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LETTER TO THE EDITOR

THE PIG AS A MODEL FOR PREMATURE INFANTS - THE IMPORTANCE OF IMMUNOGLOBULIN SUPPLEMENTATION FOR GROWTH AND DEVELOPMENT

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Preterm human neonates, contrary to preterm piglets, obtain immunoglobulins from their mothers via the placenta during intrauterine development. However, one should note that the majority of trans-placental transfer of immunoglobulins in humans takes place during the last trimester of pregnancy. It is also known that the feeding of limited amounts of colostrum or systemic infusion of small amounts of serum improves the survival of preterm and full-term piglets. Full-term piglets deprived of their mother's immunoglobulins exhibit strong apathy and develop watery diarrhoea, often resulting in death. The aim of the current study was to determine if provision of immunoglobulins using different approaches would be beneficial for survival outcomes. To reach the immunological sufficient level we infused immunoglobulins intravenously in amount mimicking the blood level in piglets fed with sow colostrum. Intravenous infusion of immunoglobulins in both preterm and full-term newborn piglets fully ensured their survival, growth and blood immunoglobulin G and protein levels similar to those observed in piglets fed colostrum. Piglets completely deprived of immunoglobulins exhibited significantly lower blood levels of immunoglobulins and protein compared to colostrum-fed animals. Piglets infused with only serum exhibited significantly lower blood immunoglobulin G level compared to those infused with immunoglobulins. In conclusion, based on the data obtained, we suggest that passive immune support provided by colostrum intake or early systemic infusion of Ig's in sufficient amounts is key to ensuring the general well-being of preterm and full-term new born piglets, used as an animal model for the human infant.

To the Editor,

Based on a WHO worldwide report, every year approximately 14.9 million infants are born before the 37th week of gestation, with a global prevalence of 11.1% of total births (1). The gestational age has an effect not only on the neonates' organ developmental stage, but also on cellular and humoral immunity, and preterm infants have lower plasma immunoglobulin levels than full-term infants, which in turn contribute to the increased risk of infections (2, 3).

Key words: pig model, colostrum, immunoglobulin infusion, preterm infants

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

A preterm pig model of about 90% gestational age, which mimics human neonates over 30 weeks of gestational age (75%), was used to study the growth and development of human preterm neonates with emphasis on sickness development and the effects of different infant formulae and nutritional recommendations (4, 5). In contrast to preterm or full-term new born infants, that are provided with maternal passive immunity *in utero*, new born piglets totally lack passive immunity transfer during the foetal period and are thus born without the protection of the circulating maternal immunoglobulins (Ig) (6).

The main aim of the current study was to investigate and prove the importance of sufficient immunoglobulin supplementation for the survival, growth and behaviour of full-term and preterm piglets. Two separate experiments were performed. In the first experiment, full-term piglets were intravenously (*i.v.*) infused with purified porcine Ig or crude porcine serum, and the growth and survival of these piglets were compared to those of piglets fed porcine colostrum containing Ig. In the second experiment, preterm piglets received purified Ig immediately after birth via injection into the umbilical vein. These piglets were fitted with an intra-gastric port which was used to feed the piglets a milk formula during a 2-week period.

MATERIALS AND METHODS

Animals and animal holding

The present study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. Efforts were made to minimize animal suffering.

The study was performed on 39 piglets. Preterm piglets (n=15) were delivered by caesarean section from 2 sows, 7 days before full gestation (115 days), corresponding to human neonates of approximately 32 weeks of gestational age. Full-term piglets (n=24), from 6 litters, born naturally, were isolated from their sows before suckling and randomly distributed into 4 experimental groups (n=6 pigs in each group).

The animals were maintained under a 12 h day-night cycle, with lights on from 08.00 am to 8.00 pm. The piglets were housed in pairs in special cages for preterm/full-term piglets. The cages were equipped with dry bedding and heating pads to maintain a temperature of between 35-37°C and humidity around 56%. The pigs were allowed to move freely within the cages and had visual and tactile contact with each other.

Treatment and feeding procedures

Following arrival of the piglets to the laboratory, vascular catheters (Silastic Laboratory Tubing 508-001, Dow Corning, Auburn MI, USA) were inserted into the umbilical vein in all pigs for immunoglobulin/swine serum infusion as well as for primary (0h) blood sampling.

Full-term pigs (Experiment 1)

At the start of experiment 1, two groups of full-term pigs were infused, via the umbilical vein (23 ml/kg b.wt), with either a sterile filtered swine serum immunoglobulin preparation (306.75 mg IgG/ml) (Group 1) or normal sterile (16 ml/pig) swine serum (SS) (25.22 mg IgG/ml) (Group 2). A third group of piglets were fed with swine colostrum (10 ml/kg b.wt.) every 2 h for the first 12 h of life. The fourth group was deprived from immunoglobulins. All piglets were fed with the same infant formula (10 ml/kg b.wt; Similac Special care 24, Abbott Nutrition, Columbus, Ohio, USA) via an orogastric tube every 2 h for up to 48 h.

The swine serum Ig preparation was obtained from a pool of blood plasma from three multiparous sows, by ammonium sulphate precipitation (7). The Ig preparation was sterile filtrated and stored at -20°C until use. The normal swine serum was prepared by centrifugation of the blood obtained from three sows, which was then pooled, sterile filtrated and stored at -20°C until use.

Preterm pigs (Experiment 2)

At the start of experiment 2, 15 preterm piglets (Group 5) were used. All preterm pigs were infused (23 ml/kg b.wt) with the swine serum Ig solution (306.75 mg IgG/ml), in the same way in which the full-term pigs were infused. During the first 2 days after delivery, the preterm piglets were fed every hour with warm (37°C) infant formula (5 mL/kg b. wt.) via the orogastric tube.

At 48 hours, due to the increasing activity of the piglets, specially designed gastric port catheters for feeding were implanted. Briefly, the pigs were anesthetized using 0.5 -1.5% air mixture of Fluothane (Zeneca, Gothenburg,

Sweden) and O_2 as a carrier gas, at approximately 0.5-1 L/min in a close-circuit respiratory system (Komesaroff Medical Developments, Melbourne, Australia). The surgery was performed under aseptic conditions. A 5-cm long incision was made posterior to the sternum, along the *linea alba*. The stomach was isolated and the specially designed gastric port catheter (Silastic, Laboratory Tubing 508-002, Dow Corning, Auburn MI, USA) was implanted via the *curvatura major* and fixed with purse string catgut sutures (KRUUSE Chromic Catgut, USP 4-0; Kruuse Svenska AB, Uppsala, Sweden). The abdomen was then stitched up using 3 layers of sutures, absorbable sutures for the muscle layers and non-absorbable sutures for the skin (Silon Monofil 2/0; CHIRANA, Prague, Czech Republic). Ampicillin (250-500 mg, Doktacillin, Astra Läkemedel, Södertalje, Sweden) was administrated at the incision site. A jugular vein catheter (Silastic, Laboratory Tubing 508-001, Dow Corning, Auburn MI, USA) for blood sampling and infusions was also implanted during the same operation.

After surgery, the preterm piglets were infused i.v. for 6 h with 5 ml/kg/h of 0.9 % NaCl containing 10% glucose, after which they were switched to the gastric port feeding with formula, at a dose of 5 ml/kg every 1 h (24 per day) or 10ml/kg every 2 h (12 per day). Throughout the experiments, the piglets received increasing amounts of formula (about 5% increase per day) to ensure liquid, energy and nutrient supply. According to the neonatology guidelines, we aimed to reach enteral feeding with 135-180 ml/kg/day of formula, however the good tolerance to feeding and the welfare of animals were paramount.

Animal observations

The animals were thoroughly monitored during the experiments. The piglets' general health status, activity/ responsiveness and gastrointestinal clinical symptoms such as vomiting, chewing, constipation, diarrhoea and abdominal distension were evaluated. Before each feeding, food retention in the stomach was examined and if reflux of formula in gastric catheters was observed, the amount of formula fed was decreased by 1-2 ml (by the end of the study the amount of formula administered was around 15 ml/kg every 2 h). Prior to feeding, piglets were examined to confirm the absence of abdominal distension and respiratory distress. The piglets wore diapers for 24-h stool and urine collection. To assess bowel function, the faecal consistency and amount were noted. The amount and intensity of the urine colour was also noted. The piglets were weighed once daily (prior to feeding at 10 a.m.) and the daily body weight gain, expressed as grams per kilogram, per day (g/kg/day), was estimated. The observation lasted 48 h for full-term piglets and 14 days for preterm piglets.

Blood sampling and analyses

Blood was collected at the start and end of the study into ice-chilled tubes coated with lithium heparin [BD Vacutainer®, 367884, Becton, Dickinson and Company (BD Medical), Franklin Lakes, New Jersey, USA] as an anticoagulant and then centrifuged at 3000 x *g* for 15 min. After centrifugation, plasma was removed and frozen at -20°C until analysis.

Total protein concentration (mg/ml) in plasma samples, swine serum and swine colostrum was estimated according to the method described by Lowry et al. (8), using bovine serum albumin (BSA, A5470, Sigma-Aldrich, St. Louis, MO, USA) as the standard.

The level of immunoglobulin G (IgG) (mg/ml) in plasma samples, swine serum and colostrum was analysed by single radial immunodiffusion (9), using anti-Pig IgG produced in rabbits (Sigma P0916, Sigma-Aldrich, St. Louis, MO, USA) and purified porcine IgG as the standard (Sigma 14381, Sigma-Aldrich, St. Louis, MO, USA).

Statistical analyses

All data are expressed as mean ± SD (standard deviation). ANOVA followed by the Tukey post-hoc test or Kruskal-Wallis test was used to indicate the statistical differences between the groups. All analyses were carried out using Statistica, version 7 (StatSoft, USA). In all statistical analyses $p<0.05$ was considered significant.

RESULTS

Weight and clinical observations

In experiment 1, only 48 h following birth, the daily body weight gain was significantly ($p < 0.05$) increased in the piglets infused with Igs (Group 1) and in the colostrum-fed piglets (Group 3) (Table I). A body weight loss was observed in piglets i.v. injected with swine serum (Group 2) from 1.43±0.28 kg to 1.33±0.52 and in the control piglets without

any supplementation (Group 4) (1.27±0.52 kg to 1.15±0.30 kg) (Table I). It is worth mentioning that all piglets from Group 4 lost weight. Following i.v. Ig supplementation, at the end of the study on the 14th day following birth, a calculated daily body weight gain of 11.9 g/kg/day was observed in the preterm piglets (Group 5) (Experiment 2). During the first postnatal week, the preterm piglets displayed weight loss.

Upon physical examination, the preterm piglets showed no symptoms of having any gastrointestinal disorders or systemic infections, such as fever, abdominal distension, bloody stools, feed retention in the stomach or vomiting. The piglets did not require respiratory or circulatory support. All piglets passed urine a few times a day and anuria was not noted. Both the preterm and full-term piglets from all treatment groups were alert and active between the feeding intervals, except the group of control pigs, fed only the infant formula (Group 4) without any supplement, which were observed to be lethargic and apathetic. The control group of piglets also had watery diarrhoea, while the piglets from the other groups passed normal stools.

Table I. *Mean body weight, daily body weight gain, plasma total protein and immunoglobulin G content during the study period.*

Pig Treatment groups	Time	Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)	Group 4 (n=6)	Group 5 (n=15)
Body weight (kg)	0 h	1.40±0.45	1.43±0.28	1.15±0.18	1.27±0.52	1.14±0.20
	48 h	1.55±0.52	1.33±0.52	1.27±0.28	1.15±0.30	1.15±0.24
	14 d	-	-	-	-	1.33±0.22
DBWG (g/kg/day)		53.6±0.78a	-34.9±0.47b	50.4±0.35c	-45.8±0.54d	11.9±0.32e
TPC (mg/ml)	0 h	18.2±2.8a	15.8±1.9a	15.9±1.3a	21.3±2.0a	17.0±2.2a
	48 h	25.0±1.9b	24.4±0.1b	36.4±3.6c	19.7±3.1a	24.5±1.2b
	14 d	-	-	-	-	23.9±2.2ab
IgGC (mg/ml)	0 h	0.13±0.03a	0.08±0.02a	0.07±0.01ab	0.08±0.02ab	0.05±0.02b
	48 h	6.60±1.30c	1.80±0.08d	9.8±0.01e	0.06±0.01ab	5.70±1.60c
	14 d	-	-	-	-	4.40±1.40c

BW, body weight; DBWG, daily body weight gain; TPC, total protein content; IgGC, immunoglobulin G content. Data expressed as mean ± SD values of BW, DBWG, TPC and IgGC at the beginning and at the end of experiment for all study groups (Group 1, full-term piglets which received an intravenous infusion of swine immunoglobulins, n=6; Group 2, full-term piglets which received an intravenous infusion of swine serum, n=6; Group 3, full-term piglets which received swine colostrum, n=6; Group 4, Similac-fed full-term piglets, n=6; Group 5, preterm piglets which received an intravenous infusion of swine immunoglobulins, n=15). Different letters given with particular results in column and in row describe significantly different between the groups and consecutive time points when $p<0.05$.

Total protein (TP) and IgG plasma levels

The plasma protein levels at the time of birth were not significantly different between the full-term and preterm piglets (Table I). Forty-eight hours following the birth of the piglets, significantly increased total plasma protein levels were observed in all treatment groups, except for that of the control full-term piglets, fed infant formula without any supplement (Group 4).

Both preterm and full-term piglets displayed trace levels of IgG in the plasma samples obtained at the time of the birth (Table I). Forty-eight hours following the birth or caesarean of the piglets, the piglets infused with Ig or fed colostrum exhibited a significant ($p<0.05$) increase in plasma IgG concentration. The infusion of swine serum (Group 2) also resulted in significantly increased plasma IgG levels, however, these increases were significantly lower than those observed in the Ig-infused or colostrum-reared piglets. The Ig deficiency was sustained in the control full-term piglets from Group 4, fed infant formula without any supplement. In the preterm piglets (Group 5), post-infusion plasma IgG levels were maintained throughout the study period (14 days).

DISCUSSION

In the current study we proved that both a preterm and full-term porcine model of human neonates need to be supported with the porcine Ig in sufficient amounts (either *via i.v.* infusion or colostrum feeding) to ensure adequate growth of the animals, and specific for piglets' alertness and vitality. In addition, the use of gastric ports simplified the feeding and allowed us to perform prolonged observations. We demonstrated that *i.v.* infusion of porcine serum Ig immediately after birth results in increased serum IgG levels in both full-term and preterm piglets, which mimics the benefits of natural colostrum intake. In a previously published study, preterm piglets were infused with sow serum to create passive immunity (11). However, the amount of infused Ig was unable to protect the animals from the development of necrotizing enterocolitis. Our results demonstrated that full-term piglets, which received porcine serum in amounts, mentioned by Shen et al. (11), displayed significantly increased plasma levels of IgG and TP in comparison to the piglets fed infant formula without any supplementation. However, the plasma IgG amounts still remained significantly lower than those observed in piglets infused with purified Ig (Group 1) and those fed colostrum (Group 3). The full-term piglets that were not provided with any form of immune support displayed low plasma levels of IgG and TP; in fact, TP levels seemed to decrease during the 48 hours following birth. This appeared to have a serious negative effect and led to the deterioration of the general health status of the piglets, including the development of apathy and watery diarrhoea. In conclusion, based on our data, we suggest that passive immune support provided by colostrum intake or early systemic infusion of Ig in sufficient amounts is key to ensuring the general well-being of preterm and full-term newborn piglets. However, more detailed research is needed to confirm the positive influence of enteral feeding with passive immunity support.

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