Maternal Acupuncture Effects on Surfactant and Antioxidant Enzymes in Preterm Rat Lungs

CHUNG-MING CHEN[1], LENG-FANG WANG[2], EIN-YIAO SHEN[3]

The objectives of this study are to evaluate the effects of maternal acupuncture treatment on lung maturation in preterm rats. Two stainless-steel needles were inserted into the Tsu-San-Li locus in the right hind leg of timed pregnant Sprague-Dawley rats for 30 min. One-day acupuncture-group mothers received electroacupuncture on day 18 of gestation. Two-day acupuncture-group mothers received electroacupuncture on days 17 and 18 of gestation. Control-group mothers received acupuncture at a site not contained in the Atlas of Human Acupuncture Points *on day 18 of pregnancy. On day 19 of gestation, pups in all dams were delivered by cesarean section. Maternal 2-day acupuncture treatment significantly increased total phospholipids in fetal lung tissue when compared with control and 1-day acupuncture-treated groups. Two-day acupuncture-treated fetuses had higher saturated phosphatidylcholine level in lung tissue although the difference did not reach statistical significance. Two-day acupuncture-treated fetuses had significantly lower superoxide dismutase, catalase, and glutathione peroxidase activities than did the control and 1-day acupuncture-treated fetuses. We conclude that maternal acupuncture treatment affects surfactant and antioxidant enzyme development in contrasting ways and may have both beneficial and potentially harmful effects on different aspects of lung development. (Acta Paediatr Tw 2005; **46**:206-11)*

Key words: acupuncture, antioxidant enzyme, lung maturation, surfactant

INTRODUCTION

Respiratory distress syndrome (RDS) is a 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 in decreasing the incidence and severity of RDS.[2] A meta-analysis of multiple randomized controlled trials showed that administration of glucocorticoids to mothers decreased the incidence of death and RDS by 50% in their preterm neonates.[3] It was suggested that there is no beneficial effect of glucocorticoid if more than 7 days have passed between treatment and delivery.[4] These findings persuaded obstetricians to repeat the course of glucocorticoid after 7 days in pregnant women at risk of preterm delivery who have 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.[5,6] Recent clinical study has revealed that repeated antenatal glucocorticoid treatment increases the incidence of neuromotor abnormalities.[7]

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.[8] This result indicates 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 be an alternative treatment for preterm labor.[9] Acupuncture at the locus, Tsu-San-Li (St-36), was found to stimulate

Department of Pediatrics, Taipei Medical University Hospital[1]; Department of Biochemistry, College of Medicine, Taipei Medical University[2]; Department of Pediatrics, Taipei Medical University Municipal Wangfan Hospital[3], Taipei, Taiwan.
Received: June 9, 2005. Revised: August 15, 2005. Accepted: September 15, 2005.
Address reprint requests to: Dr. Chung-Ming CHEN, Department of Pediatrics, Taipei Medical University Hospital, No. 252, Wu-Hsing Street, Taipei, Taiwan.
TEL: 886-2-27372181
FAX: 886-2-27360399
E-mail: cmchen@tmu.edu.tw

adrenocorticotropic hormone (ACTH) release and to enhance adrenal production of corticosterone in rats.[10,11] Fetal ACTH infusion accelerates lung maturation in preterm lambs.[12,13] Electrophysiological and histochemical studies indicate that acupuncture has significant influences on endocrinological organs.[14] Those studies support the notion that maternal acupuncture treatment may play a regulatory role in pulmonary surfactant system and antioxidant enzyme (AOE) development. We hypothesized that maternal acupuncture treatment will induce lung maturation in preterm rats and attempted to quantify the treatment effects by measuring pulmonary surfactant and AOE activities.

MATERIALS AND METHODS

Animals

The study was approved by the Institutional Animal Use Committee at Taipei Medical University and was performed with timed pregnant Sprague-Dawley rat mothers (vaginal smear positive, day 0; term, day 22). Rats subjected to electroacupuncture were anesthetized using intraperitoneal pentobarbital (25 mg/kg). Acupuncture-group mothers received electroacupuncture by inserting two stainless-steel needles into the Tsu-San-Li (St-36) in the right hind leg. 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.[15] A continuous current of 2 Hz was applied through the needles for 30 min. One-day acupuncture-group mothers received electroacupuncture on day 18 of gestation. Two-day acupuncture-group mothers received electroacupuncture on days 17 and 18 of gestation. Control-group mothers (non-point group) received acupuncture at a site not contained in the *Atlas of Human Acupuncture Points* on day 18 of pregnancy. On day 19 of gestation, the pups of all dams were delivered by cesarean section under intraperitoneal pentobarbital anesthesia (50 mg/kg). At delivery, the fetuses were weighed, and a blood sample was taken from each pregnant female before sacrifice. Plasma was immediately separated from blood cells by centrifugation and kept at–20℃ for ACTH measurements. Plasma ACTH was measured using a commercially available radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, TX, USA). The fetal lungs were then dissected free and weighed to the nearest 0.1 mg. Results are expressed as both lung weight and the ratio (%) of lung/body weight.

Measurement of Saturated Phosphatidylcholine in Lung Tissue

Lungs were homogenized and extracted with chloroform-methanol.[16] Lipid extracts from lung homogenates were treated with osmium tetroxide, and saturated phosphatidylcholine was recovered by alumina column chromatography and quantified by a phosphorus assay.[17,18] The values are expressed as mmoles per kilogram body weight.

Measurement of Antioxidant Enzyme Activities

Lungs were removed and frozen in liquid nitrogen without performing perfusion of the vasculature. The fetuses had been bled so that minimal residual blood remained in the lung tissue. Total superoxide dismutase activity was assayed by inhibition of formazan dye production (Randox Laboratories, 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 the hydrogen peroxide substrate, followed by spectrophotometric detection at 240 nm.[19] One unit of catalase equals 1 mmol hydrogen peroxide decomposed/min at 25℃.

Glutathione peroxidase activity was assayed spectrophotometrically at 340 nm as the rate of oxidation of reduced nicotinamide adenine dinucleotide phosphate.[20] Values of antioxidant enzyme activity are expressed as units per gram protein of lung tissue.

Statistical Analysis

Results are presented as means ± SEM. Statistically significant differences were analyzed by Kruskal-Wallis tests. Between-group comparisons were made by Mann-Whitney *U* tests. Differences were considered significant at $P < 0.05$.

RESULTS

There were 48 fetuses from 3 rats in the control group, 60 fetuses from 4 rats in the 1-day acupuncture group, and 65 fetuses from 4 rats in the 2-day acupuncture group. There were no significant differences 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 weighs ratio (%) are presented in Table 1. Body weights were comparable

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)	60	2.10±0.05	0.072±0.003	3.45±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.
**$P < 0.01$ vs. the control group.

among the 3 study groups. Premature rats treated with acupuncture twice (2 days) before delivery had a lower lung weight than did the control and 1-day acupuncture-treated animals. When adjusted for body weight, 2-day acupuncture-treated fetuses had a significantly lower lung/body weight ratio than did the control animals.

Adrenocorticotropic Hormone Levels in Maternal Serum Taken at Delivery

Maternal plasma ACTH levels were comparable between the 2 acupuncture-treated groups (Fig. 1), and were ~30% higher than that of the control group.

Effects of Maternal Acupuncture Treatment on Fetal Lung Phospholipid Contents

Maternal acupuncture treatments significantly increased total phospholipids in fetal lung tissue when compared with control animals (Table 2). Only 1-day acupuncture treatment significantly increased the level of saturated phosphatidylcholine in fetal lung tissue when compared with the control animals.

Effects of Maternal Acupuncture Treatment on Antioxidant Enzyme Activities in Fetal Lung Tissues

Lung superoxide dismutase and glutathione peroxidase activities were comparable between the control and 1-day acupuncture-treated fetuses (Fig. 2). Two-day acupuncture-treated fetuses had significantly lower superoxide dismutase, catalase, and glutathione peroxidase activities than did the control and 1-day

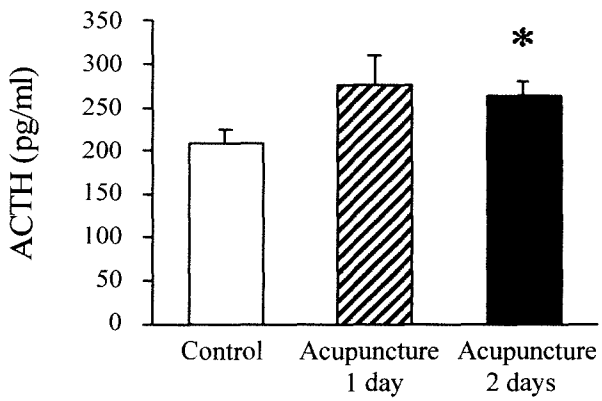

Fig. 1. Plasma adrenocorticotropic hormone (ACTH) levels in control and acupuncture- treated rats. Acupuncture-treated dams had higher ACTH levels than control dams, and the value reached statistical significance in the 2-day acupuncture group only (*$P < 0.05$).

acupuncture-treated fetuses. One-day acupuncture-treated fetuses had significantly lower catalase activities than did the control animals.

DISCUSSION

Neonatal respiratory failure is a serious clinical problem associated with high morbidity, mortality, and costs.[21,22] The major risk factor is premature birth and its associated RDS. The pathophysiology of RDS

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/kg)	Total phospholipid (μmol/kg)
Control	29	45.0±1.8	288.5±11.8
Acupuncture (1 day)	26	48.9±1.5*	311.5±8.9*
Acupuncture (2 days)	33	47.0±1.5	315.1±9.6*

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

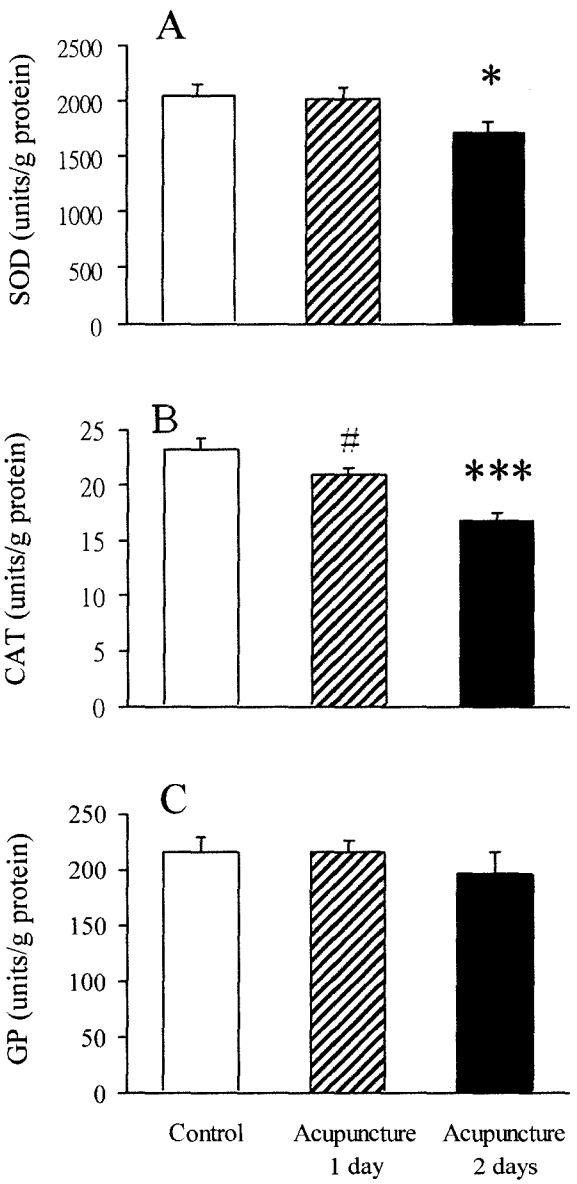

Fig. 2. Effects of maternal acupuncture treatment on (A) total superoxide oxidase (SOD) (B) catalase (CAT), and (C) glutacythione peroxidase (GP) of fetal lung tissue in the control (n=15), 1-day acupuncture-(n=34), and 2-day acupuncture (n=24)-treated groups (*$P < 0.05$, ***$P < 0.001$ vs. the control and 1-day acupuncture-treated groups. #$P < 0.05$ vs. the control group).

involves the immaturity of the lung structure and a deficit of surfactants. Glucocorticoids have been reported to accelerate fetal lung maturation and surfactant production.[23] Acupuncture at the locus, Tsu-San-Li (St-36), was found to stimulate ACTH release, and to enhance adrenal production of corticosterone in rats.[10,11] Therefore, we investigated the effects of maternal acupuncture treatment on preterm rat lungs, which have been shown to be a suitable model for the study of acute neonatal lung disease.[24] This study found a higher lung content of saturated phosphatidylcholine and total phospholipids in fetuses of mothers administered acupuncture than in control mothers. However, fetal lung antioxidant enzyme activities decreased after maternal 2-day acupuncture treatment.

Administration of dexamethasone (0.2 mg/kg/day) to pregnant rats on days 19 and 20 of gestation and delivery of fetuses on day 21 of gestation resulted in significantly decreased fetal body weight and organ/body weight ratios.[25] In this study, we found that maternal acupuncture treatment tended to decrease body weight and lung/body weight ratio in fetuses and caused a higher plasma ACTH level in dams. These results suggest that acupuncture has similar effects on the fetal body and lung growth as those of corticosteroids.

A deficiency in surfactants is central to the pathophysiology of RDS.[26] Pulmonary surfactants stabilize 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. The 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 the surfactant by weight. Therefore, we measured the saturated phosphatidylcholine content in fetal lung tissue. In this study, we found that the 2 maternal acupuncture (1-day and 2-day) treatments increased total phospholipids levels in fetal lung tissue. Plasma ACTH concentrations and total lung saturated phosphatidylcholine contents were higher in acupuncture-exposed mothers and fetuses, respectively, compared with control rats. The effects of ACTH on lung maturation are not mediated only by cortisol since fetal ACTH infusion restores lung distensibility and increases alveolar surfactant in the hypophysectomised ovine fetus while cortisol does not.[13] Our present data suggest that maternal acupuncture treatment can enhance fetal lung surfactant production, and that the mechanisms might operate through ACTH or corticosterone.

Developmental expression of AOE is critically important for neonates because lung cells are exposed to a sudden several-fold increase in oxygen concentration at birth, and premature newborns may be ventilated with high oxygen concentrations that can increase the risk

of toxicity to lung cells. The surfactant system and the AOE system of the fetal lung exhibit chronologically similar developmental patterns; both lung phospholipid contents and AOE activities increase dramatically during the final 10% to 15% of the gestation period in the rat.[27] The premature infant with surfactant deficiency may be further compromised by an underdeveloped AOE system when exposed to high concentrations of oxygen. Accelerated maturation of the AOE system in the fetal lung may be of clinical benefit. The principal intracellular AOEs are superoxide dismutase, catalase, and glutathione peroxidase. In this study, we found that maternal 2-day acupuncture treatment depressed AOE maturation in premature fetal lung. The clinical impact and exact mechanism of this depression is not known. Possible mechanisms are decreased enzyme synthesis, increased enzyme turnover, or inactivation of enzymes.

In this study, changes in maternal plasma ACTH levels after acupuncture treatment were similar to those reported by Pan et al.[11] These results suggest that acupuncture has some hormonal effects and indicate that the lung maturational effect of acupuncture might be related partly to corticosterone. Maternal acupuncture treatment significantly increased the phospholipids content in the fetal lung, but in contrast to corticosteroid treatment, it decreased AOE activities of lung tissue. These results suggest that acupuncture and corticosteroids accelerate fetal lung maturation through different mechanisms. Acupuncture causes the release of some of the anterior pituitary hormones and has significant influences on the thyroid and adrenal glands, while thyroid hormone has been reported to depress fetal AOE system development.[13,28]

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

ACKNOWLEDGEMENTS: This work was supported by a grant from the National Science Council, Taiwan (NSC92-2314-B-038 -024).

REFERENCES

1. Guyer B, Martin JA, MacDorman MF, Anderson RN, Strobino DM. Annual summary of vital statistics-1996. Pediatrics 1996; **100**:905-18.
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**:322-35.
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**:401-10.
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**:157-67.
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**:213-8.
7. 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**:581-5.
8. 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**:1656-61.
9. 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.
10. 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**:362-71.
11. 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 b-endorphin and increasd hormone release. Brain Res Bull 1996; **40**:175-82.
12. Kendal JZ, Rivera-Alcina ME, Plopper CG, Weir AJ, Randall GC, Saldana-Gautier L. A morphometric study of lung maturation in the adrenocortitrophin-infused fetal lamb. Biol Neonate 1991; **60**:258-72.
13. Liggins GC, Kitterman JA, Campos GA, Clements JA, Forster CS, Lee CH, Creasy RK. Pulmonary maturation in the hypophysectomised ovine fetus. Differential responses to adrenocorticotrophin and cortisol. J Dev Phsyiol 1981; **3**:1-14.
14. Xie QW. Endocrinological basis of acupuncture. Am J Chin Med 1981; **9**:298-304.
15. 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.
16. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; **37**:911-7.
17. Barlett GR. Phosphorous assay in column chromatography. J Biol Chem 1959; **234**:466-8.
18. Mason RJ, Nellenbogen J, Clements JA. Isolation of disaturated phosphatidylchioline with osmium tetroxide. J Lipid Res 1976; **17**:281-4.
19. Aebi H. Catalase in vitro. In: Bergmeyer HU, ed. Methods of enzymatic analysis. 2nd ed. New York and London: Academic Press, 1974. 673-7.
20. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; **70**:158-69.
21. 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**:104-10.

22. 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**:1154-60.
23. Farrell PM, Avery ME. Hyaline membrane disease. Am Rev Resp Dis 1975; **111**:657-88.
24. 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**:525-9.
25. Frank L, Roberts RJ. Effects of low-dose prenatal corticosteroid administration on the premature rat. Biol Neonate 1979; **36**:1-9.
26. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 1959; **97**:517-23.
27. Frank L, Sosenko IRS. Prenatal development of lung antioxidant enzymes in four species. J Pediatr 1987; **10**: 106-10.
28. Walther FJ, Ikegami M, Warburton D, Polk DH. Corticosteroids, thyroid-releasing hormone, and antioxidant enzymes in preterm lamb lungs. Pediatr Res 1991; **30**:518-21.

母鼠針灸治療對早產老鼠肺臟肺表面張力素及抗氧化酵素的作用

陳中明[1]　汪棱芳[2]　沈淵瑤[3]

這項研究的目的是評估母鼠針灸治療對早產老鼠肺成熟度的影響。針灸治療組以二支不鏽鋼針針灸懷孕大白鼠的後腿穴道-足三里(Tsu-San-Li)30分鐘。針灸一天組的母鼠在懷孕第18天時接受針灸。針灸兩天組的母鼠在第17及18天時接受針灸。對照組母鼠則在懷孕第18天時於不包含在人體穴位圖集裡的一個位置接受針刺。在懷孕第19天時，以剖腹產手術取出小鼠。針灸兩天組的母鼠其胎鼠的肺組織總phospholipids量顯著地比對照組和針灸一天組母鼠的胎鼠高；而其胎鼠肺組織的superoxide dismutase、catalase和glutathione peroxidase活性卻顯著地比對照組和針灸一天組母鼠的胎鼠低。我們推定母鼠針灸治療對肺臟肺表面張力素及抗氧化酵素的作用是相反的，並且可能同時在肺臟發育上具有有利和潛在有害的作用。

關鍵字：針灸，抗氧化酵素，肺成熟，肺表面張力素