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ORIGINAL ARTICLE

Birth oxidative stress and the development of an antioxidant system in newborn piglets

J. Yin[1,2], W. Ren[1,4], G. Liu[1,2], J. Duan[1,2], G. Yang[3], L. Wu[1,2], T. Li[1] & Y. Yin[1]

[1]*Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Hunan Provincial Engineering Research Center of Healthy Livestock, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, P. R. China,* [2]*University of Chinese Academy of Sciences, Beijing, P. R. China,* [3]*School of Food Science, Washington State University, Pullman, WA, USA, and* [4]*Laboratory of Immunology, Virginia- Maryland Regional College of Veterinary Medicine, and Maryland Pathogen Research Institute, University of Maryland, College Park, MD, USA*

Abstract

Birth oxidative stress is an oxidative response to a sudden transition process from maternal mediated respiration in uterus to autonomous pulmonary respiration outside the uterus. Meanwhile, oxidative stress has been demonstrated to be associated with various pathologies recorded in newborns. So, this research aimed to study the oxidative stress and the development of antioxidant system in newborn piglets. The measured variables include plasma lipid, protein and DNA oxidant injury, the activities of plasma antioxidant enzymes and the jejunal and ileal antioxidant gene expressions at 1, 7, 14, and 21 days after birth. Meanwhile, the nuclear factor erythroid 2-related factor 2 (Nrf2), transcription factor p65, and tumor protein 53 (p53) were determined by western blot. The results showed that newborn piglets suffered seriously from birth oxidative stress because of the naive antioxidant system. In addition, oxidant injury activated Nrf2 signaling pathway, resulting in the expression of antioxidant genes and release of antioxidant enzymes. With the development of antioxidant system, the oxidative balance gradually recovered on Day 7 after birth. In conclusion, birth caused oxidative stress and the oxidative balance gradually recovered with the development of antioxidant system.

Keywords: birth oxidative stress, antioxidant system development, newborn piglet

Introduction

Generally, oxidative stress is considered to be an imbalance between the production and the ability to clear the reactive oxygen species (ROS) [1,2]. ROS, such as O_2^-, H_2O_2, and OH, can interact with various molecules (i.e., lipid, protein, and DNA) in the cells and cause oxidative injury, including modification of protein structure, inactivation of biological activity, DNA strand breaks, point mutations, aberrant DNA cross-linking, and mutations in proto-oncogenes and tumor suppressor genes, thus promoting neoplastic transformation [3,4]. However, these ROS can be scavenged to maintain oxidative balance by many antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase, catalase, and other nonenzymatic anti-oxidants (i.e., glutathione, vitamins C and D) [5]. If increased ROS from mitochondria respiratory chain overwhelms antioxidant defenses, then oxidative stress occurs. In recent decades, various well-designed investigation indicated that oxidative stress is involved in the development of many diseases, including diabetes [6], cancer [7], aging, inflammation [8], cardiovascular disease [9], neurological disease [10], Parkinson's disease [11], obesity [12], and acute respiratory distress syndrome [13]. So it is clear that oxidative stress is closely associated with human health.

Many studies have shown that oxidative stress may be caused by various factors, such as xenobiotics, pathogens, and inflammatory cytokines [14]. However, to the best of authors' knowledge, little is known about the birth oxidative stress. Human and mammalian birth is characterized by a sudden transition process from maternal mediated respiration in uterus to autonomous pulmonary respiration outside the uterus [15]. In other words, infants would face an abrupt environment change from an intrauterine hypoxia with a pO_2 of 20–25 mmHg to an extrauterine relatively hyperoxia with a pO_2 of 100 mmHg [16]. In response to the changes of extracellular environmental conditions, newborns cells generate large amount of ROS, leading to the occurrence of birth oxidative stress [17]. It is known that oxidative stress may be associated with various pathologies recorded in newborns, such as hypoxic-ischemic

Correspondence: Tiejun Li and Yulong Yin, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Hunan Provincial Engineering Research Center of Healthy Livestock, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan 410125, P. R. China. E-mail: tjli@isa.ac.cn; yinyulong@isa.ac.cn.

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encephalopathy, intraventricular hemorrhage, necrotizing entero-colits, and brochopulmonary dysplasia [18,19].

Thus, supplementation with antioxidants after birth is essential for antioxidant system in newborns [20]. Meanwhile, before the application of antioxidants in clinics to mediate the oxidative stress after birth, it is critical to have a clear illustration about the development of antioxidant system after birth. So, the aim of conducting this study was to investigate the development of antioxidant system in order to make a recommendation for antioxidant therapy for newborns. According to similarities to humans regarding morphology, function of organs, and metabolic rate, newborn piglets was used as the in vivo model [21,22]. Furthermore, in this particular study the jejunum and ileum were selected as target organs because small intestine is one of the oxidative stress sites [23], and differed from some other organs (i.e., lung, liver, heart, and kidney) which have been investigated in previous research [24,25].

Material and methods

Experimental design

This study was conducted according to the guidelines of the Declaration of Helsinki and all procedures involving animal subjects were approved by the Animal Welfare Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Thirty-two newborn piglets of similar birth weight (Landrace × Large White) (ZhengHong Co., China) were randomly divided into four groups ($n = 8$), and separately slaughtered on Days 1, 7, 14, and 21 after birth. The experiment was arranged as a randomized design, and pigs were allowed free access to milk from sows throughout the whole experimental period. Before slaughter, plasma was sampled from a jugular vein for SOD, catalase, GSH-Px, malondialdeyhde (MDA), protein carbonyl, and total 8-hydroxyguanosine (8-OHG) and 8-hydroxydeoxyguanosine (8-OHdG) determination. Meanwhile, jejunal and ileal samples (approximately 2 g, in the middle place) were immediately frozen in liquid nitrogen and stored at −70°C for subsequent gene expression and western blot analysis.

Measurement of plasma oxidant injury products

Plasma protein carbonyl content was determined by an enzyme-linked immunosorbent assay (ELISA) kits which started by derivitization of the carbonyl group with dinitrophenylhydraine, followed by immunoblotting with an anti-DNP antibody (OxiSelect Protein Carbonyl ELISA Kit; Cell Biolabs, San Diego, CA, USA). Plasma MDA level was quantified by an enzyme immunoassay via probing with an anti-MDA antibody, and then followed by a horseradish peroxidase-conjugated secondary antibody (OxiSelect MDA Adduct ELISA Kit; Cell Biolabs). Total 8-OHG and 8-OHdG amount in the plasma was quantified by competitive enzyme immunoassay [26] (OxiSelect Oxidative DNA Damage Quantification Kit; Cell Biolabs).

Measurement of plasma antioxidant enzymes activities

Plasma SOD activity was measured using an ELISA kit in accordance with the manufacturer's instructions (Cell Biolabs). The SOD activity assay uses a xanthine/xanthine oxidase system to generate $O_2^{\bullet -}$, while the included chromagen produces a water-soluble formazan dye upon reduction by $O_2^{\bullet -}$, and the activity of SOD was determined as the inhibition of chromagen reduction. GSH-Px and catalase activities were measured using spectrophotometric kits in accordance with the manufacturer's instructions (Nanjing Jiangcheng Biotechnology Institute, China) [27].

cDNA synthesis and quantification mRNA by real-time PCR analysis

Total RNA was isolated from liquid nitrogen pulverized tissues with TRIZOL regent (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA) according to the manufacturer's instructions. Synthesis of the first strand (cDNA) was performed with oligo (dT) 20 and Superscript II reverse transcriptase (Invitrogen, USA).

Primers were designed with Primer 5.0 according to the gene sequence of pig (http://www.ncbi.nlm.nih.gov/pubmed/) to produce an amplification product (Table I). β-actin was used as a housekeeping gene to normalize target gene transcript levels. Real-time PCR was performed according to our previous study [28]. Briefly, 1 μL cDNA template was added to a total volume of 10 μL containing 5 μL SYBR Green mix, 0.2 μL Rox, 3 μL dH2O, and 0.4 μmol/L each of forward and reverse primers. We used the following protocol: (i) pre-denaturation program (10 s at 95°C); (ii) amplification and quantification program, repeated 40 cycles (5 s at 95°C, 20 s at 60°C); (iii) melting curve program (60–99°C with heating rate of 0.1°C S-1and fluorescence measurements). The relative expression was expressed as a ratio of the target gene to the control gene using the formula $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct = (Ct_{Target} - Ct_{\beta\text{-actin}})_{treatment} - (Ct_{Target} - Ct_{\beta\text{-actin}})_{control}$. Relative expression was normalized and expressed as a ratio to the expression in control group. Therefore, relative expression of target genes in control group was 1.0. Relative gene expressions represented the comparison versus control group and reported as a fold change from the control value.

Nuclear proteins extraction and western bolt analysis

Jejunum and ileum nuclear proteins were extracted with nuclear and cytoplasmic extraction reagents in accordance with the manufacturer's instructions (Thermo Fisher Scientific Inc., USA).

Western bolts were performed using antibodies specific to Lamin B, nuclear factor erythroid 2-related factor 2 (Nrf2), and p65 (Abcam Inc., USA). Briefly, the equal

Table I. Primers used for quantitative reverse transcription-PCR.

Name of target gene	Accession No.	Nucleotide sequence of primers (5′–3′)	Size (bp)
β-Actin	DQ845171.1	F: CTGCGGCATCCACGAAACT R: AGGGCCGTGATCTCCTTCTG	147
MnSOD	NM_214127	F: GGACAAATCTGAGCCCTAACG R: CCTTGTTGAAACCGAGCC	159
CuZnSOD	NM_001190422	F: CAGGTCCTCACTTCAATCC R: CCAAACGACTTCCASCAT	255
GPx1	NM_214201	F: TGGGGAGATCCTGAATTG R: GATAAACTTGGGGTCGGT	183
GPx4	NM_214407.1	F: GATTCTGGCCTTCCCTTGC R: TCCCCTTGGGCTGGACTTT	172
Ucp2	NM_214289	F: CCAATGTCGCTCGTAATG R: TGGCAGGGAAGGTCATC	109
p53	NM_213824	F: CTGCTTCCTGAAAACAACC R: AAGGGACAAAGGACGACA	199

F, forward; S, reverse; MnSOD, manganese-containing superoxide dismutase; CuZnSOD, copper- and zinc-containing superoxide dismutase; GPx1, glutathione peroxidase 1; GPx4, glutathione peroxidase 4; Ucp2, uncoupling protein 2; p53, tumor protein 53. All these primer sequence was designed based on the sequence corresponding to the accession number described above.
Birth oxidative stress and the development of antioxidant system in newborn piglets-Figure.

amounts of proteins obtained from nuclear fractions were separated by a reducing SDS-PAGE electrophoresis. The proteins were transferred onto polyvinylidene fluoride membranes (Millipore, MA, USA) and blocked with 5% non-fat milk in Tris-Tween buffered saline buffer (20 mM Tris, pH 7.5, 150 mM NaCl, and 0.1% Tween-20) for 3 h. The primary antibodies were incubated overnight at 4°C; the horse radish peroxidase-conjugated secondary antibodies were subsequently incubated for 1 h at room temperature before developing the blots using Alpha Imager 2200 software (Alpha Innotech Corporation, CA, USA). We digitally quantified the resultant signals and normalized the data to the Lamin B abundance. Lamin B was used as an internal loading control for nuclear protein fractions.

Statistical analysis

All data were standardized by Gaussian distribution and then analyzed using SPSS 17.0 software. Group comparisons were performed using the one-way ANOVA's Duncan (D)-test. Data are expressed as the mean ± standard error of the mean. Values in the same row with different superscripts are significant ($P < 0.05$), while values with same superscripts are not significantly different ($P > 0.05$).

Results

Birth caused lipid, protein, and DNA oxidant injury

To investigate the oxidative damage after birth, plasma MDA, protein carbonyl, and total 8-OHG and 8-OHdG levels were analyzed. MDA is the most common product of lipid peroxidation. On day 1, plasma MDA was exhibited at a very high level (914.99 ± 78.94 pmol/mg) (Figure 1), but it was decreased significantly ($P < 0.05$) on Days 7 (179.10 ± 15.74 pmol/mg) and 21 (78.74 ± 13.06 pmol/mg) after birth, indicating that lipid was exposed to a high degree of oxidation on Day 1 after birth. Furthermore, protein and nucleic acid are also normal targets for oxidative attack to produce protein carbonyl, and 8-OHG or 8-OHdG, respectively. The peak of total 8-OHG and 8-OHdG level occurred on Day 1 and threshold point occurred on Day 7 (Figure 2). Interestingly, the abundance of total 8-OHG and 8-OHdG significantly increased on Day 21 compared with that on Day 7 ($P < 0.05$). Meanwhile, protein carbonyl level was obviously higher on Day 1 than that on Days 7, 14, and 21 after birth, while no significant difference was found on Days 7, 14, and 21 after birth (Figure 3). These results suggested that newborn piglets suffered an obvious oxidative stress on Day 1, then this injury gradually ameliorated from Day 7 to Day 21 after birth.

Plasma antioxidant enzyme's activities and its development in the newborns piglets

Having observed fluctuation of oxidation products curve, antioxidant enzymes were next examined. The complex

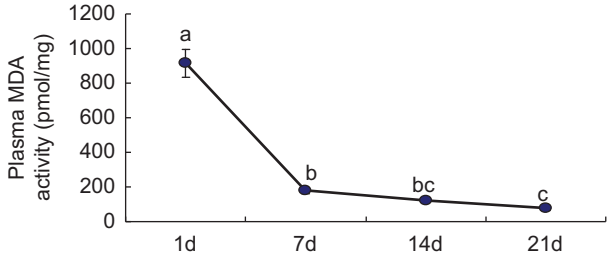

Figure 1. The fluctuation of plasma MDA in newborn piglets during 21 days. 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 8$). Values are means ± SE. [a,b,c]Significantly different ($P < 0.05$) by ANOVA. The same as below.

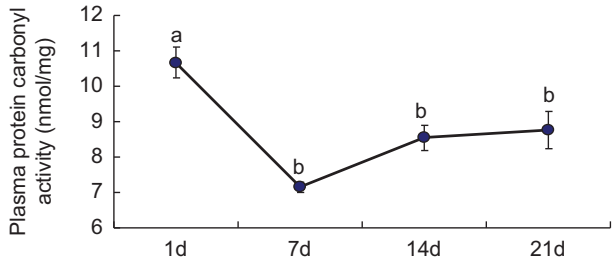

Figure 2. The fluctuation of plasma protein carbonyl in newborn piglets during 21 days. 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 8$).

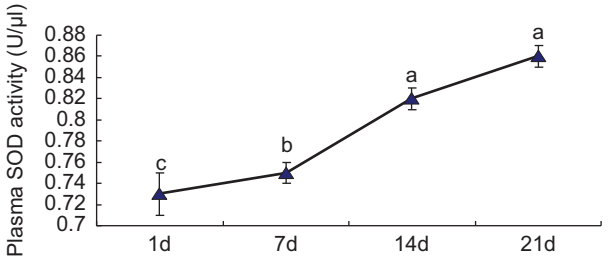

Figure 4. The fluctuation of plasma SOD activity in newborn piglets during 21 days. 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 8$).

enzymatic antioxidants (i.e., SOD, GSH-Px, and catalase) act as a major part of antioxidant system. The change of plasma antioxidant enzyme activities during 21 days after birth is illustrated in Figures 4–6. SOD and GSH-Px activities were significantly increased ($P < 0.05$) on Days 7, 14, and 21 compared with those on Day 1. But GSH-Px activity curve was occurred peak on Day 14 and declined slightly on Day 21 ($P > 0.05$). Although catalase activity has a similar fluctuation curve as GSH-Px activity, there is no difference in catalase curve.

Development of jejunum and ileum antioxidant gene expression

To demonstrate the results of plasma antioxidant enzymes activities, we studied the mRNA expression of several antioxidant related genes in the jejunum and ileum (Figure 7). In the jejunum (Figure 7A), CuZnSOD mRNA levels were 2.18-fold on Day 7 ($P < 0.05$), 2.47-fold on Day 14 ($P < 0.05$), and 3.56-fold on Day 21 ($P < 0.05$) compared with that on Day 1. Meanwhile, there was a significant ($P < 0.05$) difference in the abundance of CuZnSOD between Day 14 and Day 21 after birth. GPx1 mRNA levels were 2.29-fold on Day 7 ($P < 0.05$), 2.01-fold on Day 14 ($P < 0.05$), 2.15-fold on Day 21 ($P < 0.05$) compared with that on Day 1, and there was no difference between the levels on Day 7 and Day 21. In addition, compared with that on Day 7 and Day 14, GPx4 was significantly decreased on Day 21 ($P < 0.05$). As a redox-active transcription factor, the expression of p53 was significantly decreased on Day 21 compared with that on Days 1 and 7 ($P < 0.05$). In the ileum (Figure 7B), compared with those on Day 1, the expressions of CuZnSOD, MnSOD, GPx1, and GPx4 were 4.43-fold ($P < 0.05$), 2.21-fold ($P < 0.05$), 1.49-fold ($P < 0.05$), and 1.42-fold ($P < 0.05$), respectively, on Day 7, then these genes' expression were significantly decreased on Day 14 ($P < 0.05$). But the fluctuation curve did not exhibit same performance between Day 14 and Day 21. For example, there was an upward trend in MnSOD and GPx1 genes ($P < 0.05$), while no difference in CuZnSOD and GPx4 genes ($P > 0.05$). Furthermore, Ucp2, a gene of ROS-feedback regulation protein, was 4.26-fold increased on Day 21 and 2.24-fold increased on Day 14 compared with that on Day 1 ($P < 0.05$). In general, the mRNA expression of antioxidant genes was increased following the birth oxidative stress.

Relative nuclear protein expression

Except for p53 signal, we also studied p65 and Nrf2 signaling pathways, which are two key signaling pathways in oxidative stress. As shown in Figure 8, compared with that in the 1d, nuclear Nrf2 level was exhibited a downtrend at 7d and 14d ($P > 0.05$), then significantly increased at 21d ($P < 0.05$) in the jejunum (Figure 8B). However, nuclear Nrf2 curve was presented an opposite phenomenon in the ileum (Figure 9). Compared with that of Day 1, nuclear Nrf2 level was 2.97-fold on Day 7 ($P < 0.05$), 5.13-fold at 14th day ($P < 0.05$), but suddenly dropped to 1.2-fold at 21st day ($P > 0.05$). In addition, we failed to find any difference in p65 level, indicating that p65 signaling might not involve in the birth oxidative stress.

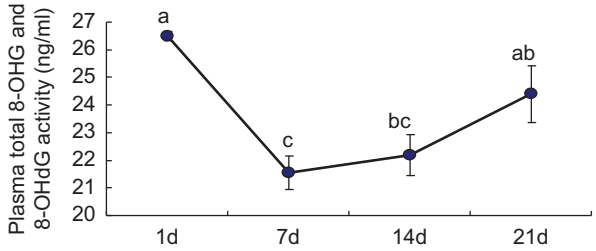

Figure 3. The fluctuation of plasma total 8-OHG and 8-OHdG in newborn piglets during 21 days. 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 8$).

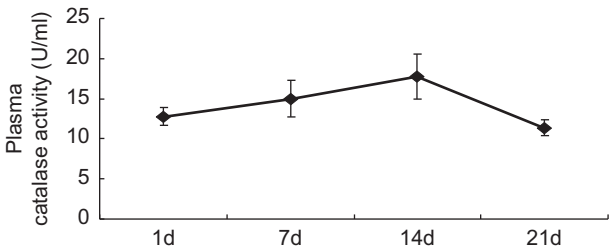

Figure 5. The fluctuation of plasma catalase activity in newborn piglets during 21 days. 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 8$).

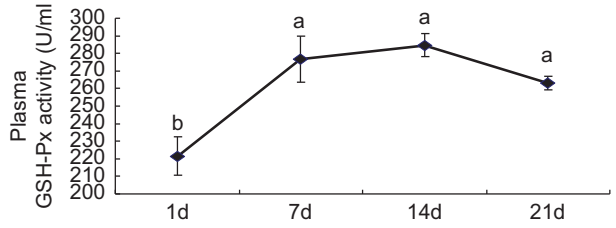

Figure 6. The fluctuation of plasma GSH-Px activity in newborn piglets during 21 days. 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 8$).

Discussion

Birth is associated with two major changes, maternal mediated respiration between autonomous pulmonary respiration and hypoxia between hyperoxia. Drastic functional changes in circulatory and respiratory during fetal-neonatal transition may cause the generation of ROS that can result in oxidative stress after birth [17]. Although the increased transfer of antioxidants (i.e., vitamins E, C, beta-carotenes, and ubiquinone) across the placenta during the last days of gestation [29], the antioxidant system is still insufficient to scavenge the excessive ROS. Kelly has reported that these excessive ROS is associated with various disorders of prematurity, such as chronic lung disease, retinopathy of prematurity, and intraventricular hemorrhage [30]. So it is clear that birth transition can induce oxidative stress and the recovery of oxidative imbalance is mainly depending on developmental antioxidant system.

MDA is the key ROS metabolic biomarker, which results from a series of reactions during lipid peroxidation caused by ROS. From the results of plasma MDA analysis, we found plasma MDA level on Day 1 was much higher than that on Days 7, 14, and 21. By further careful observation, we found that the plasma MDA level was gradually decreased during 21 days. Meanwhile, we also monitored the protein oxidation, which is defined as the covalent modification of a protein induced either directly by ROS, or indirectly by reaction with byproducts of oxidative stress. The most common product of protein oxidation is the protein carbonyl derivatives of Pro, Arg, Lys, and Thr. Many reports took protein carbonyl as protein oxidation biomarker, and found that oxidative stress can induce protein carbonyl production [31,32]. In our study, we found that plasma protein carbonyl level on Days 7 and 14 was significantly changed compared with that on the 1st day. Interestingly, plasma protein carbonyl level had an uptrend after Day 7 and it was significantly higher on Day 21 compared with that in Day 7. Furthermore, to understand the

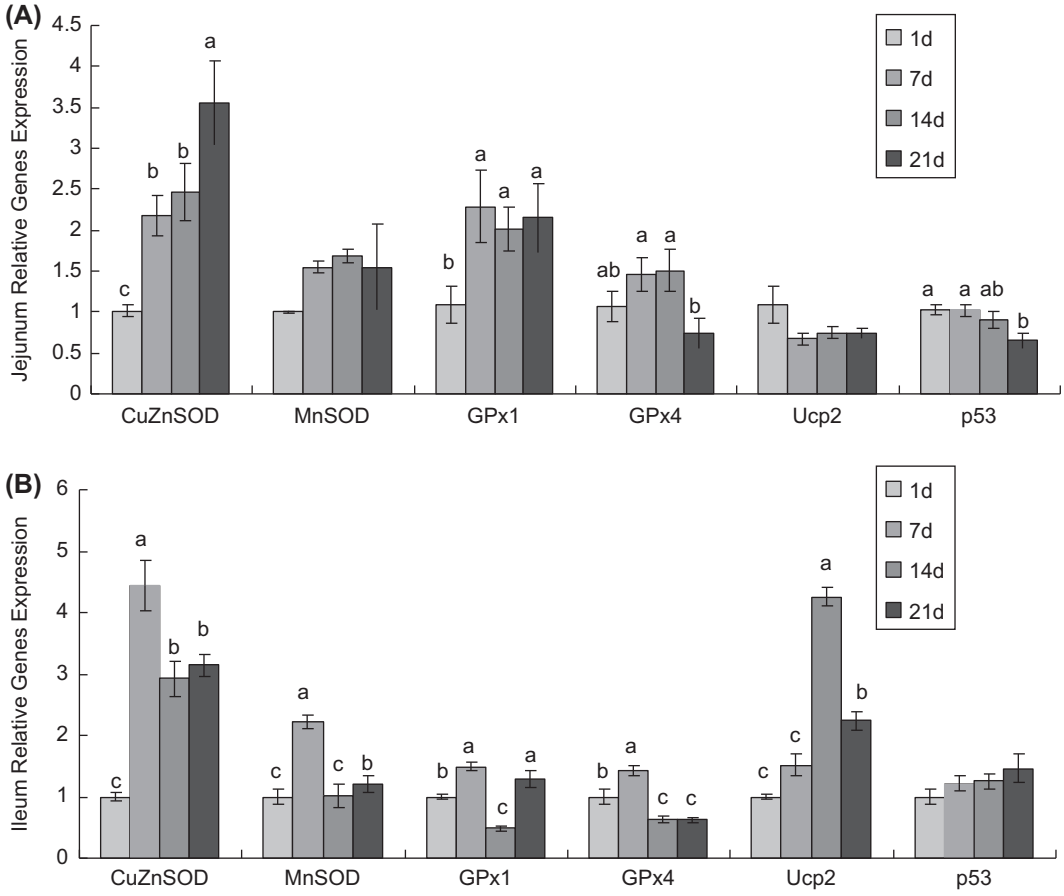

Figure 7. Oxidative stress-related gene expression in the jejunum (A) and ileum (B). SOD = manganese-containing superoxide dismutase; CuZnSOD = copper- and zinc-containing superoxide dismutase; GPx1 = glutathione peroxidase 1; GPx4 = glutathione peroxidase 4; Ucp2 = uncoupling protein 2; p53 = tumor protein 53; 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 8$).

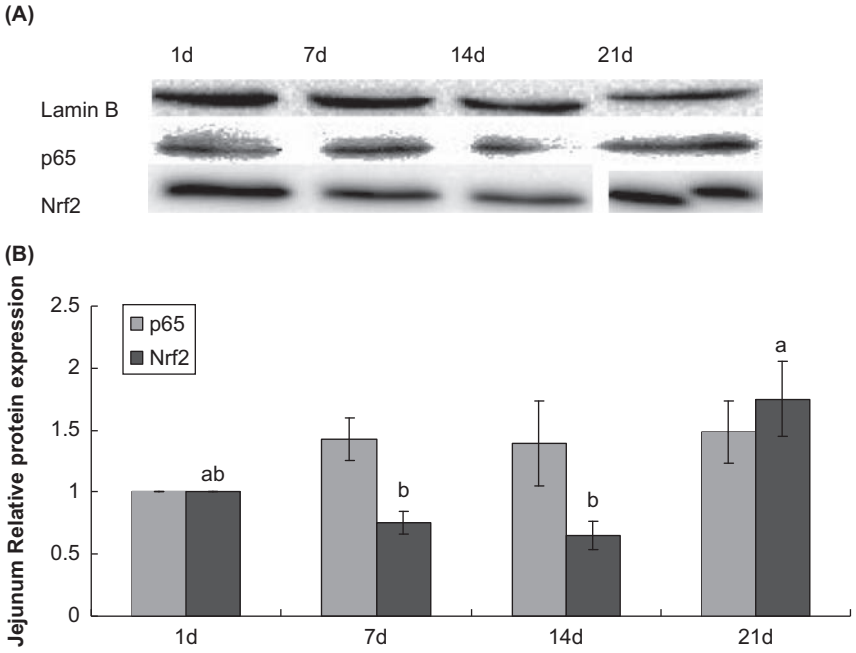

Figure 8. p65 and Nrf2 protein expression in the jejunum on Days 7, 14, 21 versus Day 1. An Immunoblotting of nuclear p65 and of Nrf2 in the jejunum during 21 days. B. Quantification of relative p65 and Nrf2 abundance from data shown in (A). 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 5$).

role of oxidative stress on nucleic acid damage, we also detected total 8-OHG and 8-OHdG levels in the plasma. Most studies have demonstrated that abundant oxidative DNA lesion can produce 8-OHdG because ROS can react with dGTP in the nucleotide pool to form 8-OHdG during DNA replication [33,34]. Based on these evidences, 8-OHdG has been widely used as a biomarker of oxidative DNA damage, and measurement of 8-OHdG level is applied to evaluate the load of oxidative stress. In our study, we found newborn piglets were seriously suffered from birth oxidative DNA damage, while this damage was gradually alleviated after birth. Through overall analysis of these three oxidation products curve, it is convincing that birth stress induces large amounts of ROS production, resulting in an obvious lipid, protein, and DNA oxidation with high level of plasma MDA, protein carbonyl, and 8-OHdG. However, these oxidative products were gradually decreased, at least, partially for the release of antioxidant enzymes, which can scavenge the excessive ROS.

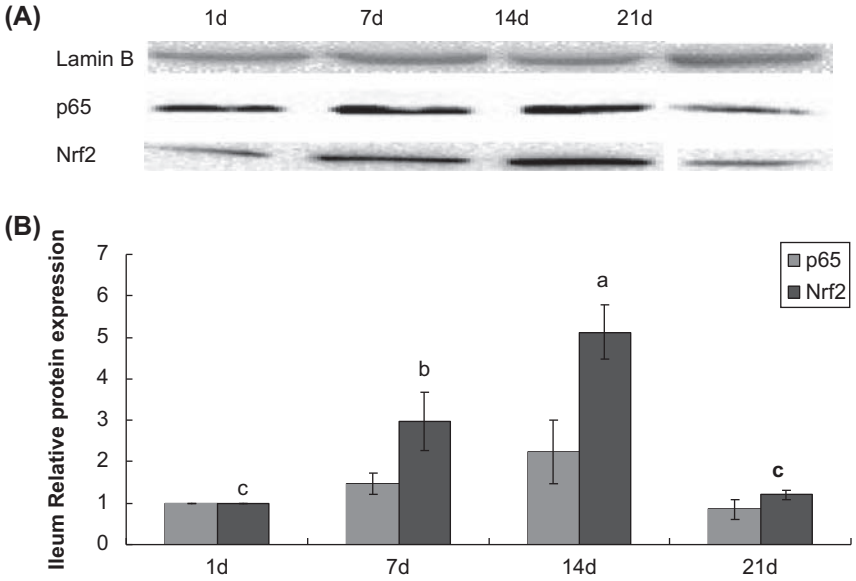

Figure 9. p65 and Nrf2 protein expression in the ileum on Days 7, 14, 21 versus Day 1. A. Immunoblotting of nuclear p65 and of Nrf2 in the jejunum during 21 day. B. Quantification of relative p65 and Nrf2 abundance from data shown in (A). 1d, 7d, 14d, and 21d mean newborn piglets are slaughtered on Day 1, 7, 14, and 21 after birth ($n = 5$).

As antioxidants, transferred from placenta, are insufficient to scavenge excessive ROS, so we also detected three kinds of antioxidant enzymes (SOD, catalase, and GSH-Px) and some antioxidant relative genes in jejunum and ileum. SOD, catalase, and GSH-Px carry out different substrate catalyzed reactions. SOD mainly scavenges $O_2^{\bullet -}$ by conversion of it to H_2O_2 [35]. In this study, we found that plasma SOD activities were gradually increased during 21d. Meanwhile, we also found that the gene expression of CuZnSOD was consistent with plasma SOD activity curve, especially in the jejunum. Although MnSOD gene expression on Days 7 and 21 is higher than Day 1 in the ileum, there is no difference in the jejunum. The reason may be that CuZnSOD is present in most parts of cells [36], so it plays a major role on SOD releasing. Most catalases are heme-containing enzymes, and they breakdown H_2O_2 to O_2 and two molecules of water. However, we did not find any difference in the plasma catalases activity among different time points in this study. GSH-Px also contributes to a partial task to eliminate H_2O_2 [36,37] and it uses several reductants to reduce H_2O_2 to two molecules of water. In this study, plasma GSH-Px activity was significantly increased on Days 7, 14, and 21 compared with that on Day 1. As the gene of GSH-Px, GPx1 mRNA expression was significantly increased during Days 7–21 as same as the change of plasma GSH-Px activity, but its expression in the ileum was decreased on Day 14. Similar observations have been reported in only a few previous studies. Rickett et al. demonstrated pre-birth can increases enzyme activity, while birth or post-birth decreases its activity [22]. These observations would again agree with current reasoning, which suggests that the decreased ROS is followed by release of antioxidant enzymes and expression of antioxidant genes. Interestingly, GPx4 expression presented a downward trend after Day 14 in the jejunum and Day 7 in the ileum. These differences also are similar with previous report that the developmental expression of antioxidant enzymes differ between tissues and time points [24].

To further explain the change of plasma oxidation injury products curve, antioxidant enzymes curve, and antioxidant relative genes development, we also explored three signaling pathways, including p53, p65, and Nrf2. Under homeostatic or non-stress conditions, Nrf2 is sequestered in the cytosol via Keap1 leading to a quick degradation; however, Nrf2-binding capacity of Keap1 is saturated and even diminished when cells are exposed to oxidative stress and excessive of ROS for the oxidation or conjugation of key cysteine residues in the Keap1 [38]. This change causes a disruption of the interaction between Nrf2 and Keap1, resulting in the decreased proteasomal degradation of Nrf2, accumulation of free Nrf2 in the cytosol, which can translocate into the nucleus and initiate the transcription of antioxidant genes [39–41]. In our study, we found that nuclear Nrf2 level gradually increased under birth oxidative stress in the ileum. Till Day 14, with activation of Nrf2 signaling, expression of antioxidant genes, and the release of antioxidant enzymes,

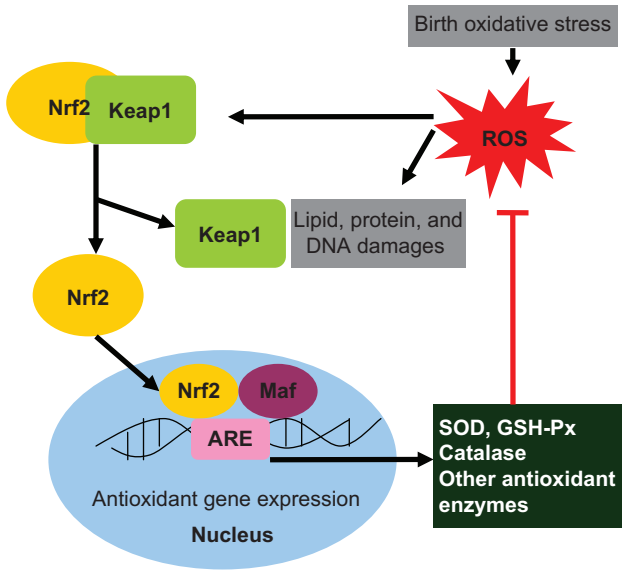

Figure 10. Nrf2 mediates birth oxidative stress. Under oxidative stress, the interaction between Nrf2 and Keap1 is disrupted, leading to decreased proteasomal degradation of Nrf2, accumulation of free Nrf2 in the cytosol, and an increase in Nrf2 translocation into the nucleus. As result, antioxidant genes expressed via ARE. In return, these genes response to oxidative stress and unbalance ROS.

excessive ROS has been scavenged and birth oxidative balance gradually recovered. So Nrf2 was sequestered in the cytosol and nuclear Nrf2 decreased at 21d. The results presented in this report also support the pervious findings that oxidative stress plays a major role in activating Nrf2 signaling pathways [42]. However, we failed to get the same result from the jejunum. The reason may be organ difference, but this still needs further study. Meanwhile, we also found that p53 involved in birth oxidative stress and antioxidant system development. P53 is a redox-active transcription factor that organizes and directs cellular responses in the face of a variety of stresses [43].

In conclusion, in newborn piglets, (1) plasma antioxidant enzymes activities curve, (2) plasma oxidative injury curve, and (3) antioxidant relative genes development figure are investigated during Day 21 after birth. These data indicated that newborn piglets suffered seriously from birth oxidative stress because of the naive antioxidant system. In addition, oxidant injury activated Nrf2 signaling pathway, resulting in the expression of antioxidant genes and release of antioxidant enzymes (Figure 10). With the polish of antioxidant system, the oxidative balance gradually recovered on Day 7 after birth. In conclusion, birth caused oxidative stress and the oxidative balance gradually recovered with the development of antioxidant system.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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