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Catch-up Growth in Intrauterine Growth-restricted Piglets Associated with the Return of Pancreatic and Intestinal Functions via Porcine Glucagon-like peptide-2 Microspheres

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Abstract

Background

Intrauterine growth restriction (IUGR) results in abnormal morphology and gastrointestinal function. As a gastrointestinal growth factor, the manner by which the porcine glucagon-like peptide-2 (pGLP-2) microsphere administration catches up with the growth of IUGR piglets was investigated.

Methods

Fourteen newborn IUGR piglets were assigned into the IUGR and pGLP-2 microsphere groups. The piglets in the pGLP-2 microsphere group were intraperitoneally administered with 100 mg of pGLP-2 microspheres on day 1 of birth.

Results

From days 15 to 26 of trial, the body weight of the IUGR piglets treated with pGLP-2 microspheres was significantly higher than that in the control group. Importantly, the weaning weight in the pGLP-2 group catches up with the body weight of normal birth weight piglets. IUGR piglets treated with pGLP-2 microspheres significantly showed increased pancreas weight, serum insulin content, and activities of digestive enzymes (lipase, trypsin, chymotrypsin, and amylase). Injection of pGLP-2 microspheres returned the intestinal absorptive capacity by significantly increasing the mRNA expression of sodium-glucose cotransporter 1 in the jejunum, glucose transporter type 2 in the duodenum and jejunum, H + -coupled transporter, and peptide transporter 1 in the jejunum and ileum. It also returned the redox balance by increasing the catalase mRNA expression and decreasing the heat shock protein 70 mRNA expression. In addition, this improvement was associated with the significant increase in gut diameter, length, and weight induced by pGLP-2.

Conclusions

Injection of pGLP-2 microspheres was a suitable therapeutic strategy for compensatory growth in low birth weight IUGR piglet.

Background

Sow litter size is increasing in porcine genetic breeding. High litter results in low uniformity in the litter and a decreased birth weight of the piglets. Increasing litter size by 45% resulted in a reduced mean birth weight of 16%. Concomitantly, the mortality rate of piglets with a birth weight below 1 kg is three to five times higher than that of piglets with a birth weight above 1 kg [1]. The pursuit of high sow productivity has indirectly increased the proportion of porcine intrauterine growth restriction (IUGR).

Pigs exhibit the most severe naturally occurring IUGR among domestic animals. An IUGR piglet is defined as having a birth weight < 1 kg and less than the low quartile of litter birth weights [2]. Most IUGR piglets

die before weaning, and those that survive suffer permanent growth restriction. Fetal growth restriction negatively and permanently affects the postnatal growth, feed utilization efficiency, and meat quality [3]. Meanwhile, the small intestine plays an important role in terminal digestion and absorption of nutrients in postnatal growth of animals. The IUGR alters the intestinal morphology causing long and thin small intestine in piglets [4]. Natural or experimentally induced IUGR is associated with abnormal gastrointestinal morphology [3].

After birth, gut growth and maturation are highly dependent on the enteral food intake and are potentially mediated via co-release of the insulino- and intestino-tropic hormones, i.e., glucagon-like peptides 1 and 2 (GLP-1 and GLP-2), from the mucosal L-cells [5]. The GLP-2 effects are mainly related to the regulation of gastrointestinal growth and function [6], e.g., mucosal growth stimulation, apoptosis inhibition, increased nutrient absorption, inhibition of gastric emptying and gastric acid secretion, intestinal permeability reduction, and intestinal blood flow stimulation. However, the need for frequent injection of porcine glucagon-like peptide-2 (pGLP-2) is the main impediment to its potential use as a therapeutic agent for intestinal dysfunction and damage in piglets because of the rapid pGLP-2 degradation by the enzyme dipeptidyl peptidase-IV (DPP-IV) [7]. For the first time, our team designed and formed pGLP-2 microspheres by using polylactic-co-glycolic acid (PLGA) [8]. One injection of pGLP-2 microspheres ameliorated weaning stress in piglets [9].

We hypothesized that infusion of pGLP-2 microspheres would stimulate the intestinal growth and body weight in newborn IUGR piglets during the suckling period. This phenomenon could prevent the potential mortality and growth restriction and is important for optimizing production efficiency.

Methods

Preparation of pGLP-2 Microspheres

Microspheres were prepared as previously described [8]. Briefly, 0.1 g PLGA and 0.01 g of polyethylene glycol 6000 were dissolved in 1 mL of methylene chloride. In addition, 1 mg of pGLP-2 (HGDGSFSDEMNTVLDNLATRDFINWLLHTKITDSL, > 98%) was dissolved in 25 mL of dimethyl sulfoxide in the organic phase and emulsified using a high-speed homogenizer at 6000 rpm for 1 min to form the solid/oil emulsion. The emulsion was poured into 40 mL 2% (wt/vol) polyvinyl alcohol aqueous phase with dissolved 5% (wt/vol) NaCl and was magnetically stirred at 2000 rpm for 3 min. The whole emulsion was quickly poured into 200 mL H_2O and magnetically stirred for 3 h to allow solvent evaporation and microsphere hardening.

Experimental Protocol

Animal studies were conducted in accordance with the guidelines of the Zhejiang Farm Animal Welfare Council of China and approved by the ethics committee of Zhejiang Academy of Agricultural Sciences. Fourteen newborn IUGR piglets (2 SD below the average birth weight within a litter) from Zhengxing Animal Husbandry Co., Ltd. from seven sows were assigned to two treatments, namely, the IUGR and the

pGLP-2 microsphere groups. Piglets with the same gender and body weight from the same sows are assigned to different groups. In addition, seven piglets from different groups were fostered on the other two sows. From delivery to day 26 postnatal, the piglets were allowed to suckle their dams freely. Piglets from the pGLP-2 group were intraperitoneally administered with 100 mg of pGLP-2 microspheres on day 1. During the experimental period, body weight was monitored at days 0, 8, 15, 21, and 26.

Sample Collection

At day 26 of the trial, 10 mL blood samples were collected from the anterior vena cava and centrifuged at 2,000 g for 10 min at 4 °C to obtain the sera. The sera were then immediately stored at − 80 °C until analysis. Subsequently, all piglets were sacrificed by injection of sodium pentobarbital (50 mg/kg BW) to collect the intestinal and pancreatic samples.

The segment from the pylorus to the Trietz ligament was considered the duodenum. The proximal segment of the rest of the small intestine was considered the jejunum, and a distal segment of 10 cm proximal to the ileocecal junction was considered the ileum. The final part of the lower gastrointestinal tract is the large intestine. The pancreas was in the duodenum loop mesentery. Immediately after opening the abdominal cavity, the length of the duodenum, jejunum, and ileum, the small intestine and the large intestine, and the weight of the pancreas, duodenum, jejunum, ileum, and small intestine were recorded. The intestinal segments (5 cm in length) were opened longitudinally, and the contents were flushed with ice-cold physiological saline solution. Mucosa was collected by scraping using a sterile glass microscope slide. The pancreas and the intestinal mucosa samples for enzyme activities were rapidly frozen in liquid nitrogen and stored at − 80 °C. One centimeter of each intestinal segment was collected for RNA isolation or histology. For RNA isolation, the segments were immediately placed in RNA locker after dissection. For histology, the segments were fixed in 10% (v/v) buffered formalin for 48 h and then embedded in paraffin by using standard techniques.

Blood Sample Analysis

The insulin, insulin-like growth factor-1 (IGF-1), and pGLP-2 contents of the serum were measured using ELISA kits (Beijing Sino-UK Institute of Biological Technology, Beijing, P.R. China) according to the manufacturer’s instructions.

Enzyme Activities

The frozen pancreas and intestinal mucosal samples were powdered in liquid nitrogen and homogenized in ice-cold 0.9% NaCl solution by using a homogenizer (1 g of sample/9 mL of 0.9% NaCl). To obtain the supernatant fluid, the homogenates were centrifuged at 2500 rpm for 15 min at 4 °C. The protein concentrations (mg protein/mL) of the homogenates were determined using a bicinchoninic acid Protein Assay Kit (Pierce Biotechnology, Inc., Rockford, IL, USA) according to the manufacturer’s instructions. Lipase, trypsin, chymotrypsin, and amylase activities in the pancreas and lactase, sucrase, maltase, and alkaline phosphatase (ALP) activities in intestinal mucosa were measured using assay kits (Beijing Sino-UK Institute of Biological Technology, Beijing, P.R. China) according to the manufacturers’ instructions.

Histology

The paraffin-embedded sections were cut at 4 μm thickness and then stained with hematoxylin and eosin (H&E). Images of small intestinal diameter, villus-plus-crypt height, and thickness of the intestinal wall, mucosal layer, submucosal layer, and muscle layer were obtained using an Olympus DP-71 digital camera (Olympus Corporation, Shinjuku, Tokyo, Japan). Approximately 12 longitudinally oriented field of view were measured from each H&E-stained section to make n = 1. All measurements were performed in a blinded manner.

Reverse transcription polymerase chain reaction

Total RNA was extracted according to the instructions of RNAiso Plus TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian. Total RNA was used for first strand cDNA synthesis according to the manual of the PrimeScript RT-reagent Kit from TaKaRa Biotechnology Co., Ltd., Dalian. The primers used are shown in Table 1. Real-time polymerase chain reaction (PCR) was carried out with ABI plus one (Life Technologies, Carlsbad, CA, USA) by using SYBR Premix Ex Taq (TaKaRa Biotechnology [Dalian] Co., Ltd., Dalian). Two-step PCR amplification was performed under the following conditions: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 62 °C for 34 s. Melting curve analysis was performed to verify the single-product generation at the end of the assay. Standard curves were generated based on the data obtained from the standards of the $2–2^{-6}$ dilution series template. The amplification efficiency ranged from 90–110%. Therefore, the relative level of gene expression between the different groups can be calculated using the $2^{-\triangle\triangle Ct}$ method.

Table 1

Primer sequences (5’ to 3’) used for the quantitative polymerase chain reaction

Gene	Forward pride	Reversed prime
ZO-1[1]	GACTTAAAGCTGCCTCAACAGA	GGTTTGTTTCAGGCGAAAGG
Claudin-1	TTCTGGGAGGTGCCCTACTT	TGGATAGGGCCTTGGTGTTG
Occludin	CCATGGCCTACTACTCGTCCAA	CGGCACCGGTGTTGATTTAT
GLP-2R[2]	TGTCCTACGTGTCGGAGATGTC	AATTGGCGCCCACGAA
SOD[3]	GAGACCTGGGCAATGTGACT	CTGCCCAAGTCATCTGGTTT
GPx[4]	GCTCGGTGTATGCCTTCTCT	AGCGACGCTACGTTCTCAAT
Catalase	TGTACCCGCTATTCTGGGGA	TCACACAGGCGTTTCCTCTC
Hsp70[5]	GCCCTGAATCCGCAGAATA	TCCCCACGGTAGGAAACG
SGLT1[6]	CCACTTTCCCTATAAAACCTCAC	CTCCATCAAACTTCCATCCTCAG
GLUT2[7]	CCTGCTTGGTCTATCTGCTGTG	TTGATGCTTCTTCCCTTTCTTT
PEPT1[8]	GATGAAATGTGAGCGTATGGG	AAAGAGGGAGGATCTGGAAAA
IGF-1R[9]	TTCGCCAGATCCTAGGGGAG	TCCCAGCTTTGATGGTCAGG
IGFBP3[10]	CTTCTGCTGGTGCGTGGATA	GATCGTGTCCTTGGCAGTCT
GADPH[11]	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

[1]ZO-1 = zonula occludens-1.

[2]GLP-2R = glucagon-like peptide-2 receptor.

[3]SOD = superoxide dismutase.

[4]GPx = guaiacol peroxidase.

[5]Hsp70 = heat shock protein 70.

[6]SGLT1 = sodium-glucose cotransporter 1.

[7]GLUT2 = glucose transporter type 2.

[8]PEPT1 = peptide transporter 1.

[9]IGF-1R = insulin-like growth factor 1 receptor.

Gene	Forward pride	Reversed prime
[10]IGFBP3 = insulin-like growth factor binding protein 3.		
[11]GADPH = glyceraldehyde-3-phosphate dehydrogenase.		

Statistical Analyses

The effects of pGLP-2 microsphere were analyzed using the independent sample T-test procedure of SAS (SAS Institute Inc., Cary, NC, USA). For all the index, the statistical unit was the pen (n = 7/treatment). Normality of data was assessed using the Shapiro–Wilk test and the hypothesis for a normal distribution of the data was not rejected. Statistical differences were considered to be significant when $p < 0.05$ and trends are discussed when $p < 0.10$.

Results

Body weight

As shown in Table 2, no significant ($p > 0.05$) differences were observed in the body weight of piglets between the non-treated IUGR and pGLP2-treated IUGR groups at D0 and D8. However, compared with the non-treated IUGR group, the body weight of animals treated with pGLP-2 microspheres for 15, 21, and 26 days significantly increased ($p < 0.05$). The weaning weight of piglets in the pGLP2-treated IUGR group catched up to normal level (7.38 kg).

Table 2
Body weight of experimental piglets

Items	IUGR[1] group	pGLP-2[2] group	SEM	p-value
Day 0, g	0.89	0.94	0.03	0.42
Day 8, g	1.73	1.99	0.10	0.21
Day 15, g	2.87^{b}	3.68^{a}	0.20	0.04
Day 21, g	4.67^{b}	5.94^{a}	0.34	0.05
Day 26, g	5.91^{b}	7.38^{a}	0.41	0.07
[1]IUGR = intrauterine growth restriction.				
[2]pGLP-2 = porcine glucagon-like peptide-2.				
[a,b]Value within a row with different superscripts were considered to be significant when $p < 0.05$, and to be trends are discussed when $p < 0.10$ (n = 7/group).				

Pancreas weight, weight and length of the intestine

As shown in Table 3, the piglets in the pGLP2-treated IUGR group showed significantly increased weight of the pancreas ($p < 0.05$), duodenum ($p < 0.1$), jejunum and ileum ($p < 0.05$), and small intestines ($p < 0.05$). The length of the jejunum, ileum, and small intestines also significantly increased ($p < 0.1$).

Table 3
Pancreas weight, weight and length of the intestine in experimental piglets

Items	IUGR[1] group	pGLP-2[2] group	SEM	p-value
Weight, g				
Pancreas	6.38b	7.97a	0.37	0.02
Duodenum	6.33b	7.76a	0.40	0.07
Jejunum and ileum	214.96[b]	269.79[a]	12.99	0.03
Small intestine	221.28[b]	277.55[a]	13.38	0.03
Length, cm				
Duodenum	17.00	18.50	0.84	0.39
Jejunum and ileum	755.14[b]	868.00[a]	31.92	0.07
Small intestine	772.14[b]	886.50[a]	32.30	0.07
Large intestine	143.50	151.00	5.60	0.53

[1]IUGR = intrauterine growth restriction.

[2]pGLP-2 = porcine glucagon-like peptide-2.

[a,b]Value within a row with different superscripts were considered to be significant when $p < 0.05$, and to be trends are discussed when $p < 0.10$ (n = 7/group).

Serum content of insulin, IGF-1, and pGLP-2

As shown in Table 4, piglets in the pGLP2-treated IUGR group showed significant increase in serum insulin content relative to control animals ($p < 0.05$). No significant differences were observed in the serum content of IGF-1 and pGLP-2 between the two groups.

Table 4
Serum content of insulin, insulin-like growth factor-1 (IGF-1), and pGLP-2 in experimental piglets

Items	IUGR[1] group	pGLP-2[2] group	SEM	p-value
Insulin, μIU/mL	14.59[b]	15.95[a]	0.43	0.04
IGF-1, ng/mL	173.39	193.39	15.06	0.48
pGLP-2, ng/mL	6.07	7.50	0.82	0.41

[1]IUGR = intrauterine growth restriction.

[2]pGLP-2 = porcine glucagon-like peptide-2.

[a,b]Value within a row with different superscripts were considered to be significant when $p < 0.05$, and to be trends are discussed when $p < 0.10$ (n = 7/group).

Pancreatic enzyme activity

Piglets in the pGLP2-treated IUGR group showed a significant increase in lipase ($p < 0.05$), trypsin ($p < 0.1$), chymotrypsin ($p < 0.1$), and amylase ($p < 0.001$) contents in the pancreas, as shown in Table 5.

Table 5
Pancreatic lipase, trypsin, chymotrypsin, and amylase activities in experimental piglets

Items	IUGR[1] group	pGLP-2[2] group	SEM	p-value
Lipase, U/mg	13.59[b]	17.43[a]	1.02	0.05
Trypsin, U/mg	18.95[b]	22.36[a]	0.98	0.08
Chymotrypsin, U/mg	26.68[b]	38.95[a]	3.84	0.10
Amylase, U/g	4532.57[b]	5178.86[a]	107.45	0.0001

[1]IUGR = intrauterine growth restriction.

[2]pGLP-2 = porcine glucagon-like peptide-2.

[a,b]Value within a row with different superscripts were considered to be significant when $p < 0.05$, and to be trends are discussed when $p < 0.10$ (n = 7/group).

Small intestinal morphology

As shown in Table 6, one injection of pGLP-2 microspheres significantly ($p < 0.05$) increased the diameter of the duodenum and jejunum and the mucosal layer thickness in the jejunum ($p < 0.1$) compared with the non-treated IUGR group. No significant ($p > 0.05$) differences were observed in other small intestinal morphologies between the two groups.

Table 6
Small intestinal morphology in experimental piglets

Items	IUGR[1] group	pGLP-2[2] group	SEM	p-value
Duodenum				
Diameter, μm	6380.40[b]	7397.38[a]	227.25	0.02
Villus height, μm	358.37	353.49	17.40	0.89
Crypt depth, μm	340.72	296.32	14.53	0.13
Villus height crypt depth ratio	1.08	1.32	0.07	0.14
Intestinal wall thickness, μm	997.36	1090.06	37.12	0.23
Mucosal layer thickness, μm	620.43	655.04	15.34	0.28
Submucosal thickness, μm	71.78	71.29	3.91	0.95
Muscle thickness, μm	259.95	279.09	16.48	0.58
Jejunum				
Diameter, μm	6290.93[b]	7155.78[a]	208.14	0.03
Villus height, μm	337.90	346.98	11.08	0.69
Crypt depth, μm	258.84	248.85	11.79	0.69
Villus height crypt depth ratio	1.39	1.44	0.08	0.79
Intestinal wall thickness, μm	914.03	955.14	44.16	0.66
Mucosal layer thickness, μm	549.92[b]	651.35[a]	29.68	0.08
Submucosal thickness, μm	52.55	47.18	5.51	0.65
Muscle thickness, μm	228.67	225.56	11.34	0.89
Ileum				
Diameter, μm	8476.24	8804.63	154.81	0.31
Villus height, μm	215.98	248.25	13.12	0.23
Crypt depth, μm	127.96	142.98	4.32	0.16

[1]IUGR = intrauterine growth restriction.

[2]pGLP-2 = porcine glucagon-like peptide-2.

[a,b]Value within a row with different superscripts were considered to be significant when $p < 0.05$, and to be trends are discussed when $p < 0.10$ (n = 7/group).

Items	IUGR[1] group	pGLP-2[2] group	SEM	p-value
Villus height crypt depth ratio	1.50	1.78	0.10	0.18
Intestinal wall thickness, μm	841.04	879.16	43.49	0.68
Mucosal layer thickness, μm	374.22	426.98	21.43	0.23
Submucosal thickness, μm	66.24	72.65	5.58	0.59
Muscle thickness, μm	269.47	316.52	27.43	0.41
[1]IUGR = intrauterine growth restriction.				
[2]pGLP-2 = porcine glucagon-like peptide-2.				
[a,b]Value within a row with different superscripts were considered to be significant when $p < 0.05$, and to be trends are discussed when $p < 0.10$ (n = 7/group).				

Activity of disaccharides and ALP

Piglets in the pGLP2-treated IUGR group showed significantly increased lactase ($p < 0.05$) and sucrose ($p < 0.1$) activities in the ileum compared with the non-treated IUGR group, as shown in Table 7. No significant differences ($p > 0.05$) were observed in the activity of all disaccharidases and ALP in the jejunum, maltase, and ALP in the ileum.

Table 7
Activities of disaccharides (maltase, lactase, and sucrase) and alkaline phosphatase (ALP) in experimental piglets

Items	IUGR[1] group	pGLP-2[2] group	SEM	p-value
Jejunum				
Maltase, U/mg	19.91	19.69	1.19	0.93
Lactase, U/mg	27.14	28.82	0.98	0.41
Sucrase, U/mg	19.90	21.93	1.20	0.42
ALP, U/g	706.08	787.50	80.74	0.63
Ileum				
Maltase, U/mg	28.30	28.38	1.49	0.93
Lactase, U/mg	26.01b	31.90a	0.42	0.04
Sucrase, U/mg	26.26b	29.36a	0.96	0.10
ALP, U/g	458.51	549.73	69.88	0.54

[1]IUGR = intrauterine growth restriction.

[2]pGLP-2 = porcine glucagon-like peptide-2.

[a,b]Value within a row with different superscripts were considered to be significant when $p < 0.05$, and to be trends are discussed when $p < 0.10$ (n = 7/group).

Gene expression of tight junction (TJ), redox, growth factor, and transport

The detected TJ protein genes include ZO-1, Claudin-1, and Occludin (Fig. 1A). Piglets in the pGLP2-treated IUGR group showed significantly increased Occludin gene expression ($p < 0.05$) in the jejunum compared with the non-treated IUGR group.

Redox-related genes include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and Hsp70 (Fig. 1B). Compared with the non-treated IUGR group, piglets in the pGLP2-treated IUGR group showed a significant increase of CAT gene expression in the duodenum ($p < 0.05$), jejunum ($p < 0.001$), and ileum ($p < 0.1$). In addition, the GPx gene expression in the ileum was significantly increased ($p < 0.05$). However, the Hsp70 gene expression in the duodenum ($p < 0.001$) and ileum ($p < 0.05$) significantly decreased.

The detected transport-related genes include SGLT1, GLUT2, and PEPT1 (Fig. 1C). Compared with the non-treated IUGR group, piglets in the pGLP2-treated IUGR group showed a significant increase of the

SGLT1 gene expression in the jejunum ($p < 0.01$), GLUT2 gene expression in the duodenum ($p < 0.1$) and jejunum ($p < 0.1$), and PEPT1 gene expression in the jejunum ($p < 0.01$) and ileum ($p < 0.01$). However, a significant decrease of the GLUT2 gene expression in the ileum was observed ($p < 0.01$).

The detected growth factor-related genes include glucagon-like peptide-2 receptor (GLP-2R), insulin-like growth factor 1 receptor(IGF-1R), and insulin-like growth factor binding protein 3 (IGFBP3) (Fig. 1D). Compared with the non-treated IUGR group, piglets in the pGLP2-treated IUGR group showed a significant decrease of the GLP-2R expression in the duodenum ($p < 0.1$) and ileum ($p < 0.001$) and IGF-1R expression in the duodenum ($p < 0.01$) and a significant increase of the IGFBP3 expression in the jejunum ($p < 0.01$).

Discussion

This study aimed to determine whether or how pGLP-2 microsphere administration to newborn IUGR piglets would compensate growth.

Administration of pGLP-2 microspheres achieved catch-up growth in IUGR piglets

Low birth weight (LBW) IUGR piglets are at high risk of postnatal morbidity and mortality and long-term compromised growth performance [3]. Poore and Fowden [10] found that in the first month of life, the LBW piglets undergo a period of catch-up growth. D'Inca et al. [4] also demonstrated that the IUGR and normal birth weight (NBW) piglets had similar relative growth rates during the first 5 days of life. Liu et al. [11] indicated that the compensatory growth of IUGR piglets is the result of an adequate nutrient intake level. Whether this kind of catch-up in BW would increase the risk of metabolic disorders in the whole growth period because of the mismatch of pre- and postnatal nutrition remains unclear. However, most studies reported that the birth weight was positively correlated with the average daily gain at suckling. Wiyaporn et al. [12] found that the LBW piglets did not catch up with NBW piglets during their early suckling period despite the similar growth rate. In this study, IUGR piglets from different groups are raised in the same litter sow with equal nutrient intake level. From days 15 to 26, the BWs of animals treated with pGLP-2 microspheres were significantly higher than those of piglets in the control IUGR group. Importantly, the weaning weight in day 26 of piglets in the pGLP-2 group catched up to the BW of the normal birth weight group, as revealed by Liu et al. [11]. This result implied that catch-up in the BW of IUGR piglets could be achieved through administration of pGLP-2 microspheres.

pGLP-2 microspheres improve the pancreatic secretion functions of IUGR piglets

The pancreas is an organ with both internal and external secretion functions. The pancreas is important for the digestion of nutrients and the regulation of blood sugar. IUGR can significantly reduce the pancreas weight and impede the pancreas development in piglets [13, 14]. This phenomenon may be related to some factors, such as the fetal restriction in the mother's womb. Fetal malnutrition may induce

physiological and/or metabolic adaptations to ensure nutrient supply to the most vital organs (such as the brain) at the expense of other organs (e.g., pancreas) [15]. Boehm et al. [16] demonstrated significantly decreased the lipase activity in a duodenal juice aspirate of IUGR infants. Pancreatic amylase and lipase activities in IUGR piglets were also significantly decreased [13].

Insulin and IGF-1 are the major driving factors of fetal growth. The absence of GH in the fetal pig elevates insulin concentration in the blood [17]. The blood insulin level of IUGR rats or piglets was significantly lower compared with that of the animals of normal weight [14]. This condition may be due to both decreased insulin production by the reduced number of pancreatic islet cells and the decreased concentrations of growth hormone [18]. Mickiewicz et al. [19] demonstrated that plasma GLP-2 in IUGR pigs tended to be low after birth but increased to values higher than those of the non-IUGR control levels during the postnatal days 28 and 188. This phenomenon presumably reflected a period of catch-up growth. The low plasma insulin and GLP-2 concentration in IUGR neonates may result in delayed maturation of intestinal mucosa in IUGR intestines. In this study, IUGR piglets treated with pGLP-2 microspheres significantly increased the pancreas weight, serum insulin content, and activities of digestive enzymes (lipase, trypsin, chymotrypsin, and amylase) in the pancreas.

IGF-1 deficiency in the gestational state may be one of the major causes of fetal growth restriction [20]. IGF-1 initiates signaling pathways by binding to the IGF-1R, and is involved in numerous biological events, such as cell proliferation, migration, and inhibition of apoptosis [21]. IGFBPs are a family of proteins that bind with high affinity to IGFs, and the binding of IGF to IGFBP inhibits the access of IGF to IGF-IR. This phenomenon results in the inhibition of IGF-stimulated signaling pathways. IGF-1 was significantly high in IUGR infants with catch-up growth with respect to IUGR infants without catch-up growth, thereby indicating its importance in early catch-up growth of IUGR babies [22]. A good correlation was also found between the birth weight and IGF-1 and IGFBP-3 levels by Özkan et al. [22]. In this study, IGF-1 serum content increased, IGF-1R expression significantly decreased in the duodenum, and IGFBP3 expression increased in the jejunum in the pGLP-2 group. These results indicated the activation of IGF-1 signaling pathways.

pGLP-2 microspheres returned the intestinal absorptive capacity and redox balance

IUGR is associated with abnormal gastrointestinal morphology and function. This phenomenon may contribute to the permanent effects of IUGR on the later growth and development of animals. As a gastrointestinal growth factor, the therapeutic effect of pGLP-2 on intestinal dysfunction and damage in weaning and diarrheal diseases of piglets has been demonstrated [6, 9]. Enteral nutrient intake can increase the plasma GLP-2 concentration [11, 23] and facilitate the intestinal development of newborn piglets through improved intestinal cell survival and proliferation [24]. To further confirm the therapeutic effect of GLP-2 on the gut, exogenous pGLP-2 microspheres were administered to the IUGR piglets during the suckling periods to examine its role in intestinal development. The injection of pGLP-2 microspheres

improved the intestinal absorptive capacity and redox balance. This improvement was associated with improved gut diameter, length, and weight induced by GLP-2.

The main nutrient transporters mediating the intestinal uptake of glucose in newborn piglets are SGLT1 and GLUT2. The gene expressions of SGLT1 and GLUT2 and active glucose transport of the jejunum increased in the IUGR piglets injected with GLP-2 [13]. Epidermal growth factor infusion significantly increased the small intestinal SGLT-1 protein expression in IUGR fetuses vs. controls but did not affect the SGLT-1 mRNA expression [25]. H⁺-coupled transporter, peptide transporter 1 (PEPT1) is responsible for the uptake of dietary dipeptides and tripeptides in the intestines; in addition, peptide transport in the small intestines is important during the first week of suckling [26]. As measured by gene expression, the SGLT1 abundance in the jejunum, GLUT2 in the duodenum and jejunum, and PEPT1 in the jejunum and ileum significantly increased in IUGR piglets via the pGLP-2 microsphere administration. The increase in the above nutrient transport protein of the GLP-2 injection group indicates the absorptive capacity improvement for IUGR piglets in a normal lactation status.

Luo et al. [27] found that IUGR piglet was associated with an increase in placental reactive oxygen species (ROS) and oxidative injury. To eliminate the harmful effects of ROS, cells are equipped with an efficient antioxidant defense system that includes enzymes, such as SOD and CAT [28]. SOD catalyzes the reduction of the superoxide anion (O^{2-}) to hydrogen peroxide (H_2O_2). In a subsequent step, CAT stimulates the H_2O_2 degradation to molecular oxygen and water [29]. The mRNA levels of CAT genes coding for antioxidant molecules were significantly lower in the IUGR group than in the controls [30]. pGLP-2 microsphere administration increased the CAT mRNA expression in the duodenum, jejunum, and ileum of IUGR piglets. This phenomenon implied that the antioxidant defense system has been activated with the pGLP-2 microsphere administration in IUGR piglets.

Hsp70 prevents and repairs damages induced in intestinal epithelial cells and organs by various stresses. Zhong et al. [31] found that the mRNA and protein expressions of Hsp70 in IUGR piglets increased in the proximal and distal jejunum and colon. The Hsp70 expression in the intestinal piglets was upregulated by IUGR, and different intestinal sites had different responses to stress. IUGR suffers from inadequate nutrient supply in the fetus, which may be one of the stimuli for high Hsp70 expression. The increased Hsp70 expression in the intestinal mucosa of IUGR piglets may be used as a marker of intestinal damage induced by IUGR. The IUGR piglets treated with pGLP-2 microspheres significantly decreased the Hsp70 expression in the duodenum and ileum. This phenomenon implied that pGLP-2 microsphere administration repaired the intestinal stress damage.

The villus height and villi/crypt ratio in the jejunum, ileum, and colon are significantly reduced compared with those of piglets with normal body weight [4, 19] in IUGR neonates at approximately 1 week after birth. Liu et al. [11] demonstrated significantly increased the villous height of IUGR piglets injected with GLP-2 compared with the control group under the condition of restricted nutrient intake. However, in this study, no significant difference was observed in the villus height and villi/crypt ratio of the small intestines between the piglets from the two groups. IUGR piglets treated with pGLP-2 microspheres

significantly increased the weight and length of the small intestines. This phenomenon may be related to the increase in the diameter of the duodenum and jejunum and the mucosal layer thickness of the jejunum. D'Inca et al. [4] demonstrated that piglets with IUGR possess thin intestines, as estimated by the ileal weight: length ratio. Thus, thick diameter of the gut in the pGLP-2 group provided histological evidence for the compensatory growth of IUGR piglets.

The intestinal effects of GLP-2 are mediated by GLP-2R, a G protein-coupled receptor [32]; this G protein-coupled receptor is highly selective for GLP-2, reacting only weakly following exposure to equimolar concentrations of structurally related peptides, such as glucagon and GLP-1 [33]. Small intestinal GLP-2R mRNA abundance was highest at birth and decreased with enteral food intake in fetal, suckling, and weaned pigs [23]. Petersen et al. [23] believed that the introduction of enteral feeding transiently increases the plasma GLP-2 concentrations and decreases the small intestinal GLP-2R mRNA levels during pig development. The downregulation of this receptor after exposure to a receptor ligand is a common phenomenon. The GLP-2R mRNA expression was downregulated in various models after GLP-2 was endogenously increased by feeding or treatment with exogenous GLP-2 [2]. Similarly, in this study, the low GLP-2R expression in the duodenum and ileum was found in piglets of the pGLP-2-treated IUGR group.

Conclusions

In conclusion, piglets treated with pGLP-2 microspheres during the suckling period could catch up to the weaning weight of NBW piglets. This improvement was associated with the improvement of the pancreatic secretion functions and return of the intestinal absorptive capacity and redox balance. Injection of pGLP-2 microspheres was a suitable therapeutic strategy for IUGR piglet.

Declarations

Ethics approval and consent to participate

Animal studies were conducted in accordance with the guidelines of the Zhejiang Farm Animal Welfare Council of China and approved by the ethics committee of Zhejiang Academy of Agricultural Sciences.

Consent for publication

Not Applicable

Availability of data and materials

All raw data are provided in supplementary file.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KK Q, J W, WJ Z, B D and XM M performed the research; KK Q designed the research, analyzed the data, and wrote the paper; ZW X revised the manuscript critically. All authors read and approved the final manuscript.

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Abbreviations

IUGR: intrauterine growth restriction; pGLP-2: porcine glucagon-like peptide-2; DPP-IV: dipeptidyl peptidase-IV; IGF-1: insulin-like growth factor-1; ALP: alkaline phosphatase; PCR: real-time polymerase chain reaction; TJ: tight junction; SOD: superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase; IGFBP3: insulin-like growth factor binding protein 3;

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Figures

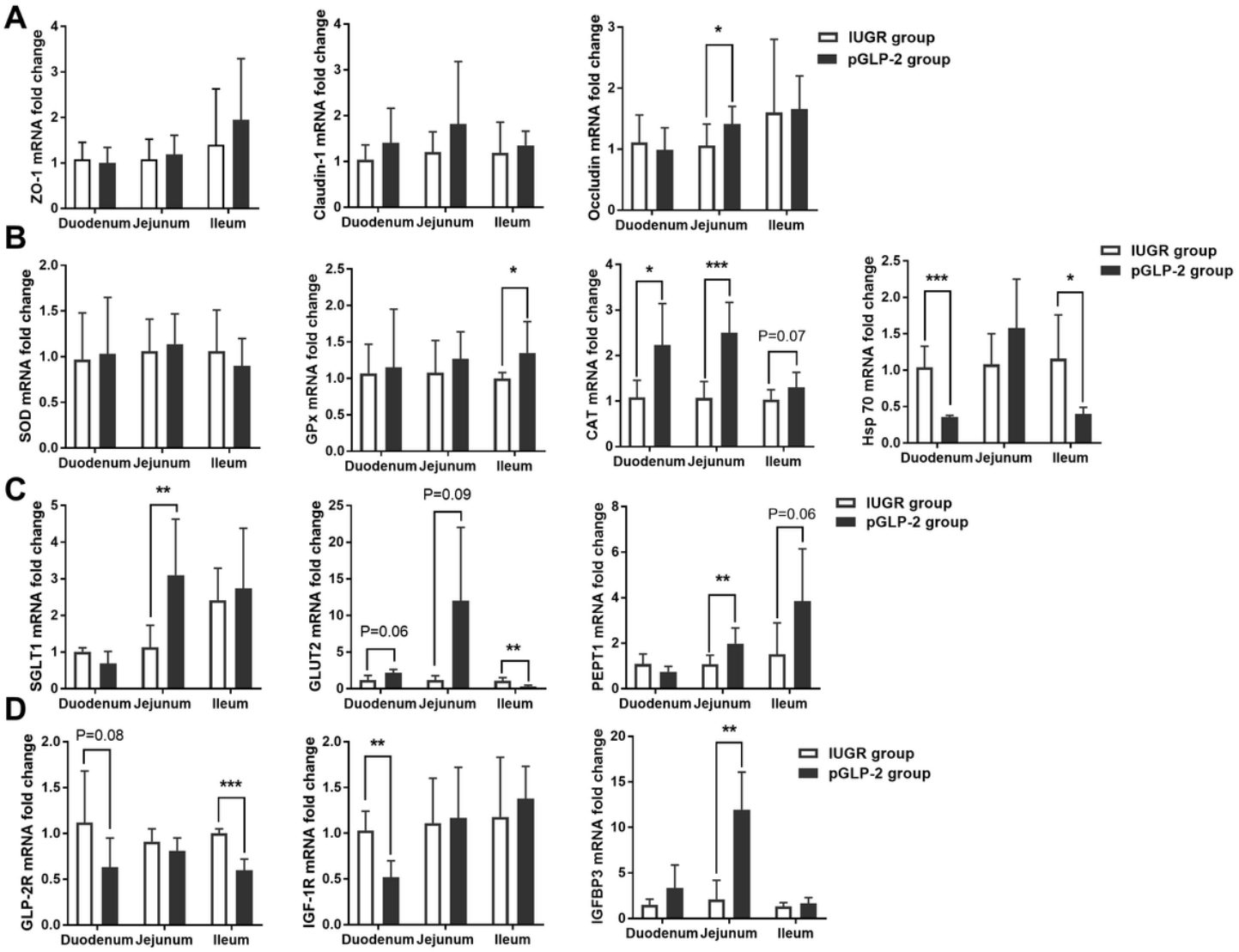

Figure 1

Relative mRNA expression of tight junction protein, redox, growth factor, and transport protein in the small intestine. Data are expressed as mean ± SEM, with n = 7 per group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate the pGLP-2 microsphere group compared with the intrauterine growth restriction (IUGR) group.

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