Inorganic Selenite Supplementation and Protection against Hyperoxic Injury in Neonates

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Summary This study was designed to determine if oral sodium selenite supplementation to Se-depleted rat pups furnishes protection against hyperoxic lung injury. Twelve female rats were bred and fed a Se-deficient (0.04 ppm Se) diet during pregnancy and lactation. Pups were supplemented either with 0 or 3.2 ng Se/g body weight daily from days 2 to 7. On day 4, two litters were mixed, with half of the pooled litter assigned to an air environment and the other half to an oxygen environment. Dams cross-fostered pups for 4 d. Selenite supplementation increased pup plasma and liver selenium concentration and the liver activity of glutathione peroxidase (GPx). However, lung GPx activity was more affected by oxygen exposure than selenite supplementation. While oral Se supplementation of the pups showed a tendency for decreased incidence of lung injury with oxygen exposure, this apparent effect was not statistically significant. Selenium-supplemented pups also showed a trend toward larger internal surface area and lung volume than selenium-depleted pups. These data indicate that early postnatal selenium repletion via direct oral selenite supplementation may be beneficial to rat pups against hyperoxic lung injury.

Key Words selenite supplementation, neonatal selenium status, hyperoxic lung injury, glutathione peroxidase

Newborn premature infants are especially prone to selenium (Se) deficiency due to their short gestational period and because they may receive negligible quantities of Se via parenteral and enteral feedings. Premature infants have a smaller storage pool of hepatic Se than full-term infants at birth (1), and the plasma Se concentration reportedly declines very rapidly during the stay in neonatal nurseries (2–5).

Normal lung development and resistance to damage by hyperoxia are especially important issues for premature infants ventilated with high O2 treatment of respiratory distress syndrome (RDS) (6). From the combination of RDS and high O2 treatment, a chronic inflammatory lung disease, bronchopulmonary dysplasia (BPD), often results. About 7,000 new cases of BPD occur every year and it is the most common form of chronic lung disease of infants in the United States (7).

Previously we reported (8) that a short period (7 d) of selenium repletion in newborn rat pups via milk from mothers fed a Se-supplemented diet enhanced pulmonary development in the pups as measured by increasing lung volume and internal surface area, providing a greater capacity for oxygen exchange (8). There was also Se-mediated protection against pulmonary damage during hyperoxia, manifested by reduced incidence and severity of pulmonary interstitial inflammation and septal attenuation in Se-repleted rat pups exposed for 4 d to >95 % O2 (8). While these results suggest that supplemental organic Se via milk would be efficacious in enhancing neonatal lung development and affording protection against oxidant stress, it is uncertain whether direct oral supplementation of Se-deficient pups with an equivalent dose of inorganic Se would yield comparable results. Thus, the present study was designed to determine whether oral sodium selenite supplementation directly to the Se-depleted rat pup would permit improved neonatal lung development and resistance to hyperoxic lung injury. Specifically, we examined the neonatal lungs of Se-deficient pups and those repleted orally for 6 d that were reared in air or a high O2 environment. Evaluations of pups included the histopathology and histomorphometry of pup lungs, and biochemical indices of the Se status.

MATERIALS AND METHODS

Nulliparous female, Sprague-Dawley rats (Harlan Industries, Indianapolis, IN, USA), weighing 180–200 g, were housed in individual, suspended, stainless-steel wire-mesh cages in a room with controlled temperature (20–22 °C) and lighting (12 h light-dark cycle). The animals were fed a commercial ration (Purina Rodent Chow,Ralston Purina Co., St. Louis, MO, USA) for a 2-wk adaptation period. At 200–240 g, rats were mated, and day 1 of pregnancy was determined by the presence of vaginal plugs and sperm. On day 1 of pregnancy, all rats were assigned to a semipurified Se-deficient diet. Demineralized water (Nanopure, Barnstead, Boston, MA, USA) and experimental diets were fed ad libitum.
Twelve female rats were bred and fed the Se-deficient diet (0.04 µg Se/g) diet during pregnancy and lactation. Food dishes were positioned so that only dams had access to food. The day after parturition (day 2), litters were culled so that there were 10 pups per dam. Pups were supplemented orally with either 0 or 3.2 mg/L Se as sodium selenite in a 0.9% saline solution (10–20 µL). Pups were weighed and sacrificed at day 8 of age. Six pups in each litter were used for biochemical assays and the remaining four pups were used for histopathology and histomorphometry. Whole blood was centrifuged (800 × g) and plasma was collected. Lungs used for biochemical analysis were first carefully perfused through the pulmonary artery with ice-cold isotonic buffer (0.1 M potassium phosphate, 0.15 M KC1, PH 7.4). Lungs and liver were surgically removed, rinsed, and blotted dry. Tissue samples were homogenized in ice-cold 5 mM phosphate buffer (pH 7.8).

Se concentrations in the diet, milk, lung, plasma, and liver were determined according to the method of McCarthy et al. (12), using a gas chromatograph equipped with an electron capture detector (Hewlett-Packard 5710A, Avondale, PA, USA). Activities of GPx (glutathione peroxidase) were determined in blood and tissue homogenates by a modified coupled assay of Paglia and Valentine (13, 14). Hydrogen peroxide was used as the substrate in the assay. The protein content of tissues and blood was determined by a modified Lowry method (15).

Lungs used for histopathology and histomorphometry were fixed via intratracheal instillation of 10% buffered formalin at an inflation pressure of 22 cm of H2O and then stored in the fixative. At least 3 d after fixation, total lung volume was measured by water displacement. Three standard cross-sections from each lung (right and left) were examined at low (10×) and high (40×) magnification by light microscopy to determine if two lesion patterns were present: septal attenuation and interstitial inflammation (16). The histological scoring was done in a double-blinded manner. Each lesion received a score ranging from 0 (no lesion) to 3 (severe lesion).

For histomorphometry, a standard integrating eyepiece was used. 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 airspace size (Lm) was calculated using the following formula (17):

Lm = (Length of line × number of lines × number of fields) / Number of tissue intercepts
Table 2. Mean plasma, liver, and lung Se concentrations and SeGPx activities of Se-deficient and Se-supplemented pups reared in air or oxygen environments for 4 d.¹

|                          | Se-deficient                      | Se-supplemented                    | ANOVA² |
|--------------------------|-----------------------------------|------------------------------------|--------|
|                          | Air | Oxygen | Air | Oxygen |            |        |
| Se concentration         |     |        |     |        |            |        |
| Plasma (μmol/L)          | 1.00±0.05 | 0.87±0.09 | 1.28±0.07 | 1.26±0.14 | Se      |
| Liver (μmol/kg)          | 2.09±0.11 | 1.84±0.13 | 2.25±0.19 | 2.50±0.18 | Se      |
| Lung (μmol/kg)           | 1.39±0.20 | 1.37±0.14 | 1.40±0.10 | 1.64±0.11 | N.S.    |
| SeGPx activity (μmol NADPH disappearance/min/g protein) |     |        |     |        |            |        |
| Plasma                   | 10.4±2.6 | 7.3±1.5 | 11.4±2.3 | 8.9±1.4 | N.S.    |
| Liver                    | 24.9±2.8 | 21.0±1.5 | 38.6±2.0 | 36.7±5.7 | Se      |
| Lung                     | 31.0±2.4 | 39.0±2.1 | 32.3±3.4 | 47.5±1.9 | O₂      |

¹ Values are means±SEM for six samples.
² Data analyzed by 2×2 factorial analysis of variance (p<0.05).
N.S., Not significant; Se, Significant effect of dietary selenium; O₂, Significant effect of oxygen environment.

Table 3. Severity scores of lung interstitial inflammation and septal attenuation from Se-deficient and Se-supplemented pups reared in air or oxygen environments for 4 d.¹

|                          | Se-deficient                      | Se-supplemented                    | ANOVA² |
|--------------------------|-----------------------------------|------------------------------------|--------|
|                          | Air | Oxygen | Air | Oxygen |            |        |
| Interstitial inflammation | 0.14±0.07 | 0.21±0.09 | 0.14±0.07 | 0.20±0.13 | N.S.    |
| Septal attenuation       | 0.43±0.12 | 0.85±0.16 | 0.39±0.14 | 0.65±0.20 | O₂      |
| Total histopathological score | 0.57±0.13 | 1.06±0.19 | 0.52±0.19 | 0.85±0.27 | O₂      |

¹ Values are means±SEM for 10–12 pups. Each lung tissue received a score ranging from 0 (no lesion) to 3 (severe lesion) in a double evaluation.
² Data analyzed by 2×2 factorial analysis of variance (p<0.05).
N.S., Not significant; O₂, Significant effect of oxygen environment.

Mean internal surface area (ISA) was calculated using the following formula (18):

\[ ISA = \frac{4 \times \text{volume of lung parenchyma}}{Lm} \]

Mean percent of airspace was calculated by:

\[ \% \text{ airspace} = \frac{Pa}{(Pa + Pt)} \]

where Pa was the number of intercept bars hitting air and Pt was the number of intercept bars hitting tissue.

Biochemical data and histomorphometrical data were evaluated using analysis of variance (2×2 factorial) statistics followed by the least significant difference test (19). Data for incidence of lung injury were assessed by the Fisher exact probability test (20). The value of p<0.05 was chosen as the level of statistical significance.

RESULTS

Maternal feed intake, body weight and tissue Se concentrations

Throughout the study, no significant differences in mean feed intake and body weight of dams were observed among the groups. The mean feed intake of all dams was 18.4±1.5 g/d. The mean body weight (g) of dams was 329.3±13.2 at the end of pregnancy and 244.6±17.8 at the end of lactation (day 8). Similarly, mean concentrations of Se in milk (mmol/mL) and liver (μmol/g) were similar between groups at the end of lactation (day 8) and were 0.67±0.10 and 4.52±0.40, respectively.

Se concentrations and SeGPx activities in pup plasma and organs

Plasma, liver, and pulmonary Se concentrations and GPx activities in pups are presented in Table 2. Se concentrations in the plasma and liver were significantly elevated by oral selenite supplementation of the pup. Oxygen exposure appeared to increase the selenium concentration in the liver and lungs of the selenium-supplemented pups, but the apparent increase was not statistically significant. Hepatic GPx activity in the pups paralleled hepatic Se concentrations and was significantly elevated by Se supplementation. The activity of GPx in the pup lung was significantly elevated by O₂ exposure and the elevation of activity was prominent in the selenium-supplemented pups.

Histopathology and histomorphometry of pup lungs

Oxygen exposure significantly increased the incidence of pulmonary damage in the pups (p<0.05), with a trend towards increased interstitial inflammation and septal attenuation (Table 3). Oral Se supplementation of the pups showed a tendency for a decreased incidence of lung injury compared to Se-deficient pups under oxygen exposure, but this apparent effect was not statistically significant. Typical microscopic characteristics of
lungs from the experimental rat pups are shown in Figs. 1 and 2. The alterations associated with septal attenuation included stunting of septal buds, a decrease in the number of alveolar septa, thinning of remaining septal buds, and enlarged alveolar spaces (Fig. 2A). In lungs with interstitial inflammation (Fig. 2B), there were multiple foci of thickening and hypercellularity of alveolar septa, mainly due to infiltration by macrophages and some neutrophils.

Lung development measured by histomorphometry is listed in Table 4. Selenium-supplemented pups showed a trend toward larger internal surface area and lung volume than selenium-depleted pups. High O2 exposure significantly decreased the lung volume of the pups by 9.1% and internal surface area of the pups by 11.4%, regardless of Se supplementation. Mean airspace size and % airspace of the pups were not affected by either Se supplementation or oxygen exposure.

DISCUSSION

The impact of inorganic selenite supplementation in selenium-depleted pups to providing protection against hyperoxic lung injury was investigated in this study. Direct, oral selenite supplementation increased the tissue selenium concentration and liver glutathione peroxidase activity in pups. There was a significant increase in pulmonary GPx activity both in selenium-depleted and -repleted pups under oxygen exposure. Incidence and severity of pulmonary damage were lower in selenium-repleted pups as evidenced by histology and histomorphometry, but statistical significance was not achieved. This is in contrast to our previous study (8) showing that neonatal Se repletion achieved by maternal Se supplementation did furnish protection via enhanced milk Se content provided to Se-repleted pups.

The reason for the diminished effectiveness of direct Se supplementation in protecting pups against hyperoxia compared to maternal supplementation is not known, but several possibilities exist. The plasma selenium concentration of selenium-repleted rat pups via dam’s milk in our previous study (8) was higher (1.32 μmol/L) than that achieved by direct selenite supplementation of pups (1.28 μmol/L) in this study, even though the quantity delivered orally was calculated to be similar. Therefore, it is possible that Se bioavailability from milk is enhanced and a greater dose of selenite is needed to protect the neonatal rat lung from hyperoxia when given directly to the neonatal pup. There is evidence to support this possibility. In several studies, organic forms of Se have been shown to be more bioavailable and potent than inorganic forms of Se, not only for the improvement of Se status but also for the treatment of certain Se deficiency related diseases in mammals (21–23). The utilization of selenite by maternal dams may have furnished a greater fraction of bioavailable selenium via milk accounting for the effectiveness of this route of Se delivery to neonatal pups.

Inorganic selenite is as well absorbed as organic Se in rats (24, 25). In contrast, the bodily retention of organic selenium, such as selenium provided by enriched yeast and selenomethionine, is greater than that of inorganic selenite in both rats and humans (21, 26). In addition, an undefined organic selenium compound from pork kidney powder is three to four times as potent.
as inorganic selenite against liver necrosis in the rat.
(22). Cantor et al. (27) found that, in chicks, selenomethionine is four times as effective as selenite in preventing pancreatic fibrosis associated with Se deficiency. Therefore, it is also possible that Se provided via milk may be metabolically more active than inorganic Se provided as selenite to the pup. That is, the organic forms in milk were not only more bioavailable, but also more effectively utilized by the lung tissue of the developing rat pup than was the inorganic selenite.

Low birth weight (LBW) infants are often in a marginally Se depleted state and a rapid supply of bioavailable Se to LBW infants with respiratory distress syndrome (RDS) may be crucial to prevent O₂-induced illness as well as to prevent other Se deficiency related symptoms. Amin et al. (2) reported that supplementation of 3 ng sodium selenite/g BW/d to infants with RDS prevented the decline in plasma Se concentration. Huston et al. (28) added selenious acid (1.34 ng/g BW/d) to a parenteral nutrition solution used in premature infants and found that the Se-supplemented infants required less O₂ ventilation time than Se-unsupple-
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Table 4. Percent airspace, mean airspace size (Lm), internal surface area (ISA), and volume of lungs from Se-deficient and Se-supplemented pups reared in air or oxygen environments for 4 d.1

|                      | Se-deficient | Se-supplemented | ANOVA2 |
|----------------------|--------------|-----------------|--------|
|                      | Air          | Oxygen          |        |
| ISA (cm²)            | 479.7±21.1   | 424.6±14.4      |        |
| Volume (mL)          | 0.82±0.02    | 0.72±0.02       |        |
| % airspace           | 75.43±1.84   | 77.49±0.80      |        |
| Lm (μm)              | 68.60±1.49   | 68.47±1.46      |        |
| Specific volume (mL/100 g BW) | 6.92±0.21 | 6.11±0.20       |        |
| Specific ISA (cm²/100 g BW) | 4061.9±178.4 | 3579.9±121.3 |        |
|                      | 507.4±24.6   | 449.7±25.0      | O₂     |
|                      | 0.84±0.03    | 0.79±0.04       | O₂     |
|                      | 76.20±0.99   | 80.64±3.34      | N.S.   |
|                      | 66.87±1.35   | 70.59±1.87      | N.S.   |
|                      | 7.08±0.29    | 6.39±0.29       | O₂     |
|                      | 4253.0±206.2 | 3656.2±202.8    | O₂     |

1 Values are means ±SEM for 10–12 pups.
2 Data analyzed by 2×2 factorial analysis of variance (p<0.05).
N.S., Not significant; O₂, Significant effect of oxygen environment.

mented infants (37 vs. 50 d). However, although the Se-supplemented group had elevated serum Se concentrations compared to the control group, the dose supplemented was not enough to maintain the plasma Se of the supplemented infants.

In future human studies, both the form of supplemental Se as well as the quantity of Se administered must be carefully evaluated so that the desired clinical outcomes can be achieved. There is every reason to believe that the maintenance of Se adequacy will be of benefit to LBW infants undergoing high O₂ ventilation. However, since the margin of safety between beneficial and toxic levels of Se is known to be relatively narrow (29), Se supplementation for LBW infants, especially those with RDS, should be undertaken with caution until the best supplemental form is identified and the correct dose of a particular form of Se is defined. To this end, additional animal studies may be required to provide the needed information for future clinical interventions with selenium. Specifically, it is essential to determine the oral or parenteral equivalents of Se to furnish the dosage that was determined previously to be effective when administered via milk. Present knowledge indicates that equivalent dosages will vary depending on the form of administered selenium.

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