, Utah, USA) containing 10% FBS (GIBCO, Carlsbad, CA, USA), 1% antibiotics (Penicillin-Streptomycin) (GIBCO, Carlsbad, CA, USA), and grown in a humidified incubator at 37°C with 5 % CO2 and 95 % air as previously described (Tang *et al*., 2018). After 80% of fusion, cells digested with 0.25% trypsin-EDTA (GIBCO, Carlsbad, CA, USA). Then, the cells (2×10^5 / well) were transferred to Transwell (Coring Inc., Coming, NY, USA), and cultured for 21-24 days until the cells naturally differentiated into a polar monolayer.

Cell treatment

Removed the medium in the culture plate, and washed the cells in Transwell for three times with D-Hanks (Solarbio, Beijing, China). The IPEC-J2 cells were treated with: (1) EGF (0 ng / mL) + LPS (0 μg / mL) (Control group), (2) EGF (100 ng / mL) + LPS (0 μg / mL) (EGF group), (3) EGF (0 ng / mL) + LPS (1 μg / mL) (LPS group), and (4) EGF (100 ng / mL) + LPS (1 μg / mL) (EGF+LPS group) for 24 h. EGF was purchased from Peprotech (Rocky Hill, NJ, USA). LPS was purchased from Sigma-Aldrich (Saint Louis, MO, USA). The EGF and LPS concentration were adopted according to Tang *et al*. (2018).

Ussing chamber assay

After a 24 h of incubation, the membrane in Transwell was took out carefully for Ussing chamber assay (VCC MC6, Physiologic Instruments, Inc., San Diego, CA, USA). The operating procedure of Ussing chamber was conducted as previously described. Briefly, the membrane was mounted between the two halves of Ussing chambers inserts, which the polar side towards to mucosal compartments and the non-polar side towards to serosal compartments. Each compartment filled with 4 mL Hepes-Tris buffer (6 g Hepes, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgSO4, 8.18 g NaC1, adjust the pH to 7.4 using 1 M Tris), circulated with carbogen gas (95% O2, 5% CO2) at a temperature of 37 °C. After an equilibration period of 10 min, 1 mL of 1 mol/L glutamine or glucose solutions was added into mucosal compartment, and 1 mL of 1 mol/L mannitol solution was added into serosal compartment. After 45 min of incubation, 0.5 mL of samples was collected from both compartments for glutamine or glucose concentration analysis. The determination of glutamine concentration was use a Glutamine measurement kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. The determination of glucose concentration was use a Glucose Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. During the experiment, TEER was monitored during the whole experimental period using a computer controlled voltage clamp device (Physiologic Instruments Inc., San Diego, CA, USA). Experiments were performed in six times. The absorption rate of glutamine and glucose was calculated by following equations:

Gln absorption rate (%) = (TGln -SGln -MGln)/TGln

Where Gln, glutamine; TGln, total Gln content; SGln, the Gln content in serosal compartment; and MGln, the Gln content in mucosal compartment.

Glu absorption rate (%) = (TGlu -SGlu -MGlu)/TGlu

Where Glu, glucose; TGlu, total Glu content; SGlu, the Glu content in serosal compartment; and MGlu, the Glu content in mucosal compartment.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Data were performed by one-way ANOVA procedure of SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA). Differences among treatment mean were determined using Duncan's multiple comparison test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Effects of EGF on TEER in IPEC-J2 induced by LPS

The intestinal epithelium is formed by a continuous monolayer of proliferating and differentiating intestinal epithelial cells (IECs), which forms a selective barrier that allows nutrients absorption and defends against toxins, allergens, and pathogens from the gut lumen into mucosal tissue (Tang *et al*., 2016). Disruption of the intestinal epithelial homeostasis has been reported to increase intestinal permeability, which cause numerous gastrointestinal diseases (Yu and Li, 2014). Thus, the normal regeneration and integrity of epithelial cells in the small intestine is important for maintaining intestinal health, and is the structural basis for the absorption of nutrients for animals. Transepithelial electrical resistance (TEER) cans reflect the cells permeability of intestine, which could be used as an important index to evaluate intestinal injury, and has been mainly applied for assessing the permeability of tight junctions or the membrane perturbation by toxicants on intestinal epithelium (Chen *et al*., 2015; Garcia-Hernandez *et al*., 2015). The Ussing chamber model is one of *in vitro* methods to measure TEER (He *et al*., 2013). In the present study, the effect of EGF on TEER in IPEC-J2 induced by LPS was showed in Figure 1. The results showed that, EGF and LPS had significantly ($P < 0.05$) effects on TEER of IPEC-J2 cells. EGF significantly ($P < 0.05$) increased the TEER of cells, and LPS significantly ($P < 0.05$) decreased the TEER of cells compared to other treatments. While, cells treated

with EGF plus LPS significantly ($P < 0.05$) increased the TEER compared to cells treated LPS, and has no difference compared to Control group. It indicated that EGF has a protective effect on IPEC-J2 cells injured by LPS. Previous studies had confirmed the protective effects of EGF acted in intestinal homeostasis, which EGF significantly reduced the LDH release and apoptosis of intestinal epithelial cells (Tang *et al*., 2018), and significantly increased the TEER and decrease epithelial paracellular permeability (Basuroy *et al*., 2006; Flores-Benitez *et al*., 2009; Garcia-Hernandez *et al*., 2015). The present study also confirmed that EGF could increase the TEER of IPEC-J2 cells, which consistent with the results of Tang *et al*. (2018), indicated that EGF plays an important role in maintaining intestinal integrity.

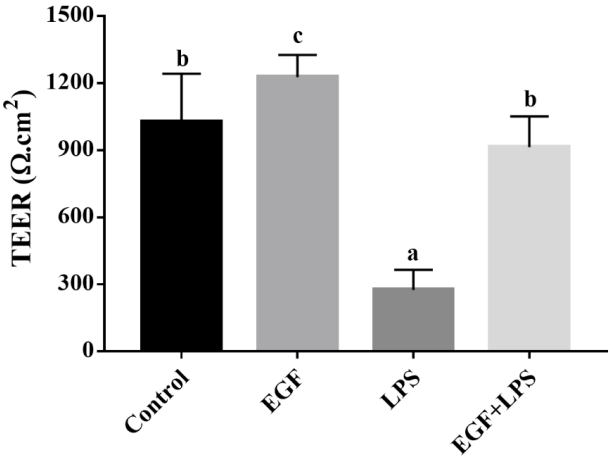

Fig. 1. Effects of EGF on TEER of IPEC-J2 cells induced by LPS. TEER, transepithelial electrical resistance; EGF, epidermal growth factor; LPS, lipopolysaccharide; a, b, c means different lowercase letters indicate significant differences ($P<0.05$).

Effect of EGF on glutamine absorption in IPEC-J2 cells induced by LPS

Glutamine is the main source of energy for intestinal epithelial cells and lymphocytes (Huang *et al*., 2007; Pochini *et al*., 2014). The absorption of glutamine is crucial for the cell survival, proliferation and differentiation (Xing *et al*., 2017; Bernard *et al*., 2018). In the present study, we used the Ussing chamber system to measure the effect of EGF on the absorption of glutamine in IPEC-J2 cells, the results are presented in Figure 2. The results showed EGF had a positive effect on glutamine absorption of IPEC-J2 cells. Cells treated with EGF significantly ($P < 0.05$) promoted glutamine absorption compared to Control group. LPS had a negative effect on glutamine absorption in IPEC-J2 cells. The glutamine absorption rate in the LPS-treated group was significantly ($P < 0.05$) lower than that in the Control group. *In vivo* and *in vitro* studies have shown that EGF can promote the absorption of glutamine in intestinal epithelial cells by increasing Na+-dependent neutral amino acid transporter (ASCT2) expression and ASCT2 transport activity, a broad-spectrum amino acid transporter, in intestinal epithelial cells (Ray *et al*., 2005; Huang *et al*., 2007; Avissar *et al*., 2008). The present study also confirmed that EGF could promote the absorption of glutamine in intestinal epithelial cells. While, LPS, a major integral component of the outer membrane of gram-negative bacteria, which can induce cell injury severely (Talavera *et al*., 2015; Tang *et al*., 2018), made the cell permeability increased, finally resulted a decreased nutrients absorption function. The present study showed that EGF plus LPS group had a higher ($P < 0.05$) glutamine absorption rate than that LPS treated group, although significantly ($P < 0.05$) lower than EGF treated group, but had no difference between the Control group. The results from the present study indirectly indicated that EGF had a function on repairing the damaged cells. This is because, in theory, during the repairing process, organs need more energy to meet the energy needs of cell proliferation and differentiation.

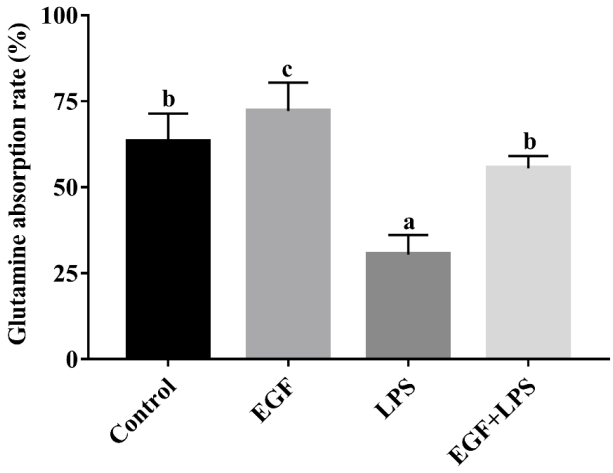

Fig. 2. Effects of EGF on glutamine absorption of IPEC-J2 cells induced by LPS. EGF, epidermal growth factor; LPS, lipopolysaccharide; a, b, c means different lowercase letters indicate significant differences ($P<0.05$).

Effect of EGF on glucose absorption in IPEC-J2 cells induced by LPS

The effect of EGF on glucose absorption in IPEC-J2 cells induced by LPS is presented in Figure 3. The results showed that EGF significantly ($P < 0.05$) increased glucose absorption, and LPS significantly ($P < 0.05$) decreased glucose absorption, EGF plus LPS group had a higher ($P < 0.05$) glucose absorption rate than that LPS treated group, although significantly ($P < 0.05$) lower than EGF treated

group, but had no difference between the control group, which is consistent with glutamine absorption. Glucose is the main carbon and energy source of eukaryotic cells, there were at least two glucose transporters, sodium/glucose cotransporter 1 (SGLT1), and glucose transporter 2 (GLU2), have been shown to be involved in glucose transport (Bedford *et al*., 2015; Xu *et al*., 2015; Huerzeler *et al*., 2020). SGLT1, a high affinity and low transport capacity glucose transporter is mainly mediates glucose transport across the brush border membrane of the small intestine (Wang *et al*., 2015; Bedford *et al*., 2015). GLU2, a low affinity and high transport capacity glucose transporter, can mediate the intracellular glucose transporter to portal vein (Chaudhry *et al*., 2012; Zheng *et al*., 2012). The up-regulated expression of SGLT1 can increase glucose absorption and improve energy level (Song *et al*., 2010). Previous studies have shown that EGF could promote the absorption of intestinal glucose by upregulating the expression of SGLT1 (Cellini *et al*., 2005; Bedford *et al*., 2015; Xu *et al*., 2015). However, the effect of EGF on the expression of GLU2 is controversial. Bedford *et al*. (2015) showed that EGF had no effect on GLUT2 mRNA expression in weaned piglets, while, Xu *et al*. (2015) and Wang *et al*. (2019) showed that EGF could promote the expression of GLUT2 mRNA in weaned piglets. Therefore, though, the present studies have demonstrated EGF can promote the absorption of glucose, it cannot clarify what way EGF to promote the glucose absorption, the further research is needed.

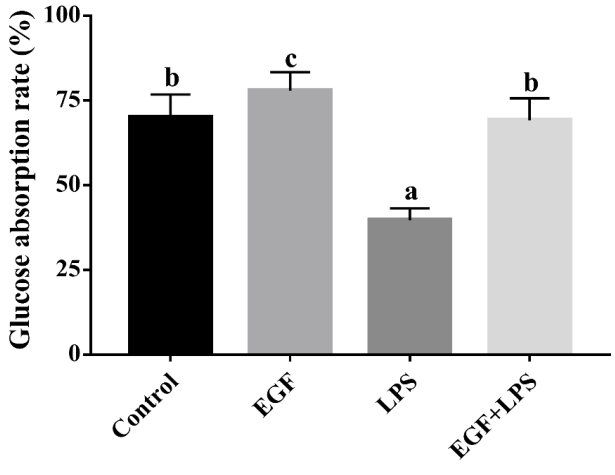

Fig. 3. Effects of EGF on glucose absorption by IPEC-J2 cells induced by LPS. EGF, epidermal growth factor; LPS, lipopolysaccharide; a, b, c means different lowercase letters indicate significant differences (P<0.05).

CONCLUSIONS

In summary, the results of the present study suggested that EGF could enhance the TEER and promote the absorption of glucose and glutamine by IPEC-J2 cells induced by LPS. It can be speculated that EGF repaired injured cells by reducing cell permeability and increasing the absorption of glutamine and glucose.

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Statement conflict of interest statement

The authors have declared no conflict of interest.

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