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Effect of Selenium on Pulmonary Glutathione Peroxidase and Alveolarization of Neonatal Rats

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Abstract - This study was designed to determine whether selenium (Se) nutrition affects pulmonary glutathione peroxidase and alveolarization in the neonatal rat. Twenty-four female Sprague Dawley rats were bred and fed a semipurified Se-deficient (0.04 ppm, Se-) or a Se-adequate (0.5 ppm, Se+) diet through pregnancy and lactation. Pulmonary DNA synthesis was slightly higher in Se+ pups than in Se- pups on d 6 and d 9 of lactation, but significant difference was not found. As pulmonary alveolarization progressed, mean air space size decreased and internal surface area and lung volume increased. No difference in pulmonary alveolarization was found between Se- and Se+ pups by age. Pulmonary Se concentration was higher in Se+ pups than in Se- pups at all age. Glutathione peroxidase activity in lung tissue reflected Se status and was lower in Se- pups than in Se+ pups. In conclusion, selenium has no significant effect on alveolarization of neonatal lungs, but it is necessary for adequate supply of pulmonary antioxidant, glutathione peroxidase.

Key words : selenium, lung, alveolarization, glutathione peroxidase, rat

INTRODUCTION

Newborn infants are at risk for the development of selenium (Se) deficiency due to their rapid rate of growth and accompanying high metabolic requirements. Low birth weight infants frequently develop respiratory distress syndrome (RDS), primarily because of structural underdevelopment of alveoli (Stark and Frantz 1986). Premature infants have diminished hepatic Se stores compared to full-term infants (Bayliss *et al.* 1985), and their plasma Se concentrations are reported to decrease dramatically during treatment for RDS in intensive care units (Amin *et al.* 1980; Lockitch *et al.* 1989; Tubman *et al.* 1990). However, the clinical significance of low Se status in low birth weight (LBW) infants has not been well investigated.

An adequate supply of dietary Se during pregnancy and lactation is critical in protecting neonatal rat pups from lung injury when they are exposed to a high O_2 (>95%) environment (Smith and Picciano 1986). Se inadequacy results in an elevated incidence of pulmonary interstitial inflammation and septal attenuation and a decrease in lung weight (Kim *et al.* 1991). The role of Se as an important antioxidant, glutathione peroxidase (GPx), has been proposed to benefit maintaining membrane integrity of the neonatal lung.

Previously, it has been suggested that selenium deficiency results in less functional pulmonary surfactant, by decreasing the pulmonary disaturated phosphatidyl choline (DSPC) to total phosphatidyl choline ratio (Kim 1994). However, whether Se deficiency impairs structural development of the lung has not been investigated. Therefore, present study was designed to determine

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Table 1. Diet composition

Ingredient	Amount (g kg^{-1})
Casein[1]	200
Sucrose	300
Cornstarch	331
Corn oil	100
Cellulose[2]	20
Vitamin mixture[3]	10
Se-free AIN-76 mineral mixture[4]	35
DL-methionine	2
Choline bitartrate	2

[1]Vitamin-free test casein (Teklad, Madison, WI)
[2]Alphacel, ICN Nutritional Biochemical Co., Cleveland, OH.
[3]AIN-76 vitamin mixture (NRC 1978).
[4]AIN-76 mineral mixture prepared without Se (NRC 1978). Sodium selenite was added to provide 0.5 ppm Se in formulation of the Se+ diet.

whether Se nutrition affects pulmonary glutathione peroxidase and alveolarization in the neonatal rat. Specifically, the developmental maturity of lungs was assessed by measuring lung volume, dry/wet lung weight ratio, incorporation of ^{3}H-thymidine into the lung DNA and by using histomorphometry to estimate mean air space size, internal surface area, and percent air space.

MATERIALS AND METHODS

1. Experimental design

Nulliparous female, Sprague-Dawley rats (Harlan Industries, Indianapolis), weighing 180~200 g, were housed in individual cages in a room with controlled temperature (20~22°C) and lighting (12 h light-dark cycle). Rats were mated at 200~240 g and pregnancy was determined by the presence of vaginal plugs and sperm. Twenty-four rats were bred and assigned to one of two experimental diets during pregnancy and lactation: Se-deficient (Se-, 0.04 ppm) or Se-adequate (Se+, 0.5 ppm). Diets (Table 1) were formulated to contain all nutrients in quantities adequate for reproduction (National Research Council 1978) except for Se. Demineralized water (Nanopure, Boston) and diets were fed *ad libitum*. The day after parturition (day 2), litters were weighed and culled so that there were 10 pups per dam. Dams were permitted to nurse their young until day 6, 9, or 16 of lactation.

2. Analytical procedures

Two pups from each litter were used for the measurement of [^{3}H]-thymidine incorporation into pulmonary DNA. [^{3}H]-thymidine (5 mCi L^{-1}) was injected into the pups intraperitoneally 90 minutes before death (10 μl g^{-1} BW) (Witsci and Saheb 1974). The syringe was weighed before and after injection to determine the amount of [^{3}H]-thymidine injected. Pups were sacrificed and lungs were carefully perfused through the pulmonary artery. Fresh whole lungs were homogenized in an ice-cold 5 mM phosphate buffer (pH 7.8) and nucleotides were extracted using 0.5 N $HClO_4$ solution. An aliquot of the extract was used to count the [^{3}H]-thymidine incorporated into the pulmonary DNA.

Two pup left lungs from each litter were used for wet/dry weight measurement. Those lungs were not perfused, excised and weighed in weighing bottles before and after oven drying (80°C) to constant weight (3 days).

Two pups from each litter were used for histomorphometrical analysis of the lungs. Lungs were fixed via intratracheal instillation of 10% buffered formalin at an inflation pressure of 22 cm of H_2O and then stored in the fixative. At least 3 days after fixation, total lung volume was measured by water displacement. Three standard cross-sections from each lung (right and left) were examined for subsequent histomorphometrical analysis. These sections were taken from the same area of apical, middle and diaphragmatic portions of each lung. Sections were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. A standard intergrating eyepiece was used for counts. The number of bars which intersected lung tissue per field and the number of times the lines were crossed by tissue septa per field were counted. Mean air space size, internal surface area and percent air space were calculated from the counts (Weibel 1963; Thurlbeck *et al.* 1981).

The remaining pups from each litter were used for biochemical analyses. Se concentrations in the diet, lung, plasma, and liver were determined according to the method of McCarthy *et al.* (1981), using a gas chromatograph equipped with an electron capture detector (Hewlett-Packard 5710A, Avondale, PA). Activities of glutathione peroxidase (GPx) were determined in blood and tissue homogenates by a modified coupled assay of

Paglia and Valentine (1967). Hydrogen peroxide was used as the substrate in the assay. Protein content of tissues and blood was determined by a modified Lowry method (Lowry *et al.* 1951).

3. Statistics

Data were evaluated using analysis of variance (2×3 factorial, Se treatment vs. time) statistics (Steel and Torrie 1980). Differences between dietary treatments at each time point were determined by the least significant difference test. A probability value of $p<0.05$ was chosen as the level of statistical significance.

RESULTS

DNA synthesis in pup lungs, as measured by incorporation of [^{3}H]-thymidine into lung DNA, is shown at Fig. 1. DNA synthesis was slightly higher in Se+ pups on d 6 and d 9, but significant difference was not found. The degree of DNA synthesis was decreased in older pups regardless of dietary Se and no difference was found between Se- and Se+ pups on d 16.

The results of histomorphometry of pup lungs are shown at Table 2. As pulmonary alveolarization progressed, mean air space size (Lm) decreased and internal surface area (ISA) and lung volume increased. Meanwhile, specific lung volume and specific ISA by 100 g-body weight decreased significantly between d 9 and d 16 of age. No difference in pulmonary alveolarization was found between Se- and Se+ pups by age.

Mean body and lung weights and dry/wet lung ratio of pups are listed at Table 3. Mean body and lung weights increased, but dry/wet lung ratio did not change with age. Dietary Se had no effect on body and lung weights, and dry/wet lung ratio in the pups.

Mean Se concentrations in pup lung, plasma and liver are shown at Table 4. Lung Se concentration was higher

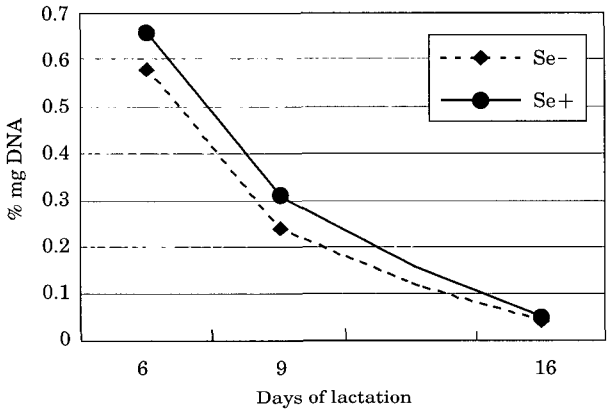

Fig. 1. [^{3}H]-thymidine incorporation into lung DNA of Se-deficient or Se-adequate pups. Data were analyzed by 2×3 factorial analysis of variance and a significant effect of pup's age was found ($p<0.001$). In each dietary Se treatment, d6 > d9 = d16.

Table 2. Mean air space size (Lm), internal surface area (ISA), lung volume and percent air space of the lung from Se-deficient (Se-) or Se-adequate (Se+) pups

	Group	Postnatal age of pups			ANOVA[1]
		d6	d9	d16	
Lm (μm)	Se-	75.4±1.9	68.7±2.3	60.2±1.9	Age
	Se+	73.2±4.3	68.6±2.5	61.2±1.0	
ISA (cm^2)	Se-	361.7±14.0	542.3±21.3	1143.3±118.8	Age
	Se+	370.6±17.4	509.3±14.4	1126.3±33.7	
Volume (ml)	Se-	0.68±0.03	0.93±0.04	1.71±0.04	Age
	Se+	0.68±0.06	0.88±0.05	1.72±0.05	
% air space	Se-	74.6±1.4	74.7±1.4	73.3±0.7	N.S.
	Se+	74.3±2.0	72.8±1.6	73.4±1.1	
Volume (ml 100 g^{-1} BW)	Se-	6.92±0.19	6.49±0.16	5.21±0.12	Age
	Se+	6.49±0.25	6.45±0.22	5.17±0.14	
Specific ISA (cm^2 100 g^{-1} BW)	Se-	3671±74	3794±109	3484±102	Age
	Se+	3576±159	3767±45	3382±101	

Values are means±SEM.
[1]Data were analyzed by 2×3 factorial analysis of variance ($p<0.05$)
Age: significant effect of age; N.S.: not significant.

Table 3. Mean body and lung weights of Se-deficient (Se-) and Se-adequate (Se+) pups

	Group	Postnatal age of pups			ANOVA[1]
		d6	d9	d16	
Body wt (g)	Se-	9.56±0.42	14.63±0.88	32.83±1.62	Age
	Se+	9.97±0.66	13.19±0.47	33.44±0.80	
Wet lung wt (g)	Se-	0.18±0.01	0.32±0.04	0.51±0.02	Age
	Se+	0.20±0.02	0.25±0.02	0.55±0.07	
Dry/wet lung wt (%)	Se-	16.55±0.69	16.26±1.32	18.08±0.59	Age
	Se+	17.79±0.23	17.78±0.78	17.63±0.76	

Values are means±SEM.
[2]Data were analyzed by 2×3 factorial analysis of variance
Age: significant effect of age ($p<0.05$)

Table 4. Se concentrations in lung, plasma and liver of Se-deficient (Se-) or Se-adequate (Se+) pups

	Group	Postnatal age of pups			ANOVA[1]
		d6	d9	d16	
			(μmol kg^{-1})		
Lung	Se-	1.30±0.18	1.51±0.09	1.48±0.07	Se, Age
	Se+	1.74±0.11	2.13±0.13	2.22±0.08	
Plasma[2]	Se-	0.92±0.11	0.96±0.07	1.12±0.03	Se, Age, Se*Age
	Se+	1.47±0.27	1.81±0.18	2.46±0.11	
Liver[3]	Se-	2.57±0.58	1.93±0.22	1.53±0.10	Se, Se*Age
	Se+	3.44±0.42	3.85±0.23	4.65±0.33	

Values are means±SEM
[2]Data were analyzed by 2×3 factorial analysis of variance ($p<0.05$)
Se: significant effect of Se; Age: significant effect of age; Se*Age: significant interaction effect between Se and age
[3]In Se+ pups: d6 = d9 < d16
[4]In Se- pups: d6 > d16; In Se+ pups: d6 < d9 = d16

in Se+ pups than in Se- pups and the concentration increased through d 16 of age. Plasma and liver Se concentrations were also higher in Se+ pups than in Se- pups. Plasma and liver Se concentrations of Se+ pups increased with age. On the other hand, liver Se concentration of Se- pups became lower with age. Mean Se concentrations in lung, plasma and liver from Se- pups were 74%, 63% and 69% of those in Se+ pups on d 6 and 67%, 46% and 33% of those in Se+ pups on d 16.

GPx activities in plasma and tissues are shown at Fig. 2. GPx activities in plasma and tissues reflected Se status in the respective tissues and were lower in Se- pups than in Se+ pups. Lung GPx activity did not change in Se+ pups by age, while the activity decreased significantly in Se- pups from d 6 to d 9 of age. Plasma and liver GPx activities of Se+ pups significantly increased from d 9 to d 16 of age, while no significant changes were found in Se- pups. Mean GPx activities in lung, plasma and liver and of Se- pups were 79%, 70% and 37% of those in Se+ pups on d 6 and 43%, 48% and 10% of those in Se+ pups on d 16.

DISCUSSION

Fetal lung development can be divided into 4 major stages (Burri 1984). During the embryonic stage, major lobes of the lungs are defined. In the pseudoglandular stage, the major airways develop from trachea up to terminal bronchioles. Then, during the canalicular stage, airway branching and elongation continue, resulting in the formation of respiratory bronchioles. During the terminal sac or saccular stage, further differentiation of the lung occurs, with the appearance of terminal air spaces called saccules. During the saccular stage, capillaries become closely approximated to the epithelium, establishing a gas exchange area.

Approximately 80~90% of alveolarization occurs dur-

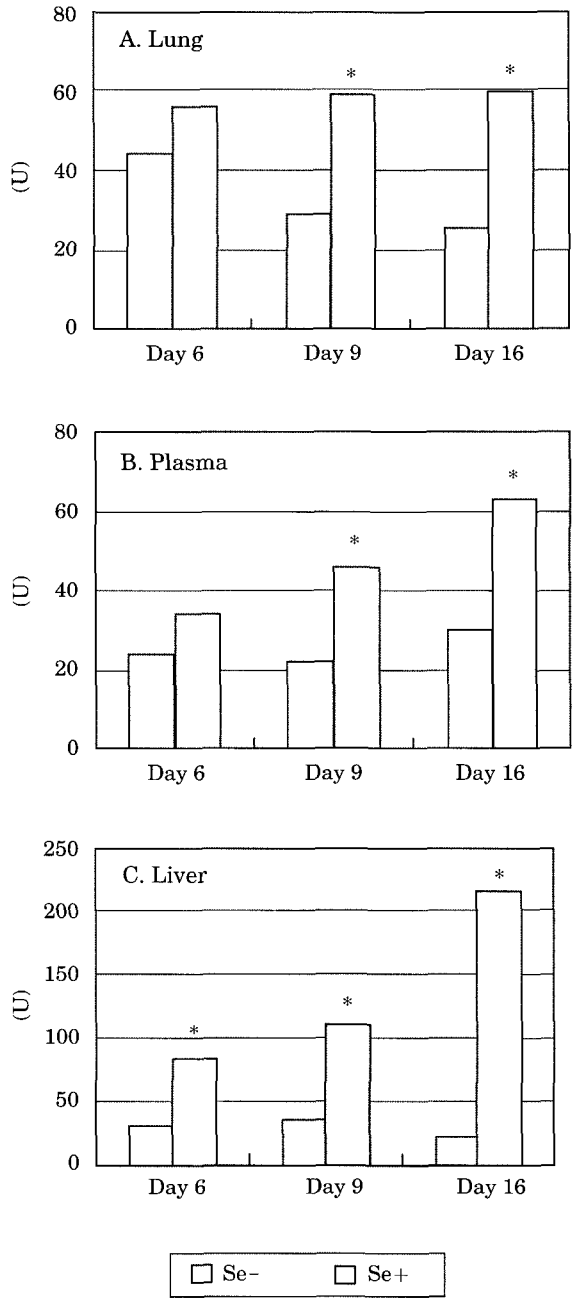

Fig. 2. Glutathione peroxidase activities in lung, plasma, and liver of Se-deficient (Se-) or Se-adequate (Se+) pups. Asterisks denote statistically significant differences in glutathione peroxidase activities between Se- and Se+ pups.

ing early postnatal period (Massaro *et al.* 1985). Compared to human beings, the rat has less developed alveoli at birth (Maloney 1984). The large, thick-walled saccules of newborn pups subdivide by outgrowth and elongation of septa in the saccule walls (Burri 1974). This subdivision is completed by the 14th postnatal day and then followed by rapid thinning of the alveolar wall, completed by the end of third week. Therefore, at the end of lactation period (3 weeks), saccules are fully developed into numerous, small, thin-walled alveoli. In this study, the structural development of the neonatal rat lung was assessed by histomorphometrical analysis to see whether Se inadequacy impairs normal pulmonary alveolarization. Even though Se concentration and GPx activity were lower in Se- pups than in Se+ pups, alveolarization was not impaired in Se- pups.

One of the most important metabolic events in perinatal lung maturation is surfactant production and secretion (Post *et al.* 1988). Surfactant is produced by the alveolar type II epithelial cell and is stored in intracellular organelles called lamellar bodies prior to secretion (Wright and Dobbs 1991). DSPC is the major surface-active component of surfactant that is responsible for decreasing the surface tension at the alveolar surface. The physiological advantages of reducing surface tension in alveoli include increasing the compliance of the lung and reducing the work of expanding it with each breath (West 1985). It has been shown that the DSPC percentage of the neonatal lung increases in Se adequacy (Kim 1994). It is possible that although structural development of the lung, as assessed by histomorphometry, was not shown to be impaired by marginal Se deficiency, the measurements used in this study might not have been sensitive enough to detect the effects of a marginally Se deficient diet.

The concentration of selenium and glutathione peroxidase activity in the pup lung directly reflected dietary Se concentration. While lung Se concentration was significantly increased in Se adequacy, similar elevation was not shown in Se inadequacy. The difference in pulmonary Se concentration between Se- and Se+ pups was enhanced over time. It is likely that continuation of low dietary Se during the neonatal period results in a shortage of Se store. The very low antioxidant enzyme, GPx, activity due to low Se storage can easily dispose pup lung to a high oxygen attack (Kim *et al.* 1992). Therefore, selenium adequacy is necessary for protection of lungs from oxidative stress, but selenium has little direct effect on pulmonary alveolarization of neonatal rats.

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