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Research Paper

Impact of glutathione supplementation of parenteral nutrition on hepatic methionine adenosyltransferase activity

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ABSTRACT

Background: The oxidation of the methionine adenosyltransferase (MAT) by the combined impact of peroxides contaminating parenteral nutrition (PN) and oxidized redox potential of glutathione is suspected to explain its inhibition observed in animals. A modification of MAT activity is suspected to be at origin of the PN-associated liver disease as observed in newborns. We hypothesized that the correction of redox potential of glutathione by adding glutathione in PN protects the MAT activity.
Aim: To investigate whether the addition of glutathione to PN can reverse the inhibition of MAT observed in animal on PN.
Methods: Three days old guinea pigs received through a jugular vein catheter 2 series of solutions. First with methionine supplement, (1) Sham (no infusion); (2) PN: amino acids, dextrose, lipids and vitamins; (3) PN-GSSG: PN+10 μM GSSG. Second without methionine, (4) D: dextrose; (5) D+180 μM ascorbylperoxide; (6) D+350 μM H_2O_2. Four days later, liver was sampled for determination of redox potential of glutathione and MAT activity in the presence or absence of 1 mM DTT. Data were compared by ANOVA, $p < 0.05$.
Results: MAT activity was 45 ± 4% lower in animal infused with PN and 23 ± 7% with peroxides generated in PN. The inhibition by peroxides was associated with oxidized redox potential and was reversible by DTT. Correction of redox potential (PN+GSSG) or DTT was without effect on the inhibition of MAT by PN. The slope of the linear relation between MAT activity and redox potential was two fold lower in animal infused with PN than in others groups.
Conclusion: The present study suggests that prevention of peroxide generation in PN and/or correction of the redox potential by adding glutathione in PN are not sufficient, at least in newborn guinea pigs, to restore normal MAT activity.

1. Introduction

The intravenous nutritional support for individuals who have impaired or immature gastrointestinal tract such as extreme premature infants is essential for their development and health. However, several hepatic complications are associated with this mode of nutrition. In adults, parenteral nutrition (PN) induces hepatic steatosis [1,2] whereas the intra-hepatic cholestasis is frequent in premature infants [3,4]. Animal data suggest that peroxides, H_2O_2 and ascorbylperoxide (2,3-diketo-4-hydroxyperoxyl-5,6-dihydroxyhexanoic acid), that contaminating parenteral nutrition [5,6] are involved in these disorders [7,8]. Peroxides can lead to perturbation of the metabolism following oxidation of the redox-sensitive thiol functions of specific proteins (Fig. 1). Hence, the activity of the hepatic methionine adenosyltransferase (MAT) is inhibited by PN or infused H_2O_2 [9].

MAT is at the crossroads of several metabolic pathways (Fig. 1). For instance, MAT catalyzes the formation of S-adenosylmethionine, the main methyl donor of the organism [10,11]. Perturbation in the generation of S-adenosylmethionine is frequently associated with hepatic disorders such as intrahepatic cholestasis [12,13]. The activity of MAT is the first step in the transformation of methionine into cysteine of which the availability is a limiting step for glutathione synthesis [14]. Intracellular concentration of GSH affects the activity of glutathione peroxidase during detoxification of peroxides [17]. Thus, peroxides generated in the PN can induce a vicious cycle by inhibiting MAT that leads to a lower GSH [9], and therefore to a lower capacity to detoxify the infused peroxides.

The oxidation of thiol into sulfenic acid (MAT-SOH) by peroxide is reversible [15]. The mixed disulfide (MAT-SSG) formed following

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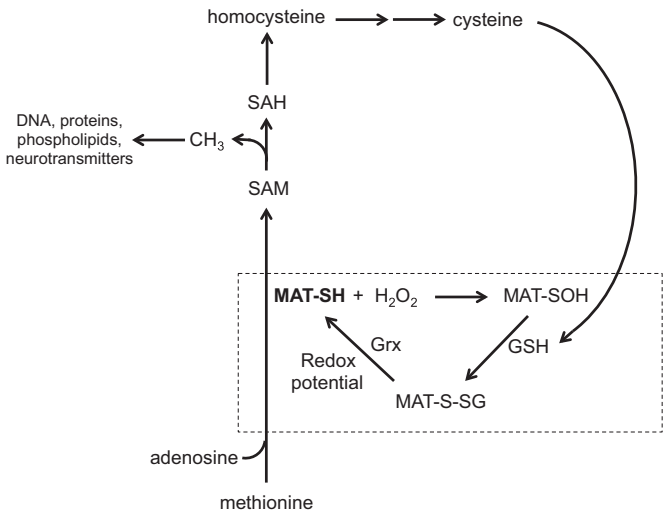

Fig. 1. Interrelation between MAT activity, peroxides and glutathione. The active form of MAT (MAT-SH) is responsible for the transformation of methionine in cysteine in order to sustain the synthesis of glutathione (GSH). In a context of PN, when MAT is inhibited (MAT-SOH) by peroxides (H_2O_2) generated into PN, a vicious cycle occurs. The low activity of MAT induces a low synthesis of GSH that is essential for the MAT recovery. By a compromised generation of the methyl donor S-adenosylmethionine (SAM), several metabolisms (proteins, DNA, phospholipids, neurotransmitters, etc.) are altered. SAH: S-adenosylhomocysteine.

interaction of MAT-SOH with GSH is recycled into the native protein by the glutaredoxin using glutathione as electron donor. We expect that the inhibition of MAT by PN occurs by this mechanism. The influence of the redox potential in the regeneration of MAT-SH, from MAT-SSG, is explained by its participation in the Gibbs equation [16] explaining a better efficiency of glutaredoxin in a more reduced environment.

Recently we have reported that addition of glutathione into PN led to a more reduced status of redox potential in lungs of newborn guinea pigs [17]. We hypothesize that the addition of glutathione in PN will decrease the redox potential value (to a more reduced status) in the liver, and consequently, will improve the regeneration of MAT activity. Therefore, the objectives of the study were (1) to compare the redox potential values as well as the hepatic MAT activities in newborn guinea pigs receiving a PN enriched or not with glutathione, or intravenous solutions containing peroxides, (2) to assess that the inhibition is caused by oxidation of thiols by using dithiothreitol (DTT), and (3) to document the relation between the redox potential and the activity of MAT.

2. Methods

2.1. Animal model

At three days of life, Hartley guinea pigs (Charles River Laboratories, St-Constant, QC, Canada) were anaesthetized by using ketamine and xylazine in order to fix a jugular catheter (Lake Villa, IL, USA). The catheter was placed and externalized in the scapular region, and connected to the infusion system. The studied solutions were infused continuously through the catheter at rate of 22 ml/100 g body weight/d. The solutions were changed daily.

2.2. Experimental designs

We examined the stability of both GSH and GSSG in parenteral nutrition solution to decide which molecule should be used in further experiments. In plasma, γ-glutamyltranspeptidase uses GSSG and GSH with the same efficiency to enrich the tissues into cysteine (essential for the cellular synthesis of GSH) [17,18]. 20 μM GSH or 10 μM GSSG (20 μM GSH equivalent) were added to PN (without lipid). After 1, 3, 5 and 24 h incubation at room temperature, samples were collected for the determination of total glutathione (GSH+GSSG) using a colorimetric method [19] as previously described [17].

Based on the report documenting that diets with different intakes in methionine influence the activity of MAT [20], two different protocols were used to assess the impact of PN or peroxides on the MAT activity and on redox potential. For the first protocol three groups of animals, in which methionine was included in the nutrition, were compared:

(1) Sham: The catheter was closed and animals were fed the regular laboratory food for guinea pigs.
(2) PN: Animal were exclusively on intravenous solution containing 2% (w,v) amino acids (Primene, Baxter, Toronto, ON, Canada) 8,7% (w,v) dextrose, 1% (v,v) multivitamin preparation (Multi-12/K_1 pediatrics, Sandoz, Boucherville, QC, Canada), 1.6% (w,v) lipid emulsion (Intralipid 20%, Fresenius Kabi, Mississauga, ON, Canada) and 1 U/ml heparin.
(3) PN+10 μM GSSG: Animals were exclusively on PN containing 10 μM GSSG. This form of glutathione was choice to avoid interactions with other components of PN [17].

For the second protocol three other groups of animals were compared. In order to isolate the effect of peroxides, the only carbon source for energy was dextrose (no amino acids or lipids):

(4) D: Animals were infused with a solution containing 8.7% (w,v) dextrose, 0.3% (w,v) NaCl and 1 U/ml heparin.
(5) AscOOH: Animals were infused with D containing 180 μM ascorbylperoxide; a concentration inducing perturbation of hepatic lipid and glucose metabolism [8] as well as redox potential in liver [8] and in lung [21] of newborn guinea pigs.
(6) H_2O_2: Animals were infused with D containing 350 μM H_2O_2; similar concentration of peroxides reported as contaminant in PN [21].

Four days later, at seven days of age, all animals were sacrificed. The liver samples were removed, processed and stored at −80 °C until biochemical determinations.

In accordance with the principles of the Canadian Council, the Institutional committee for good practice with animals in research of CHU Sainte-Justine approved the present protocol.

2.3. Determinations of redox potential of glutathione:

Briefly, as previously described [9,21], 0.5 g of liver was mixed with 5 volumes of 5% (w/v) freshly prepared metaphosphoric acid and homogenized on ice during 20 s with Polytron (Biospec Products, Bartlesville, OK, USA). After centrifugation 3 min at 10,000 RPM, supernatants were isolated for glutathione determination and pellets were used for protein measurement. GSH and GSSG were separated by capillary electrophoresis (Beckman Coulter). Assuming a density of 1.0 for the liver, the redox potential was calculated (25 °C, pH 7) by using the Nernst equation.

2.4. Determination of MAT activity

The activity of MAT was quantified on the cytosolic fraction of liver as previously described [9]. Briefly, 300 μg of protein (measured by the Bradford essay with BSA as standard) were suspended in the buffer (75 mM Tris/HCL, 250 mM KCl, 9 mM $MgCl_2$, pH 7.8) that contained substrates (5 mM methionine+5 mM [2,8-^{3}H]ATP (1 Ci/mol)) for a final volume of 150 μl, and were incubated