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Clinical studies

Trace elements and antioxidant enzymes in extremely low birthweight infants

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ABSTRACT

Oxygen radicals are believed to contribute to typical diseases of prematurity, such as bronchopulmonary dysplasia (BPD), intraventricular haemorrhage (IVH), retinopathy of prematurity (ROP) and necrotising enterocolitis (NEC). Our aim was to investigate whether these disorders are associated with disturbances in antioxidant enzyme activities and with low trace elements, which are co-factors of antioxidant enzymes. 209 infants with birthweight less than 1000 g were enrolled into a European multicentre randomised erythropoietin (rhEPO) trial; 155 developed one or more of the above mentioned diseases. We analysed Zn, Cu, Fe, Se in plasma and red blood cells (RBCs), superoxide dismutase (CuZn-SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in RBCs on the 3rd and 68th day of life. Zn, Fe, Se in plasma, and Se in RBCs decreased ($p < 0.01$), and Zn in RBC ($p < 0.001$), CuZn-SOD ($p < 0.01$) and CAT increased ($p < 0.05$), whereas GSH-Px remained unchanged. No differences were observed between the rhEPO and control groups. Antioxidant enzyme activities did not correlate with gestational age. In infants with BPD, IVH, ROP, or NEC, CuZn-SOD and CAT ($p < 0.05$) were higher at day 68 than in infants without these diseases. CuZn-SOD and GSH-Px at 3 days and CuZn-SOD at 68 days correlated positively ($p < 0.05$) with the duration of oxygen treatment. In conclusion, in ELBW infants, trace element concentrations decreased over the first 10 weeks of life. Lower trace element concentrations, did not affect the activities of CuZn-SOD, GSH-Px, and CAT. Typical diseases of prematurity were not associated with decreased antioxidant enzyme activities.

Introduction

Excessive formation of toxic reactive oxygen species (ROS) has been proposed as one pathogenetic mechanism of diseases of prematurity. In bronchopulmonary dysplasia (BPD), inflammation increases oxidative stress, and causes lung fibrosis and surfactant inactivation [1]. Immature oligodendroglia is vulnerable to oxygen and ROS. Following asphyxia and reoxygenation, lipid peroxidation in the brain results in intraventricular haemorrhage (IVH) and apoptosis [2]. Increased retinal blood flow, immature autoregulation, oxygen treatment and immature antioxidants contribute to retinopathy of prematurity (ROP) [3]. In necrotising enterocolitis (NEC), ischaemia, reperfusion and inflammation increase ROS formation, leading to enterocytic apoptosis [4].

Superoxide dismutase (CuZn-SOD) transforms the hazardeous superoxide anion O^{2-} into H_2O_2 and O_2, which are further transformed into O_2 and H_2O by glutathione peroxidase (GSH-Px) and catalase (CAT). Iron (Fe) and copper (Cu) promote the formation of free radicals by catalysing the oxidation of physiological compounds, drugs and chemicals by molecular oxygen as shown mostly in in vitro assessment [5]. Low antioxidant enzyme activities have been described in preterm infants [6]. BPD was predicted by low RBC Se on the 3rd day [7] but could not be prevented by Se substitution [8].

To prevent anaemia of prematurity, early treatment with rhEPO and iron has been proposed [9]. During this treatment, CuZn-SOD, GSH-Px and CAT activities were reported to be uncompromised [10]. However, a meta-analysis found severe ROP more frequently following early rhEPO and iron treatment [11]. This prompted us to analyse antioxidant enzymes and trace elements in ELBW infants participating in a European rhEPO trial

Abbreviations: Se, selenium; Zn, zinc; Cu, copper; Fe, iron; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; RBC, red blood cells; ELBW, extremely low birthweight; BPD, bronchopulmonary dysplasia; IVH, intraventricular haemorrhage; ROP, retinopathy of prematurity; NEC, necrotising enterocolitis

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with respect to the occurrence of BPD, IVH, ROP and NEC. We hypothesised:

(1) In ELBW infants concentrations of trace elements shortly after birth are lower than published reference values and decrease further until 10 weeks of life.
(2) ELBW infants who develop BPD, IVH, ROP or NEC have lower antioxidant enzyme activities after birth and a smaller increase in their activities in the first 10 weeks than infants without these diseases.

Materials and methods

Patients

Patients were recruited from May 1998 to June 1999 within the 4th European randomised controlled 14-centre trial on rhEPO in ELBW infants (ClinicalTrials.gov ID NCT00593801). The study, including the investigations described here, was approved by the local ethics committees and written parental consent was obtained. Previously published haematopoietic results showed that early rhEPO treatment effectively reduces the need for transfusion in ELBW infants [9].

209 infants completed the study, 155 of whom developed BPD, IVH, ROP or NEC. The infants were allocated to the following study groups (total number of infants/infants with typical diseases of prematurity): early rhEPO (71/53), late rhEPO (71/53) and controls (67/49). Small for gestational age was defined according to Fenton [12], BPD according to Shennan et al. [13], IVH according to Papile et al. [14], ROP according to ICROP [15] and NEC according to Walsh and Kliegman [16].

Treatment and nutrition

rhEPO, iron treatment, and transfusion were regulated by the study protocol [9]. Infants assigned to one rhEPO group received 750 IU per kg per week of rhEPO β intravenously or subcutaneously. rhEPO treatment was initiated between days 3 and 5 of life in the early rhEPO group and 3 weeks later in the late rhEPO group and was discontinued at 37 weeks corrected age or at discharge, whichever occurred first. All infants received enteral iron supplementation at a daily dose of 3 mg/kg starting from day 3 to 5. Iron was increased to 6 mg/kg/d at day 14 and to 9 mg/kg/d at day 26, if transferrin saturation was $<30\%$. At a transferrin saturation of 30–80% the iron dose was kept at 6 mg/kg/d and at $>80\%$ iron supplementation was interrupted. Parenteral iron was not administered.

Further management of the infants followed European standards, but was not regulated by the protocol. Each centre used its own standard preterm formula and human milk fortifier. As our centre randomised 20% of the infants, we describe our nutritional protocol. Infants were fed with mother's own or donated human milk, fortified with 5% FM 85 (Nestlé, Frankfurt, Germany), or with preterm formula Humana 0 (Humana GmbH, Herford, Germany). The fortifier FM 85 is a powder, which provides additional protein, minerals, trace elements and vitamins to human milk. Preterm formulas contain protein, minerals, trace elements and vitamins in higher concentrations than term formulas. On day 3 amino acids, lipids, trace elements and vitamins were added to the infusion. When 50% of the scheduled enteral intake was reached, parenteral nutrition was reduced. Nutrition aimed for 120–135 kcal/kg/d and 3.0–4.0 g/kg/d protein. Days of parenteral nutrition, time to regain birthweight, body length and head circumference, and data on the medical treatment were recorded prospectively up to day 68 of life.

Sample collection and analysis

Blood samples were taken on days 3 and 68 (250 μL EDTA blood). They were centrifuged for 10 min at 2000*g* and plasma was separated. RBCs were washed three times with 0.9% NaCl solution. All samples were stored at $-20\,^{\circ}C$ and thawed before analysis. For the present study, analyses were performed for Zn, Cu, Se (in plasma and RBC lysate), for Fe (in plasma), and for CuZn-SOD, GSH-Px, and CAT (in RBC lysate) at the Department of Trace Elements in Health and Nutrition (Hahn-Meitner Institute Berlin, Germany). Also haemoglobin analysis was carried out by Drabkin's cyanmethemoglobin method at the same institute. Plasma ferritin was measured by nephelometry, turbidimetry, or chemiluminescence, depending on the availability at the centres.

Trace element analyses

Zn, Cu, Fe, and Se were analysed in triplicate by inductively coupled plasma mass spectrometry (Elan 6000 Perkin Elmer, Ontario, Canada) as described previously [17]. Suprapur or ultrapur reagents, internal standards (germanium, rhodium, and iridium), and certified reference materials (NIST toxic elements in urine, Seronorm Serum, Accunorm Serum normal IP, Ghent Serum and IAEA 155 whole blood) were used. The values of reference materials measured for quality control were in good agreement with a within-day and day-to-day variation coefficient of less than 5%. To characterise low trace element status, the following serum threshold values for preterm infants were used: Zn, 458 μg/L [18]; Cu, 250 μg/L [19]; Fe, 0.17 mg/L [20]; ferritin, 48 μg/L (day 3)/35 μg/L (day 68) [20]; and Se, 10 μg/L [22]. Reference values for trace element concentrations in serum of preterm infants are given in Table 2 [18,20,21,23,24].

Enzyme analyses

Enzyme activities were measured spectrophotometrically (Beckmann DU 650, Munich, Germany) with 5 replicates/sample. CuZn-SOD was measured in the clear supernatant using pyrogallol in TRIS buffer at pH 8.0 according to Beutler's method [25]. SOD from Sigma was used for calibration. GSH-Px activity was analysed according to Paglia and Valentine [26] using the RANSEL® assay (Randox Laboratories GmbH, Krefeld, Germany). A control sample from Randox gave a value of 440 ± 47 U/L ($n=24$, control range 364–492 U/L). CAT was measured using Aebi's method [27]. Bovine CAT from Sigma was used for calibration.

Statistics

Statistical analysis was carried out with SPSS software 12.0 (SPSS Inc., Chicago, USA). Data are expressed as median (centiles, quartiles) or *n* (%). Group differences were identified with the Wilcoxon test, the chi-square test, and the Kruskal–Wallis test; all tests were two-sided and non-parametric. Spearman rank correlation coefficient and regression analysis were used to relate trace element concentrations, enzyme activities and clinical data. $p<0.05$ was set as the threshold of significance.

Results

Parenteral nutrition was applied in 209 ELBW infants for the following median (quartiles) duration: glucose for 26 (16/38) days, amino acids for 22 (13/34) days, lipids for 12 (3/22) days, trace elements for 14 (1/26) days, and vitamins for 19 (10/37) days. Mother's own or donor milk was given exclusively for