Influence of N-acetyl-5-methoxytryptamine on Fetal Lung Maturation in Experimental Preterm Delivery Model*

Önder ÇELİK[1], Şeyma HASÇALIK[1], Mustafa TAMSER[2], Hale KIRIMLIOĞLU[3], M. Atahan GÜVEN[4], M. İlker DOĞRU[5], A. Kocagün DOĞRU[5], Yusuf TÜRKÖZ[6], Muhittin YÜREKLİ[5]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Inönü University, Malatya, Turkey
[2] Department of Physiology, Faculty of Veterinary, Fırat University, Elazığ, Turkey
[3]Department of Pathology, Faculty of Medicine, Inönü University, Malatya, Turkey
[4]Department of Obstetrics and Gynecology, Faculty of Medicine, Sütçüimam University, K. Maraş, Turkey
[5]Department of Molecular Biology, Faculty of Arts & Science, Inönü University, Malatya, Turkey
[6]Department of Clinical Biochemistry, Faculty of Medicine, Inönü University, Malatya, Turkey

Abstract

Objective: The study was planned to investigate the effect of pharmacological concentrations of melatonin on maturation of the fetal lung in preterm delivered rats.

Materials and Methods: Thirty pregnant rats were divided into three groups. The study group received either betametasone (n=10) or melatonin (n=10) between 17th and 18th days of gestation, whereas the third group (n=10) served as control. Pregnancies were terminated on the 21st days of gestation in the control group and on the 19th days of gestation in the betametasone and melatonin groups. Amniotic fluid adrenomedullin, nitric oxide (NO) concentrations and lamellar body count were determined and results compared with each other.

Results: The adrenomedullin levels of the melatonin, betametasone and control groups were found to be 29.84±3.45 pg/ml, 43.15±6.63 pg/ml and 49.39±12.93 pg/ml, respectively. The mean lamellar body count was found to be five fold higher in the control group than that in the melatonin group; and, it was 4.1 fold higher in the betametasone group than that in the melatonin group. There were no significant difference among the groups regarding the NO levels. Lamellar body counts of the betametasone and control groups were higher than those which have received melatonin, but the difference was not statistically significant ($p>0.05$). Masson's trichrome stained section were similar in all groups.

Discussion: Melatonin was found to maintain the 19th day amniotic fluid adrenomedullin, lamellar body and NO levels within the 21st day limits. It is possible that adrenomedullin secretion was modulated by exogenous melatonin administration and melatonin may have been involved in the regulation of fetal lung maturation.

Keywords: fetal lung maturation, melatonin, betametasone, adrenomedullin, NO, lamellar body

Özet

Deneysel Erken Doğum Modelinde N-asetil-5-metoksitriptaminin Fetal Akciğer Matürasyonu Üzerine Etkisi

Amaç: Bu çalışma, sıçanların erken doğum modelinde, farmakolojik doz melatoninin fetüste akciğer matürasyonu üzerine etkisini araştırmak için planlandı.

Materyal ve Metot: Otuz Wistar-albino gebe sıçan üç gruba bölündü. 1. Grup: Melatonin (n=10), 2. Grup: Betametazon (n=10), 3. Grup: Kontrol (tedavi almayan term gebeler) (n=10). Melatonin ve betametazon grubundaki sıçanların her biri gebeliğin 17. ve 18. günlerinde (preterm dönem) iki doz tedavi aldılar. Kontrol grubunda gebelikler 21. günde (term), betametazon ve melatonin grubunda ise 19. günde histeretomi ile sonlandırıldı. Tüm sıçanların amniyotik sıvıları alınarak adrenomedulin, NO ve lamellar cisim düzeyleri ölçüldü. Fetal akciğerler de histolojik değerlendirme için cerrahi olarak çıkarıldı.

Corresponding Author: Dr. Önder Çelik
İnönü Üniversitesi Tıp Fakültesi, Kadın Hastalıkları ve Doğum AD,
44069 Malatya, Türkiye
Phone : +90 422 341 06 60
Fax : +90 422 341 07 28
E-mail : oncelik@inonu.edu.tr

350

Çelik Ö, Hasçalık Ş, Tamser M et al., Melatonin and Fetal Lung Maturation, J Turkish-German Gynecol Assoc, Vol. 8(4); 2007:350-355

Sonuçlar: Melatonin, betametazon ve kontrol grubunda adrenomedulin düzeyleri sırası ile 29.84±3.45 pg/ml, 43.15±6.63 pg/ml ve 49.39±12.93 pg/ml olarak saptandı. Melatonin ve betametazon grubunda gebelikler preterm dönemde sonlandırılmasına rağmen, gruplar arasında adrenomedulin konsantrasyonları açısından istatistiksel anlamlılık saptanmadı (*p*>0.05). Benzer şekilde NO düzeyleri de üç grupta aynı olup aralarında fark saptanmadı (*p*>0.05). Kontrol ve betametazon gruplarının lamellar cisim düzeyleri melatonin grubundan, sırasıyla 5 ve 4.1 kat yüksek bulundu. Masson trikrom boyalı kesitlerde alveol kolajen içerikleri her grupta eşit derecede azalmış olarak saptandı.

Tartışma: Melatonin uygulaması ile 19. gün amniyotik sıvı adrenomedulin, lamellar cisim ve NO düzeylerinin 21. gün limitlerinde tutulması sağlanmıştır. Melatonin, olasılıkla adrenomedulin salınımını düzenleyerek fetal akciğer matürasyonunun gelişimine katkıda bulunmaktadır.

Anahtar sözcükler: fetal akciğer matürasyonu, melatonin, betametazon, adrenomedulin, nitrik oksit, lamellar cisim

Introduction

Adrenomedullin is a hypotensive peptide, first identified in extracts of phaeochromocytoma (1). Pregnancy is associated with increased circulating adrenomedullin concentrations in both the rat (2) and man (3,4). The plasma concentration of adrenomedullin has been reported to increase progressively from the first to third trimester (5,6). In addition to those in the peripheral plasma, significant levels of adrenomedullin have been measured in umbilical vein (3) and amniotic fluid (3). Adrenomedullin has been localized to the epithelium of the amnion, chorion and extravillous trophoblasts (7,8). Adrenomedullin has a profound and prolonged vasodilating effect in the fetal pulmonary circulation (9) and mRNA for adrenomedullin has been detected in the columnar epithelium of normal human lung (10). It is also possible that vasodilation induced by adrenomedullin may be mediated via nitric oxide (NO) release from the endothelium. Studies have shown that NO mediates responses to adrenomedullin in the rat pulmonary vascular areas (11,12). Using the isolated perfused rat lung, it has been shown that human adrenomedullin decreases in preconstricted vascular tone (13).

To date, no ideal substance has been found to increase fetal lung maturation in preterm delivery. Adrenomedullin production by vascular smooth muscle cells is increased by a range of cytokines, growth factors, and hormones, including dexamethasone (14,15). Production of adrenomedullin in bronchial cells during fetal lung development suggests a role of this peptide in the regulation of bronchial tone and neonatal alveolar ventilation. The pineal secretory product, melatonin (N-acetyl-5-methoxytryptamine), is known to exhibit free radical scavenging ability (16). As melatonin is easily administered and absorbed, its administration leads to effective therapeutic tissue levels with significant systemic drug distribution with a high therapeutic index (17,18). On the other hand, no one has investigated the effect of pharmacological concentrations of melatonin on adrenomedullin secretion and fetal lung maturation. To investigate whether treatment with melatonin improve the fetal lung maturation we measured adrenomedullin, NO and lamellar body count in amniotic fluid of preterm delivered rats.

Materials and Methods

This experimental study was carried out on 30 Wistar albino female rats of 250-300 g body weight, maintained in individual cages under controlled temperature (21-23°C) at relative humidity, and day light, and fed *ad libitum*. Attempts at conception were undertaken for up to 5 consecutive days (one estrus cycle). The timed conception was identified as gestational day 0 by the presence of a copulatory plug during a 2 hours exposure with the male. The pregnant females were then housed individually throughout gestation. At 15th day of gestation, the gravid rats (n=30) were randomly assigned to one of the three groups as shown in Table 1: control, betametasone and melatonin groups. We used betametasone (Celestone Chronodose; Eczacıbaşı Pharmaceuticals Co., Istanbul, Turkey) formulation, used in animal and human investigations, composed of an equivalent molar weight mixture of the sodium phosphate and acetate forms (19,20). The 6 mg/ml solution of betametasone was diluted with 0.9% sodium chloride solution to prepare a 0.1 mg dose in 0.25 ml. Melatonin® (Sigma Chemical Co, St. Louis, MO, USA), used in this study, was dissolved in ethanol and diluted in saline to give a final concentration of 5% ethanol. Each rat received either intraperitoneally (ip) ethanol or betametasone (0.25 ml dose drawn into 1 ml syringes, im) or melatonin (5 mg/kg, ip). We administered single dose of betametasone,

Table 1. Study groups

Groups	n	Medication	Hysterotomy day
1. Control	10	Ethanol (ip) between 17th and 18th days	Pregnancies were terminated by hysterotomy on 21st days
2. Betametasone	10	0.1 (mg/kg) betamethasone (IM) between 17th and 18th days	Pregnancies were terminated by hysterotomy on 19th days
3. Melatonin	10	5 (mg/kg) melatonin intraperitoneally between 17th and 18th days	Pregnancies were terminated by hysterotomy 19th days

Table 2. Amniotic fluid adrenomedullin, NO and lamellar body count of each group

Groups	AF-adrenomedullin pg/ml	Lamellar body count/µL	NO µmol/L
1. Control	49.39±12.93	13888.77±590	27.70±1.20
2. Betametasone	43.15±6.63	11444.44±633	24.49±0.57
3. Melatonin	29.84±3.45	2777.77±521	28.10±1.60
p values			
1 vs 2	0.614	0.733	0.150
1 vs 3	0.121	0.130	0.826
2 vs 3	0.285	0.233	0.119

melatonin and ethanol on 17th day of gestation and second doses of betametasone, melatonin and ethanol were injected on 18th day of gestation. Pregnancies were terminated by hysterotomy on the 19th day of gestation before the onset of spontaneous labour in the betametasone and melatonin groups. On the other hand, pregnancies of the control group were terminated by hysterotomy on the 21st day of gestation. All surgical procedures were performed while the rats were under intraperitoneal ketamine HCl (50 mg/kg) and xylazine HCl (10 mg/kg) anesthesia combination. The permission for the animal tests and experiments was given by the Bioethical Board of Inönü University Medical Faculty.

Samples of amniotic fluid (AF) were collected during the hysterotomy. Fetuses were delivered with a longitudinal incision to the uterus and then they were removed from their amniotic sacs. Afterwards they were decapitated and their lungs were harvested for pathological examination. AF samples were immediately centrifuged after collection at 1500 g for 10 minutes at 4°C; the supernatants were divided into aliquots. Fetal lungs were processed after fixing with 10% formaldehyde solution. Four micrometer of tissue sections were prepared from paraffin embedded blocks and stained with hematoxylene and eosin (H&E), Masson's trichrome and PAS and examined under light microscope.

Adrenomedullin analysis
Amniotic fluid adrenomedullin concentration was measured by using reverse-phase high-performance liquid chromatography (HPLC). Adrenomedullin samples were subjected to reverse-phase HPLC (C-18 column, 4.6x250 mm, Cecil 1100, Supelco, Cambridge, UK), with a linear-gradient dilution of CH_3CN from 10% to 60% in a solution of 0.1% trifluoroacetic acid. Rat adrenomedullin (1-50 pmol/ml) was used as the standard (Phoenix Pharmaceutical, Mountain View, CA, USA), the absorbance being read at 210 nm as described before (21,22)

NO analysis
First, all amniotic fluid samples were deproteinized. Briefly, for every 200 µl sample, 400 µl of 10% zinc sulfate and 400 µl of 0.5 N sodium hydroxide was added. The samples were then vortexed and centrifuged for 5 minute at 4°C. For this study, 200 µl deproteinized aliquot or water blank was

incubated in a final volume of 750 µl containing 75 µl of 0.32 mol/L potassium phosphate buffer (pH 7.5), 25 µl of nitrate reductase (10 U/ml, Sigma) with NADPH (50 µmol/L, Sigma) and FAD (50 µmol/L, Sigma) as coenzyme, and 650 µl of water for 2 hours. After reducing nitrate to nitrite with nitrate reductase, total nitrite was determined with Greiss reagent by mixing equal volumes of the reduced samples with Greiss reagent, 1:1 0.1% α-naphtylamine in water/1% p-aminobenzene sulfamide in 5% phosphoric acid. The samples were allowed to stand for 15 minutes and then read in a spectrophotometer at an absorbance of 548 nm. A range of sodium nitrite standards (0-100 µmol/L) was prepared, and a standard curve was used to convert sample measurements to micromoles per liter of nitrite. The reaction was linear from 0.25 to 100 µmol/L. Assays were performed as duplicates (23).

Lamellar body count
Lamellar body presence was quantified by electronic cell counter (Sysmex K800 Medical Electronics, Hamburg). The equipment was set for the size parameters used for platelet counts between 2 and 20 fl in volume. The diameter of
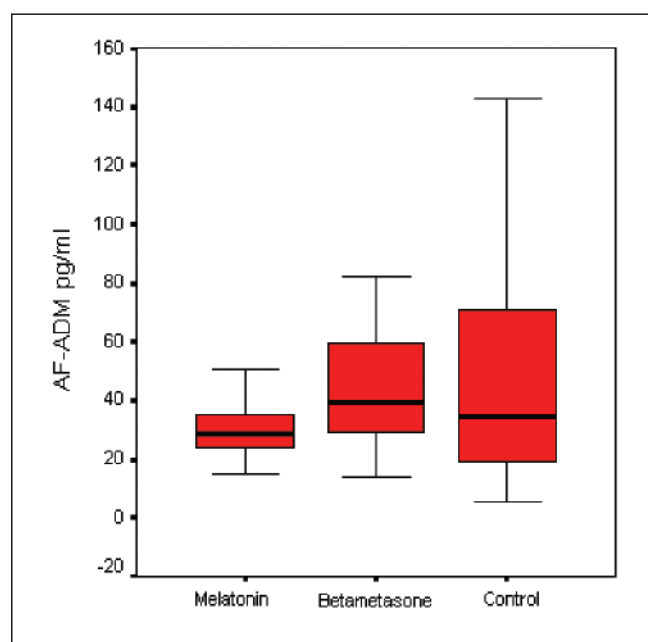

Figure 1. Graphic represantation of amniotic fluid adrenomedullin concentration of all groups.
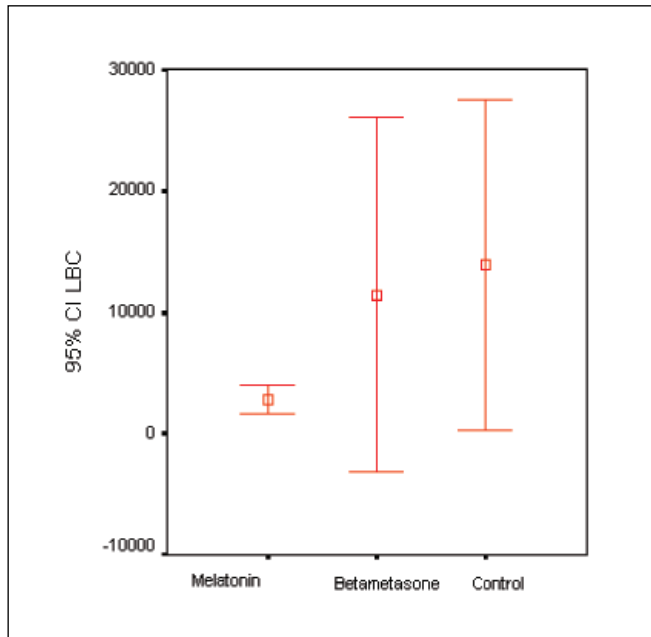
Figure 2. Lamellar body count in the melatonin, betametasone and control groups.

lamellar bodies ranges from 0.2-2 μm. Because amniotic fluid usually contains only cells larger than 7 μm or cellular debris the counter is able to discriminate between these particles. It operates according to the principle of electronic resistive-pulse counting (24). The samples were analysed immediately after collection. Samples were mixed by gentle inversion and the fluid was transferred to a clear test tube. Care was taken not to process bloody fluids and fluids containing obvious mucus. The sample test tubes were placed on a tube rocker for 5 minutes at 300 g to remove cellular debris. The supernatant was then measured in the electronic cell counter.

Statistical analysis

Statistical analyses were performed using The Statistical Package for Social Sciences (SPSS). Normality of the distribution was confirmed using Kolmogorov-Smirnov z-test. Results are given in the text as mean ± standard error. Difference between the experimental groups with respect to adrenomedullin levels, lamellar body counts and NO measurements were tested using one-way anova and *post-hoc* multiple comparisons (LSD). Highest acceptable significance level was defined as 0.05.

Results

The results of adrenomedullin and NO estimations and lamellar body counts are shown in Table 2. Adrenomedullin levels of the melatonin, betametasone and control groups were found to be 29.84±3.45 pg/ml, 43.15±6.63 pg/ml and 49.39±12.93 pg/ml, respectively. There were no significant differences between the groups regarding the adreno-medullin concentrations (Figure 1). Despite terminating the pregnancies of the melatonin-treated group on the 19th day, adrenomedullin levels of these rats were found to be similar to those of the control group whose pregnancies were termi-
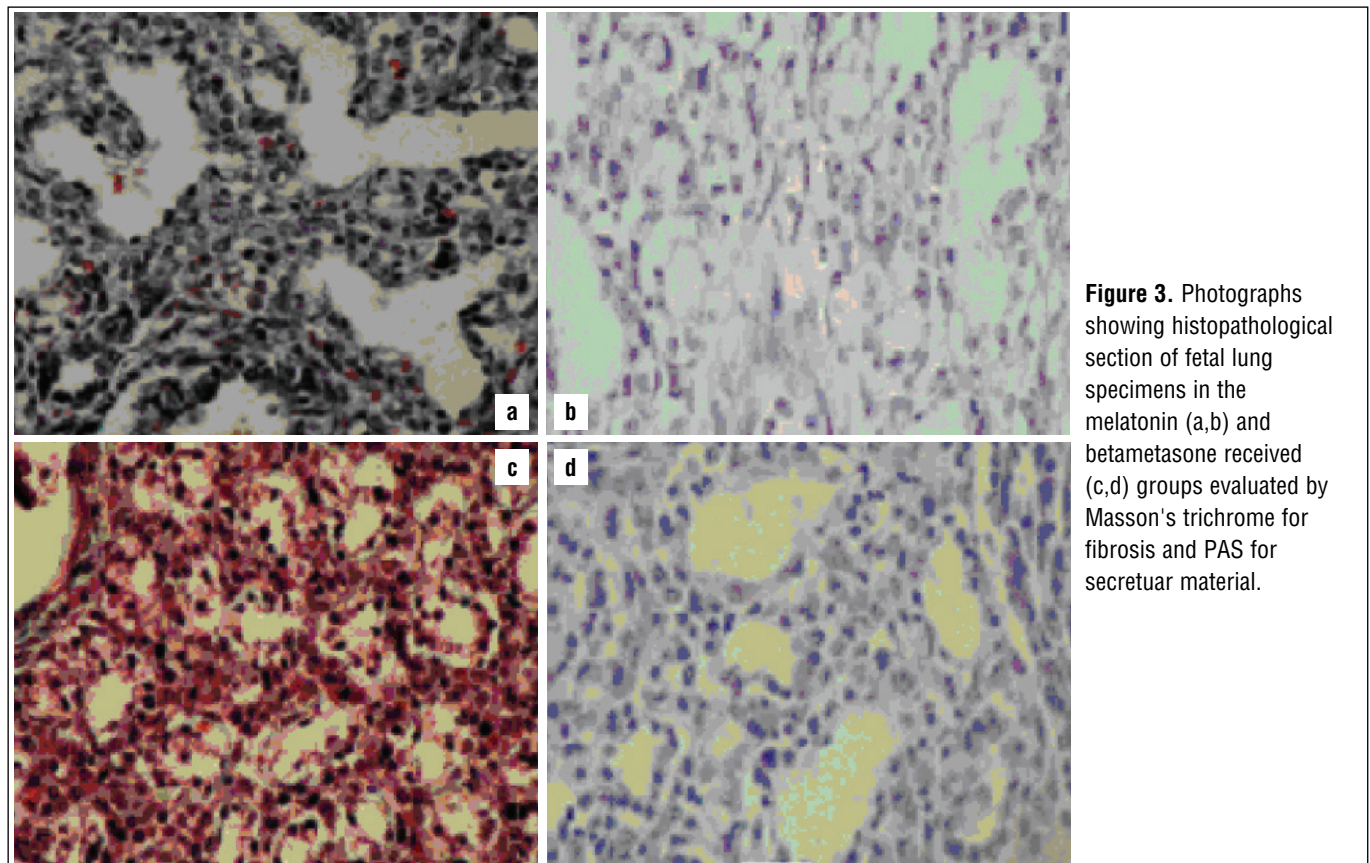

Figure 3. Photographs showing histopathological section of fetal lung specimens in the melatonin (a,b) and betametasone received (c,d) groups evaluated by Masson's trichrome for fibrosis and PAS for secretuar material.

nated on the 21th day. The mean NO levels of the betametasone, melatonin and control groups were similar. The mean lamellar body count in the control group was found to be five fold higher than that in the melatonin group (Figure 2). Lamellar body count in the betametasone group was 4.1 fold higher than that in the melatonin group but the difference between groups was not significant. Melatonin treatment was found to maintain adrenomedullin and NO levels and lamellar body counts within the control limits. Masson's trichrome and PAS staining of the fetal lung specimens of the melatonin-treated and the betametasone-treated rats were similar (Figure 3).

Discussion

Normal development of the human lung can be divided into five stages: embryonic, pseudoglandular, canalicular, saccular, and alveolar (25-28). Although these periods of lung development are similar across mammalian species, the relative timing and length of each stage varies between species. In the rat, alveolarization begins during late gestation but primarily occurs during the first 2 weeks after birth (29). Mechanisms that regulate alveolarization are poorly understood, but multiple stimuli modulate distal lung growth (29-31). Glucocorticoids can interfere with alveolarization while at the same time inducing structural maturation by thinning the mesenchyme and inducing the surfactant system (32,33). Also, it is well established that adrenomedullin secretion is stimulated by glucocorticoids (15).

Recent studies have revealed that adrenomedullin preferentially dilates pulmonary vessels and that the main clearance site of adrenomedullin is the lung (9). It has been shown that adrenomedullin increases intracellular cAMP, which affects fetal lung maturity by promoting glycogen degradation, essential for the synthesis of surfactant and lung growth. To date there have been no investigations on the effect of melatonin on adrenomedullin secretion and fetal lung maturation. Melatonin is a powerful free radical scavenger that inhibits nitric oxide synthase (NOS), reducing the NO which it generates which leads to a hydroxyl radical or peroxynitrite. Both the hydroxyl radical and the peroxynitrite can easily damage tissue (18). Although, cells of the developing lung express the nitric oxide synthatases I and II, it is unknown whether NO plays a role in lung development. To address this question, Young et al. (34) exposed fetal rat lung explants to NO donors and NOS inhibitors and observed that donors increased and inhibitors decreased airway branching. Similarly, the mechanisms involved in the adrenomedullin effects in the fetal sheep lung depend largely on NO release (35). In the present study, NO levels of melatonin treated rats were found similar to those of the control goup. Moreover, melatonin was found to maintain adrenomedullin levels within the limits seen in the betametasone and control groups. This means that melatonin increases adrenomedullin and NO levels. Our data support the idea that melatonin-induced adrenomedullin may play an important role in regulating pulmonary vascular tone and lung maturation through the stimulation of NO production. Because the vasodilating action of adrenomedullin has been shown to depend largely on endogenous NO production (35), melatonin may play a role in the mechanisms of fetal lung maturation.

Pulmonary surfactant, a mixture of lipids, proteins, and carbohydrates, synthesized and secreted by alveolar type II cells and it prevents lung collapse at end-expiration (36). Two factors may promote oxidation of surfactant lipids in the rats with preterm delivery (melatonin and betametasone groups). First, antioxidant defenses may be exhausted by an excess of reactive oxygen products and may fail to provide protection. Secondly, the major antioxidant species in the alveoli may be excluded from the micro-environment in which preterm delivery occurs. Although, we did not evalute the lipid peroxidation products in fetal lungs, there is strong evidence that melatonin increases the activity of a number of antioxidative enzymes (17,18). One recent study supports our idea that melatonin was best in quenching the superoxide radicals, thereby, reducing the lipid peroxidation level of the lung surfactant (37).

Lamellar bodies originate from the fetal alveoli and are released into amniotic fluid by fetal breathing movements. They contain "packed" surfactant, mainly consisting of phospholipids. Due to their size (0.2-2 μm) which corresponds to that of the platelets (1-3 μm), they can easily be quantified by electronic cell counters (24). Although, lecithin-phingomyelin ratio and phosphatidylglycerol assay are accepted tests to assess fetal lung maturity, these tests are expensive, time consuming and require trained laboratory personnel. The lamellar body count has been used for more than a decade and performs as well as traditional phospholipid analyses as an assay for evaluating fetal lung maturity (38,39). Lamellar body counting offers the advantage of being rapid, objective, inexpensive and available to all hospitals. A recent study has reported that cortisol causes a marked stimulation of synthesis and accumulation of phosphatidylcholine in the lamellar bodies in lung explants from fetal rabbits (40). Our finding is 4 to 5 fold lower lamellar bodycount in the melatonin group as compared to that of the control and betametasone groups suggests that melatonin does not increase lamellar body production.

Finally, melatonin was found to be effective in the development of fetal lung maturation. The 19th day (preterm) adrenomedullin levels of the melatonin group were similar to the 21st day (term) adrenomedullin levels of the control group. Since adrenomedullin is localized in the fetal membranes and the placenta (7,8) the possible sources of adrenomedullin may be the fetal membranes, placenta and the fetal lung. Taken together, our own findings and data from literature strongly suggest that, melatonin-induced adrenomedullin production in fetal membranes and epithelial cells of bronchial system may also have an important function in the complex process leading to fetal lung maturation. Further study should be undertaken to clarify the effect of exogenous melatonin on lung differentiation and maturation.

References

1. Kitamura K, Kangawa K, Kawamoto M et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993;192:553-60.

2. Jerat S, Kaufman S. Effect of pregnancy and steroid hormones on plasma adrenomedullin levels in the rat. Can J Physiol Pharmacol 1998;76:463-6.

3. Di Iorio R, Marinoni E, Letizia C et al. Adrenomedullin, a new vasoactive peptide, is increased in preeclampsia. Hypertension 1998;32:758-63.

4. Nagata N, Kato J, Kitamura K et al. Dissociation of adrenomedullin concentrations in plasma and cerebrospinal fluid in pregnant and non-pregnant women. Eur J Endocrinol 1998;139:611-4.

5. Hata T, Miyazaki K, Matsui K. Decreased circulating adrenomedullin in pre-eclampsia.Lancet 1997;350:1600.

6. Di Iorio R, Marinoni E, Cosmi EV. Adrenomedullin in pre-eclampsia. Lancet 1998;351:676-7.

7. Macri CJ, Martinez A, Moody TW et al. Detection of adrenomedullin, a hypotensive peptide, in amniotic fluid and fetal membranes. Am J Obstet Gynecol 1996;175:906-11.

8. Marinoni E, Di Iorio R, Letizia C et al. Immunoreactive adrenomedullin in human fetoplacental tissues. Am J Obstet Gynecol 1998;179:784-7.

9. Heymann MA. Control of the pulmonary circulation in the fetus and during the transitional period to air breathing. Eur J Obstet Gynecol Reprod Biol 1999;84:127-32.

10. Martinez A, Miller MJ, Unsworth EJ et al. Expression of adrenomedullin in normal human lung and in pulmonary tumors. Endocrinology 1995;136:4099-105.

11. Majid DS, Kadowitz PJ, Coy DH, Navar LG. Renal responses to intra-arterial administration of adrenomedullin in dogs. Am J Physiol 1996;270:F200-5.

12. Nossaman BD, Feng CJ, Kaye AD et al. Pulmonary vasodilator responses to adrenomedullin are reduced by NOS inhibitors in rats but not in cats. Am J Physiol 1996;270:L782-9.

13. Heaton J, Lin B, Chang JK et al. Pulmonary vasodilation to adrenomedullin: a novel peptide in humans. Am J Physiol 1995;268:H2211-5.

14. Imai T, Hirata Y, Iwashina M, Marumo F. Hormonal regulation of rat adrenomedullin gene in vasculature. Endocrinology 1995;136:1544-8.

15. Minamino N, Shoji H, Sugo S et al. Adrenocortical steroids, thyroid hormones and retinoic acid augment the production of adrenomedullin in vascular smooth muscle cells. Biochem Biophys Res Commun 1995;211:686-93.

16. Reiter RJ, Melchiorri D, Sewerynek E et al. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 1995;18:1-11.

17. Reiter RJ, Guerrero JM, Garcia JJ, Acuna-Castroviejo D. Reactive oxygen intermediates, molecular damage, and aging. Relation to melatonin. Ann N Y Acad Sci 1998;854:410-24.

18. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. J Biomed Sci 2000;7:444-58.

19. Celestone Soluspan suspension In: Physicians' Desk Reference, 50th ed. Medical Economics, Montvale, NJ, 1996;pp. 2130-31.

20. Ballard PL, Ballard RA. Scientific basis and therapeutic regimens for use of antenatal glucocorticoids. Am J Obstet Gynecol 1995;173:254-62.

21. Sato K, Hirata Y, Imai T et al. Characterization of immunoreactive adrenomedullin in human plasma and urine. Life Sci 1995;57:189-94.

22. Evereklioglu C, Yurekli M, Er H et al. Increased plasma adrenomedullin levels in patients with Behcet's disease. Dermatology 2000;201:312-5.

23. Ozbek E, Turkoz Y, Gokdeniz R et al. Increased nitric oxide production in the spermatic vein of patients with varicocele. Eur Urol 2000;37:172-5.

24. Dubin SB. Characterization of amniotic fluid lamellar bodies by resistive-pulse counting: relationship to measures of fetal lung maturity. Clin Chem 1989;35:612-6.

25. Burri PH. Structural aspects of prenatal and postnatal development and growth of the lung. In: Lung Growth and Development. McDonald JA ed., Dekker, New York, 1997;pp. 1-35.

26. Demello DE, Reid LM. Pre- and postnatal development of the pulmonary circulation. In: Basic Mechanisms of Pediatric Respiratory Disease: Cellular and Integrative. Chernick V, Mellins RB eds., Philadelphia, PA, Decker, 1991;pp. 6-54.

27. Emery JL, Mithal A. The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. Arch Dis Child 1960;35:544-7.

28. Langston C, Kida K, Reed M, Thurlbeck WM. Human lung growth in late gestation and in the neonate. Am Rev Respir Dis 1984;129:607-13.

29. Massaro D, Massaro GD. Dexamethasone accelerates postnatal alveolar wall thinning and alters wall composition. Am J Physiol 1986;251:R218-24.

30. Roman J. Cell-cell and cell-matrix interactions in development of the lung vasculature. In: Lung Growth and Development. McDonald JA ed., Dekker, New York, 1997;pp. 365-400.

31. Shannon JM, Deterding RR. Epithelial-mesenchymal interactions in lung development. In: Lung Growth and Development. McDonald JA ed., Dekker, New York, 1997;pp. 81-118.

32. Bunton TE, Plopper CG. Triamcinolone-induced structural alterations in the development of the lung of the fetal rhesus macaque. Am J Obstet Gynecol 1984;148:203-15.

33. Pinkerton KE, Willet KE, Peake JL et al. Prenatal glucocorticoid and T4 effects on lung morphology in preterm lambs. Am J Respir Crit Care Med 1997;156:624-30

34. Young SL, Evans K, Eu JP. Nitric oxide modulates branching morphogenesis in fetal rat lung explants. Am J Physiol Lung Cell Mol Physiol 2002;282:L379-85.

35. Takahashi Y, de Vroomen M, Gournay V et al. Mechanisms of adrenomedullin-induced increase of pulmonary blood flow in fetal sheep. Pediatr Res 1999;45:276-81.

36. Lewis JF, Jobe AH. Surfactant and the adult respiratory distress syndrome. Am Rev Respir Dis 1993;147:218-33. Review. Erratum in: Am Rev Respir Dis 1993;147:following 1068.

37. Bouhafs RK, Jarstrand C. Effects of antioxidants on surfactant peroxidation by stimulated human polymorphonuclear leukocytes. Free Radic Res 2002;36:727-34.

38. Neerhof MG, Dohnal JC, Ashwood ER et al. Lamellar body counts: a consensus on protocol. Obstet Gynecol 2001;97:318-20.

39. Celik O, Hascalık S, Kazezoglu G. Lamellar body counts in amniotic fluid and the relation with Dubowitz scoring systems. Gynecol Obstet Reprod Med 2002;8:205-207.

40. Mendelson CR, Snyder JM. Effect of cortisol on the synthesis of lamellar body glycerophospholipids in fetal rabbit lung tissue *in vitro*. Biochim Biophys Acta 1985;834:85-94.