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IN VITRO AND *IN VIVO* EFFECTS OF THE ANTIOXIDANT L-CARNITINE ON OXIDATIVE STRESS IN MAPLE SYRUP URINE DISEASE

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Introduction: Maple syrup urine disease (MSUD) is an inborn error of metabolism caused by deficiency of the activity of the mitochondrial enzyme complex branched-chain L-2 ketoacid dehydrogenase (BCKD). The metabolic defect leads to accumulation of the branched chain amino acids (BCAA) leucine (Leu), isoleucine (Ile) and valine (Val) and the corresponding branched-chain α-keto acids (BCKA), α-ketoisocaproic acid (KIC), α-keto-β-methylvaleric acid and α-keto isovaleric acid. The clinical features of MSUD include ketoacidosis, seizures, coma, psychomotor delay and mental retardation. Treatment consisted in Leu, Val and Ile restricted diet. Studies in animals have demonstrated that lipid peroxidation is stimulated by BCAA and BCKA in brain of rats and these metabolites reduce *in vitro* and *in vivo* the cerebral capacity of modulate the damage associated to increased free radical production. Also, there is evidence that oxidative stress occurs in MSUD patients at diagnosis and during treatment and that this is not correlated with the amino acids accumulating in this disease. 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, through its action against peroxidation in different tissues by various mechanisms, a "scavenger" of reactive oxygen species and the stabilizing effect of damage to cell membranes.
Objective: Considering that the pathophysiology of MSUD is still poorly understood this research aim to investigate the *in vitro* and *in vivo* effect of L-car on oxidative stress in MSUD and its correlation with Leu, Ile, Val and their metabolites.
Materials and Methods: The alkaline comet assay, used to investigate *in vitro* effects of different concentrations of Leu (100,250,500,1000,2500,3000 μM/L) and KIC (30,60,150,600,1200,2000 μM/L) on DNA damage from human leukocytes was performed using silver staining and visual scoring for further evaluation of antioxidant effect of L-car. A chemically-induced model of MSUD in Wistar rats of 15 days of life produced by subcutaneous administration of BCAA pool (15.8μL/g body weight containing 190 mmol/L Leu, 59 mmol/L Ile, and 69 mmol/L Val) and concomitant intraperitoneal injection of L-car (100 mg/Kg body weight) to investigate the mechanisms of brain damage characteristic of this disorder and the effect of L-car. The reactive species of thiobarbituric acid (TBA-RS), sulfhydryl content and the enzyme activity catalase (CAT) were determined in rat cortex.
Results and Discussion: In this study it was investigated the protective effects of L-car upon oxidative stress caused by BCAA and their metabolites *in vitro* and *in vivo*. Previous studies shows the *in vitro* effect of LEU and KIC on oxidative damage in MSUD. We verified that in all tested concentrations of LEU and KIC the DNA damage index was significantly increased compared to control ($p < 0.01$) and there were no differences in DNA damage between the second to largest concentration of these metabolites, except for 3000 μmol/L of LEU, which was significantly different from all concentrations ($p < 0.01$). This preliminary results shows that LEU and KIC leads to in vitro DNA damage and the effect of L-car upon this process will be evaluated in further studies. On the other hand, L-car prevented lipoperoxidation, measured by TBA-RS, protein damage, measured by sulfhydryl content and alteration on CAT activity ($p<0,05$) in rat cortex from a chemically-induced model of MSUD.
Conclusions: These results may contribute to the understanding of the mechanism of action of the cytotoxic effect of metabolites accumulated in MSUD and evidences of the role of oxidative stress in the pathophysiology of MSUD and the effect of L-car upon this process. The study of antioxidants like L-car, can open an additional therapeutic approach to the currently employed for MSUD patients, which is primarily dietary and therefore difficult to handle.

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