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ORIGINAL ARTICLE

The effect of diet on total antioxidant status, erythrocyte membrane Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in patients with classical galactosaemia

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Summary *Objective*: Classical galactosaemia is characterized by high levels of galactose-1-phosphate (Gal-1-P), galactose and galactitol. In vitro studies have shown modulation of the rat brain Na^+,K^+-ATPase and Mg^{2+}-ATPase activities by Gal-1-P. The aim of this study was to evaluate the erythrocyte membrane Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in galactosaemic patients and to correlate them to Gal-1-P, total antioxidant status (TAS) and membrane protein content (PC).

Patients and methods: Nine patients ($N = 9$) originally on "loose diet" (group B) were requested to follow their diet strictly (group A). Twelve healthy children were the controls (group C). The activities of the enzymes, TAS and Gal-1-P in blood were determined spectrophotometrically. In the in vitro study, erythrocyte membranes from controls were preincubated with Gal-1-P (300 μM), and then with L-cysteine (0.83 mM) or reduced glutathione (0.83 mM) whereas these from the patients with the antioxidants only.

Results: Na^+,K^+-ATPase, Mg^{2+}-ATPase, TAS and PC were significantly ($P < 0.001$) reduced (0.31 ± 0.03, 1.7 ± 0.2 μmol Pi/hxmg protein, 0.89 ± 0.02 mmol/l, 36.8 ± 2.0 g/l, respectively) in group B as compared with those of group A (0.58 ± 0.06, 2.5 ± 0.2 μmol Pi/hxmg protein, 1.41 ± 0.11 mmol/l, 51.5 ± 3.1 g/l, respectively) and controls (0.67 ± 0.05, 3.2 ± 0.2 μmol Pi/hxmg protein, 1.65 ± 0.12 mmol/l, 64.0 ± 3.5 g/l, respectively). Gal-1-P levels in group B was significantly higher than those in group A and controls. Positive correlation coefficients were found between the enzyme activities, PC and TAS whereas Gal-1-P inversely

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correlated to the enzyme activities. Incubation of the erythrocyte membranes from the patients with the antioxidants failed to restore the activities of inhibited enzymes, whereas the inhibition by Gal-1-P in controls was reversed.

Conclusions: High blood Gal-1-P conentrations resulted in low TAS and PC. The inhibition of Na^+,K^+-ATPase and Mg^{2+}-ATPase may be due to the presence of free radicals and/or the elevated Gal-1-P.

Introduction

Galactose-1-phosphate uridyltransferase (GALT) deficiency is an autosomal recessive disorder of metabolism. Infants with the ''classical galactosaemia'' phenotype display abnormal galactose tolerance, absent or barely detectable GALT activity in erythrocytes and liver, and marked elevation of galactose (Gal), galactose-1-phosphate (Gal-1-P) and galactitol in the blood resulting in a life-threatening multiorgan system disease following lactose ingestion.[1,2] Despite dietary lactose restriction, patients usually manifest a persistent elevation in Gal-1-P blood levels and urinary galactitol excretion possibly due to endogenous production and/or hidden in the food sources.[1] In addition and most importantly, many patients develop clinical complications involving the CNS. The aetiology of this encephalopathy is still unknown.[3]

Free radicals could represent one of the main causes of cellular dysfunction in brain.[4] The same could apply for some other tissues, which utilize one-fifth of the total oxygen demand of the body[5] and are not particularly enriched in any of the antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase).[6,7]

In our previous in vitro studies[8,9] Gal-1-P was found to inhibit rat brain Na^+,K^+-ATPase and to modulate Mg^{2+}-ATPase activity. The alterations of the enzyme activities were strongly mediated by free radicals.[10] It is likely, therefore, that in vivo poor dietary control of classical galactosaemic patients may result in altered neural excitability,[11] metabolic energy production, as well as in changes in their catecholaminergic and serotoninergic system,[12] their Mg^{2+} cellular concentration and the Mg^{2+}-dependent enzyme activities.[13]

Therefore, we aimed to evaluate Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in the erythrocyte membranes from patients with classical galactosaemia and to correlate the enzyme activities with their Gal-1-P blood concentration as well as with their blood total antioxidant status (TAS).

Furthermore, in an in vitro study, we attempted to find out whether the addition of antioxidants could restore a probable modulation of the studied erythrocyte membrane enzyme activities.

Subjects and methods

Subjects

The study population consisted of 9 patients, mean age 6.8 ± 1.2 years, with classical galactosaemia, who did not adhere strictly to their therapeutic diet (group B) as evidenced by their high blood Gal+Gal-1-P levels. The patients were requested to follow their special therapeutic diet strictly for 30 days and then they were re-evaluated (group A). Twelve ($N = 12$) healthy children of comparable age were the controls (group C). All galactosaemic patients were followed up to the Inborn Errors of Metabolism Dpt (Institute of Child Health) in Athens.

Methods

(A) Erythrocyte membrane preparation and protein content (PC) evaluation

Venous blood (5.0 ml) samples were collected into heparinized blood collection tubes from galactosaemic patients and controls. Within 2 h of collection the erythrocytes were sentimented by centrifugation at 2000*g* for 30 min at 4 °C, washed three times, after similar centrifugations, with a buffer solution (2.50 mM, tris (hydroxymethyl) aminomethane–HCl (Tris–HCl), pH 7.4, 140 mM NaCl, 1 mM $MgCl_2$ 10 mM glucose). The erythrocytes were then resuspended in 1.0 ml of the above buffer and stored at 4 °C for up to 24 h before erythrocyte membrane preparation. The washed erythrocytes were lysed after five times of freezing (−80 °C) and thawing (50 °C), as described by Galbraith and Watts[14] and Kamber et al.[15] The hemolysate was centrifuged at 35.000*g* for 30 min with 40–60 vol of cold 0.1 mol/l Tris–HCl, pH 7.4 four times until a white pink color appeared. Membranes were suspended in 0.1 mol/l. Tris–HCl, pH 7.4 to a final concentration of 2 mg per ml. The PC was determined, as described by Lowry et al.[16] Membranes stored at −40 °C retained the enzyme activities, for at least 2 weeks. The minor Hb that remained attached to the membrane surface was measured with the kit 527-A (Sigma Chemicals Co.,