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Respiratory distress syndrome (RDS) is a major cause of morbidity and mortality in preterm neonates. Maternal glucocorticoid treatments given to women at high risk of preterm delivery have been extensively evaluated for their efficacy to decrease the incidence and severity of RDS. Acupuncture at the locus Tsu-San-Li (St-36) was found to enhance adrenal production of corticosterone, and to stimulate adrenocorticotropic hormone release in the rats. Maternal glucocorticoid could not prevent completely the incidence of RDS and have untoward neurological side effects after multiple doses. This study was performed with timed pregnant Sprague-Dawley rat mothers. Rats subjected to electroacupuncture were inserted two stainless-needles into the Tsu-San-Li (St-36) in the right hindleg for 30 min. Acupuncture group mothers (1 day) received electroacupuncture on day 18 of gestation. Acupuncture group mother (2 days) received electroacupuncture on days 17 and 18 of gestation. Control group mother (non-point group) received acupuncture at site not contained in the Atlas of Human Acupuncture Points on day 18 of pregnancy. On day 19 of gestation, all the dams were delivered by cesarean section. We quantify the treatment effects on lung maturation in preterm rats by biochemical (surfactant phospholipids, antioxidant enzyme) and morphological (lung morphometry) analyses. The aims of this study are to evaluate the effects of acupuncture on lung maturation in preterm rats and to investigate the mechanisms of acupuncture acceleration of fetal lung maturation. Maternal acupuncture treatment (2 days) significantly increased lung tissue saturated phosphatidylcholine and total phospholipid content when compared with control animals. Acupuncture (2 days)-treated rats had lower activities of three lung antioxidant enzymes (AOE) (superoxide dismutase, catalase, and glutathione peroxidase) when compared with control and acupuncture (1 day)-treated rats and the values reached statistical significance for catalase only. These findings indicate that acupuncture affects surfactant and AOE development in opposite ways and may have beneficial and potentially harmful effects on different aspects of lung development.
Keywords ¡GAcupuncture, antioxidant enzyme, lung maturation, surfactant

Introduction

Respiratory distress syndrome (RDS) is major cause of morbidity and mortality in preterm neonates (1). Maternal glucocorticoid treatments given to women at high risk of preterm delivery have been extensively evaluated for their efficacy to decrease the incidence and severity of RDS (2). The meta-analysis of the multiple randomized controlled trials showed that maternal glucocorticoid decreased the incidence of death and RDS by 50% (3). It is suggested that the beneficial effect of glucocorticoid was absent if there was an interval of over seven days between treatment and delivery (4). These findings persuaded obstetricians to repeat the course of glucocorticoid after seven days in the pregnant women at risk of preterm delivery had not yet given birth. However, there is considerable evidence from experimental animals that glucocorticoids have an adverse effect on the growth and development of the immature brain. Uno et al. gave dexamethasone in doses of 0.5, 5, or 10 mg/kg to pregnant rhesus macaques on day 132 and delivered at 135 days of gestation, they found that decreased cell numbers of neurons in the hippocampus (5). The loss of cells was increased by multiple doses. Huang et al. showed a dose and four doses of betamethasone (0.5 mg/kg) to pregnant sheep at about 70% of gestation was followed by a reduction in brain weight of 10% and 21% at term, respectively (6). Cerebrum, cerebellum, and brain stem were all reduced in weight. Recent clinical studies revealed that repeated antenatal gludocorticoid and early postnatal dexamethasone treatment increased the incidence of neuromotor abnormalities (7-9).

Surfactant phospholipids and protein synthesis in fetal lung tissue is regulated both in vivo and in vitro by a number of hormones and factors, including glucocorticoids, thyroid hormones, prolactin, estrogen, androgens, growth factors, cytokines, and insulin. Jobe et al. demonstrated that remarkable early lung maturation occurred independently of cortisol (10). These results indicate that there are other potent lung maturation factors that remain to be identified. Acupuncture has been reported to suppress uterine contractions induced by oxytocin and this may an alternative treatment for preterm labor (11). Acupuncture at the locus Tsu-San-Li (St-36) was found to enhance adrenal production of corticosterone, and to stimulate adrenocorticotropic hormone release in the rats (12, 13). Maternal glucocorticoid could not prevent completely the incidence of RDS and have untoward neurological side effects after multiple doses. We hypothesized that acupuncture would induce early and fast lung maturation in preterm rats and quantified the treatment effects by morphological, biochemical, and molecular analyses. The aims of this study are to evaluate the effects of acupuncture on lung maturation in preterm rats and to investigate the mechanisms of acupuncture acceleration of fetal lung maturation.

Materials and Methods

Animals

The study is approved by the Institutional Animal Use Committee at Taipei Medical University and is performed with timed pregnant Sprague-Dawley rat mothers (vaginal smear positive, day 0; term, day 22). Rats subjected to electroacupuncture were anesthetized by intraperitoneal pentobarbital (25 mg/kg). Acupuncture group mothers received electroacupuncture by inserting two stainless-needles into the Tsu-San-Li (St-36) in the right hindleg. The point is located in the rat below the capitulum fibulae, between the tibia and fibula approximately 5 mm lateral to the anterior tubercle of the tibia. (14). Continuous current of 2 Hz was applied through the needles for 30 min. Acupuncture group mother (1 day) received electroacupuncture on day 18 of gestation. Acupuncture group mother (2 days) received electroacupuncture on days 17 and 18 of gestation. Control group mother (non-point group) received acupuncture at site not contained in the Atlas of Human Acupuncture Points on day 18 of pregnancy. On day 19 of gestation, all the dams were delivered by cesarean section under intraperitoneal pentobarbital anesthesia (50 mg/kg). At delivery, the fetuses were weighed and a blood sample is taken from each pregnant female and fetus before killing.

Plasma is immediately separated from blood cells by centrifugation and kept at –20¢J for hormone measurements. The fetal organs of interest were then dissected free and weighed to the nearest 0.1 mg as soon as possible. Results are expressed as both individual organ weight and the ratio (%) of organ weight/body weight.

Measurement of saturated phosphatidylcholine in lung tissue

Lungs were homogenized and extracted with chloroform-methanol (15). Lipid extracts from lung homogenates were treated with osmium tetroxide, and saturated phosphatidylcholine was recovered by alumina column chromatography and was quantified by phosphorus assay (16, 17). The values are expressed as μmoles per gram lung weight.

Measurement of antioxidant enzyme activities

Total superoxide dismutase activity was assayed by inhibition of formazan dye production (Randox Laboratories Ltd., Antrim, UK). This method employs xanthine and xanthine oxidase to generate superoxide radicals that react with p-iodonitrotetrazolium salts to form a red formazan dye. The superoxide dismutase activity was measured by the degree of inhibition of this reaction. One unit of superoxide dismutase equaled 50% inhibition of p-iodonitrotetrazolium reduction.

Catalase activity was measured by the rate of reduction of hydrogen peroxide substrate, followed spectrophotometrically at 240 nm (18). One unit of catalase equals 1 μmol hydrogen peroxide decomposed/min at 25¢J.

Glutathione peroxidase activity was assayed spectrophotometrically at 340 nm at the rate of oxidation of NADPH (19). The assay mixture for measurement of this cytosolic enzyme includes cumene hydroperoxide as primary substrate, with sodium azide added to inhibit contributing activity form catalase enzyme.

Morphological analysis

For morphological studies, 2-3 pups' lungs from each dam were collected. A ventral midline thorax incision was made and the heart and lungs were removed *en bloc* and storage in fixative. Lung tissue was examined microscopically for the assessment of lung maturation following staining with hematoxylin and eosin.

Statistical analysis

Results are presented as the means±SEM. The between-group comparisons were made using analysis of variance (ANOVA) followed by Scheffe post-hoc multiple comparisons tests. Significance was accepted at $p<0.05$.

Results

There were 48 fetuses from 3 rats in the control group, 67 fetuses from 4 rats in the acupuncture (1 day) group, and 65 fetuses from 4 rats in the acupuncture (2 days) group. There was no significant difference in litter size among the 3 study groups

Effects of maternal acupuncture treatment on fetal body weight, lung weight, and the ratio (%) of lung/body weight

Maternal treatment effects on fetal body weight, lung weight, and the lung/body weigh ratio (%) are presented in Table 1. The body weights were comparable among the 3 study groups. Premature rats exposed to acupuncture (2 days) before delivery had significantly lower lung weight and lung/body weight ratio than did the acupuncture (1 day)-treated animals.

Effects of maternal acupuncture treatment on fetal lung phospholipid contents

Maternal acupuncture treatment (2 days) significantly increased saturated phosphatidylcholine and total phospholipids in fetal lung tissue when compared with control animals (Table 2). The ratios (%) of saturated phosphatidylcholine to total phospholipid were comparable among the 3 study groups. The ratios (%) were 15.6±0.3, 15.7±0.3, and 14.9±0.3 in the control, acupuncture (1 day)-, and acupuncture (2 days)-treated groups, respectively.

Effects of maternal acupuncture treatment on antioxidant enzyme activities in fetal lung

tissues

Lung superoxide dismutase and glutathione peroxidase activities were comparable between control and acupuncture (1 day)-treated groups (Fig. 1). Acupuncture (2 days)-treated rats had lower superoxide dismutase and glutathione peroxidase activities than acupuncture (1 day)-treated rats but the values did not reach statistical significance. Catalase activity exhibited a similar pattern as do superoxide dismutase and glutathione peroxidase, its value decreased with 1-day acupuncture treatment and the activity further decreased significantly with 2-day acupuncture treatment.

Histology

The histological appearance of the lungs is illustrated in Figure 2. Examination of random fields under a light microscope revealed denser mesenchyme and more epithelial tubules in the acupuncture-treated groups as compared to control animals.

Discussion

Neonatal respiratory failure is a serious clinical problem associated with high morbidity, mortality, and costs (20, 21). The major risk factor is premature birth and it's associated RDS. The pathophysiology of RDS is the immature lung structure and a deficit of surfactants. Glucocorticoids have been reported to accelerate fetal lung maturation and surfactant production (22). Acupuncture at the locus Tsu-San-Li (St-36) was found to enhance adrenal production of corticosterone, and to stimulate adrenocorticotropic hormone release in the rats (12, 13). Therefore, we investigated the effects of maternal acupuncture treatment in preterm rats, which have been shown to be a suitable model for the study of acute neonatal lung disease (23). This study found a higher lung content of saturated phosphatidylcholine and more-mature lung histology in fetuses of mothers administered acupuncture than in control mothers. These results show that the acupuncture has lung maturational effects similar to those of dexamethasone.

A deficiency in surfactants is central to the pathophysiology of RDS (24). Pulmonary surfactant stabilizes the lung by producing a surface-active monolayer that reduces the surface tension at the air-liquid interface of the terminal airways. This reduction in surface tension contributes to mechanical stability by preventing alveolar and bronchiolar collapse during expiration. Surfactant is composed of approximately 90%lipids and 10% proteins. The surface activity property is due primarily to dipalmitoyl phosphatidylcholine, which is approximately 45% of surfactant by weight. Therefore, we measured the saturated phosphatidylcholine content in fetal lung tissue. In this study, we found that maternal acupuncture treatment increased saturated phosphatidylcholine and total phospholipids levels in fetal lung tissue.

The antioxidant enzyme system and the surfactant system of the fetal lung exhibit chronologically similar developmental patterns; both lung phospholipid content and AOE activities increase dramatically during the final 10 to 15% of gestation in the rat (25). The premature infant with surfactant deficiency may be further compromised by an underdeveloped antioxidant enzyme system when exposed to high concentrations of oxygen. Accelerated maturation of the antioxidant enzyme system in the fetal lung may be of clinical benefit. The principal intracellular antioxidant enzymes are superoxide dismutase, catalase, and glutathione peroxidase. In this study, we found that maternal acupuncture treatment (2 days) depresses antioxidant enzyme maturation in premature fetal lung. The mechanism of this depression is not known. Possible mechanisms were decreased enzyme synthesis, increased enzyme turnover, or inactivation of enzyme.

In conclusion, these findings indicate that acupuncture affects surfactant and AOE development in opposite ways and may have beneficial and potentially harmful effects on different aspects of lung development. Further studies are needed to investigate the hyperoxic AOE response in the newborn animals prenatally treated with acupuncture.

References

1. Guyer B, Martin JA, MacDorman MF, Anderson RN, Strobino DM. Annual summary of vital statistics-1996. Pediatrics 1996;100:905-918.
2. Ballard PL. Hormones and lung maturation: monographs on endocrinology, Vol. 28. New York: Springer-Verlag, 1986.
3. Crowley P. Antenatal corticosteroid therapy: a meta-analysis of the randomized trials¡－1972-1994. Am J Obstet Gynecol 1995;173:322-335.
4. Gamsu HR, Mullinger BM, Donnai P, Dash CH. Antenatal administration of betamethasone to prevent respiratory distress syndrome in preterm infants: report of a UK multicentre trial. Br J Obstet Gynecol 1989;96:401-410.
5. Uno H, Lohmiller L, Thieme C, Kemnitz JW, Engle MJ, Roecker EB, Farrel PM. Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. 1. Hippocampus. Dev Brain Res 1990;53:157-167.
6. Huang WL, Beazley LD, Quinlivan JA, Evans SF, Newnham JP, Dunlop SA. Effect of corticosteroids on brain growth in fetal sheep. Obstet Gynaecol 1999;94:213-218.
7. Yeh TF, Lin YJ, Huang CC, Chen YJ, Lin CH, Lin HC, Heish WS, Lien YJ. Early dexamethasone therapy in preterm infants: a follow-up study. Pediatrics 1998;101:E7.
8. O'Shea TM, Kothadia JM, Klinepeter KL, Goldstein DJ, Jackson BG, Weaver RG 3rd, Dillard RG. Randomized placebo-controlled trial of a 42-day tapering course of dexamethasone to reduce the duration of ventilator dependency in very low birth weight infants: outcome of study participants at 1-year adjusted age. Pediatrics 1999;104:15-21.
9. Modi N, Lewis H, Al-Naqeeb N, Ajayi-Obe M, Dore CJ, Rutherford M. The effects of repeated antenatal glucocorticoid therapy on the developing brain. Pediatr Res 2001;50:581-585.
10. Jobe AH, Newnham JP, Willet KE, Moss TJ, Ervin MG, Padbury JF, Sly P, Ikegami M. Endotoxin-induced lung maturation in preterm lambs is not mediated by cortisol. Am J Respir Crit Care Med. 2000;162:1656-1661.
11. Pak SC, Na CS, Kim JS, Chae WS, Kamiya S, Wakatsuki D, Morinaka Y, Wilson L. The effect of acupuncture on uterine contraction induced by oxytocin. Am J Chin Med 2000;28:35-40.
12. Liao YY, Seto K, Saito H, Fujita M, Kawakami M. Effect of acupuncture on adrenocortical hormone production: I. Variation in the ability for adrenocortical hormone production in relation to the duration of acupuncture stimulation. Am J Chin Med 1979;4:362-371.
13. Pan B, Castro-Lopes JM, Coimbra A. Activation of anterior lobe cortitrophs by electroacupuncture or noxious stimulation in the anaesthetized rat, as shown by colocalozation of Fos protein with ACTH and β-endorphin and increasd hormone release. Brain Res Bull 1996;40:175-182.
14. Lee JH, Beitz AJ. The distribution of brain-stem and spinal cord nuclei associated with different frequencies of electroacupuncture analgesia. Pain 1993;52:11-28.
15. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-917.
16. Barlett GR. Phosphorous assay in column chromatography. J Biol Chem 1959;234:466-468.
17. Mason RJ, Nellenbogen J, Clements JA. Isolation of disaturated phosphatidylchioline with osmium tetroxide. J Lipid Res 1976;17:281-284.
18. Aebi H. Catalase in vitro. In: Bergmeyer HU, ed. Methods of Enzymatic Analysis, 2nd ed. New York & London: Academic Press, 1974: 673-677.
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-169.

20. Walsh-Sukys MC, Bauer RE, Cornell DJ, Friedman HG, Stork EK, Hack M. Severe respiratory failure in neonates: mortality and morbidity rates and neurodevelopmental outcomes. J Pediatr 1994;125:104–110.
21. Angus DC, Linde-Zwirble WT, Clermont G, Griffin MF, Clark RH. Epidemiology of neonatal respiratory failure in the United States: projections from California and New York. Am J Respir Crit Care Med 2001;164:1154–1160.
22. Farrell PM, Avery ME. Hyaline membrane disease. Am Rev Resp Dis 1975;111:657-688.
23. Tanswell AK, Wong L, Possmayer F, Freeman BA. The preterm rat: a model for studies of acute and chronic neonatal lung disease. Pediatr Res 1989;25:525-529.
24. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 1959;97:517-523.
25. Frank L, Sosenko IRS. Prenatal development of lung antioxidant enzymes in four species. J Pediatr 1987;10:106-110.

Table 1. Effects of maternal acupuncture treatment on fetal body weight and lung weight

Treatment	n	Body weight (g)	Lung weight (g)	Lung/body weight (%)
Control	48	2.12±0.05	0.078±0.003	3.66±0.08
Acupuncture (1 day)	67	2.20±0.05	0.087±0.003	3.86±0.09
Acupuncture (2 days)	65	2.04±0.07	0.069±0.003***	3.39±0.06***

Values are expressed as means±SEM. n is the number of fetuses tested.
***p<0.001 vs. acupuncture (1 day) group.

Table 2. Effects of maternal acupuncture treatment on saturated phosphatidylcholine and total phospholipids in fetal lung tissue of preterm rats

Treatment	n	Saturated phosphatidylcholine (μmol/g lung)	Total phospholipids (μmol/g lung)
Control	22	1.20±0.04	7.73±0.30
Acupuncture (1 day)	20	1.32±0.05	8.43±0.32
Acupuncture (2 days)	33	1.39±0.06*	9.29±0.27**

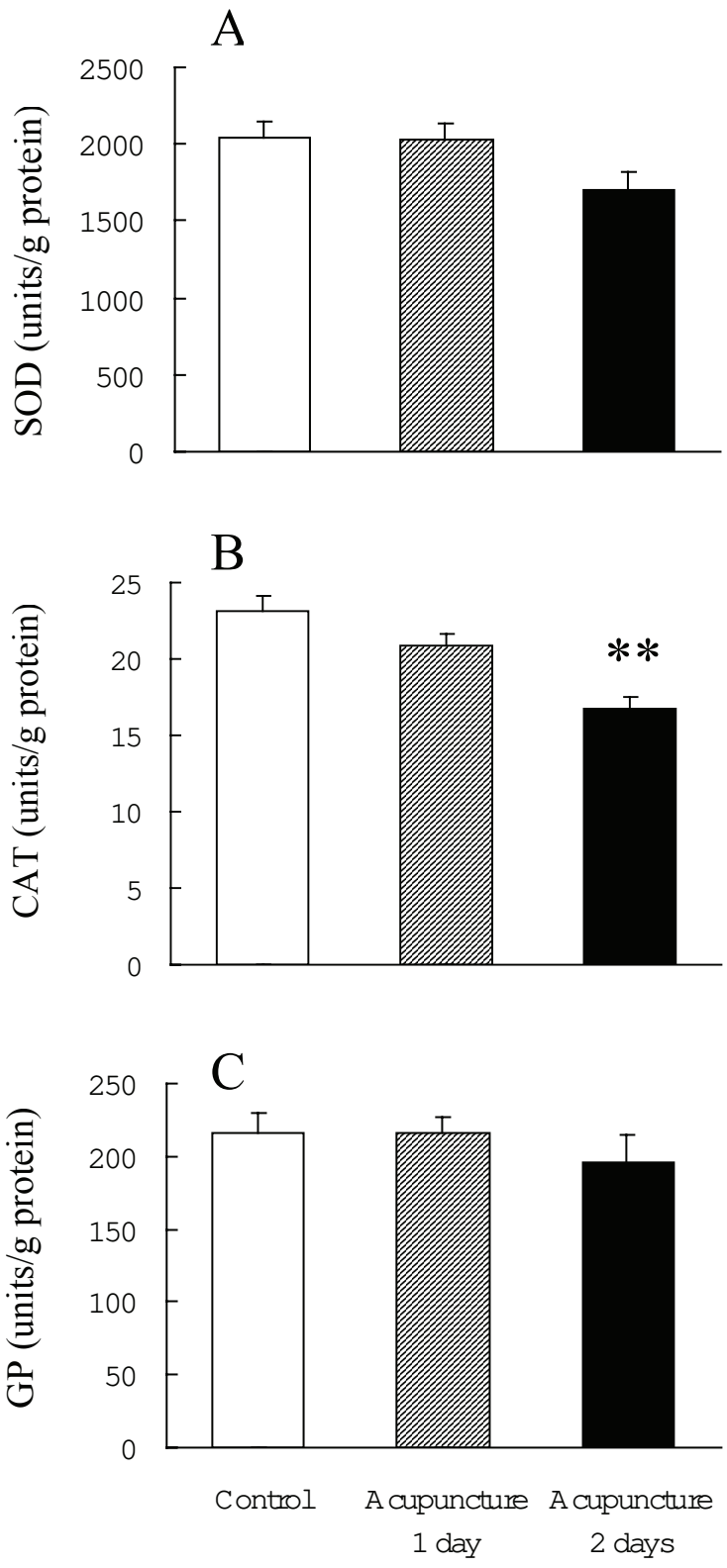
Values are expressed as means±SEM. n is the number of fetuses tested.
*p<0.05, **p<0.01 vs. control group.

Figure legends

Fig. 1. Effects of maternal acupuncture treatment on (A) total superoxide oxidase (SOD) (B) catalase (CAT), and (C) glutacythione peroxidase (GP) of fetal lung tissue in control (n=15), acupuncture (1 day, n=34), and acupuncture (2 days, n=24)-treated group. **p < 0.01 compared to the control and acupuncture (1 day)-treated groups.

Fig.2 Histological appearance of lungs in preterm rats delivered to (A) control, (B) acupuncture (1 day), and (C) acupuncture (2 days)-treated group (original magnification ×200, H&E stain). Extensive branching of intermediate airways and more epithelial tubules were noted in the acupuncture-treated groups.

Figure 1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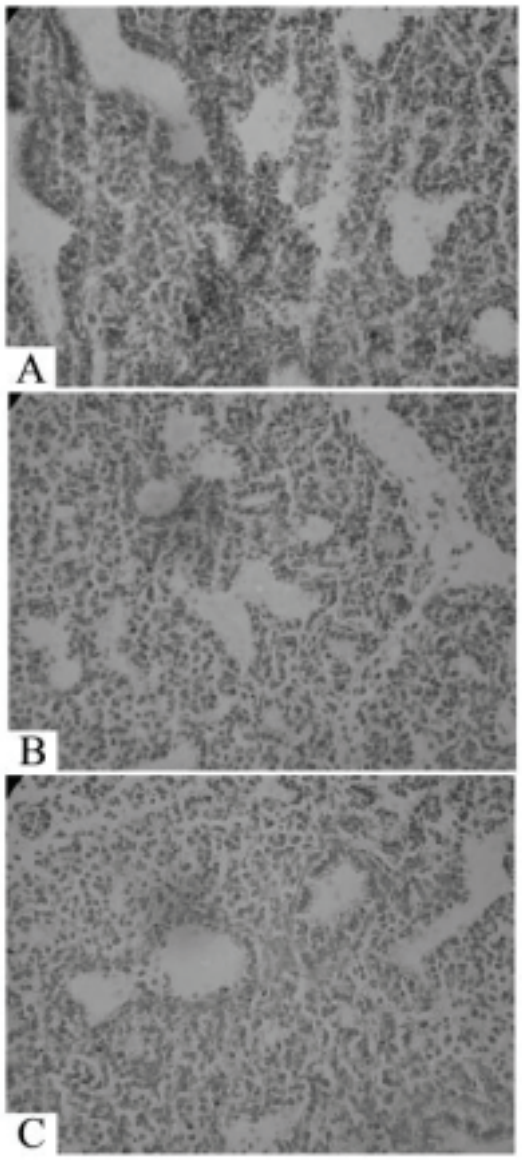
Figure 2