INTRODUCTION

Phenylketonuria (PKU) is an inborn error of amino acid metabolism, caused by deficient or absent of phenylalanine hydroxylase activity, enzyme responsible for hydroxylation of phenylalanine (Phe) to tyrosine (SCRIVER and KAUFMAN, 2001). As consequence, the accumulation of Phe and its toxic metabolites, phenylacetic (PAA), phenyllactic (PLA) and phenylpyruvic (PPA) acids, in blood and tissues of the affected patients (SCRIVER and KAUFMAN, 2001; CASEY, 2013).

Previous studies showed a decrease of plasma L-carnitine (L-car) levels in PKU patients (SITTA et al. 2009a). Also, L-car antioxidant property have been increasingly postulated (SOLARSKA et al., 2010).

Although Phe and its metabolites are considered neurotoxic in PKU, there is no consensus about the mechanism responsible for the central nervous system (CNS) related problems.

In this context, evidence indicate that oxidative stress plays an important role in the pathophysiology, since the accumulation of Phe and/or its metabolites can lead to the excessive free radical production or to a reduction of antioxidant status interfering in the normal development of the CNS (VAN SPRONSEN et al. 2001; SITTA et al. 2009a,b; DEON et al. 2015).
In the present work, we investigated whether Phe, PAA, PLA and PPA separately or in combination (Pool), in two concentrations (A and B), could provoke *in vitro* DNA damage in peripheral leukocytes of healthy individuals. We also aimed to study the potential protective *in vitro* effects of L-car, in two concentrations, on Pool A and Pool B.

**OBJECTIVE**

**MATERIALS AND METHODS**

Venous blood samples were collected into heparinized vials under sterile conditions from three healthy volunteers and used for the control group (without metabolites), and tested metabolites groups (Phe, PAA, PLA, PPA and L-car).

Leukocytes from each individual were separately treated with two concentrations of Phe (600 and 1200 μM), PAA (5 and 10 μM), PLA (10 and 20 μM) and PPA (10 and 15 μM), beyond of Pool A (Phe 600 μM + PAA 5 μM + PPA 10 μM + PLA 10 μM) and Pool B (Phe 1200 μM + PAA 10 μM + PPA 15 μM + PLA 20 μM) which have also been co-treated with two concentration of L-car (30 and 100 μM) for 6 h at 37 °C following comet assay protocol (TICE *et al*., 2000; HARTMANN *et al*., 2003).

All participants gave informed consent and this study was approved by the Ethics in Research Committee of Hospital de Clínicas de Porto Alegre.
RESULTS

Figure 1 *In vitro* effect of (a) Phe (600 μM and 1200 μM), (b) PAA (5 μM and 10 μM), (c) PLA (10 μM and 20 μM) and (d) PPA (10 μM and 15 μM) on DNA damage (comet assay) in leukocytes from whole blood of healthy subjects. Results represent mean ± SEM (standard error of the mean). (a) ***p<0.001 compared to control group; ###p<0.001 compared to 600 μM Phe group. (b) ***p<0.001 compared to control group; ##p<0.01 compared to 5 μM PAA group. (c) **p<0.01 and ***p<0.001 compared to control group; #p<0.05 compared to 10 μM PLA group and (d) ***p<0.001 compared to control group; ##p<0.01 compared to 10 μM PPA group. One-way analysis of variance ANOVA followed by Tukey post hoc test.
RESULTS

Figure 2 *In vitro* effect of L-car (30 μM and 100 μM) on DNA damage (comet assay) induced by (a) Pool A (b) Pool B in leukocytes from whole blood of healthy subjects. Results represent mean ± SEM (standard error of the mean). (a) ***p<0.001 compared to control group; ## p<0.01 and ### p<0.001 compared to Pool A group; #### p<0.001 compared to Pool A + 30 μM group. (b) ***p<0.001 compared to control group; ### p<0.001 compared to Pool B group; #### p<0.001 compared to Pool B + 30 μM group. One-way analysis of variance ANOVA followed by Tukey post hoc test.
In this study, both tested Phe, PAA, PLA, PPA and Pool concentrations have resulted in DNA damage index (DI) significantly higher than the control group. We observed that the co-treatment with 100 μM L-car reduced significantly the DNA damage induced by Pool A and Pool B. 30 μM L-car did not decrease the DI in the Pool B group.

Probably the ability of L-car to activate the DNA repair enzyme (ADPribose) polymerase (RIbas et al., 2014; Mescka et al., 2015), may have contributed significantly to the effects found preventing damage to DNA, as well as the antioxidant and free radical scavenger capacity already mentioned (Gülçin, 2006).

Thus, considering that the current treatment of phenylketonuric patients involves a protein restricted diet that may lead to decreased antioxidant defenses, L-car supplementation is a very important to protect patients against the deleterious consequences of oxygen reactive species in order to avoid CNS injury.

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