行政院國家科學委員會補助專題研究計畫成果報告

產前金線連和皮質類固醇對早產老鼠肺臟成熟的作用

計畫類別：√ 個別型計畫　　整合型計畫
計畫編號：NSC90-2314-B-038-011
執行期間：90 年 8 月 1 日至 91 年 7 月 31 日

計畫主持人：陳中明
協同主持人：鄭可大,許政成,蘇柏誠

本成果報告包括以下應繳交之附件：
- 赴國外出差或研習心得報告一份
- 赴大陸地區出差或研習心得報告一份
- 出席國際學術會議心得報告及發表之論文各一份
- 國際合作研究計畫國外研究報告書一份

執行單位：臺北醫學大學

中　華　民　國九十一年十月三十日

計畫中文摘要：

關鍵詞：金線連，皮質類固醇，肺臟成熟度，肺表面張力素

雖然近年來周產期及新生兒照護有相當的進步，但是呼吸窘迫症候群仍然是造成早產兒罹病率和死亡率的主要原因。母親有早產的徵兆時，給予注射皮質類固醇，已經被廣泛研究認為可以減輕及降低早產兒呼吸窘迫症候群的嚴重度及發生率。一項隨機、多中心的研究結果，指出母親皮質類固醇的治療可以降低呼吸窘迫症候群一半的死亡率及發生率。但是動物實驗卻發現，母體皮質類固醇的治療對於胎兒尚未成熟腦部的生長及發育有不好的影響。最近的臨床試驗也發現，早產兒出生後給予皮質類固醇以預防慢性肺疾病，會增加早產兒日後發生神經異常的機會。金線連（*Anoectochilus formosanus*）有抗發炎，治療高血壓、糖尿病的療效。老鼠實驗發現金線連的萃取物有極高的抗氧化及保護肝臟的作用。臨床上金線連也有促進小孩子生長及發育的作用。我們的研究人員之一發現金線連的萃取物可以使小老鼠腦下垂體中分泌生長激素的細胞數目增加。由於母體皮質類固醇的治療並不能完全百分之百地預防呼吸窘迫症候群的發生，加上它有神經性的副作用，這些因素促使我們從中藥材中找尋可以刺激肺臟成熟的物質。這計畫中我們假設金線連可以刺激胎兒的肺臟成熟，並且與已知有肺臟成熟作用的皮質類固醇比較。我們使用配對懷孕的大白鼠（未曾懷孕過，陰道抹片陽性為第 0 天，足月 22 天），金線連組由懷孕第 12 天至 18 天止共七天，每天餵食金線連（300 毫克/公斤體重）；皮質類固醇組在懷孕第 18 天時由腹腔注射皮質類固醇（0.2 毫克/公斤體重）；控制組只注射等量的生理食鹽水。在懷孕第 19 天時，所有母鼠經由剖腹產取出胎兒，我們使用型態學及生化學來評估各組的治療效果。金線連及皮質類固醇治療可增加胎兒肺臟飽和磷脂酸以及母鼠血清生長激素的量，組織切片發現金線連及皮質類固醇組有較成熟的肺臟。這些結果顯示產前金線連治療有促進胎兒肺臟發育的作用。

計畫英文摘要：

Respiratory distress syndrome is a major cause of morbidity and mortality in preterm neonates. *Anoectochilus formosanus* has been used to promote growth and development of children. We hypothesized that antenatal *A. formosanus* treatment would induce early lung maturation. This study was performed with timed pregnant Srague-Dawley rat mothers. *A. formosanus* group mothers were tube fed *A. formosanus* extract (300 mg/kg/day) for 7 days from days 12 to 18 of gestation. Dexamethasone group mothers were injected intraperitoneally with dexamethasone (0.2 mg/kg) in saline on day 18 of pregnancy. Control group mothers were similarly injected with saline alone. On day 19 of gestation, the fetuses were delivered by cesarean section. *A. formosanus* treatment significantly increased fetal lung/body weight ratio than dexamethasone treatment. Saturated phosphatidylcholine levels in fetal lung tissue and growth hormone levels in maternal serum were significantly increased in the *A. formosanus* and dexamethasone-treated groups compared with controls. Histological appearance of the preterm rat lungs revealed that extensive branching of intermediate airways and denser mesenchyme, more epithelial tubules and PAS-positive vacuoles were noted in the dexamethasone and *A. formosanus* groups when compared with the control group. These results suggest that antenatal *A. formosanus* treatment may play a role in accelerating fetal rat lung maturation.

Key words: *Anoectochilus formosanus*, glucocorticoid, 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 used extensively to decrease the incidence and severity of RDS.[2,3] 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] However, there is considerable evidence from experimental animals that glucocorticoids have an adverse effect on the growth and development of the immature brain.[5-7]

A. formosanus has the effects of anti-inflammation and liver protection and has been reported to treat underdeveloped child, hypertension, diabetes mellitus, tuberculosis, and osteopenia in ovariectomized rats.[8,9] Intraamniotic injection of dexamethasone induced lung maturation and this effect was independent of cortisol level.[10] This result indicates that there are other potential lung maturation factors that remain to be identified. One of the authors found that water extract of *A. formosanus* increased the numbers of somatotrophin producing cells in the pituitary gland of mice. We hypothesized that *A. formosanus* would increase the secretion of pituitary hormones and then induce lung maturation in the preterm rats.

Materials and Methods

Animals

This study was performed with timed pregnant Srague-Dawley rat mothers. *A. formosanus* group mothers were tube fed the extract of *A. formosanus* (300 mg/kg/day) for 7 days from days 12 to 18 of gestation. Dexamethasone group mothers were injected intraperitoneally with dexamethasone (0.2 mg/kg) in saline on day 18 of pregnancy. This dexamethasone dose was previously shown to improve postnatal lung function of preterm rats.[11] Control group mothers were similarly injected with saline alone. On day 19 of gestation, all the dams were anesthetized with pentobarbital and delivered by cesarean section.

Preparation of the extract of *Anoectochilus formosanus*

The species of the plant was authenticated and a voucher specimen (Accession No. SP 9703010) was deposited in the Herbarium of the Taipei Medical University (Cheng et al., 1998). The extract was obtained by filtration through the cheesecloth to remove the debris, followed by centrifugation at 1,2000 rpm for 1 hour at 4℃.

Biochemical analysis

Pieces of the lung were homogenized and aliquots were extracted with chloroform-methanol[13] and treated with osmium tetroxide and saturated phosphatidylcholine was recovered by alumina column chromatography and was quantified by phosphorus assay.[14,15]

Morphological analysis

Serial lung sections were cut at 4-μm thickness and stained with hematoxylin and eosin.

Results

There were 27 fetuses from 3 rats in the control group, 40 fetuses from 3 rats in the dexamethasone group, and 50 fetuses from 5 rats in the *A. formosanus* group.

Table 1. Effects of maternal treatment on fetal body weight and organ weight/body weight ratio.

Treatment	n	Body weight (g)	Brain/ Body weight (%)	Heart/ Body weight (%)	Lung/ Body weight (%)	Kidney/ Body weight (%)	Liver/ Body weight (%)
Control	27	2.48 ± 0.07	4.47 ± 0.12	0.48 ± 0.01	3.91 ± 0.09	0.81 ± 0.02*	8.67 ± 0.21
Dexamethasone	40	2.43 ± 0.05	4.56 ± 0.11	0.49 ± 0.01	3.78 ± 0.09	0.76 ± 0.02	8.18 ± 0.19
A. formosanus	50	2.38 ± 0.06	4.78 ± 0.15$^{\#}$	0.51 ± 0.02	4.11 ± 0.07*	0.83 ± 0.02*	8.23 ± 0.19

Values are expressed as mean ± SEM.

$^{*}p < 0.05$ compared to dexamethasone group, $^{\#}p < 0.05$ compared to control group.

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

Treatment	n	Saturated phosphatidylcholine (μmol/kg)	Total phospholipid (μmol/kg)
Control	19	69.7 ± 2.7	405.8 ± 21.4
Dexamethasone	30	77.2 ± 3.0*	465.1 ± 26.4
A. formosanus	36	78.8 ± 1.7*	483.9 ± 13.0*

Values are expressed ad mean ± SEM. n is number of fetuses tested.
$^*p < 0.05$ compared to control group.

Figure 1. Effects of maternal treatment of *A. formosanus* on total protein contents of fetal lung tissue in preterm rats. Numbers in parentheses are numbers of fetuses tested. *p<0.05 compared to dexamethasone-treated group.

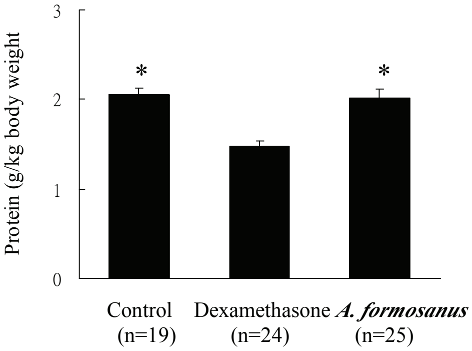


Figure 2. Growth hormone levels in maternal serum taken at delivery. *p < 0.05 compared to control group.

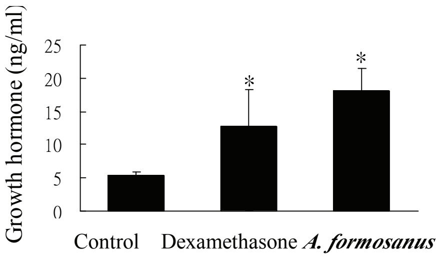
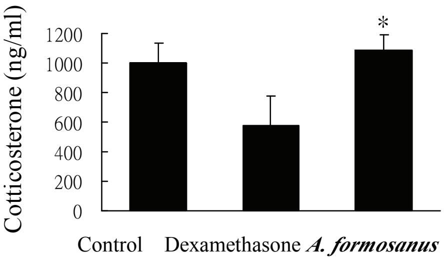
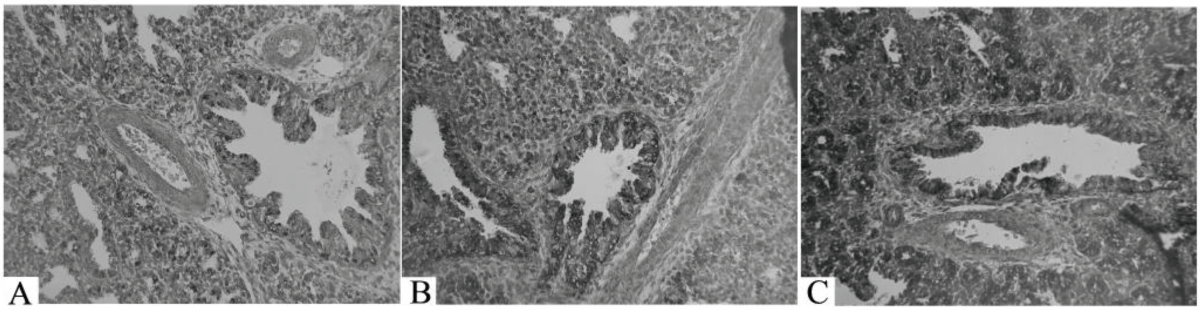


Figure 3. Corticosterone levels in maternal serum taken at delivery. *p < 0.05 compared to dexamethasone group.





Histology (Figure 4)
Histological appearance of lungs in the preterm rats delivered to (A) control, (B) dexamethasone, and (C) *A. formosanus* group mothers (original magnification ×200, H&E stain). Extensive branching of intermediate airways and denser mesenchyme, more epithelial tubules and PAS-positive vacuoles were noted in the dexamethasone and *A. formosanus* groups compared with the control group.

Discussion

Neonatal respiratory failure is a serious clinical problem associated with high morbidity, mortality, and cost.[16,17] The major risk factor is premature birth and its associated RDS. The pathophysiology of RDS is immature lung structure and deficient surfactant. Glucocorticoids have been reported to accelerate fetal lung maturation and surfactant production.[18] However, there is considerable evidence that glucocorticoids have an adverse effect on the growth and development of the immature brain.[5-7] *A. formosanus* extract has been reported to promote the growth of underdeveloped child and increase the numbers of somatotrophin producing cells in the pituitary gland of mice (personal communication). Therefore, we investigated the effects of antenatal *A. formosanus* treatment in preterm rat that has been shown to be a suitable model for study of acute neonatal lung disease.[19] This study found that higher lung saturated phosphatidylcholine content and more mature lung histology in the fetus of mothers administered *A. formosanus* and dexamethasone than in control mothers. These results show that *A. formosanus* has effects similar to dexamethasone.

Administration of dexamethasone (0.2 mg/kg/day) to the pregnant rat on days 19 and 20 of gestation and delivered the fetus on day 21 of gestation resulted in significantly decreased body weight and organ/body weight ratios.[20] In this study, we found that antenatal dexamethasone treatment decreased body weight, kidney/body weight, liver/body weight, and lung/body weight ratios in the fetuses, but this was not statistically significant compared to control animals. The disparity between these studies might be due to different glucocorticoid dosage. Antenatal *A. formosanus* treatment has the same effects as dexamethasone on body weight and liver/body weight ratio but it significantly increased lung/body weight and kidney/body weight ratios compared with the dexamethasone-treated group. It appears that *A. formosanus* promotes kidney and lung growth in this premature animal model.

This study we found that total protein content was significantly decreased in fetal lung tissue of dexamethasone-treated mothers compared with control and *A. formosanus*-treated mothers. A similar finding was noted in the rats delivered on day 18 of gestation whose mothers was treated with dexamethasone on day 17 of gestation.[21] The reduced protein content reflects decreased protein leak in fetal lungs.[22] We speculated that the reduced total protein content and lung/body weight ratio in the dexamethasone-treated group in this study was partly due to decreased capillary-alveolar permeability.

Deficiency of surfactant is central to the pathophysiology of RDS.[23] Lung alveoli are lined with surfactant, which prevent their collapse on expiration and which is essential for normal postnatal lung function. Therefore, we measured saturated phosphatidylcholine content in fetal lung tissue because it is the major surface tension lowering component of surfactant and made up approximately 45% of lung surfactant by weight. In this study, we found that antenatal *A. formosanus* treatment increased total phospholipid in fetal lung tissue. Dexamethasone treatment tended to have similar effect. Saturated phosphatidylcholine levels in fetal lung tissue were significantly increased by antenatal dexamethasone and *A. formosanus* treatments. These results suggest that *A. formosanus* can enhance surfactant production as glucocorticoid does.

Glucocorticoids regulate growth hormone secretion by modulating both hypothalamic and pituitary function.[24] In vitro studies have shown that glucocorticoids increase growth hormone gene expression and enhance growth hormone release from cultured rat pituitary somatotrophs.[25,26] Administration of dexamethasone at late gestation induced growth hormone expression in fetal rat pituitary gland.[27] We found that *A. formosanus* treatment significantly increased growth hormone levels to that of dexamethasone treatment. Growth hormone appears to have important effects on fetal metabolism and development. We speculated that the increased growth hormone might stimulate the production of pulmonary surfactant. Maternal corticosterone levels were comparable in the control and *A. formosanus* groups. This result indicates that lung maturational effect of *A. formosanus* occurred independently of corticosterone. Antenatal *A. formosanus* treatment significantly increased lung saturated phosphatidylcholine content, but in contrast to dexamethasone treatment it increased lung/body weight ratio and total protein content of lung tissue. These results suggest that *A. formosanus* and dexamethasone accelerate fetal lung maturation through different mechanisms.

In conclusion, antenatal treatment of *A. formosanus* extract increased saturated phosphatidylcholine content and improved morphology in the fetal lung. These results suggest that antenatal *A. formosanus* may play a role in accelerating fetal rat lung maturation. Future studies are needed to measure in vivo the pulmonary function of antenatal *A. formosanus* extract treatment. The potential therapeutic advantages of this extract seem worthy of further investigation in larger experiments.

References

1. Guyer, B., Martin, J.A., MacDorman, M.F., Anderson, R.N., Strobino, D.M.: Annual summary of vital statistics-1996. Pediatrics 100: 905-918, 1996.
2. Ballard, P. L.: Hormonal regulation of pulmonary surfactant. Endocr. Rev. 10: 165-181, 1989.
3. Crowley, P.: Antenatal corticosteroid therapy: a meta-analysis of the randomized trials－1972-1994. Am. J. Obstet. Gynecol. 173: 322-335, 1995.
4. Gamsu, H.R., Mullinger, B.M., Donnai, P., Dash, C.H.: Antenatal administration of betamethasone to prevent RDS in preterm infants: report of a UK multicentre trial. Br. J. Obstet. Gynecol. 96: 401-410, 1989.
5. Uno, H., Lohmiller, L., Thieme, C., Kemnitz, J.W., Engle, M.J., Roecker, E.B., Farrel, P.M.: Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. 1. Hippocampus. Dev. Brain Res. 53: 157-167, 1990.
6. Huang, W.L., Beazley, L.D., Quinlivan, J.A., Evans, S.F., Newnham, J.P., Dunlop, S.A.: Effect of corticosteroids on brain growth in fetal sheep. Obstet. Gynaecol. 94: 213-218, 1999.
7. French, N.P., Hagan, R., Evans, S.F., Godfrey, M., Newnham, J.P.: Repeated antenatal corticosteroids: size at birth and subsequent development. Am. J. Obstet. Gynecol. 180: 114-121, 1999.
8. Lin, C.C., Huang, P.C., Lin, J.M.: Antioxidant and hepatoprotective effects of *Anoectochilus formosanus* and *Gynostemma pentaphyllum*. Am. J. Chin. Med. 28: 87-96, 2000.
9. Shih, C.C., Wu, Y.W., Lin, W.C.: Ameliorative effects of Anoectochilus formosanus extract on osteopenia in ovariectomized rats. J. Ethnopharmacology 77: 233-238, 2001.
10. Jobe, A.H., Newnham, J.P., Willet, K.E., Moss, T.J., Ervin, M.G., Padbury, J.F., Sly, P., Ikegami, M.: Endotoxin-induced lung maturation in preterm lambs is not mediated by cortisol. Am. J. Respir. Crit. Care Med. 162: 1656-1661, 2000.
11. Post, M., Barsoumian, A., Barry, T.S.: The cellular mechanism of glucocorticoid

acceleration of fetal lung maturation. J. Biol. Chem. 261: 2179-2184, 1986.

12. Cheng, K.T., Fu, L.C., Wang, C.S., Hsu, F.L., Tsay, H.S.: Identification of *Anoectochilus formosanus* and *Anoectochilus koshunensis* species with RAPD markers. Planta Med. 64: 46-49, 1998.
13. Bligh, EG., Dyer, W.J.: A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917, 1959.
14. Barlett, G.R.: Phosphorous assay in column chromatography. J. Biol. Chem. 234: 466-468, 1959.
15. Mason, R.J., Nellenbogen, J., Clements, J.A.: Isolation of disaturated phosphatidylchioline with osmium tetroxide. J. Lipid Res. 17: 281-284, 1976.
16. Angus, D.C., Linde-Zwirble, W.T., Clermont, G., Griffin, M.F., Clark, R.H.: Epidemiology of neonatal respiratory failure in the United States: projections from California and New York. Am. J. Respir. Crit. Care Med. 164: 1154–1160, 2001.
17. Walsh-Sukys, M.C., Bauer, R.E., Cornell, D.J., Friedman, H.G., Stork, E.K., Hack, M.: Severe respiratory failure in neonates: mortality and morbidity rates and neurodevelopmental outcomes. J. Pediatr. 125: 104–110, 1994.
18. Farrell, P.M., Avery, M.E.: Hyaline membrane disease. Am. Rev. Resp. Dis. 111: 657-688, 1975.
19. Tanswell, A.K., Wong, L., Possmayer, F., Freeman, B.A.: The preterm rat: a model for studies of acute and chronic neonatal lung disease. Pediatr. Res. 25: 525-529, 1989.
20. Frank, L., Roberts, R.J.: Effects of low-dose prenatal corticosteroid administration on the premature rat. Biol. Neonate 36: 1-9, 1979.
21. Schellenberg, J.C., Liggins, G.C., Stewart, A.W.: Growth, elastin concentration, and collagen concentration of perinatal rat lung: effects of dexamethasone. Pediatr. Res. 21: 603-607, 1987.
22. Ikegami, M., Berry, D., ElKady, T., Pettenazzo, A., Seidner, S., Jobe, A.: Corticosteroids and surfactant change lung function and protein leaks in the lungs of ventilated premature rabbits. J. Clin. Invest. 79: 1371-1378, 1987.
23. Avery, M.E., Mead, J.: Surface properties in relation to atelectasis and hyaline membrane disease. Am. J. Dis. Child 97: 517-523, 1959.
24. Thakore, J.H., Dinan, T.G.: Growth hormone secretion: the role of glucocorticoids. Life Sci. 55: 1083-1099, 1994.
25. Evans, R.M., Bimberg, N.C., Rosenfeld, M.G.: Glucocorticoid and thyroid hormones transcriptionally regulate growth hormone gene expression. Proc. Natl. Acad. Sci. USA 79; 7659-7663, 1992.
26. Miller, T.L., Mayo, K.E.: Glucocorticoids regulate pituitary growth hormone-releasing hormone receptor messenger ribonucleic acid expression. Endocrinology 138: 2458-2465, 1997.
27. Nogami H., Tachibana T. Dexamethasone induces advanced growth hormone expression in the fetal rat pituitary gland in vivo. Endocrinology 132: 517-523, 1993.