

Butyrate promotes the recovering of intestinal wound healing through its positive effect on the tight junctions¹

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ABSTRACT: Postweaning diarrhea is one of the most common causes of morbidity and mortality in weanling piglets. Feeding sodium butyrate to weanling piglets decreased the incidence of diarrhea, but the mechanism has not been fully elucidated. The present study was to evaluate the effect of sodium butyrate on diarrhea in relation to wound healing of intestinal barrier using IPEC-J2 cell model. Cultured cells were scratched to induce wound and then were treated with 4 mM sodium butyrate. The results showed that supplementation of the cells with sodium butyrate significantly promoted the process of wound healing, indicating the protective effects of butyrate on the intestinal mucosa. Butyrate treatment enhanced mRNA expression of the intestinal mucosal tight junction proteins occludin and zonula

occludin protein-1 ($P < 0.05$), which suggested that the promotion of wound healing by butyrate is related to the maintenance of the function of the intestinal barrier. In addition, in the butyrate-treated group, intestinal total superoxide dismutase and glutathione peroxidase ($P < 0.05$), two of the main antioxidant enzymes, as well as glutathione ($P < 0.05$), one of the nonenzymatic antioxidant components, were enhanced whereas the malondialdehyde level, a marker of free radical mediated lipid peroxidation injury, was decreased ($P < 0.05$) compared with the control group. Collectively, these results indicate that dietary sodium butyrate might, at least partly, play an important role in recovering the intestinal tight junctions having a positive effect on maintaining the gut integrity.

Key words: butyrate, intestinal barrier, wound healing

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INTRODUCTION

For young animals, weaning is a critical stage because of alterations in the gastrointestinal tract architecture and function, often challenged by postweaning stresses including diarrhea, low feed intake, and body weight loss, and these stresses can adversely affect intestinal health and function (Song et al., 2011). The gastrointestinal tract is a complex and dynamic balanced ecosystem comprising an interaction between the epithelial barrier, immune mediators, and a myriad of microbial species (McCracken and Lorenz, 2001). Newborn infants and young animals generally have not established an appropriate balance in the gastrointestinal tract and therefore often suffer with oxidative stress (McCracken and Lorenz, 2001).

Butyrate is produced by bacterial fermentation of undigested carbohydrates in the intestine of human and animals and recent studies have shown effects on natural immunity by inducing host defense peptides expression (Sunkara et al., 2011). However, to our knowledge, no research has been performed to reveal the mechanism of butyrate on weaning diarrhea and the intestinal barrier. In the present study, using scratched IPEC-J2 cells as an in vitro intestinal wound model, we evaluated the effects of sodium butyrate in controlling the process of wound healing.

MATERIALS AND METHODS

Cell Culture and Treatment

Porcine small intestinal epithelial cells (IPEC-J2, passages 8–14) were cultured at 37°C in a 5% CO₂ incubator. The maintenance cell media was Dulbecco's minimum essential medium, which was supplemented with 10% fetal bovine serum (Invitrogen,

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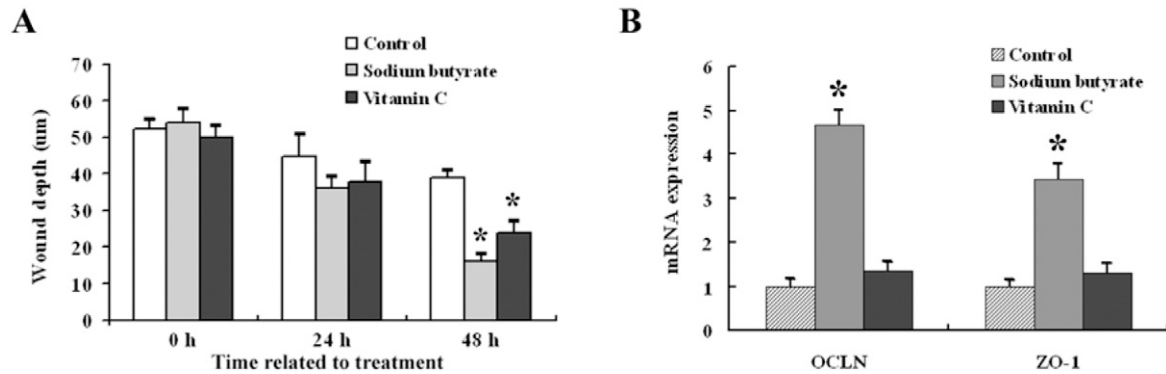


Figure 1. Effects of sodium butyrate on the wound depth and the mRNA expression of mucosal tight junction proteins of IPEC-J2 cells during wound healing. (A) The effect on the wound depth. * $P < 0.05$. (B) Quantitative real-time PCR analysis of occludin (OCLN) and zonula occludens protein-1 (ZO-1). * $P < 0.05$.

Carlsbad, CA). The media was changed 3 times a week according to standard culture protocols.

Before treatment, approximately 1.0×10^5 IPEC-J2 cells per well were seeded into 24-well culture plates. After 36 h, 90 to 95% confluence was achieved. Cells in each well were scratched along the diameter using 10 μ L pipet tips to induce wound. Just after provoking the wound, the wound depth was measured using microscope and served as the baseline of the 0 h. Then cells in different wells were fed with fresh culture medium either unsupplemented (control) or supplemented with 4 mM sodium butyrate (Sigma, St. Louis, MO) for 48 h, with 10 mM vitamin C as a positive control for the antioxidant properties.

Observation of Cell Morphology

The IPEC-J2 cells from different groups were immediately fixed in polyformaldehyde for morphology determination under a regular Olympus microscope. Representative photographs of the cell morphology of the wound healing were collected using Visitron Systems (Puchheim). The wound depth was measured as described by Song et al (2011).

Determination of Antioxidant Indices

The antioxidant index and protein content of the cell samples were determined using assay kits (Nanjing Jiancheng, Nanjing, China) as described by Song et al. (2011). Briefly, cells were lysed in ice-cold sodium-potassium phosphate buffer (0.01 M; pH 7.4). Homogenate was centrifuged at $3000 \times g$ for 10 min at 4°C and the resultant supernatants were analyzed for antioxidant enzyme activity including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione (GSH) as well as malondialdehyde (MDA) using the following methods: SOD activity was measured by the xanthine oxidase method, GSH-Px activity was detected with 5,50-dithiobis-p-nitrobenzoic acid and the change in absorbance at 412 nm was

measured, MDA level was analyzed with 2-thiobarbituric acid and the change in absorbance was read at 532 nm, and GSH can react with 5,5'-dithiobis (nitrobenzoic acid to produce oxidized glutathione and chromophore 5-thio-2-nitrobenzoic acid, which can be detected at 412 nm. Total protein concentration of the supernatants was measured using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL). All absorbance levels were measured using a Synergy4 Multifunction Microplate Reader (Bio-Tek Instruments, Winooski, VT).

Quantitative Real-Time PCR and Statistical Analyses

The IPEC-J2 cells were collected and total RNA was prepared using TRIzol reagent and reverse transcription was performed with the ThermoSCRIPT RT-PCR System (Invitrogen, Carlsbad, CA). Quantitative real-time PCR analysis of occludin (OCLN) and zonula occludens protein-1 (ZO-1) were carried out using the TaqMan Sequence Detection System (Roche, Basel, Switzerland) and the DNA double-strand specific SYBR Green I dye according to the manufacturer's instructions. The gene-specific primers for OCLN and ZO-1 were as follows: forward 5'- CTT ATA GGC CTG ATG AAT -3' and reverse 5'- CAT AGA CAG AAT CCG AAT -3' for OCLN (179 bp) and forward 5'- TAG CTC TGG CAT TAT TCG -3' and reverse 5'- ACA CCC TGC TTA GAA TCC -3' for ZO-1 (156 bp). Results were normalized to 18S rRNA levels. Each experiment was performed in

Table 1. Effects of sodium butyrate on antioxidant indices in the IPEC-J2 cells¹

Item ²	Control	Sodium butyrate	Vitamin C
SOD, units/mg protein	12.8 \pm 0.9 ^a	25.1 \pm 2.9 ^b	28.4 \pm 2.1 ^b
GSH-Px, units/mg protein	27.3 \pm 4.4 ^a	64.9 \pm 6.1 ^b	70.6 \pm 5.5 ^b
GSH, mg/g protein	3.7 \pm 2.2 ^a	11.9 \pm 1.9 ^b	19.5 \pm 2.3 ^c
MDA, nmol/mg protein	5.5 \pm 0.5 ^a	2.0 \pm 0.6 ^b	3.1 \pm 0.3 ^c

^{a,b,c}Values within the same row not sharing common lowercase letters are significantly different ($P < 0.05$).

¹Values are mean \pm standard deviations (n = 6).

²SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; GSH = glutathione; MDA = malondialdehyde.

triplicate with each treatment placed into 3 PCR tubes. All data were analyzed using ANOVA procedure of SAS (Statistical Analysis System, Inc., Cary, NC).

RESULTS AND DISCUSSION

Effects of Sodium Butyrate on IPEC-J2 Cell Morphology

As shown in Figure 1A, there was no significant difference among different groups at 0 h just after provoking the wound ($P > 0.05$) as well as when the cells were treated for 24 h ($P > 0.05$). However, the wound depth was significantly decreased in cell incubated with sodium butyrate compared with the control at 48 h ($P < 0.05$), which suggested sodium butyrate significantly promoted the process of wound healing of IPEC-J2 cells at 48 h. The performance of sodium butyrate treatment was better than vitamin C ($P < 0.05$).

These results provided evidence about the effects of butyrate on intestinal epithelial cells in vitro. Although other studies indicated that dietary supplementation of sodium butyrate can increase animal performance (Sunkara et al., 2011), it is not clear the effect that butyrate has against *Escherichia coli* in the small intestine and therefore in the prevention of diarrhea.

Effect on Expression of Intestinal Mucosal Tight Junction Proteins

To reveal the effects of sodium butyrate supplementation on intestinal mucosal tight junction, the expression of OCLN and ZO-1 was measured at the mRNA level. As shown in Figure 1B, the mRNA level of both OCLN and ZO-1 was much higher in cells incubated with sodium butyrate than untreated cells of the control group ($P < 0.05$) whereas vitamin C had little influence on the expression of OCLN and ZO-1 ($P > 0.05$).

Sodium butyrate significantly increased the expression of OCLN and ZO-1 in the intestinal mucosa epithelial cells at the mRNA level. In combination with previous studies in vivo, these data suggest that sodium butyrate may reduce diarrhea through a reduction in intestinal permeability and increasing the expression of mucosal tight junction proteins in a manner similar to the effects of ZnO on the intestinal mucosa (Song et al., 2011).

Effect on Intestinal Antioxidant Indices

After treatment with sodium butyrate, SOD ($P < 0.05$) and GSH-Px ($P < 0.05$), the two main antioxidant enzymes, as well as GSH ($P < 0.05$), one of the nonenzymatic antioxidant components, increased in the scratched IPEC-J2 cells whereas MDA, a source of free radical mediated lipid peroxidation injury, decreased compared with the control ($P < 0.05$; Table 1). The effects of butyrate on SOD and GSH-Px were similar to those of vitamin C ($P > 0.05$).

The alteration in antioxidant indices by sodium butyrate, including the amount of MDA, GSH, and antioxidative enzymes, suggest an improvement in the level of oxidative stress in the intestinal mucosa cells, which may result in improved wound healing. In contrast, although improving antioxidant indices, vitamin C has little influence on the process of wound healing. All these results suggest that the mechanism by which butyrate promotes wound healing may not be fully due to the antioxidant stress (Song et al., 2011). Our in vitro data suggest that sodium butyrate improves the intestinal tight junction and depresses permeability by improving antioxidant ability, a mechanism distinct from the previous studies (Sunkara et al., 2011).

In summary, the present study demonstrated that treatment with butyrate in the scratched IPEC-J2 cells can significantly promote the process of wound healing accompanied by improved antioxidative stress ability and increased mucosal tight junction proteins. In support of previous studies in vivo (Sunkara et al., 2011), sodium butyrate play an important role in recovering the intestinal tight junctions having a positive effect on maintaining the gut integrity.

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