KEMIN
Technical Literature

1900 Scott Avenue • Des Moines, Iowa, USA 50317 • tel: 515.559.5100 • www.kemin.com

Protective Effects of Butyric Acid and Zinc on Equine Intestinal Cell Function Under Hypoxic Conditions

Abstract

Spheroids were isolated from small intestinal crypts to evaluate the effect of butyric acid and zinc on dye leakage under hypoxic conditions. Spheroids were luminally injected with butyric acid and zinc-FD4 or FD4 only and cultured in normoxic or hypoxic conditions for 17 hours. Images were taken at the start and end of the experiment and corrected total cell fluorescence (CTCF) was calculated to determine FD4 dye loss. Treatment with butyric acid and zinc decreased dye loss in both normoxic (p=0.09) and hypoxic conditions (p=0.039) compared to control spheroids. Results support that supplementing equine intestinal spheroids prior to cellular injury with butyric acid and zinc may benefit the intestinal epithelium in horses undergoing gastrointestinal stressors.

Introduction

Intestinal epithelial cells are essential in absorbing nutrients needed by the host while simultaneously creating a barrier that excludes harmful toxins and pathogens from entering the host. Environmental and disease stressors can play a major role in compromising these functions which can lead to intestinal and systemic inflammatory challenges. Butyric acid[1-3] and zinc[4,5] have been shown to improve intestinal barrier function via tight junction expression, a key component in maintaining intestinal integrity. Previous *in vitro* studies in swine and poultry have demonstrated a protective effect with the combination of butyric acid and zinc on barrier function in heat stress and inflammatory conditions.[6,7]

The current study evaluated the protective effects of butyric acid and zinc on intestinal barrier function under normoxic and hypoxic conditions in primary equine spheroids.

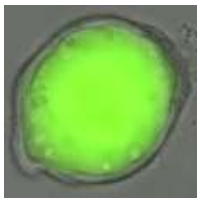
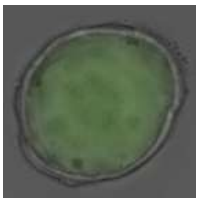
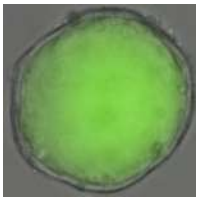
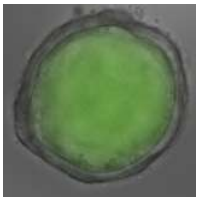
Materials and Methods

Equine spheroids were isolated from small intestinal crypts and cultured in a three-dimensional extracellular matrix (Matrigel® Matrix, Corning Inc., Corning, NY) supplemented with growth factors and media. FITC-dextran 4 kDa (FD4), a fluorescent marker, was used to quantify permeability of the spheroids. Spheroids were luminally injected with butyric acid and zinc-FD4 or FD4 only (3-5 spheroids/ treatment/group). Successful injection criteria included: the entire lumen needed to be filled with dye, no dye could be seen outside of the spheroids and initial exposure time on the microscope needed to be low. After injection, spheroids were placed in normoxic (1% O2) or hypoxic (1% O2) conditions for 17 hours. Fluorescence images were taken at the start of the experiment (0 hour) and 17 hours after and corrected total cell fluorescence (CTCF) was calculated to determine FD4 dye loss. Multiple t-tests were performed with corrections for multiple comparisons using the Holm-Sidak method to compare percentage of dye loss of the treatment (butyric acid and zinc) to control in normoxic and hypoxic conditions.

1900 Scott Avenue • Des Moines, Iowa, USA 50317 • tel: 515.559.5100 • www.kemin.com

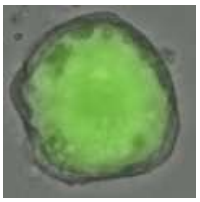
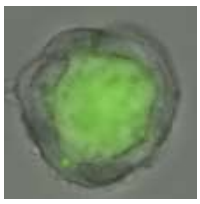
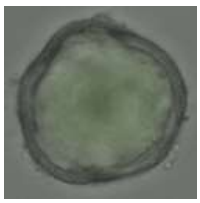
Results

Figure 1 and 2 are representative images of the spheroids in normoxic and hypoxic conditions, respectively. In normoxic conditions, the spheroids injected with butyric acid and zinc showed a reduction (15% difference) in dye loss compared to the control (Figure 3). There was a significant reduction (13% difference) in dye loss with butyric acid and zinc spheroids compared to control in hypoxic conditions.

Treatment	0 hours	17 hours
Control*		
Butyric acid and zinc*		

*Dye loss for control was 70%; butyric acid and zinc was 44%

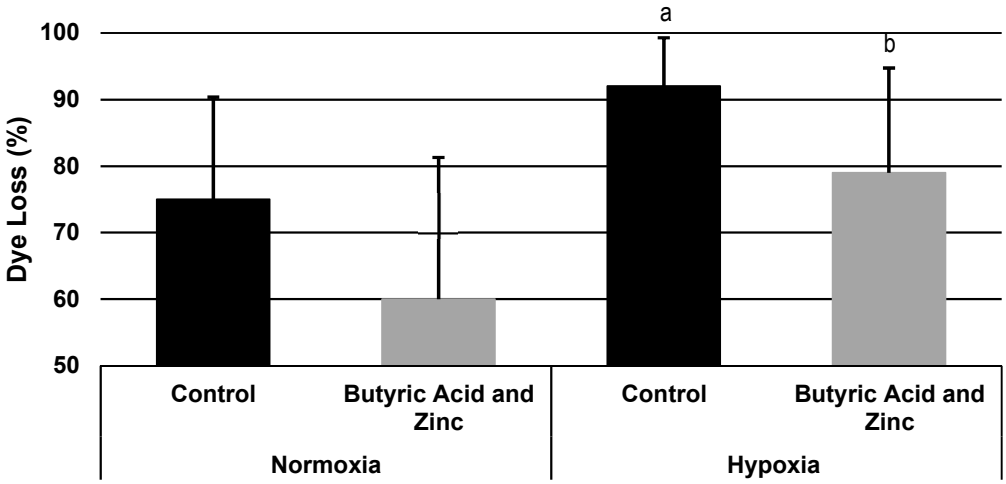
Figure 1. Representation of percent dye loss of spheroids in normoxic conditions.

Treatment	0 hours	17 hours
Control*		
Butyric acid and zinc*		

*Dye loss for control was 92%; butyric acid and zinc was 55%

Figure 2. Representation of percent dye loss of spheroids in hypoxic conditions.

1900 Scott Avenue • Des Moines, Iowa, USA 50317 • tel: 515.559.5100 • www.kemin.com



Differing superscripts indicate statistical significance within culturing conditions P ≤ 0.05.
Error bars represent standard deviation.
Treatments were replicated N=11 control, normoxia; N=10 butyric acid and zinc, normoxia; N=control, hypoxia; N=18 butyric acid and zinc, hypoxia.

Figure 3. Percent dye loss of spheroids in normoxic and hypoxic conditions

Conclusions

Results of this study demonstrate that supplementing equine intestinal spheroids with butyric acid and zinc may have a protective effect on intestinal barrier function under hypoxic conditions. Further research using this novel model will provide an avenue to develop and investigate additional solutions to protect against intestinal injury.

References

1. Peng, L., et al., Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr. 2009. 139(9): p. 1619-25.
2. Ma, X., et al., Butyrate promotes the recovering of intestinal wound healing through its positive effect on the tight junctions. J Anim Sci, 2012. 90 Suppl 4: p. 266-8.
3. Wang, H.B., et al., Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. Dig Dis Sci, 2012. 57(12): p. 3126-35.
4. Hu, C., et al., Diosmectite-zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs. Br J Nutr, 2013. 110(4): p. 681-8.
5. Roselli, M., et al., Zinc Oxide Protects Cultured Enterocytes from the Damage Induced by *Escherichia coli*. J. Nutr., 2003. 133: p. 4077–4082.
6. Vignale, K, Koltes D., Weil J., West S., Weimer S.L., Iseri V. and Christensen K.D 2017. The effect of encapsulated butyric acid and zinc on performance and gut integrity in heat stressed male broiler chickens. 2017 International Poultry Scientific Forum. Atlanta, GA. Abstract: T180, page 53.
7. Mani, V., Rubach, J.K., Koltes, D.A., Gabler, N.K., and Poss, M.J. 2016. The protective effects of ButiPEARL™ Z during heat stress as measured through in vitro studies with swine intestinal epithelial cells and an in vivo swine trial. 2016 Midwest animal science meeting. Journal of Animal Science, Volume 94, Issue suppl. 2, Page 150.
8. Kemin Internal Document, 18-00652.