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Adding glutathione to parenteral nutrition prevents alveolar loss in newborn Guinea pig ☆

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ABSTRACT

Bronchopulmonary dysplasia, a main complication of prematurity, is characterized by an alveolar hypoplasia. Oxidative stress is suspected to be a trigger event in this population who has a low level of glutathione, a main endogenous antioxidant, and who receives high oxidative load, particularly ascorbylperoxide from their parenteral nutrition. Hypothesis: the addition of glutathione (GSSG) in parenteral nutrition improves detoxification of ascorbylperoxide by glutathione peroxidase and therefore prevents exaggerated apoptosis and loss of alveoli.

Methods: Ascorbylperoxide is assessed as substrate for glutathione peroxidase in Michaelis-Menten kinetics. Three-days old guinea pig pups were divided in 6 groups to receive, through a catheter in jugular vein, the following solutions: 1) Sham (no infusion); 2) PN(-L): parenteral nutrition protected against light (low ascorbylperoxide); 3) PN(+L): PN without photo-protection (high ascorbylperoxide); 4) 180 μM ascorbylperoxide; 5) PN(+L)+10 μM GSSG; 6) ascorbylperoxyde+10 μM GSSG. After 4 days, lungs were sampled and prepared for histology and biochemical determinations. Data were analysed by ANOVA, $p < 0.05$

Results: The Km of ascorbylperoxide for glutathione peroxidase was 126 ± 6 μM and Vmax was 38.4 ± 2.5 nmol/min/ U. The presence of GSSG in intravenous solution has prevented the high GSSG, oxidized redox potential of glutathione, activation of caspase-3 (apoptosis marker) and loss of alveoli induced by PN(+L) or ascorbylperoxide.

Conclusion: A correction of the low glutathione levels observed in newborn animal on parenteral nutrition, protects lungs from toxic effect of ascorbylperoxide. Premature infants having a low level of glutathione, this finding is of high importance because it provides hope in a possible prevention of bronchopulmonary dysplasia.

1. Introduction

Bronchopulmonary dysplasia (BPD) is a main complication of prematurity. The exact aetiology of this chronic lung disease, characterized by alveolar hypoplasia, is unknown. Nonetheless there is a general consensus that oxidative stress is a triggering event. In this population, the two main oxidative sources are oxygen supplement because pulmonary immaturity and parenteral nutrition (PN) because gastrointestinal track immaturity. The link between oxygen supplement and development of BPD is well described [1]. In the last two decades, physicians were more careful with oxygen administration. Despite this caution in the use of supplemental oxygen, the incidence of BPD remains high [2].

The other main source of oxidant molecules is PN. The interactions between electron donors such as vitamin C and dissolved oxygen in the intravenous solution generate peroxides [3, 4]. A typical PN administered to premature infants, containing 1% of multivitamin preparation generates between 300 and 400 μM peroxides [3, 5], of which close to 80% are hydrogen peroxide [3]. The absence of adequate protection against ambient light accelerates the reaction. By using the light energy, the photo-excited riboflavin favours the transfer of electrons between ascorbate and oxygen. Full light protection reduces by half the generation of peroxides [5, 6]. Since the photo-protection of PN has been reported to reduce the incidence of BPD [7, 8], these peroxides are suspected to involve in BPD development. We have reproduced the effect of light exposure of PN on pulmonary alveolarization in newborn animal [9, 10]. The administration on four days of PN that

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is devoid of light protection induces a loss of alveoli following an exaggerated apoptosis [10, 11]. Recently we have shown that this loss is not the effect of H_2O_2 but of a new molecule named ascorbylperoxide (2,3-diketo-4-hydroxyperoxyl-5,6-dihydroxyhexanoic acid). This molecule is derived from peroxidation of dehydroascorbate by H_2O_2[12, 13].

Because an adequate photo-protection of PN, from pharmacy service, where the intravenous solution is compounded, until the bedside is practically impossible in clinical routine, we have investigated the possibility to improve the *in vivo* capacity to detoxify the ascorbylperoxide. The peroxide characteristic of this molecule suggested that it could be reduced by the action of glutathione peroxidase. Therefore, the first objective was to demonstrate the relationship between ascorbylperoxyde and glutathione peroxidase. The success of this approach suggested that the tissue concentration of glutathione (GSH) is important for this detoxification.

At birth, the level of glutathione is dependent on gestational age [14]. In extreme premature newborns, the level of glutathione remains low for at least the first three weeks of life [14]. A lack of substrate for the *de novo* synthesis of glutathione explains this fact [15]. A low cellular availability of cysteine is a known limiting factor for glutathione synthesis [16]. This condition can be explained by the immaturity of capture of cysteine by cells [17] and by low capacity of liver to deliver glutathione in bloodstream. An important role of glutathione is to serve as pool of cysteine for cells [18]. γ-Glutamyltranspeptidase on cellular membranes transfers the γ-glutamyl moiety of glutathione to another amino acid in circulation thereby generating two dipeptides that are uptaken by the cells. Following dipeptidase action, amino acids are released for a new synthesis of glutathione. The activities of γ -glutamyltranspeptidase and of synthetic enzymes are mature in preterm infants [19]. Glutathione present in bloodstream derives from the liver. This organ has high capacity to synthesize glutathione because it uses the transformation of methionine to generate cysteine. However, the first enzyme of this transformation, the methionine adenosyltransferase, is inhibited by peroxides from PN as shown in animal [20] and the last enzymatic step, the cystationase, is immature in premature infants [21, 22]. We hypothesized that the addition of glutathione in PN bypasses the liver process of glutathione production, increases the cellular capacity to detoxify ascorbylperoxide and consequently prevents the loss of alveoli following infusion of PN. Therefore, the second aim of the study was to test in newborn animals, the impact of an addition of glutathione in PN on the pulmonary level of glutathione (GSH, GSSG and redox potential), apoptosis and alveolar development.

2. Materials

Guinea pigs were purchased from Charles River (St-Constant, Montréal, QC, Canada). Catheters were obtained from SAI Infusion Technologies (Lake Villa, IL, USA). Intralipid20% was acquired from Fresenius Kabi Canada (Mississauga, ON, Canada). Multivitamin preparation (Multi-12/K_1 paediatrics), amino acids (Primene) and dextrose were provided by Baxter (Toronto, ON, Canada). GSH, GSSG, L-ascorbic acid, riboflavin, catalase and glutathione peroxidase were bought from Sigma-Aldrich (Oakville, ON, Canada). Glutathione reductase was obtained from Roche Diagnostics (Indianapolis, IN, USA). L-2-oxo-thiazolidine 4-carboxilic acid (OTC) was acquired from Clintec (Deerfield, IL, USA). Hydrogen peroxide 30%, and ammonium acetate HPLC grade were purchased from Fisher (Fair Lawn, NJ, USA). Boric acid was obtained from J.T.Baker Chemical (Phillipsburg, NJ, USA). The Enzyme Immunoassay Kit for the determination of 8-Isoprostane-F2α was provided by Cayman Chemical (Ann Arbor, MI, USA). Sep Pak C18 column were purchased from Water limited (Mississauga, On, Canada).

3. Methods

3.1. In vitro study

Glutathione peroxidase reduces peroxides in their corresponding alcohol. Into the reaction, two electrons from GSH are transferred to the peroxide. The formed GSSG is recycled by glutathione reductase using NADPH as electron donor. In our assay, the loss of NADPH monitored at 340 nm is used to assess the activity of glutathione peroxidase. The enzymatic kinetic of glutathione peroxidase corresponds to the Michaelis-Menten model. Such kinetic using increasing concentrations of ascorbylperoxide allows the confirmation that ascorbylperoxide is detoxified by glutathione peroxidase. The reaction medium contained 50 mM GSH, 5 mM NADPH, 0 to 540 μM ascorbylperoxide, 0.25 U glutathione reductase in a buffer (250 mM TRIS, 0.1 mM EDTA-Na_2) at pH 7.6. After 3 minutes at 25 °C, the reaction was started with the addition of 0.1 U glutathione peroxidase (from human erythrocytes-according to Sigma-thus glutathione peroxidase-1). After one minute of reaction, the loss of NADPH was monitored at 340 nm. The velocity of the reaction (v_0) was obtained using the molar extinction coefficient of NADPH (6.22 mM^{-1} cm^{-1}) and was expressed as μmol NADP generated / minute / U glutathione peroxidase.

3.2. In vivo study

A catheter was fixed in jugular vein of 36 guinea pigs aged of 3 days as previously described [10, 20]. The studied intravenous solutions were infused continuously through the catheter at a rate of 200 mL/kg/d. The solutions were changed daily. Animals were divided in six groups as followed:

- Sham: the catheter was closed by a node. Animals did not receive any intravenous solution. They were fed with regular food for guinea pig.
- PN(-L): animal were fed exclusively by intravenous solution containing 4 g/kg/d amino acids, 17.4 g/kg/d dextrose, 2 mL/kg/d multivitamin preparation, 3.2 g/kg/d lipid emulsion and 1 U/mL heparin [20]. The solution was photo-protected (-L) with opaque materials. We previously reported that this solution contains about 20 μM ascorbylperoxide [11].
- PN(+L): PN without photo-protection (about 75 feet-candle). We previously reported that this solution contains about 35 μM ascorbylperoxide [11].
- PN(+L)+GSSG: PN(+L) enriched with 10 μM GSSG.
- Ascorbylperoxide: animals were fed exclusively by intravenous solution containing 17.4 g/kg/d dextrose, 0.6 g/kg/d NaCl, 1 U/mL heparin and 180 μM ascorbylperoxide. This concentration of ascorbylperoxide is about five time higher than the measured concentration in PN(+L) and served to test the robustness of the concentration of GSSG used.