The Effect of Epidermal Growth Factor (EGF) on Lung Phosphatidylcholine, Phosphatidylglycerol and Superoxide Dismutase Activity

AKCİĞER FOSFATİDİLKOLİN, FOSFATİDİLGLİSEROL VE SÜPEROKSİT DİSMUTAZ AKTİVİTESİ ÜZERİNE EPİDERMAL GROWTH FAKTÖRÜNÜN ETKİSİ

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Summary

// is known that lung maturation is regulated with epidermal growth factor (EGF). The effect of EGF on the maturation of lung is not clear, hut the different doses of EGF may regulate the phospolipids and antioxidant enzymes in lung.

In the present study, we treated the newborn rat with 10 and 20 pg/kg EGF for one week and we measured phosphatidylcholine and phosphatidylglycerol with thinlayer chromatography and measured superoxide dismutase (SOD) activity with the method ofnitroblue tetrazolium (NBT) reduction in the lung tissue.

When compared with controls, phosphatidylcholine and phosphatylglycerol levels were decreased in parallel doses of EGF. On the other hand SOD activity was decreased in 10 fig/kg doses of EGF and was increased in 20 /Jg/kg doses of EGF.

Our results showed EGF may enhance the hydrolysis of phosphatidylcholine and phosphatidylglycerol but increase the SOD activity in only high doses.

Key Words: Epidermal Growth factor, Lung maturation, Newborn rat, Phosphatidylcholine, Phosphatidylglycerol, Superoxide dismutase

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Özet

Akciğer maturasyonunun epidemial growth faktörü (EGF) aracılığıyla düzenlendiği bilinmektedir. Ancak bu etkinin mekanizması açık değildir. Farklı doz/ardaki EGF akciğer fos folipidleri, antioksidan enzimleri üzerinde etkili olabilmektedir.

Bu çalışmada biz 10 ve 20 /Jg/kg EGF'yi bir hafta süre ile yenidoğan rallara vererek ince tabaka kromotografisi ile akciğer dokusunda fosfatidilkolin ve fosfatidilgliserol miktarlarını ve nitro blue tetrazolium (NBT) indirgenme yöntemiyle siiperoksit dismutaz (SOD) aktivitesini ölçtük.

Kontrollerle karşılaştırıldığında, uygulanan EGF'nin dozuna paralel olarak fosfatidilkolin, fosfatidilgliserol miktarları azaldı. Buna karşı SOD aktivitesi 10 flg/kg dozda azalırken 20 pig/kg dozda artış gösterdi.

Bizim sonuçlarımız EGF"nin fosfatidilkolin ve fosfatidilgliserol hidrolizini arttırabildiğini, SOD aktivitesini ise sadece yüksek dozda yükseltebileceğini ortaya koydu.

Anahtar Kelimeler: Epidemial growth faktörü. Akciğer maturasyonu, Yenidoğan rat, Fosfatidilkolin, Fosfatidilgliserol, Supcroksit dismutaz

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Epidermal growth factor (EGF) is a polypeptide hormon which has been shown to promote lung growth and maturation (1-3). The administration of EGF in vivo appears to stimulate epithelial hyperplasia of the airways, DNA synthesis and, maturation of lung function (1,4). The effect of EGF on other parameters of lung maturation including surfactant synthesis is of particular interest. Deficiency of surfactant is a central feature of the neonatal respiratory distress syndrome (1,5).

Oxygen metabolites play an important role in many different biological processes including oxygen induced lung injury and ischemia-repcrfusion injury (6).

Superoxide dismutase enzyme (SOD) which disunites superoxide anions into oxygen and H_2O_2, is known to play a pivotal role in protecting cells from free radical damage (7,8).

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In all mammalian species studied to date there is a late gestational increase in surfactant and a parallel increase in antioxidant enzymes of the lungs (9,10). It has been proposed that similar to the development of an efficient surfactant system, the development of a protective antioxidant defense system may be a necessary "preparation for birth" phenomenon (11).

Little is known about the mechanism of the EGF effects on surfactant and antioxidant systems of neonatal rat lung. To investigate this mechanism we have measured phosphaditylcholinc and phosphatidylglycerol content, an important surfactant for lung, and superoxide dismutasc activity which is an antioxidant enzyme in lungs of neonatal rat after treatment with EGF at low and high doses.

Material and Methods

Animal Procedure

Locally bred 21 pups weighing 35 g were used in this study. Three study groups were formed. In order to prevent stress, animals were housed continuously with their mothers. Animals were housed in an air conditioned and light-controlled room and mothers were fed a commercial pelleted diet and water ad libitum. To establish the effects of EGF on the parameters, animals were divided into 3 groups. Pups were given EGF [10 Ug/kg sc. (n:7, study group I) and 20 Ug/kg sc. (n:7, study group II)] dissolved in saline or with an equal volume (0.1 ml) of saline only (n:7, control group), once daily, begining in the first 24 h. following the birth at 11.00-12.00 am. for 8 days and killed 24 hr. after the last treatment. Immediately after cervical dislocation, tissue samples were rapidly removed and put into liquid nitrojen to be stored at -70°C.

SOD Assay

Tissue samples were homogenized in the ratio of 1/10 (w/v) in phosphate buffer (pH: 7.4) and the supernatant was carefully seperated by adding 3/5 (v/v) choloroform and ethanol. This mixture was centrifuged at 5000xg for 2 hours. The supernatant was used for assay of superoxide dismutasc. This assay for superoxide dismutasc activity involves inbilion of nitroblue tetrazolium (NBT) reduction (12). One unit of SOD activity is defined as the protein that inhibits the rate of NBT reduction by 50%.

The supernatant protein was measured by the method of Lowry et al (13) and the results were expressed as unit per mg protein tissue.

Phosholipid Assay

The lung phosphatidylcholine and phosphatidylglycerol were detemined by thinlaycrchromotography (TLC) in silical gel 60 plates from Merck. Tissue samples were homogenized in choloroform: methanol 2:1 (v/v) to a final dilution 20 fold the volume of the tissue sample and the phospholipids were extracted with this mixture (14). Then this extract was washed 2 times with distile water and evoporated under a nitrojen stream; the dry residue was dissolved with one volume of chloroform: methanol 2/1 (v/v). These samples were applied to silica gel plates.

After treatment with chloform: methanol: water (70:30:5), the plates were dried at 80°C. Lipid fractions were visualized on the plate using iodine vapors; the areas containing the seperated phospholipids were scraped off and transferred into glass test tubes. Acid digestion was performed using sulphuric acid/perchloric acid at 100°C for 1 hour and phosphorus was measured on each sample with amonium molibdate. Assuming that lu. mol phosphorus is cqivalcnt to lu\ mol phospholipid, the results were converted to mol phospholipid / g tissue (15).

Results

The effect of EGF, on SOD activity in different doses is shown in table 1. EGF decreased SOD ac-

Table 1. Superoxide Dismutase (SOD) activities of all groups.

	SOD (U/mg protein)
Control (n:7)	36.69±4.24
Group 1 (n:7)	29.06±3.09
Group 2 (u:7)	55.37i3.50*

*Statistical analysis: *)<(). 005 between control and EGF treated groups.*

Group 1: 10 pg/kg dose of EGF
Group 2: 20 pg/kg dose of EGF

Table **2.** Phosphatidyl glycerol and Phosphatidylcholine levels of all groups.

	Phosphatidylglyccrol (mmol/g tissue)	Phosphatidylcholine (mmol/g tissue)
Control (n:7)	3.05±0.48	10.18±0.58
Group 1 (n:7)	2.40±1.00§	5.38Ü.12*
Group 2 (n:7)	0.07±0.04*	1.28±0.61*

*Statistical analysis: *p<0.0()5 between control and EGF treated groups.*
§p>0.05 between control and EGF treated groups.

Group I: 10 pg/kg dose of EGF
Group 2: 20 pg/kg dose of EGF

tivity at a dose of 10 pg/kg, whereas EGF at a dose of 20 pg/kg increased SOD activitiy significantly in comparison with the control group. The effect of EGF treatment on neonatal lung phosphatidyl choline and phosphatidylglyccrol is shown in Table 2. Both phosphatidyl choline and phosphatidylglyccrol content decreased in a dose depended manner.

Discussion

In our experimental study we investigated the activity of SOD enzyme and phosphatidylcholine and phosphatidylglycerol content in lungs of newborn rat following EGF treatment.

Sosenko et al (16) postulated from their data that the mechanism involved in antioxidant enzyme and surfactant development have similarities in animal model and these systems also share a common controlling mechanism in the fetal lung. The present study revealed that the administrated EGF to the neonate decreased the phosphatidylcholine and phosphatidyl glycerol content in a dose depended manner, while SOD activity was increased at only high dose. These results differ from findings of Price et al (17) where EGF was observed to increase phosphatidylcholine synthesis. But they performed their study in vitro lung cultures and under hypcroxic conditions.

Mallampalli cl al (18), have observed an increase in phosphatidylcholine and a decrease in phosphatidylglycerol content in neonatal lung cytosol with a dose of 1.25 pg i.p. EGF on day 2 and 3 of life. Morover our data is consistent with recent evidences suggesting that phosphatidyl choline hydrolysis may be important in the control of cell growth (19,20). Reynolds et al (21) have found the EGF induced phosphatidylcholine hydrolysis in fibroblast.

It has been postulated that EGF decreases the rate of 3H-cholinc incorporation into disaturated phosphatidylcholine in air but increased phosphatidyl choline synthesis in response to hyperoxia (17). Our animals were kept under normal air conditions. In our study SOD activity decreased in lungs of newborn rats treated with low doses of EGF compared to controls. Whereas the high dose EGF treated lungs demonstrated significantly increased SOD activity. SOD is an important enzyme in protecting from superoxide anions which is a free radical produced by molecular oxygen (6-8). At birth; the newborn animals are exposed to a relatively 0_2 rich environment.

The EGF at low dose may not be protective on 0_2 rich environment. The ability of EGF to induce increased antioxidant enzyme appears to occur with high doses.

In summary two conclusion emerge from our study. First our results suggest that EGF may protect the neonatal lung when exposed to air immediately after birth from the effect of superoxide anion radicals. Second the increased surfactant synthesis during fetal development may be decreased and modulated by the effect of EGF depending on dose after birth under normal air conditions.

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