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Original article

A preliminary study on fetal lung injury in a rat model of acute pancreatitis in pregnancy

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ABSTRACT

Acute pancreatitis in pregnancy (APIP), which was thought to be rare, is becoming more frequent. In addition, high perinatal mortality among fetuses has been reported. Our research aimed to investigate and assess fetal lung injury in a rat model of APIP and its possible mechanisms. The APIP model was induced by sodium taurocholate in Sprague-Dawley rats during the third trimester. Sham-operated (SO) rats in late gestation were used as controls, and dynamic observation and detection in the SO and acute pancreatitis (AP) groups were performed at 3 time-points. Histological changes in the fetal lungs, as well as the maternal pancreas and placenta were assessed. The levels of serum amylase, lipase, TNF-α and IL-1β were detected in maternal rats, and the expression of surfactant proteins A, B, C and D as well as their mRNA were determined. In this study, fetal lung injury as well as maternal pancreas and placenta injuries occurred in a time-dependent manner. The levels of serum amylase, lipase and TNF-α were markedly increased in maternal rats, and the levels of surfactant proteins A, B, C and D in fetal lungs were significantly decreased in the fetal lungs of the AP group. Ultrastructure injuries and the dysregulated synthesis and secretion of pulmonary surfactant proteins were observed in the AP group.

Our research suggests that fetal lung injury is involved in the rat model of APIP and that the dysregulated synthesis and secretion of pulmonary surfactant proteins play a critical role in fetal lung injury during APIP.

1. Introduction

Acute pancreatitis in pregnancy (APIP) is a rare but dangerous disease that has been estimated to occur in 1/12,000 to 1/1000 pregnancies [1–3]. APIP frequently occurs in the third trimester, and gallstones are the most common cause [1]. A retrospective cohort study showed that maternal outcomes have improved in the past decade [4], but a high prenatal mortality rate has been reported for fetuses (3.6%) [1,3]. A 23-year, single-center retrospective analysis showed that 58% of women underwent cesarean delivery and that 47% of the surviving fetuses were delivered preterm [5]. Fetal outcomes associated with APIP include preterm delivery (35.4%), jaundice (29.2%), small for gestational age 22.8%), respiratory distress syndrome (4.45%), and intrauterine fetal death (1.85%) [1]. Among these complications, the present study focused on respiratory dysfunction in neonates after APIP.

Neonatal respiratory distress syndrome is a life threatening respiratory disease in newborns that frequently occurs in premature infants (6%–7%); it is also the most common cause of admission to the neonatal intensive care unit [6,7]. It has been thought that the etiology of respiratory distress syndrome is related to the developmental immaturity of lungs, particularly of the surfactant synthesizing system [6,8]. The etiology, pathogenesis and methods of preventing and treating near-term and full-term infants with respiratory distress syndrome have attracted increasing attention [6,9–11]. However, at present, there is no animal experimental study on fetal lung injury in APIP.

In this study, we established the APIP rat model using a modified model based on our previous research [12] to assess the pathological changes and levels of surfactant proteins in fetal lung, and discuss the possible mechanisms.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley rats (n = 36; gestation days 17–19, first gestation, weighting 370–450 g) were obtained from the experimental

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animal center of Wuhan University. The study was approved by the Ethics Committee of Wuhan University (Wuhan, Hubei, China). The animal experiments were conducted in accordance with EEC regulations (Official Journal of European Community L35812/18/1986) and NIH standards (Guide for the Care and Use of Laboratory Animal, National Institutes of Health's publication85-23, revised 1996). Rats were housed in a controlled environment and provided standard rodent chow and water. For 12 h before surgical procedures, the rats were deprived of food but were allowed free access to water.

2.2. Experimental modeling and design

All rats were anesthetized with isoflurane (induction with 4%–5% isoflurane, maintaining 2%–3%; oxygen flow, 1.5 l/min). The acute pancreatitis (AP) model was established by a standard retrograde infusion (0.1 ml/min; average, 2 min) of 5% sodium taurocholate (Sigma, USA) saline solution into the biliary-pancreatic duct (0.6 ml/kg), and both sides of the biliary-pancreatic duct were clipped for 5 min [12]. After closure, saline solution (2 ml/100 g body weight) was compensated subcutaneously for fluid loss associated with the surgical procedure. Rats were randomized into 2 groups: (1) the sham-operated (SO) group (n = 18), (2) the AP group (n = 18), both groups were further subdivided into 3 time points (3 h, 6 h, and 12 h, n = 6 in each subgroup). All rats in the SO group underwent a sham surgery in which the pancreas and duodenum were manually flipped several times without STC infusion. Rats were sacrificed at the indicated time points after the induction of pancreatitis, and all adult fetal rats were obtained by cesarean delivery. All rats after harvesting were euthanized by cervical dislocation, and all pups were euthanized by rapid freezing.

2.3. Maternal serum assay

All blood samples were collected from the post cava of each maternal rat using vacuum serum separator tubes (BD, USA), allowed to naturally clot for 30 min, and centrifuged at $1500g \times 10$ min. The Supernate was collected and stored at −80 °C in individual aliquots. Serum amylase (AMY) and lipase (LIP) were measured using standard techniques with a fully automatic chemistry analyzer (Olympus, Japan). Levels of TNF-α and IL-1β in maternal serum were measured using commercial ELISA kits (Elabscience, China), and all procedures were conducted according to instructions from the manufacturer and duplicated 3 times.

2.4. Histological examination

Fetal lung, the head of the maternal pancreas and the placenta were harvested, trimmed at proper sizes, fixed in 4% phosphate-buffered formaldehyde and embedded in paraffin. The paraffin-embedded tissues were sequentially sliced into 4 μm sections and stained with hematoxylin-eosin (H & E). Blinded morphometric assessments were performed by 2 independent pathologists under a light microscope (Olympus, Japan). Pancreatic histological assessment was determined by evaluating edema, necrosis, hemorrhage and inflammation according to the scale described by Schmidt [13]. The criteria of the fetal lung pathological score were based on the method of Bhatia [14].

2.5. Reverse transcriptase polymerase chain reaction (RT-PCR) assay

Total lung RNA was extracted from frozen fetal lung tissue using Trizol reagent and reverse-transcribed to complementary DNA using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) according to the manufacturer's instructions. Polymerase chain reaction was performed with the primers for GAPDH (sense primer 5′-TTCCTACCCCCAATGTATCCG-3′ and anti-sense primer 5′-CATGAGGTCCACCACCTGTT-3′, NM_017008.4), SP-A (sense primer 5′-CCTTCACCCTCTTCTTGACTGTTG-3′ and anti-sense primer CAGGCTCTCCCTTGTCTCCAC-3′, 001270645.1), SP-B (sense primer 5′-AAAGCCTGGAGCAAGCGATAC-3′ and anti-sense primer GAAAGCGTCTTCCTTGGTCATC-3′, NM_138842.1), SP-C (sense primer 5′-TTGTCGTCGTGGTGATTGTAGG-3′ and anti-sense primer GAAGGTAGCGATGGTGTCTGTG-3′, NM_017342.2) and SP-D (sense primer 5′-ATGAAGACCCTTTCGCAGAGAT-3′ and anti-sense primer AGGCAACCCTGAGAGTCCCA-3′, NM_012878.2). RT-PCR reactions were performed using 2.5 μl of cDNA, 12.5 μl of SYBR green mix (Roche, Germany) and 7.5-μM concentrations of each primer in a total volume of 25 μl. A CFX Real-Time PCR Detection System (Bio-Rad, USA) was programed with an initial step of 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. All reactions were run in duplicate, averaged, and normalized to the GADPH gene to quantify the relative gene expression using the $2^{-\Delta\Delta CT}$ method.

2.6. Immunofluorescence assay

Immunofluorescence assays were used to detect the expression of surfactant protein A (SP-A) in fetal lung. After deparaffinization and rehydration, serial sections (4 μm) were boiled in a pressure cooker (121 °C, 4 min) with citrate buffer (10 mM, pH 6.0) for epitope retrieval, cooled to room temperature and rinsed in phosphate-buffered saline(PBS). Sections were incubated in 10% donkey serum (Jackson, USA) to avoid nonspecific staining and then incubated with rabbit anti-rat polyclonal antibodies against SP-A, SP-B, SP-C, SP-D (Santa Cruz, USA) in a moisture box overnight at 4 °C. Then, slides were reacted with donkey anti-rabbit fluorescent antibody (Abcam, UK) and mounted with fluoroshield containing DAPI (Abcam, UK).

Immunofluorescence slides were photographed using a fluorescence microscope in a blinded fashion, analyzed by Image Pro Plus 6.0 system (Media Cybernetics, USA) for quantitative analysis. Briefly, the lung tissues were selected as the area of interest (AOI). Integrated optical density (IOD) of the AOI was adopted as the measurement parameter. The IOD of SP-A, SP-B, SP-C and SP-D expression in fetal lung from all groups was examined and compared.

2.7. Transmission electron microscopy examination

Fetal lung alveolar type II epithelial cells were examined using a transmission electron microscope. A small portion (approximately 1 mm^3) of fresh tissue was excised from the fetal lungs and fixed in 2.5% glutaraldehyde (0.1 mol/L phosphate buffer, pH 7.4) overnight at 4 °C, followed by post fixation with 1% osmium tetroxide in the same buffer for 1 h at 4 °C. The tissues were dehydrated in a graded series of ethanol and acetone. Ultrathin sections were cut on a Leica EMUC7 ultra microtome and stained with lead citrate and uranyl acetate. Changes in the alveolar type II cells were examined by using an HT7700 transmission electron microscope (Hitachi, Japan).

2.8. Statistical analysis

All data were expressed as the mean ± standard deviation, and analyzed with SPSS 20.0 statistical software (SPSS Inc., USA). Statistical analyses between groups were performed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. A value of $P < 0.05$ was considered a statistically significant difference.

3. Results

3.1. Evaluation of the severity of pancreatitis

Pancreatic injury was estimated, based on edema, inflammatory cell infiltration, hemorrhage, and necrosis [13]. The pancreatic histopathological score gradually increased after the induction of pancreatitis in the AP group ($P < 0.05$), and these scores were higher than those of the SO group at the respective time points ($P < 0.05$) (Fig. 1).

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