Effects of Cecal Infusion of Propionate on Colonic Mucosal Gene Expression in Growing Pigs: Post-print

Authors: Chen Huizi, Zhang Yanan, Kaifan Yu, Zhu Weiyun

Date: 2018-12-24T00:00:00+00:00

Abstract

This study aimed to investigate the effects of propionic acid on gene expression in the colonic mucosa of growing pigs through cecal infusion and transcriptome sequencing. Sixteen Duroc × Landrace × Yorkshire castrated male pigs were selected and randomly divided into a control group and a treatment group, with 8 pigs in each group. During the experimental period, pigs in the control and treatment groups received cecal infusion of equal volumes of physiological saline and propionic acid solution prepared with physiological saline, respectively, for a total of 28 days. After the experimental period, the pigs were slaughtered and colonic mucosa was collected for transcriptome sequencing analysis. The results showed that after propionic acid infusion, a total of 121 differentially expressed genes (FC$ $2, P<0.05) were detected in the colonic mucosa of growing pigs, including 78 upregulated genes and 43 downregulated genes. Among them, propionic acid significantly increased the expression of genes related to glucose metabolism and energy metabolism, such as peptide YY (PYY), insulin-like growth factor binding protein 7 (IGFBR7), fructose-bisphosphate aldolase (ALDOB), ATP synthase 5I (ATP5I), NADH dehydrogenase subunit 4L (ND4L), and cytochrome P450 (CYP39A1) (P<0.05), and significantly promoted the expression of immune-related genes such as Occludin-1, somatostatin (SST), and G protein-coupled receptor 5A (GPRC5A) (P<0.05). GO and KEGG pathway analysis revealed that upregulated genes were significantly enriched in GO terms mainly including glycogen compound metabolism, carbohydrate derivative metabolism, etc., and significantly enriched pathways included oxidative phosphorylation, glycosaminoglycan biosynthesis, etc. Significantly enriched GO terms for downregulated genes mainly included cardiac chamber development, stem cell population maintenance, etc., and significantly enriched pathways included taurine and hypotaurine metabolism, folate biosynthesis, etc. In conclusion, cecal infusion of propionic acid altered the expression of genes related to colonic glucose metabolism and energy metabolism, as well as intestinal

barrier and immune function, indicating that propionic acid has a certain regulatory effect on colonic metabolism and host health.

Full Text

Effects of Cecal Infusion of Propionate on Gene Expression in Colon Mucosa of Growing Pigs

CHEN Huizi, ZHANG Yanan, YU Kaifan*, ZHU Weiyun
Laboratory of Gastrointestinal Microbiology, Jiangsu Key Laboratory of Gastrointestinal Nutrition and Animal Health, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

Abstract

This study investigated the effects of cecal infusion of propionate on gene expression in the colon mucosa of growing pigs using RNA sequencing. Sixteen Duroc×Landrace×Large White barrows were randomly divided into a control group and an experimental group (n=8 per group). During the experimental period, pigs in the experimental group received cecal infusions of propionate solution (25 mL, 2 mol/L, pH 5.8) prepared with saline, while control pigs received equivalent volumes of saline alone, twice daily (07:00 and 18:00) for 28 days. On day 29, all pigs were slaughtered and colon mucosa samples were collected for transcriptome sequencing analysis. The results revealed 121 differentially expressed genes (DEGs) in the colon mucosa following propionate infusion (FC$ $2, P<0.05), with 78 genes upregulated and 43 downregulated. Propionate significantly increased expression of genes related to glucose and energy metabolism, including peptide YY (PYY), insulin-like growth factor binding protein 7 (IGFBP7), fructose-bisphosphate aldolase (ALDOB), ATP synthase 5I (ATP5I), nicotinamide adenine dinucleotide dehydrogenase subunit 4L (ND4L), and cytochrome P450 (CYP39A1) (P<0.05). Additionally, propionate significantly enhanced expression of immune-related genes such as Occludin-1, somatostatin (SST), and G protein-coupled receptor 5A (GPRC5A) (P<0.05). GO and KEGG pathway analyses showed that upregulated DEGs were significantly enriched in GO terms including glycosyl compound metabolism and carbohydrate derivative metabolism, and in pathways such as oxidative phosphorylation and glycosaminoglycan biosynthesis. Downregulated DEGs were significantly enriched in GO terms like cardiac chamber development and stem cell population maintenance, and in pathways including taurine and hypotaurine metabolism and folate biosynthesis. In conclusion, cecal infusion of propionate altered the expression of genes associated with glucose metabolism, energy metabolism, intestinal barrier function, and immunity, indicating that propionate plays a regulatory role in colonic metabolism and host health.

Keywords

propionate; pigs; colon; metabolism; gene expression

Introduction

Intestinal inflammation, particularly colonic diseases, has become a major threat to human health in recent years, drawing considerable attention to colonic health [1]. Short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, are produced through anaerobic bacterial fermentation of undigested carbohydrates such as non-starch polysaccharides (NSP), oligosaccharides, and resistant starch in the gut, reaching their highest concentrations in the colon [2]. SCFAs perform multiple physiological functions in the body [3-4], serving as substrates for de novo synthesis of glucose and lipids [5] while also inhibiting pro-inflammatory cytokine production and exerting anti-inflammatory effects [6]. Thus, SCFAs play crucial roles in both host metabolism and intestinal health.

Previous research has primarily focused on the metabolic effects of acetate and butyrate, with relatively fewer studies investigating propionate. However, emerging evidence has confirmed propionate' s regulatory effects on host metabolism. Propionate serves as a precursor for intestinal gluconeogenesis and can activate neural pathways related to intestinal gluconeogenesis in the host [7], thereby improving glucose tolerance and insulin sensitivity [8] while reducing lipid synthesis and serum cholesterol levels [9]. Propionate also promotes secretion of the gastrointestinal hormone peptide YY (PYY) through its receptors, subsequently regulating host appetite and gastrointestinal motility to confer metabolic benefits [10]. Furthermore, propionate can reduce lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNF-α) secretion and decrease expression of immune-related genes such as interleukin-6 (IL-6), thereby improving host immune status [11]. Using growing pigs as a model, this study investigated the effects of cecal propionate infusion on colonic mucosal gene expression through transcriptome sequencing to provide theoretical insights into propionate' s role in colonic metabolism.

Materials and Methods

1.1 Experimental Animals and Design

Sixteen 60-day-old Duroc×Landrace×Large White barrows weighing approximately 25 kg were individually housed with ad libitum access to water and feed. After a 3-day acclimation period, all pigs underwent T-fistula surgery in the cecum. Following a 2-week recovery, the pigs were randomly assigned to two groups (n=8 per group): a control group and an experimental group. During the 28-day experimental period, pigs in the experimental group received cecal infusions of propionate solution (25 mL, 2 mol/L, pH 5.8) prepared with saline, while control pigs received equivalent volumes of saline, administered twice daily

at 07:00 and 18:00. On day 29, all 16 pigs were slaughtered, and colon mucosa samples were immediately collected and snap-frozen in liquid nitrogen for subsequent analysis.

1.2 RNA Extraction and Sequencing

1.2.1 Total RNA Extraction RNase decontamination spray was used to eliminate RNases from equipment surfaces. Total RNA was extracted from colon mucosa samples using the TRIzol method according to the manufacturer' s protocol. RNA concentration and purity were determined using a NanoDrop 2000 spectrophotometer.

1.2.2 Transcriptome Sequencing Extracted total RNA was sent to Beijing Annoroad Gene Technology Co., Ltd. for transcriptome sequencing. After DNase treatment to remove genomic DNA contamination, double-stranded cDNA was synthesized. PCR amplification was performed to enrich target fragments, followed by quality assessment and sequencing. Low-quality reads and reads containing >10% unknown bases were filtered out to obtain clean reads [12].

1.2.3 Screening of Differentially Expressed Genes (DEGs) DEGs were identified from the colon mucosa transcriptome data using the criteria of $P<0.05$, false discovery rate (FDR)<0.01, and fold change (FC)$ $2.

1.2.4 GO and KEGG Pathway Analysis The Omicsbean system was used to perform GO and KEGG pathway analyses of DEGs to understand their biological functions. GO is a functional classification system that uses standardized vocabulary to comprehensively describe gene attributes across species through three primary categories: biological process, cellular component, and molecular function. KEGG pathway analysis identifies enriched metabolic and signal transduction pathways among DEGs to further elucidate their functional roles.

1.2.5 Protein-Protein Interaction (PPI) Analysis PPI analysis was also conducted using the Omicsbean system, which establishes connections among DEGs based on the STRING database and constructs interaction networks according to the biological pathways in which these genes are primarily involved.

1.3 Quantitative Real-Time PCR (qPCR)

Seven DEGs related to glucose metabolism and immunity were selected for qPCR validation: PYY, Occludin-1, IGFBP7, glutathione S-transferase A1 (GSTA1), gamma-glutamyltransferase 1 (GGT1), GPRC5A, and ribosomal protein S28 (RPS28). qPCR was performed using SYBR Green Mix reagents according to the manufacturer' s instructions. The porcine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene served as the internal reference. Primer sequences for each gene are listed in .

Results

2.1 Differentially Expressed Genes

Following propionate infusion, 121 DEGs were identified in the colon mucosa of growing pigs (FC$ $2, P<0.05), comprising 78 upregulated and 43 downregulated genes [Figure 1: see original paper]-A. Functional annotation revealed that 49 upregulated and 30 downregulated genes could be annotated [Figure 1: see original paper]-B. Among these DEGs were several genes involved in glucose metabolism (e.g., IGFBP7, ALDOB, GGT1), energy metabolism (e.g., peroxisome assembly factor LOC100624129, ND4L, CYP39A1), immunity (e.g., Occludin-1, SST, GPRC5A), and the gastrointestinal hormone PYY .

2.2 GO Analysis of DEGs

GO analysis was performed to identify the biological processes in which DEGs were primarily involved. For upregulated genes, 12 GO terms were significantly enriched, including glycosyl compound metabolism, carbohydrate derivative metabolism, carbohydrate metabolism, and generation of precursor metabolites and energy [Figure 2: see original paper]-A. Specifically, 4 DEGs were enriched in glycosyl compound metabolism, 7 in carbohydrate derivative metabolism, and 5 in carbohydrate metabolism. For downregulated genes, 8 GO terms were significantly enriched, including cardiac chamber development, stem cell population maintenance, negative regulation of cell motility, and positive regulation of response to cytokine stimulus [Figure 2: see original paper]-B.

2.3 KEGG Pathway Analysis of DEGs

KEGG pathway analysis revealed that DEGs were significantly enriched in several pathways, including oxidative phosphorylation, glutathione metabolism, metabolic pathways, folate biosynthesis, and glycosaminoglycan biosynthesis. Separate analysis of upregulated and downregulated genes showed that upregulated genes were significantly enriched in 5 pathways, including oxidative phosphorylation, glycosaminoglycan biosynthesis, and glycosphingolipid biosynthesis [Figure 3: see original paper]-A, while downregulated genes were significantly enriched in taurine and hypotaurine metabolism and folate biosynthesis [Figure 3: see original paper]-B.

2.4 PPI Analysis of DEGs

A PPI network was constructed using the Omicsbean system [Figure 4: see original paper]. The metabolic pathway formed the central hub of this network, connecting with proteins including ATP5I, intestinal alkaline phosphatase (ALPI), cysteine dioxygenase 1 (CDO1), GGT1, mitochondrial ND4L (MT-ND4L), and mitochondrial ATP synthase 8 (MT-ATP8). Through these proteins, the metabolic pathway interconnected with glutathione metabolism, oxidative phosphorylation, glycosphingolipid biosynthesis, and glycosaminoglycan biosynthesis pathways, collectively regulating intestinal metabolism.

2.5 Quantitative Real-Time PCR Validation

qPCR was performed to validate the expression levels of selected DEGs in colon mucosa, including six upregulated genes (PYY, Occludin-1, IGFBP7, GSTA1, GPRC5A, RPS28) and one downregulated gene (GGT1) [Figure 5: see original paper]. The results demonstrated that expression trends from qPCR were consistent with those from RNA-seq, confirming the reliability of the transcriptome sequencing data.

Discussion

This study employed transcriptome sequencing to investigate the effects of cecal propionate infusion on gene expression in the colon mucosa of growing pigs. The findings revealed that propionate infusion influenced expression of genes involved in glucose and energy metabolism (e.g., PYY, IGFBP7, ALDOB, LOC100624129, ND4L, CYP39A1) and altered expression of genes related to intestinal barrier function and immunity (e.g., Occludin-1, SST, GPRC5A). GO and KEGG pathway analyses demonstrated that propionate induced significant changes in biological processes and metabolic pathways in colon mucosa, primarily involving glucose metabolism and energy metabolism, with additional enrichment in immune regulation-related pathways. These results suggest that propionate modifies expression levels of glucose metabolism- and immune-related genes in colon mucosa, potentially playing an important regulatory role in colonic and systemic metabolism.

GO terms enriched among DEGs related to glucose and energy metabolism included glycosyl compound metabolism, carbohydrate derivative metabolism, carbohydrate metabolism, glycosaminoglycan biosynthesis, glycosphingolipid biosynthesis, and oxidative phosphorylation. In the glycosyl compound metabolic process, propionate significantly upregulated expression of PYY, IGFBP7, and ALDOB. PYY is involved in numerous digestive processes, enhancing insulin sensitivity, inhibiting gastric acid secretion, reducing gastrointestinal motility, and participating in maintenance of energy balance and glucose homeostasis [4,14-15]. Previous studies have shown that SCFAs stimulate colonic PYY secretion to regulate intestinal motility [10]. IGFBP7, a member of the insulin-like growth factor binding protein family, can bind to the IGF-1 receptor and block its activation by insulin-like growth factors [16]. ALDOB is a crucial enzyme in gluconeogenesis that catalyzes the conversion of glyceraldehyde-3-phosphate to fructose-1,6-bisphosphate. These alterations in gene expression indicate that propionate promotes intestinal glucose production and influences intestinal glucose metabolism and gastrointestinal digestive function.

Furthermore, propionate infusion significantly enriched the oxidative phosphorylation process in colon mucosa, with marked upregulation of ATP5I, LOC100624129, ND4L, and CYP39A1. Oxidative phosphorylation represents a vital pathway for energy acquisition in the body. LOC100624129 is a

peroxisome assembly factor, ND4L is an NADH dehydrogenase subunit, and CYP39A1 is a cytochrome P450 enzyme—all essential components of biological oxidation that oxidize nutrients including carbohydrates, fats, and proteins to generate energy for vital activities [17-18]. These findings suggest that propionate exerts regulatory effects on colonic energy metabolism.

Beyond its effects on glucose and energy metabolism, this study also revealed significant changes in expression of numerous immune-related genes. Previous research has demonstrated that SCFAs regulate colonic immune cell homeostasis and intestinal immune system function [19-21]. The present study found that propionate significantly upregulated expression of Occludin-1, SST, and GPRC5A—genes associated with intestinal barrier function and immunity. Occludin-1 is a critical tight junction protein that maintains cellular permeability and ensures intestinal barrier integrity [22-23]. SST inhibits production and release of inflammatory factors, thereby suppressing inflammation development [24-25]. Additionally, SST has been shown to influence intestinal epithelial tight junctions and ameliorate endotoxin-induced reductions in tight junction protein expression, favoring maintenance of intestinal barrier function [26]. GPRC5A is a G protein-coupled receptor whose knockout activates nuclear factor- B (NF- B) in mouse epithelial cells, promoting inflammation and tumorigenesis [27-28]. GSTA1 catalyzes conjugation of endogenous or exogenous toxic substances with reduced glutathione to form non-toxic derivatives for elimination, thereby exerting detoxification effects that help maintain host immune status [29]. Thus, propionate' s regulation of these genes reveals its role as an SCFA in modulating host intestinal barrier and immune function.

Conclusion

Cecal infusion of propionate in growing pigs altered expression of genes related to colonic glucose metabolism, energy metabolism, intestinal barrier function, and immunity, demonstrating that propionate plays a regulatory role in colonic metabolism and host health.

References

[1] ZHANG G Y, HAMAKER B R. Cereal carbohydrates and colon health[J]. Journal of Food Science & Biotechnology, 2012, 87(4): 331–341.
[2] WONG J M W, DE SOUZA R, KENDALL C W C, et al. Colonic health: fermentation and short chain fatty acids[J]. Journal of Clinical Gastroenterology, 2006, 40(3): 235–243.
[3] DEN BESTEN G, VAN EUNEN K, GROEN A K, et al. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism[J]. Journal of Lipid Research, 2013, 54(9): 2325–2340.
[4] CHAMBERS E S, MORRISON D J, FROST G. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms?[J]. Proceedings of the Nutrition Society, 2015, 74(3): 328–336.

[5] WOLEVER T M, BRIGHENTI F, ROYALL D, et al. Effect of rectal infusion of short chain fatty acids in human subjects[J]. American Journal Gastroenterology, 1989, 84(9): 1027-1033.
[6] 刘小华, 李舒梅, 熊跃玲. 短链脂肪酸对肠道功效及其机制的研究进展 [J]. 肠外与肠内营养, 2012, 19(1): 56-58.
[7] DELAERE F, DUCHAMPT A, MOUNIEN L, et al. The role of sodium-coupled glucose co-transporter satiety effect portal glucose sensing[J]. Molecular Metabolism, 2012, 2(1): 47-53.
[8] DE VADDER F, KOVATCHEVA-DATCHARY P, GONCALVES et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits[J]. Cell, 2014, 156(1/2): 84-96.
[9] TODESCO T, RAO A V, BOSELLO O, et al. Propionate lowers blood glucose and alters lipid metabolism healthy subjects[J]. The American Journal Clinical Nutrition, 1991, 54(5): 860-865.
[10] PSICHAS A, SLEETH M L, MURPHY K G, et al. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents[J]. International Journal of Obesity, 2015, 39(3): 424-429.
[11] DE SILVA A, SALEM V, LONG C J, et al. The gut hormones PYY3-36 and GLP-17-36 amide reduce food intake and modulate brain activity in appetite centers in humans[J]. Cell Metabolism, 2011, 14(5): 700-706.
[12] TEDELIND S, WESTBERG F, KJERRULF M, et al. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease[J]. World Journal of Gastroenterology, 2007, 13(20): 2826-2832.
[13] 李建平, 秦贵信, 赵志辉, 等. 不同脂肪源饲粮育成猪肝脏转录组差异分析 [J]. 动物营养学报, 2015, 27(7): 2128-2139.
[14] SIMPSON K, PARKER J, PLUMER J, et al. CCK, PYY and PP: the control of energy balance[M]//JOOST G. Handbook experimental pharmacology. Berlin: Springer, 2012: 209-230.
[15] DE SILVA A, BLOOM S R. Gut hormones and appetite control: a focus on PYY and GLP-1 as therapeutic targets in obesity[J]. Gut and Liver, 2012, 6(1): 10-20.
[16] EVDOKIMOVA V, TOGNON C E, BENATAR T, et al. IGFBP7 binds to the IGF-1 receptor and blocks activation of insulin-like growth factors[J]. Science Signaling, 2012, 5(255): ra92.
[17] ORTIZ DE MONTELLANO P R. Cytochrome P450: structure, mechanism, and biochemistry[M]. 3rd ed. Berlin: Springer, 2005.
[18] BLANCO A, BLANCO G. Biological oxidations: bioenergetics[M]//BLANCO A, BLANCO G. Medical biochemistry. Amsterdam: Elsevier, 2017.
[19] MEIJER K, DE VOS P, PRIEBE M G. Butyrate and other short-chain fatty acids as modulators of immunity: what relevance for health?[J]. Current Opinion in Clinical Nutrition and Metabolic Care, 2010, 13(6): 715-721.
[20] SMITH P M, HOWITT M R, PANIKOV N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis[J]. Science, 2013, 341(6145): 569-573.

[21] ARPAIA N, CAMPBELL C, FAN X Y, et al. Metabolites produced by commensal bacteria promote peripheral regulatory Tcell generation[J]. Nature, 2013, 504(7480): 451-455.
[22] SUZUKI T. Regulation of intestinal epithelial permeability by tight junctions[J]. Cellular and Molecular Life Sciences, 2013, 70(4): 631-659.
[23] VAN ITALLIE C M, ANDERSON J M. Architecture of tight junctions and principles of molecular composition[J]. Seminars in Cell & Developmental Biology, 2014, 36: 157-165.
[24] TEN BOKUM A M, HOFLAND L J, VAN HAGEN P M. Somatostatin and somatostatin receptors in the immune system: a review[J]. European Cytokine Network, 2000, 11(2): 161-176.
[25] CHOWERS Y, CAHALON L, LAHAV M, et al. Somatostatin through its specific receptor inhibits spontaneous and TNF-alpha- and bacteria-induced IL-8 and IL-1 beta secretion from intestinal epithelial cells[J]. Journal of Immunology, 2000, 165(6): 2955-2961.
[26] 雷姗. 生长抑素对肠上皮细胞屏障功能的保护作用及机制研究 [D]. 博士学位论文. 广州: 南方医科大学, 2014.
[27] BARTA P, VAN PELT C, MEN T Y, et al. Enhancement of lung tumorigenesis in a Gprc5a Knockout mouse with chronic extrinsic airway inflammation[J]. Molecular Cancer, 2012, 11(1): 4.
[28] DENG J, FUJIMOTO J, YE X F, et al. Knockout of the tumor suppressor gene Gprc5a in mice leads to NF- B activation in airway epithelium and promotes lung inflammation and tumorigenesis[J]. Cancer Prevention Research, 2010, 3(4): 424-437.
[29] COLEMAN J, BLAKE-KALFF M, DAVIES E. Detoxification of xenobiotics in plants: chemical modification and vacuolar compartmentation[J]. Trends Plant Science, 1997, 2(4): 144-151.

Note: Figure translations are in progress. See original paper for figures.