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Inhibition of fetal lung maturation by indomethacin in pregnant rabbits

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It has been previously shown that maternal or fetal administration of steroids induces fetal lung maturation in certain animal species [3, 7, 13, 15].

Studies by LIGGINS and HOWIE [12] have demonstrated an acceleration of fetal lung maturation in humans following the parenteral administration of betamethasone (a corticosteroid) to women 48–72 hours before delivery, evidenced by a reduction in the incidence of respiratory distress syndrome (RDS) in these infants.

On the other hand, it has been suggested that maternal administration of Indomethacin can produce uterine inhibition in humans when the pregnant woman starts a premature labor [22].

VANE [20] demonstrated that Indomethacin simultaneously inhibited prostaglandins (PGs) release and spontaneous contractions of pregnant rat uteri in vitro.

The role of PGs in parturition is suggested by the observed increase of PGs concentration in human blood [10] and amniotic fluid [9] during labor and by the observation that exogenously administered PGs can induce labor [11] or abortion [17].

Since PGs are involved in many metabolic processes, we have studied the action of Indomethacin given to pregnant rabbits, on fetal lung maturation.

1 Material and methods

Thirty four time-dated pregnant Californian rabbits (14 control and 20 Indomethacin-treated animals)

were used in this study. They were mated between 10 am and 2 pm, so that gestational age could be calculated arbitrarily to within 4 hours.

Experimental procedures were performed at the same hours.

The does were treated three days before delivery, with i/m administration of Indomethacin (10 mg/kg/day) or solvent (placebo) twice daily, until delivery (e.g. those delivered on the 27th day of gestation received treatment from day 24).

The rabbits were fed ad libitum with water and rabbit food. Daily observations were made to assure that Indomethacin-treated animals were in good condition. No obvious difference was observed between both groups.

The fetuses were delivered by hysterotomy at 27, 28, 29 and 30 days of gestation, using i/v pentobarbital. The time between pentobarbital injection and hysterotomy was about 1–2 minutes. No fetal wastage was observed.

The newborns were allowed to breathe room air placed in an incubator at 37° C, for 30 minutes before they were weighed. At this time, they were killed with 30 mg of intra-peritoneal sodium pentobarbital. Exsanguination by cutting the abdominal aorta and tracheostomy was performed in five newborns of the litter.

Once tracheostomized, lung washes were performed as follows. Each pair of lungs was gently expanded with air from a syringe connected to the trachea by a polyethylene tube. This was followed by five gentle lavagings, each with 2 ml of 0.9 saline

(20° C). The washings which totalled 10 ml for each pair of lungs were pooled by litter groups and the cells and debris in the lavage fluid removed by centrifugation (1000 xg, 5 min).

After the lavages, the lungs were dried in a desiccator at 100° C until their weight was constant for the purpose of unit expression of alveolar wash phospholipid content.

The blood collected after exsanguination of the newborns was pooled by litter groups, allowed to coagulate and Radioimmunoassay (RIA) for cortisol in serum was performed.

The saline alveolar wash was stirred with an equal volume of methanol and the lipids extracted with two volumes of chloroform (Folch) [6].

The extracted lipids were plated and run on heat-activated thin layers of silica gel H containing 5% ammonium sulphate. The plates were first developed upwards using chloroform-methanol-acetic acid-H₂O (390-150-48-24 v/v) then from right to

left with tetrahydrofuran-methylal-methanol-2NH₄OH (400-285-78-42 v/v). Between the individual runs the plates were heated at 90° C for 5 minutes.

After development of the chromatograms the plates were charred at 280° C for 10 minutes to visualize compounds. This method allows a very good identification of lecithin, sphingomyelin, phosphatidylinositol, phosphatidylserina, phosphatidylglycerol on the plate [2].

Lecithin was eluted and phosphorus determination was performed by the method of BARTLETT [1].

2 Results

The P-Lecithin concentrations in lung washes of rabbits in both groups (control and treated) are shown in Fig. 1.

The level of P-Lecithin increases in both groups with gestational age, since day 27 until day 30.

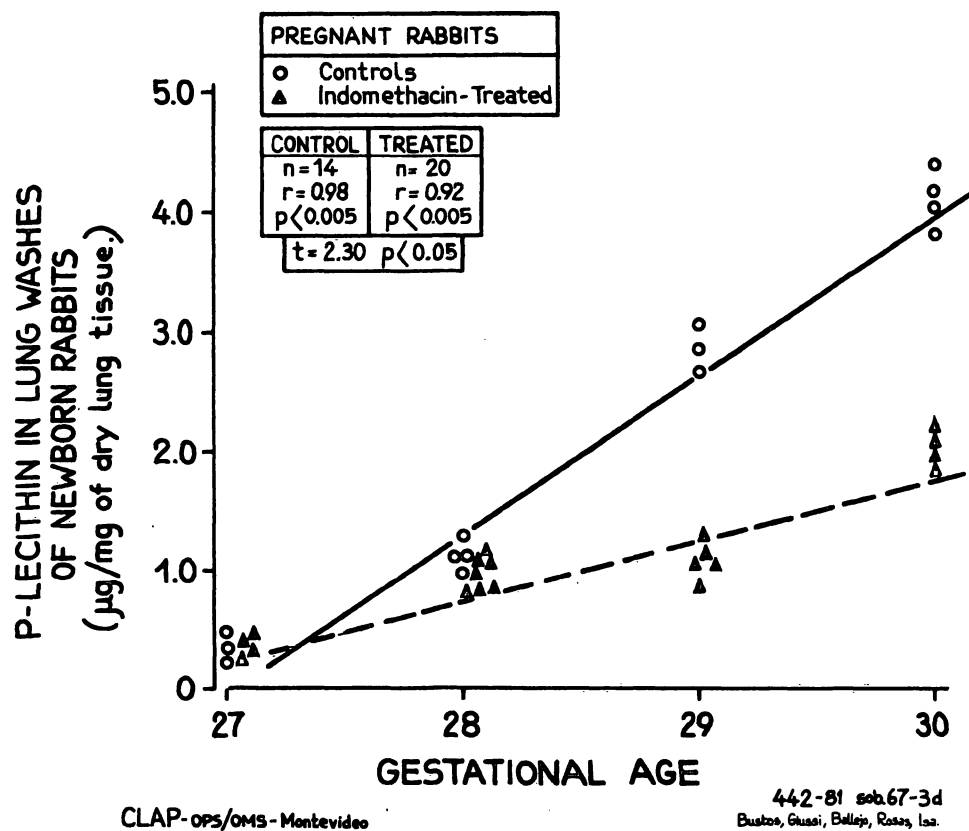


Fig. 1. P-lecithin contents in lung washes of newborn rabbits at different gestational ages. The indomethacin-treated group has lower values at days 29 and 30 than the control group.

Although the increment of P-Lecithin is observed in both groups, the control group has a sharp increase on day 29, which is not seen in the Indomethacin-treated group. In the same way, on day 30 the amount of P-Lecithin in the control group is higher than the level obtained in the Indomethacin group. Lineal regression analyses were done for both groups. Their slopes were

statistically different ($p < 0.05$). Mean values with standard deviations and statistical analyses are shown on Tab. I. These results suggest that Indomethacin in these experimental conditions produces a decrease in the amount of lecithin collected from lung washes.

The data of serum cortisol in the newborn rabbits for both groups are shown on Fig. 2 and Tab. II.

Tab. I. P-lecithin washes $\mu\text{g}/\text{mg}$ dry lung weight.

Gestational age (days)	Control			Treated			Level of Significance*
	n	\bar{x}	SD	n	\bar{x}	SD	
27	3	0.34 \pm 0.06		4	0.34 \pm 0.05		NS**
28	4	1.12 \pm 0.10		7	0.98 \pm 0.11		NS**
29	3	2.83 \pm 0.20		5	1.06 \pm 0.14		$p < 0.005$
30	4	4.10 \pm 0.24		4	2.02 \pm 0.15		$p < 0.005$

*Student "t" test

**Not significant

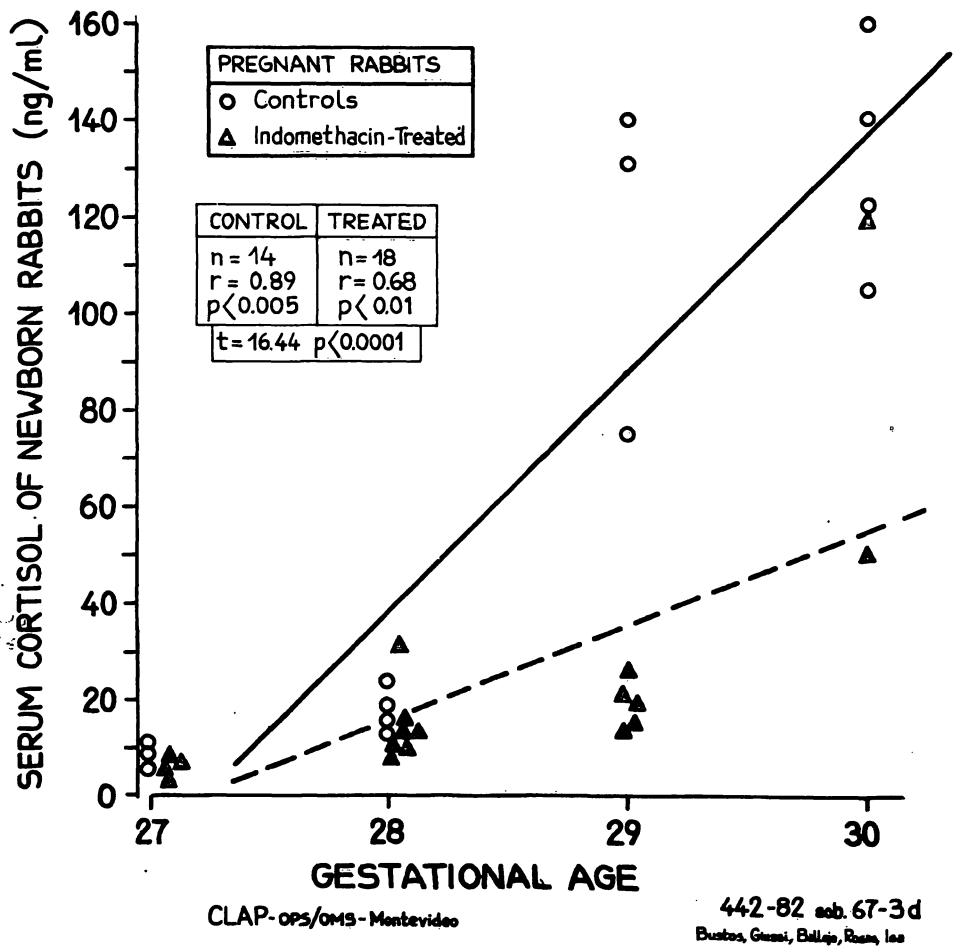


Fig. 2. Serum cortisol levels of newborn rabbits at different gestational ages. The increment of cortisol along gestational age in the indomethacin-treated group is lower than the control group.

Tab. II. Cortisol in newborn serum ng/ml.

Gestational age (days)	Control			Treated			Level of Significance*
	n	\bar{x}	SD	n	\bar{x}	SD	
27	3	8.26 ± 0.97		4	5.50 ± 0.83		p < 0.01
28	4	17.7 ± 4.66		7	14.6 ± 7.75		NS**
29	3	115.6 ± 35.4		5	18.5 ± 4.88		p < 0.005
30	4	131.75 ± 23.6		2	85.0 ± 49.4		NS**

* Student "t" test

** Not significant

Cortisol levels increase with gestational age in both groups. An important increase, six-fold at day 29, is observed in the control group, while the Indomethacin group remains at the same level obtained at days 27 and 28.

The lineal regression analysis of serum cortisol levels along gestational age of the control group and the Indomethacin-injected group shows that the slopes of both lines are statistically different ($p < 0.0001$).

This indicates that Indomethacin interferes with the events that determine the levels of cortisol in fetal blood.

3 Discussion

Previous reports have shown that the lecithin content of the alveolar fluid correlates well with functional maturation of the lung.

To prove this, we have shown that there is a progressive increment in the lecithin content of alveolar wash with increasing gestational age. Through maternal treatment with Indomethacin, a significantly lower lecithin content in alveolar washes at 29 and 30 days of gestation is observed, indicating an inhibition of the surfactant production.

Although there was no clear difference in the breathing pattern between both groups, it is important to point out that the reduction in the amount of lecithin found in the Indomethacin group was less than half that of the control group.

The decreased lecithin production was associated with a diminution in the levels of serum cortisol on day 29 and 30.

The action of corticoids as inductors of surfactant is very well known [7]. Probably the lower levels

of cortisol obtained on days 29 and 30 could partially explain the diminished production of lecithin observed on these same days.

The cortisol titers in fetal serum of control rabbits during the last 4 days of gestation (Tab. II and Fig. 2) show that cortisol increased abruptly on day 29th. This fact may be due to an increased secretion of cortisol by the fetal adrenal, perhaps in response to increased secretion of ACTH by the adenohypophysis.

The lower levels of cortisol found in the Indomethacin group suggest that Indomethacin inhibited at some points of the hypothalamo-hypophysoadrenal axis, the secretion of cortisol by the fetal adrenal.

In this sense it has been published that PGs stimulates the release of hypothalamic Corticotrophin Releasing Hormone [14], ACTH from adenohypophysis [4, 8] and the steroidogenesis in the adrenal cortex [5, 18].

Also, it has been published that thyroxine stimulates the production of surfactant [16, 21]. A recent paper demonstrated that PGs could act promoting the liberation of T4 [19].

The inhibition of surfactant production found in our experiment could also be partially explained by a reduction in T4 secretion.

As has been pointed out at the beginning of this paper, Indomethacin has been used as a utero-inhibitor drug. Our results advise about the possibility of an undesirable effect of Indomethacin on fetal lung maturation (inhibition of the surfactant production).

To our knowledge, this has not yet been published. Therefore we believe that other experiments should be performed before any conclusion may be extrapolated to clinical use.

Summary

It has been suggested that premature labor is successfully treated with Indomethacin. As lung maturation is influenced by many drugs affecting metabolic processes, we investigated the effects of Indomethacin administration to pregnant rabbits upon the biochemical maturation of the fetal lung.

The rabbits were treated either with Indomethacin, 10 mg/kg/day given i/m or solvent (placebo), i/m at the same dose, twice daily for 3 days before the fetuses were prematurely delivered on days 27, 28, 29 and 30 of gestation.

We quantified the lecithin phosphorus in alveolar washes of the newborns and cortisol levels in serum, since it has been established that endogenous glucocorticoids are essential for the biochemical development of the lung.

We found that lecithin phosphorus concentration in alveolar washes increases with gestational age in the control group (Fig. 1). In the Indomethacin-treated group, lecithin phosphorus concentration also increases, but reaching lower levels than those of the control group.

This difference was greater at days 29 and 30, where mean values of $2.83 \mu\text{g}/\text{mg} \pm 0.20$ (SD) and $4.10 \mu\text{g}/\text{mg} \pm 0.24$ (SD) dry lung weight respectively for the control group and $1.06 \mu\text{g}/\text{mg} \pm 0.14$ (SD) and $2.02 \mu\text{g}/\text{mg} \pm 0.15$ (SD) for the Indomethacin-treated group were found.

Also, on all the days considered in this study, the concentration of cortisol in fetal serum of the control group was higher than that found in the treated group.

Our results indicate that the administration of Indomethacin to pregnant rabbits had a deleterious effect on the maturation of the fetal lung.

As the diminished concentration of lecithin in the alveolar washes was associated with lower levels of cortisol in fetal serum, this effect may be due in part to the inhibitory action of Indomethacin on the hypothalamo-hypophyso-adrenal axis.

These results must be confirmed in other species before reaching a conclusion regarding its applicability in clinical use.

Keywords: Cortisol, fetal, indomethacin, lecithin, lung, maturation, prostaglandins, rabbits, surfactant.

Zusammenfassung

Hemmung von fetaler Lungenreifung mit Indomethacin bei schwangeren Kaninchen.

Vorzeitige Wehen können erfolgreich mit Indomethacin behandelt werden. Da die Lungenreifung durch manche stoffwechselaktiven Arzneimittel beeinflusst wird, erforschten wir die Wirkungen von Indomethacin-Gaben an schwangere Kaninchen auf die biochemische Reifung der fetalen Lunge.

Die Kaninchen wurden entweder mit Indomethacin (10 mg/kg/Tag i.m.) oder Lösung (placebo) i.m. in derselben Dosierung 2 × täglich 3 Tage lang behandelt. Anschließend wurden die Feten frühreif am 27., 28., 29. oder 30. Tag der Schwangerschaft geboren.

Wir bestimmten den Phosphorlezithinspiegel in der alveolaren Spülflüssigkeit der Neugeborenen. Außerdem den Kortisol-Spiegel im Serum, da festgestellt worden ist, daß die endogenen Glukokortikoide wesentlich für die biochemische Entwicklung der Lunge verantwortlich sind.

Wir fanden in der Kontrollgruppe (Abb. 1), daß die Phosphorlezithinkonzentration in den alveolaren Spülflüssigkeiten anwächst mit dem Schwangerschaftsalter. In der mit Indomethacin behandelten Gruppe wird die Konzentration des Phosphorlezithin auch größer, aber sie erreicht niedrigere Spiegel als jene in der Kontrollgruppe. Dieser Unterschied war am 29. und 30. Tage größer, an denen Mittelwerte von $2,83 \mu\text{g}/\text{mg} \pm 0,20$ (SD) und $4,1 \mu\text{g}/\text{mg} \pm 0,24$ (SD) trockenes Lungen-Gewicht für die Kontrollgruppe und $1,06 \mu\text{g}/\text{mg} \pm 0,14$ (SD) und $2,02 \mu\text{g}/\text{mg} \pm 0,15$ (SD) für die mit Indomethacin behandelte Gruppe gefunden wurden. An allen in dieser Studie gemessenen Tagen war auch die Konzentration von Kortisol im fetalen Serum der Kontrollgruppe höher als der Spiegel in der behandelten Gruppe.

Die Ergebnisse zeigen, daß die Gabe von Indomethacin an schwangere Kaninchen eine schädliche Wirkung auf die Reifung der fetalen Lunge hat.

Schlüsselwörter: Fet, Indomethacin, Kortisol, Lezithine, Lungenreifung, Prostaglandine, Surfactant.

Résumé

Inhibition de la maturation pulmonaire foetale par indométhacine chez les lapins gravides

Ayant entendu parler d'un traitement réussi du travail prématuré par indométhacine et sachant que la maturation pulmonaire est influencée par bien des médicaments affectant les processus métaboliques, nous avons décidé d'étudier les effets de l'indométhacine sur la maturation biochimique du poumon foetal chez des lapins gravides.

Les lapins ont reçu soit de l'indométhacine, 10 mg/kg/jour i/m, ou un solvant (placebo), i/m avec la même dose, deux fois par jour pendant les trois jours précédant l'ac-

couchement prématuré des foetus le 27, 28, 29 ou 30ème jour de gestation.

Sachant que les glucocorticoïdes endogènes sont indispensables pour le développement biochimique du poumon, nous avons déterminé la quantité de lécithine phosphoreuse dans les lavages alvéolaires des nouveau-nés et les niveaux de cortisol dans le sérum.

Nous avons pu établir que la concentration de lécithine phosphoreuse dans les lavages alvéolaires augmente avec l'âge de gestation dans le groupe de contrôle (Fig. 1). Dans le groupe traité à l'indométhacine, la concentration

de lécithine phosphoreuse a aussi augmenté mais à des degrés moindres, la différence ayant été plus accusée aux 29 et 30èmes jours où les valeurs moyennes ont été respectivement de 2,83 $\mu\text{g}/\text{mg} \pm 0,20$ (SD) et de 4,10 $\mu\text{g}/\text{mg} \pm 0,24$ (SD) de poids pulmonaire sec pour le groupe de contrôle et de 1,06 $\mu\text{g}/\text{mg} \pm 0,14$ (SD) et de 2,02 $\mu\text{g}/\text{mg} \pm 0,15$ (SD) pour le groupe traité à l'indométhacine. En conclusion, on peut dire que pour tous les jours pris en considération dans notre étude, la concentration de cortisol dans le sérum foetal du groupe de contrôle a été plus élevée que celle relevée dans le groupe traité à l'indométhacine.

Mots-clés: Cortisol, foetal, indométhacine, lapins, lécithine, maturation, poumon, prostaglandine, surfactant.

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