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Short communication

Protein and lipid damage in maple syrup urine disease patients: L-carnitine effect

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ABSTRACT

Maple syrup urine disease (MSUD) is an inborn error of metabolism biochemically characterized by elevated levels of the branched chain amino acids (BCAA) leucine, isoleucine, valine and the corresponding branched-chain α-keto acids. This disorder is clinically characterized by ketoacidosis, seizures, coma, psychomotor delay and mental retardation whose pathophysiology is not completely understood. Recent studies have shown that oxidative stress may be involved in neuropathology of MSUD. L-Carnitine (L-Car) plays a central role in the cellular energy metabolism because it transports long-chain fatty acids for oxidation and ATP generation. In recent years many studies have demonstrated the antioxidant role of this compound. In this work, we investigated the effect of BCAA-restricted diet supplemented or not with L-Car on lipid peroxidation and in protein oxidation in MSUD patients. We found a significant increase of malondialdehyde and of carbonyl content in plasma of MSUD patients under BCAA-restricted diet compared to controls. Furthermore, patients under BCAA-restricted diet plus L-Car supplementation presented a marked reduction of malondialdehyde content in relation to controls, reducing the lipid peroxidation. In addition, free L-Car concentrations were negatively correlated with malondialdehyde levels. Our data show that L-Car may have an antioxidant effect, protecting against the lipid peroxidation and this could represent an additional therapeutic approach to the patients affected by MSUD.

1. Introduction

Maple syrup urine disease (MSUD) is an inborn error of metabolism caused by deficiency of mitochondrial enzyme complex branched-chain α-keto acid dehydrogenase (BCKD) activity. The metabolic defect leads to accumulation of the branched chain amino acids (BCAA) leucine (Leu), isoleucine and valine and the corresponding branched-chain α-keto acids (BCKA), α-ketoisocaproic acid (KIC), α-keto-β-methylvaleric acid and α-keto isovaleric acid (Chuang and Shih, 2001). Individuals with MSUD usually present poor feeding, convulsions, ketoacidosis, hypoglycemia, coma, ataxia, psychomotor delay and mental retardation, as well as generalized edema and hypomyelination/demyelination on magnetic resonance imaging studies of the central nervous system. The treatment consists of a dietary restriction of BCAA (very few natural proteins and a BCAA-free amino acid mixture (Barschak et al., 2008; Chuang and Shih, 2001)).

Animals studies have demonstrated that lipid peroxidation is stimulated by BCAA and BCKA in rat brains and that these metabolites reduce the in vitro capacity of cerebral tissue to modulate the damage associated with increased free radical production (Bridi et al., 2003; Fontella et al., 2002).

L-Carnitine (L-3-hydroxy-4-N,N,N trimethylaminobutyrate) is a small quaternary amine highly polar and water-soluble, which may be biosynthesized by humans (25%) or derived from dietary sources (75%), as for example meats and nuts (Derin et al., 2004; Gulcin, 2006). Usually it is present in plasma in the form of free carnitine, which plays the function to support the transport of long-chain fatty acids across the inner mitochondrial membrane for utilization in metabolism through β-oxidation (Tastekin et al., 2007). L-Carnitine (L-Car) has antioxidant properties, which may protect cells from toxic oxygen metabolites. These properties are scavenging hydroxyl radicals, superoxide anion and hydrogen peroxide as well as inhibition of hydroxyl radical production in the Fenton reaction system (Derin et al., 2004). In agreement, it has been shown

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that L-Car supplementation may prevent oxidative damage in cerebral cortex of young rats induced by MSUD acute model (Mescka et al., 2011).

The aim of the present study was to evaluate parameters of protein oxidative damage and lipid peroxidation in plasma of MSUD patients under therapy with BCAA restricted diet supplemented or not with L-Car in order to investigate the possible effect of L-Car on oxidative stress in treated MSUD patients.

2. Materials and methods

2.1. Patients and controls

Plasma samples from seven patients (mean age 8.28 ± 2.87 years) with classical MSUD under protein restricted diet protocol from the Medical Genetic Service of Hospital de Clínicas de Porto Alegre (HCPA), Brazil were studied. The most common clinical symptoms presented by MSUD patients at diagnosis were convulsions, ketoacidosis, poor feeding, hypoglycemia and psychomotor delay. Dietary treatment (median 0.95 year – range 15 days to 9.83 years) consisted of a protein-restricted diet supplemented with a semi-synthetic formula of essential amino acids (except leucine, isoleucine and valine), vitamins and minerals and not containing L-Car (MSUD 2-Milupa). The diet contained the following amounts of Leu (before 12 months of age: 40–80 mg kg^{-1} day^{-1}; after 1 year of age: 275–535 mg day^{-1}), Ile (before 12 months of age: 20–50 mg kg^{-1} day^{-1}; after 1 year of age: 165–325 mg day^{-1}) and Val (before 12 months of age: 20–60 mg kg^{-1} day^{-1}; after 1 year of age: 190–375 mg day^{-1}). In addition, for this study, MSUD patients were supplemented with L-Car capsules, at a dose of 50 mg kg^{-1} day^{-1}, not exceeding 1.5 g day^{-1} for 2 months. Oxidative stress parameters and free L-Car levels were analyzed in blood of MSUD patients before (group A) and after one (group B) and 2 months (group C) of L-Car supplementation. Blood amino acids levels, expressed in μM/L, were measured by HPLC according Joseph and Marsden (1986), with slight modifications (Wajner et al., 2000). The control group consisted of samples from six aged-matched healthy children (mean age 6.0 ± 3.12 years). The biochemical parameters were determined in plasma from controls and MSUD patients. Malondialdehyde (MDA) was measured by method described by Karatepe (2004) and the results were expressed in μM/L of MDA. Protein carbonyl formation was measured according to Levine et al. (1990) and the results were represented as protein carbonyl content (nmol/mg protein). Free L-Carnitine levels were determined in blood spots by liquid chromatography electrospray tandem mass spectrometry (LC/MS/MS), using the multiple reaction monitoring (MRM) mode (Chace et al., 1997) and the results were reported in μM/L. For the statistical analysis, comparison between means was analyzed by one-way ANOVA followed by the Duncan multiple range test when the F value was significant ($p < 0.05$). Correlations were carried out using the Pearson correlation coefficient.

The study was approved by the Ethics Committee of HCPA, RS, Brazil. All parents of the patients included in the present study gave informed consent.

3. Results

Table 1 exhibits blood Leu and free L-Car concentrations in MSUD patients before and after L-Car supplementation and also in controls. The results show that L-Car levels were significantly reduced in MSUD patients before supplementation when compared to controls and that the supplementation was able to reverse this deficiency.

Fig. 1 shows that MDA was markedly increased in patients before L-Car supplementation (group A) and this therapy was able to reduce this process reverting to control levels (group C) [$F(3.29) = 19.541$, $p < 0.05$]. Moreover, as can be seen in Fig. 2, a significant negative correlation was observed between free L-Car levels and MDA content ($r = -0.56$; $p < 0.05$) in treated MSUD patients.

Fig. 3 shows that the carbonyl content was significantly enhanced in MSUD patients (groups A, B and C) when compared to

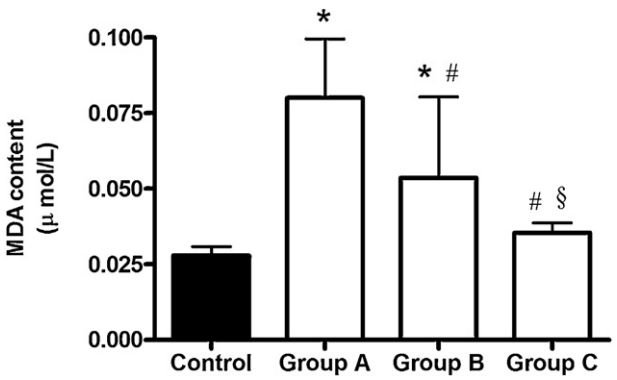

Fig. 1. Malondialdehyde content in plasma from MSUD patients and controls. Group A: patients before supplementation with L-carnitine. Group B: patients after 1 month of L-Car supplementation. Group C: patients after 2 months of L-Car supplementation. Data represent the mean ± SD (controls: $n = 14$; group A: $n = 7$; group B: $n = 7$; group C: $n = 5$). $^{*}p < 0.05$, compared to controls. $^{\#}p < 0.05$, compared to group A. $^{\S}p < 0.05$, compared to group B (ANOVA, followed by the Duncan multiple range test).

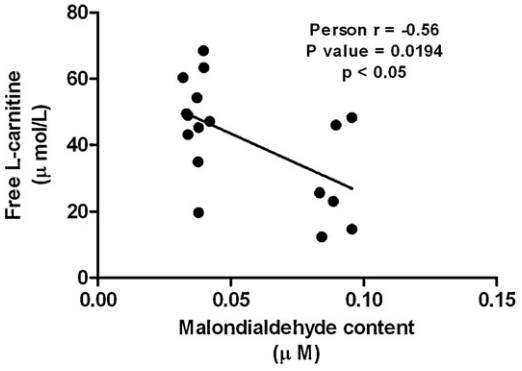

Fig. 2. Correlation between MDA and free L-carnitine levels in MSUD patients. Graphs show the Pearson correlation coefficient and probabilities.

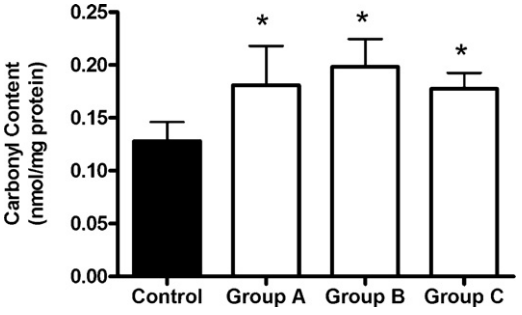

Fig. 3. Protein carbonyl in plasma from MSUD patients and controls. Group A: patients before supplementation with L-carnitine. Group B: patients after 1 month of L-Car supplementation. Group C: patients after 2 months of L-Car supplementation. Data represent the mean ± SD (controls: $n = 6$; group A: $n = 7$; group B: $n = 7$; group C: $n = 5$). $^{*}p < 0.05$, compared to controls (ANOVA, Duncan multiple range test).

Table 1
Blood leucine (Leu) and free L-carnitine (L-Car) concentrations in controls and in MSUD patients before and after supplementation with L-Car.

	Controls ($n = 6$)	MSUD patients before supplementation ($n = 7$)	MSUD patients after 1 month supplementation ($n = 7$)	MSUD patients after 2 months supplementation ($n = 5$)
Leu (μmol/L)	130.75 ± 27.16	178.18 ± 58.59	264.57 ± 136.74	278 ± 105.55
Free L-carnitine (μmol/L)	43.85 ± 8.89	24.28 ± 10.83*	49.01 ± 8.60	53.18 ± 10.79

* $p < 0.05$ different from controls.