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Establishment of a porcine model of indomethacin-induced intestinal injury

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1. ABSTRACT

A useful animal model of intestinal injury is pivotal for studying its pathogenesis and developing nutritional interventions (e.g., amino acid supplementation). Here, we propose the use of indomethacin (IDMT) to induce intestinal inflammation in neonatal pigs. Fourteen-day-old piglets fed a milk replacer diet receive intraperitoneal administration of IDMT (5 mg/kg body weight) for 3 consecutive days. On day 4, blood and intestinal samples are obtained for physiological and biochemical analyses. IDMT increases blood DAO activity, I-FABP concentration, neutrophil and eosinophil numbers; intestinal MMP3 mRNA levels, MPO activity, and MDA concentration; but reduces the plasma concentration of citrulline (synthesized exclusively by enterocytes of the small intestine), intestinal GSH-Px activity, and mRNA levels for *villin*, *I-FABP*, *TRPV6*, *AQP10*, and *KCNJ13*. Moreover, extensive hemorrhagic spots, thinned intestinal wall, and ulcers in the distal jejunum and ileum are observed in IDMT-challenged piglets. Furthermore, IDMT decreases intestinal villus height and villus surface area in the piglet jejunum. Collectively, this work establishes a porcine model of intestinal injury for designing novel nutritional means to improve gut function in pigs and humans.

2. INTRODUCTION

One of the critical problems in pig production is intestinal dysfunction, which could be induced by various stresses, such as weaning and bacterial or viral infections. To date, the underlying mechanisms responsible for stress-induced intestinal dysfunction remain largely unknown. A useful porcine model of intestinal injury is pivotal for studying intestinal dysfunction, but it is not readily available. The most commonly used porcine model of intestinal inflammation is the piglet infected with enterotoxigenic *E.coli* (1,2), but this model required high environmental control and was complex since the intestinal injury was induced by multiple factors (3). To simplify the bacteria-infected model, lipopolysaccharide (LPS)-induced intestinal inflammation and injury was alternatively used in piglets (4,5). However, LPS was reported to have no noticeable effect on the viability or morphology of cultured Caco-2 cells (6), indicating inconsistent effects of LPS on intestinal function. Therefore, it is a challenge to establish a stable and repeatable animal model of intestinal injury.

Indomethacin (IDMT) is a non-steroidal anti-inflammatory drug (NSAID) and is well known to cause severe inflammation and ulceration of the small intestine in the rat via inhibiting prostaglandin synthesis (7). Clinically, the rodent model of IDMT induced intestinal injury was extensively used to study the mechanisms responsible for bowel disease and to develop nutritional interventions (8,9). However, we are not aware of any report of a porcine model of IDMT-induced intestinal

injury. Owing to the overwhelming physiological or nutritional similarities between pigs and humans, the pig provides a uniquely relevant animal model for studying bowel disease (10). Therefore, in the present study, we attempted to establish a porcine model of IDMT-induced intestinal injury, thereby providing a useful tool for studying intestinal dysfunction and developing nutritional interventions in pigs and humans.

3. IDMT INDUCED INTESTINAL INJURY OF PIGLETS

3.1. General study protocol

Sixteen 7-day-old healthy piglets (Durc × Landrace × Yorkshire, 2.9.6 ± 0.2.4 kg) were housed individually in pens that were placed in a temperature-controlled nursery barn (28-30^{o}C). Piglets were fed a milk replacer diet and had free access to drinking water. After a 7-day adaptation, piglets were randomly assigned to one of the 2 groups (8 pigs per group), either the control group (piglets were treated with a vehicle ($NaHCO_3$-ethanol) solution) or the IDMT-challenged group). IDMT was obtained from Sigma Chemicals and dissolved in a $NaHCO_3$-ethanol solution (5% $NaHCO_3$, w/v). On day 1 of the trial, piglets in the IDMT group received intraperitoneal administration of IDMT (5 mg/kg BW once daily) for 3 consecutive days, whereas those in the control group received intraperitoneal administration of the same volume of the $NaHCO_3$-ethanol solution. The dosage of 5 mg/kg BW IDMT was chosen according to the results of a preliminary experiment, in which piglets were administered with 2.5., 5 and 10 mg IDMT/kg BW once daily. On day 4 of the trial, 10% D-xylose (1 mL/kg BW) was orally administered to piglets after their body weights were recorded. At 1 h post administration of D-xylose, blood samples were collected from the anterior vena cava and centrifuged to collect the plasma. All pigs were killed under sodium pentobarbital anesthesia to obtain intestinal tissues (4).

All assay were performed using the previously published methods (3,5,11). Plasma biochemical indexes (TP, BUN, Creatinine, CHOL, TG, TBIL, ALT, and AST) were determined by a Hitachi automatic biochemistry analyzer 7100 with WAKO chemical reagents (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Blood cell indexes (WBC, RBC, PLT, NEUT, LYM, MONO, EOS, and BASO) were determined by a hematology analyzer (Siemens ADVIA 2120i, Germany). Additionally, serum citrulline were determined as described by Xie *et al*. (12). Briefly, serum samples (1 ml each) were mixed well with salicylsulfonic acid (1 ml) and then placed in an ice bath for 15 min. The mixture was centrifuged (10,000 *g*) for 15 min and then he supernatant fluid was transferred to a new tube. After adjusting the pH to 7.0. by the addition of a lithium hydroxide solution, the supernatant fluid was filtered through a 0.2.2 μm membrane, and then analyzed for citrulline by an automatic amino acid analyzer (S433D, Sykam GmbH, Eresing, Germany).

The activity of diamine oxidase (DAO) was determined by using spectrophotometry with a commercially available kit (Nanjing Jiancheng Biological Product, Nanjing, China). D-xylose in the plasma was measured as described by Wang *et al*. (11). Briefly, plasma samples were added to the phloroglucinol color regent solution and then heated for 4 min. Meanwhile, a xylose standard solution was prepared by dissolving D-xylose in saturated benzoic acid to obtain concentrations of 0, 0.7., 1.3., and 2.6. mmol/L. The xylose standard solutions or plasma samples were added to the color reagent solution to determine the absorbance (554 nm) by using a spectrophotometer (Model 6100, Jenway Ltd., UK). In addition, the plasma I-FABP was determined by an enzyme-linked immunosorbent assay kit (Hycult Biotech Inc., PA, USA) according to the manufacturer's guidelines.

To evaluate intestinal damage, the parameters of hemorrhagic spots and ulcers were scored. A score of 0-3 was assigned to the parameters according to the extent and severity of the lesions: 0, no visible change; 1, sporadic hemorrhagic spots (local and mild); 2, extensive and moderate hemorrhagic spots; 3, extensive and severe hemorrhagic spots (6,13). When the pig abdomen was opened from the sternum to the pubis, the intestinal lesions was visually judged according to the scoring method mentioned above.

To determine intestinal histology, paraformaldehyde-fixed intestinal segments (from jejunum and ileum) were dehydrated and embedded in paraffin. Four-μm sections were cut and then stained with hematoxylin and eosin stain. Intestinal histology was determined using a light microscope (Leica, Germany) with Leica Application Suite image analysis software (Leica, Germany). Only vertically oriented villi and crypts were measured (4). Values are expressed as means from ten adjacent villi. Both the villus: crypt ratio and villous surface area were calculated.

Activities of SOD, MPO, and GSH-Px, as well as the levels of MDA and H_2O_2 in plasma and intestinal mucosa were determined using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Assays were performed in triplicate.

The expression of genes in the jejunum and ileum were quantified using real-time RT-PCR (5). For total mRNA extraction, a frozen intestinal sample (~ 100 mg) was powdered and total RNA was isolated using the TRIzol Reagent protocol (Invitrogen, Carlsbad, CA). Total RNA was further quantified using

Table 1. Sequences of the primers used for quantitative real-time PCR analysis

Genes	Forward	Reverse
Villin	TATTATTGGTGTTCGTGCTA	TCTGGAGGAATAGGATACTAA
I-FABP	AGATAGACCGCAATGAGA	TCCTTCTTGTGTAATTATCATCAGT
MMP3	GATGTTGGTTACTTCAGCAC	ATCATTATGTCAGCCTCTCC
pBD-1	ACCGCCTCCTCCTTGTATTC	CACAGGTGCCGATCTGTTTC
KCNJ13	ATGGATGTGTCGCTGGTCTTT	CACAACTGCTTGCCTTTACGAG
TRPV6	AGGAGCTGGTGAGCCTCAAGT	GGGGTCAGTTTGGTTGTTGG
AQP10	TGTCTGCTTTCTGTGCCTCTG	GGATGCCATTGCTCAAGGATAGATAA
IL-8	TTCGATGCCAGTGCATAAATA	CTGTACAACCTTCTGCACCCA
RPL4	GAGAAACCGTCGCCGAAT	GCCCACCAGGAGCAAGTT
HPRT1	AACCTTGCTTTCCTTGGTCA	TCAAGGGCATAGCCTACCAC
GAPDH	CGTCCCTGAGACACGATGGT	CCCGATGCGGCCAAAT

the NanoDrop® ND-1000A UV-VIS spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and its purity was assessed by using 1% denatured agarose gel electrophoresis. Moreover, total RNA was reverse-transcribed using a PrimeScript® RT reagent kit with gDNA Eraser (Takara, Dalian, China) according to the manufacturer's instructions. cDNA was synthesized and stored at -20 °C until use.

To amplify intestinal cDNA fragments, the primer pairs (Table 1) were used for qPCR. The qPCR was performed using the SYBR® Premix Ex Taq™ (Takara, Dalian, China) on an Applied Biosystems 7500 Fast Real-Time PCR System (Foster City, CA). The specificity of the qPCR reactions were assessed by analyzing the melting curves of the products and verifying the size of the amplicons (5). To ensure the sensitivity and accuracy of the results obtained by qPCR, intestinal samples were normalized internally by simultaneously using the average cycle threshold (Ct) of ribosomal protein L4 (RPL4) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (14) as a reference in each sample to avoid any artifacts from variation in the target gene. Results were analyzed by using the $2^{-\Delta\Delta Ct}$ method (15). Each biological sample was run in triplicate.

All the data are expressed as means ± SD. According to the $2^{-\Delta\Delta Ct}$ method, the mean value of intestinal gene expression in the control group was set to 1.00. Differences between treatment means were determined by the Student's unpaired t-test. Possibility values < 0.0.5 were taken to indicate statistical significance.

3.2. Changes in the body weight of piglets challenged with IDMT

In the present study, IDMT administration did not affect the growth performance of the piglets since the final body weight or ADG was similar between the control and IDMT (Table 2). However, Byun *et al*. (16) reported that oral administration of 6 mg/kg IDMT for 2 weeks resulted in body weight reduction in rats. Similarly, Filaretova *et al*. (17) showed that rats exhibited a reduced body weight on day 3 post IDMT injection (35 mg/kg, S.C.). Therefore, the differences in the IDMT dosage, as well as in the duration of treatment and animals, may have contributed to the discrepancy of results among these studies. Of particular note, it seems that the sensitivity of pig intestinal tract to IDMT challenge is lower than that of the rat intestinal tract (18).

3.3. Changes in the hematological indexes of piglets challenged with IDMT

Compared with the control group, IDMT treatment increased (p < 0.0.5) the diamine oxidase (DAO) activity, as well as the levels of blood urea nitrogen (BUN), triglyceride (TG), and intestinal fatty acid-binding protein (I-FABP), and the numbers of neutrophils (NEUT) and eosinophils (EOS), but decreased (p < 0.0.5) the number of lymphocytes (LYM) and citrulline concentration in the plasma of piglets (Table 3). Plasma BUN is an indicator of amino acid utilization and has a negative correlation with ADG and gain/feed ratio (19). IDMT could possibly alter nitrogen utilization and lipid metabolism since the increases in plasma BUN and TG concentrations were observed in piglets treated with IDMT (Table 3). Besides, the increase in plasma BUN may be explained by decreased excretion in kidneys, since IDMT could inhibit PG synthesis and consequently reduce renal blood flow (20). In addition, IDMT can regulate lipoprotein lipase, which catalyzes the hydrolysis of plasma TG (21). The reason for IDMT-induced elevation of plasma TG is unknown, but may be related to its reduced uptake by extra-intestinal tissues. Interestingly, IDMT administration may benefit liver function since IDMT tended to decrease the activities of ALT (P = 0.0.86) and AST (P = 0.0.90), which serve as sensitive indicators of liver damage (22).

Regarding blood cell indexes, IDMT challenge increased the numbers of neutrophils

Table 2. Effects of intraperitoneal administration of indomethacin on the body weights of piglets

Items	Control group	IDMT group	p-value
Initial body weight at 7 days of age (kg)	2.96 ± 0.27	2.96 ± 0.25	0.985
Final body weight at 17 days of age (kg)	3.86 ± 0.43	3.79 ± 0.27	0.700
Average daily gain (g)	86.9 ± 9.78	87.7 ± 14.7	0.908

Data are means ± SD, *n* = 8.

Table 3. Effects of indomethacin on the hematological indexes of piglets

Items	Control group	IDMT group	p-value
Plasma biochemical indexes			
Total protein (g/L)	46.1 ± 3.22	44.4 ± 3.11	0.315
Urea (mmol/L)	0.99 ± 0.[1]52	1.44 ± 0.27[1]	0.001
Creatinine (mmol/L)	66.3 ± 3.73	65.6 ± 8.21	0.862
Cholesterol (mmol/L)	2.63 ± 0.41	2.32 ±0.52	0.203
Triglycerides (mmol/L)	0.43 ± 0.07[2]	0.55 ± 0.11[1]	0.019
Total bilirubin (μmol/L)	6.08 ± 1.37	7.51 ± 1.59	0.074
Alanine transaminase (U/L)	42.7 ± 6.34	36.2 ± 7.71	0.086
Aspartate transaminase (U/L)	44.6 ± 7.87	38.7 ± 4.59	0.090
Blood cells indexes			
White blood cells (10^9/L)	11.4 ± 1.58	11.9 ± 2.14	0.596
Red blood cells (10^{12}/L)	6.29 ± 0.31	6.22 ± 0.24	0.631
Platelets (10^9/L)	396 ± 85.8	390 ± 77.7	0.879
Neutrophils (10^9/L)	3.99 ± 0.64[2]	4.94 ± 0.83[1]	0.023
Lymphocytes (10^9/L)	6.22 ± 0.68[1]	5.11 ± 0.90[2]	0.015
Monocytes (10^9/L)	0.70 ± 0.24	0.72 ± 0.31	0.915
Eosinophils (10^9/L)	0.24 ± 0.03[2]	0.32 ± 0.05[1]	0.001
Basophils (10^9/L)	0.05 ± 0.01	0.05 ± 0.01	0.667
Concentration of D-xylose, I-FABP, and citrulline in the plasma			
D-xylose (mmol/L)	0.75 ± 0.19	0.89 ± 0.16	0.728
I-FABP (pg/mL)	58.9 ± 11.9[2]	123 ± 31.1[1]	< 0.001
Citrulline (nmol/mL)	75.3 ± 16.7[1]	58.9 ± 11.5[2]	0.038
Activity of enzyme in the plasma			
Diamine oxidase (U/L)	1.66 ± 0.42[2]	3.89 ± 0.65[1]	< 0.001

Data are means ± SD, *n* = 8. I-FABP, intestinal fatty acid-bonding protein. [1,2] Means within rows with different superscripts differ ($p < 0.05$)

and eosinophils, but decreased the numbers of lymphocytes (Table 3). Previous studies demonstrated that IDMT readily caused severe acute inflammation and disruption of intestinal barrier function with a consequential increase in the enterobacterial translocation (7,9). Therefore, the IDMT-induced increase in blood immune cell numbers may result from the intestinal inflammation and bacterial translocation. Alternatively, IDMT could induce a direct rapid shape change response in the number of eosinophils but not the number of neutrophils (23).

Plasma DAO serves as a biomarker for the severity of mucosal injury (11). In mammals, DAO is extensively expressed in the epithelium and can shed off into the intestinal lumen under stress, leading to an increase in circulating DAO (24). Similarly, I-FABP is located predominantly in the enterocytes of the small intestine and is released into the blood stream when intestinal ischemic diseases and necrosis occur (11). Therefore, I-FABP in the plasma and the intestine is another useful biomarker for intestinal damage. In the present study, the administration of IDMT increased the plasma DAO activity and I-FABP levels (Table 3), but reduced the I-FABP mRNA levels in the jejunum and ileum of piglets, indicating that IDMT administration induced intestinal injury. This notion was substantiated by the IDMT-induced reduction in blood citrulline, since blood citrulline, which is exclusively synthesized by enterocytes in pigs (25), is also regarded as a valid biomarker of intestinal failure (26,27).

Table 4. The numbers of piglets with and without intestinal lesions (n = 8 per group)

Grade	Grade parameters	Control group	IDMT group
0	No visible change	5 (8)	1 (8)
1	Sporadic hemorrhagic spots (localized and mild)	2 (8)	1 (8)
2	Extensive and moderate hemorrhagic spots	1 (8)	1 (8)
3	Extensive and severe hemorrhagic spots, and ulcers	0 (8)	5 (8)

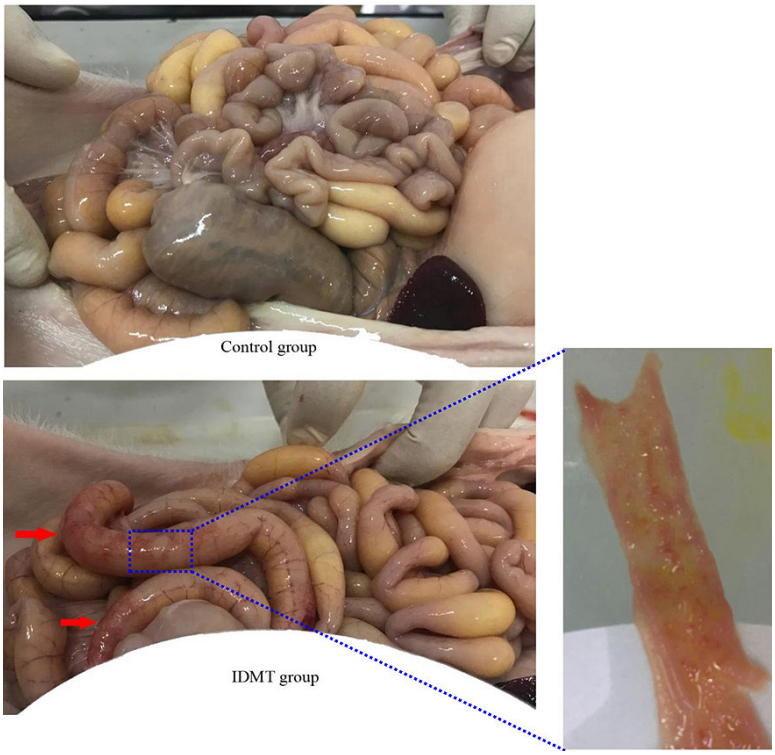

Figure 1. Indomethacin (IDMT) induced intestinal lesions in piglets. There were extensive hemorrhagic spots (red arrows) in the distal jejunum and ileum of piglets in the IDMT group. Of note, thinned intestinal wall and ulcers were observed in these lesion sections when opened longitudinally.

3.4. Intestinal lesions and alterations of intestinal histology in piglets challenged with IDMT

Although 1 of 8 piglets did not show any alterations of intestinal histology, 5 of 8 piglets exhibited severe intestinal lesions and 2 of 8 piglets exhibited moderate intestinal lesions post IDMT administration (Table 4). Therefore, almost all piglets (7/8) exhibited intestinal lesions after IDMT administration duo to the fact that extensive hemorrhages, thinned intestinal walls, and ulcers in the jejunum and ileum of piglets were observed (Figure 1). This was consistent with the previous studies within human patients (9), rats (7), and guinea-pigs (28). Results of the analysis of intestinal morphology indicated that IDMT induced severe degeneration of the epithelium (red arrow) in the villus of jejunum (Figure 2B) and ileum (Figure 2D). The findings that IDMT decreased the jejunal villus height and villus surface area in piglets further supported the notion that IDMT could induce intestinal injury (Table 5). As mentioned above, IDMT can cause severe gut inflammation and ulceration. Specifically, IDMT reduced the production of intestinal endogenous prostaglandins, leading to microcirculatory disturbances, the decrease of intestinal mucus, the disruption of intercellular junctions, and the increase of mucosal permeability (9). Mucosal injury can be attributed to the penetration of bile acid, proteolytic enzymes, enterobacteria, and toxins. Meanwhile, inflammatory cytokines were induced and neutrophil and eosinophil infiltration occured (7,9). Of particular importance, IDMT administration could inhibit inducible NO synthase, and subsequently lead to acute intestinal microvascular injury (29), which may also contribute to intestinal hemorrhaging.

3.5. Redox status in the plasma and intestine challenged with IDMT

Compared with the control group, IDMT challenge increased (p < 0.0.5) plasma hydrogen peroxide (H_2O_2) levels, as well as

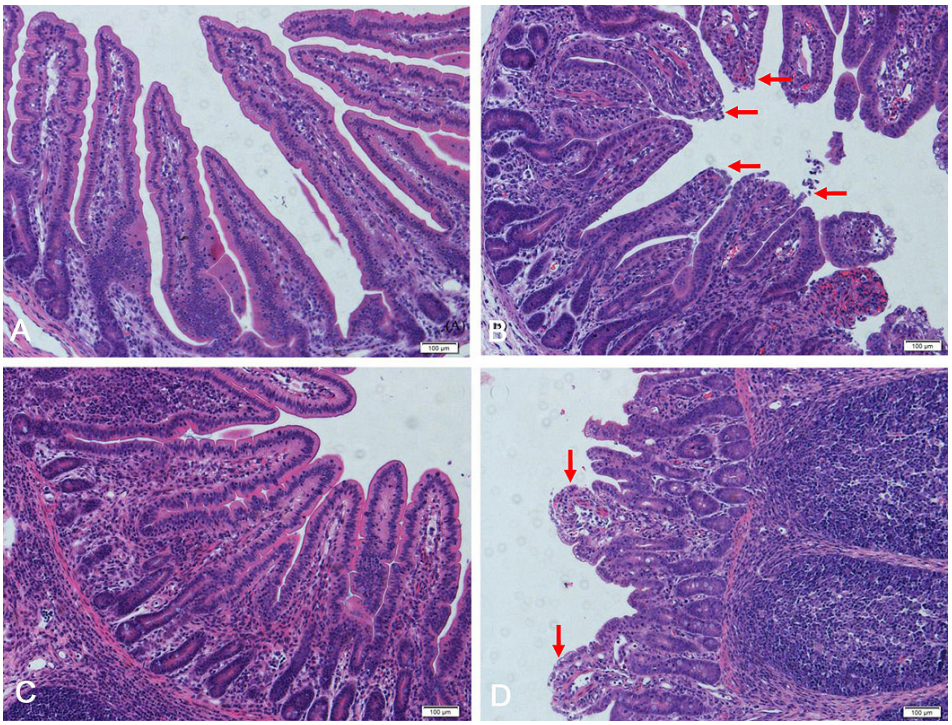

Figure 2. Intestinal histology of piglets in the control and indomethacin (IDMT) groups. Intestinal sections were stained with haematoxylin and eosin (×100). (A) the jejunal histology of piglets in the control group. (B) the jejunal histology of piglets in the IDMT group. (C) the ileal histology of piglets in the control group. (D) the ileal histology of piglets in the IDMT group. No obvious pathological changes were found in the jejunum and ileum of piglets in the control group. However, IDMT induced severe degeneration of the epithelium (red arrow) in the villus of piglet jejunum and ileum.

Table 5. Effects of indomethacin on the intestinal morphology of piglets

Items	Control group	IDMT group	p-value
Jejunum			
Villus height (VH, μm)	304 ± 70.2[1]	195 ± 47.4[2]	0.043
Crypt depth (CD, μm)	95.1 ± 16.2	88.0 ± 12.4	0.498
VH/CD	3.40 ± 0.75	2.44 ± 0.51	0.268
Villus width (μm)	79.4 ± 13.1	70.7 ± 12.9	0.232
Villus surface area (μm^2)	5334 ± 1141[1]	3384 ± 696[2]	0.044
Ileum			
Villus height (VH, μm)	280 ± 60.2	275 ± 45.3	0.444
Crypt depth (CD, μm)	98.6 ±4.41	82.9 ± 20.9	0.110
VH/CD	3.03 ± 0.36	3.01 ± 0.40	0.762
Villus width (μm)	90.8 ± 3.89	85.2 ± 9.04	0.716
Villus surface area (μm^2)	6007 ± 1421	5317 ± 957	0.683

Data are means ± SD, n = 8. VH/CD, villus height/crypt depth. [1,2] Means within rows with different superscripts differ ($p < 0.05$)

jejunal malonaldehyde (MDA) concentration and myeloperoxidase (MPO) activity, but reduced ($p <$ 0.0.5) the activity of jejunal glutathione peroxidase (GSH-Px) in piglets (Table 6). As an NSAID, IDMT could solubilize enterocyte membrane phospholipids and damage the epithelial mitochondria which could result in intercellular calcium efflux and the induction of free radical production. This could cause the peroxidation of the intestine and mucosal oxidative stress (9). In support of this view, we found that the IDMT challenge elevated the jejunal MDA concentration, but reduced jejunal GSH-Px activity (Table 6). Of note, IDMT also increased the jejunal MPO activity. Previous studies demonstrated that oral administration of IDMT caused progressive small intestinal ulceration which was associated with a significant increase in MPO activity in the small intestine (7).

Table 6. Effects of indomethacin on redox status in the plasma and intestine of piglets

Items	Control group	IDMT group	p-value
Plasma			
Superoxide dismutase (U/ml)	86.1 ± 12.2	79.4 ± 12.8	0.735
Glutathione peroxidase (U/ml)	300 ± 71.7	270 ± 66.9	0.330
Myeloperoxidase (U/l)	62.8 ± 11.3	52.0 ± 12.9	0.572
H_2O_2 (nmol/mg protein)	44.5 ± 10.1[2]	67.0 ± 16.5[1]	0.035
Malonaldehyde (nmol/mg protein)	12.0 ± 3.03	11.3 ± 2.92	0.908
Jejunum			
Superoxide dismutase (U/mg protein)	11.4 ± 3.30	12.0 ± 3.60	0.240
Glutathione peroxidase (U/mg protein)	35.7 ± 8.26[1]	20.7 ± 4.97[2]	0.008
Myeloperoxidase (U/mg protein)	0.28 ± 0.06[2]	0.48 ± 0.10[1]	0.016
H_2O_2 (μmol/mg protein)	18.0 ± 4.42	21.1 ± 5.06	0.077
Malonaldehyde (nmol/mg protein)	0.86 ± 0.19[2]	1.56 ± 0.38[1]	0.002

Data are means ± SD, n = 8. [1,2] Means within rows with different superscripts differ ($p < 0.05$

3.6. Variation of gene expression profiles in the intestinal mucosa challenged with IDMT

To clarify the molecular actions of IDMT, we determined the expression of genes associated with mucosal growth, immunity, and nutrient transport by using the qRT-PCR method. The results showed that IDMT administration reduced the mRNA levels for villin, I-FABP, TRPV6, AQP10, and KCNJ13 in the ileum, while increasing the mRNA levels for MMP3, pBD-1, and IL-8 in the jejunum and ileum of piglets (Table 7). Since villin is a marker of villus cell differentiation (11), the down-regulation of villin indicated the inhibition of cell differentiation and enterocyte death induced by IDMT. As previously mentioned, the increase in plasma I-FABP levels and the down-regulation of intestinal I-FABP also indicated there was IDMT-induced intestinal damage. Moreover, MMP3, an important mediator of gut damage, including immune-mediated intestinal injury (30,31), was up-regulated by IDMT, which further confirmed IDMT-induced intestinal injury. Additionally, in the present study, IDMT treatment caused intestinal inflammation since the IL-8 and pBD-1 mRNA levels in the jejunum and ileum were elevated. A previous study revealed that IDMT could induce IL-8 production by enterocytes via the NF-κB signaling pathway (32).

It is noteworthy that IDMT could down-regulate the expression of intestinal nutrient transporters, such as a calcium transporter (TRPV6), a potassium channel (KCNJ13), and a water channel (AQP10) (Table 7). TRPV6 is an epithelial-specific calcium channel and is involved in the intestinal transcellular calcium transport (33), whereas the AQP10 is a water channel and is expressed in the brush-border membrane of the absorptive epithelial cells (34). It was reported that the transcript of AQP10 was absent in the duodenal biopsies of celiac patients. Similarly, results of our previous study indicated that both AQP 3 and AQP10 were down-regulated in the piglet jejunum by lipopolysaccharide challenge (5). Therefore, IDMT not only induced intestinal injury, but also compromised the intestinal capacity of nutrient transport.

4. CONCLUSION AND PERSPECTIVES

In the present study, piglets that received intraperitoneal administration of 5 mg IDMT/kg body weight exhibited increased numbers of circulating neutrophils and eosinophils, as well as extensive hemorrhages and ulcers in the distal jejunum and ileum. IDMT increased the plasma DAO and I-FABP, and intestinal MMP3, MDA and MPO. Additionally, IDMT reduced plasma citrulline concentration, as well as intestinal villus height and villus surface area, GSH-Px activity, and the expression of genes for villin, I-FABP, and several nutrient transporters (Figure 3). Overall, IDMT administration could readily induce intestinal injury in piglets. This useful porcine model of intestinal injury is expected to facilitate the development of new dietary means (e.g., dietary supplementation with amino acids (35-38), prebiotics or probiotics (39-42)) to effectively prevent and treat gut inflammation in animals and humans.

5. ACKNOWLEDGEMENTS

Dan Yi and Wenkai Liu contributed equally to this work and should be considered as co-first authors. The animal use protocol for this research was approved by the Institutional Animal Care and Use Committee at Wuhan Polytechnic University. Our work was jointly supported by National Key R&D Program of China (2016YFD0501210, 2017YFD0500505), the Program of National Agricultural Research Outstanding Talents of China (2015), Hubei Provincial Technology and Innovation Program (2016ABA121, 2017AHB062), Natural Science Foundation of Hubei Province (2016CFA070), the Hubei Hundred Talent Program, Agriculture and Food Research Initiative Competitive

Table 7. Effects of indomethacin on gene expression in the jejunum and ileum of piglets

Items	Control group	IDMT group	p-value
Jejunum			
Villin	1.00 ± 0.24[1]	0.67 ± 0.12[2]	0.003
I-FABP	1.00 ± 0.16[1]	0.71 ± 0.18[2]	0.004
Matrix metalloproteinase-3	1.00 ± 0.25[2]	2.50 ± 0.59[1]	< 0.001
Porcine β-defense 1	1.00 ± 0.21[2]	1.42 ± 0.36[1]	0.015
KCNJ13	1.00 ± 0.25	0.90 ± 0.22	0.397
TRPV6	1.00 ± 0.17[1]	0.73 ± 0.17[2]	0.006
Aquaporin 10	1.00 ± 0.25[1]	0.48 ± 0.11[2]	< 0.001
Interleukin-8	1.00 ± 0.20[2]	4.48 ± 0.35[1]	< 0.001
Ileum			
Villin	1.00 ± 0.20[1]	0.52 ± 0.12[2]	< 0.001
I-FABP	1.00 ± 0.24[1]	0.54 ± 0.14[2]	< 0.001
Matrix metalloproteinase-3	1.00 ± 0.25[2]	3.15 ± 0.44[1]	< 0.001
Porcine β-defense 1	1.00 ± 0.18[2]	1.60 ± 0.32[1]	< 0.001
KCNJ13	1.00 ± 0.21[1]	0.73 ± 0.17[2]	0.014
TRPV6	1.00 ± 0.19[1]	0.62 ± 0.10[2]	< 0.001
Aquaporin 10	1.00 ± 0.20[1]	0.33 ± 0.04[2]	< 0.001
Interleukin-8	1.00 ± 0.23[2]	3.85 ± 0.39[1]	< 0.001

Data are means ± SD, n = 8. I-FABP, intestinal fatty acid-bonding protein; KCNJ13, potassium inwardly-rectifying channel, subfamily J, member 13; TRPV6, transient receptor potential cation channel, subfamily V, member 6. 1,2 Means within rows with different superscripts differ (p < 0.05)

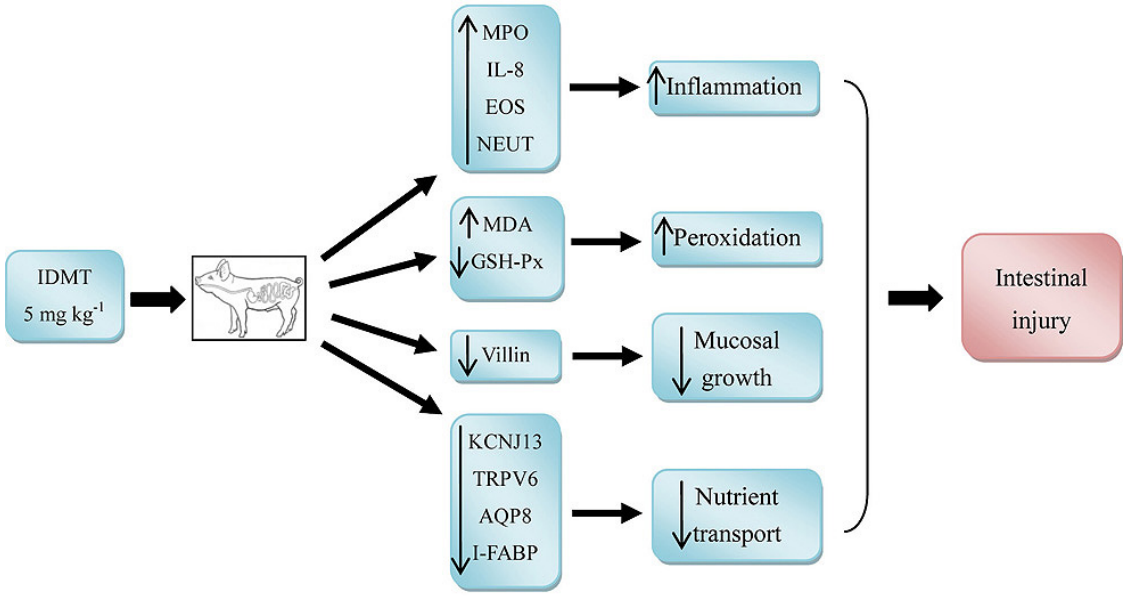

Figure 3. Proposed mechanisms whereby indomethacin induces intestinal injury in piglets. Indomethacin enhances the expression of proinflammatory genes in the small intestine and the levels of eosinophils and neutrophils in the blood. This results in intestinal inflammation, oxidative stress, peroxidation, and damage in piglets, as well as reduced intestinal growth and impaired intestinal transport of nutrients (e.g., water, lipids, calcium, and potassium). AQP8, aquaporin 8; EOS, eosinophils; GSH-Px, glutathione peroxidase; I-FABP, intestinal fatty acid-bonding protein; IL-8, interleukin 8; KCNJ13, potassium inwardly-rectifying channel, subfamily J, member 13; MDA, malonaldehyde; MPO, myeloperoxidase; NEUT, neutrophils; TRPV6, transient receptor potential cation channel, subfamily V, member 6.

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Abbreviations: ADG, average daily gain; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; AQP10, aquaporin 10; BASO, basophils; CHOL, cholesterol; DAO, diamine oxidase; EOS, eosinophils; GSH-Px, glutathione peroxidase; H2O2, hydrogen peroxide; I-FABP, intestinal fatty acid-bonding protein; IL-8, interleukin 8; KCNJ13, potassium inwardly-rectifying channel, subfamily J, member 13; LYM, lymphocytes; MDA, malonaldehyde; MMP3, matrix metalloproteinase-3; MONO, monocytes; MPO, myeloperoxidase; NEUT, neutrophils; pBD-1, porcine β-defense 1; PLT, platelets; RBC, red blood cells; SOD, superoxide dismutase; TBIL, total bilirubin; TG, triglycerides; TP, total protein; TRPV6, transient receptor potential cation channel, subfamily V, member 6; WBC, white blood cells.

Key Words: Hematology, Indomethacin, Inflammation, Intestinal injury, Animal Model, Piglets, Review

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