Metab Brain Dis (2015) 30:925–933
DOI 10.1007/s11011-015-9649-1

RESEARCH ARTICLE

Protective effect of L-carnitine on Phenylalanine-induced DNA damage

Marion Deon · Sharon S. Landgraf · Jessica F. Lamberty · Dinara J. Moura · Jenifer Saffi · Moacir Wajner · Carmen R. Vargas

Received: 10 October 2014 / Accepted: 7 January 2015 / Published online: 20 January 2015

Abstract The pathogenesis and the progression of phenylketonuria (PKU), an inborn error of phenylalanine (Phe) metabolism, have been associated with oxidative damage. Moreover, it has been increasingly postulated the antioxidant properties of L-Carnitine (LC). The aim of this study was to verify the effect of LC on Phe-induced DNA damage. The in vitro effect of different concentrations of LC (15, 30, 120 and 150 μM) on DNA damage-induced by high phenylalanine levels (1000 and 2500 μM) was examined in white blood cells from normal individuals using the comet assay. Urinary 8-hydroxydeoguanosine (8-OHdG) levels, a biomarker of oxidative DNA damage, and plasmatic sulfhydryl content were measured in eight patients with classical PKU, under therapy with protein restriction and supplemented with a special formula containing LC, and in controls individuals. Both in vitro tested Phe concentrations (1000 and 2500 μM) have resulted in DNA damage index significantly higher than control group. The in vitro co-treatment with Phe and LC reduced significantly DNA damage index when compared to Phe group. The urinary excretion of 8-OHdG and plasmatic sulfhydryl content presented similar levels in both groups analyzed (controls and treated PKU patients). In treated PKU patients, urinary 8-OHdG levels were positively correlated with blood Phe levels and negatively correlated with blood LC concentration and plasmatic sulfhydryl content. The present work yields experimental evidence that LC can reduce the in vitro DNA injury induced by high concentrations of phenylalanine, as well as, allow to hypothesize that LC protect against DNA damage in patients with PKU.

Keywords Phenylalanine · L-carnitine · DNA damage · Phenylketonuria · Antioxidant

M. Deon · S. S. Landgraf · J. F. Lamberty · M. Wajner · C. R. Vargas
Serviço de Genética Médica, HCPA, Rua Ramiro Barcelos, 2350, CEP 90035-003 Porto Alegre, RS, Brazil

M. Deon (✉) · C. R. Vargas
Programa de Pós-Graduação em Ciências Farmacêuticas, UFRGS, Av. Ipiranga, 2752, CEP 90610-000 Porto Alegre, RS, Brazil
e-mail: marion_deon@yahoo.com.br

S. S. Landgraf
Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, IFRJ, Rua Pereira de Almeida, 88, CEP 20260-100 Rio de Janeiro, RJ, Brazil

D. J. Moura · J. Saffi
Laboratório de Genética Toxicológica, Universidade Federal de Ciências de Saúde de Porto Alegre, UFSPA, Rua Sarmento Leite, 245, CEP 90050170 Porto Alegre, RS, Brazil

M. Wajner · C. R. Vargas (✉)
Programa de Pós-Graduação em Ciências Biológicas, Bioquímica, UFRGS, Rua Ramiro Barcelos, 2600, CEP 90035-003 Porto Alegre, RS, Brazil
e-mail: crvargas@hcpa.ufrgs.br

Introduction

Phenylalanine (Phe) is an essential amino acid in humans and a dietary source is necessary to maintain Phe homeostasis as well as is required for endogenous protein synthesis (Scriver and Kaufman 2001; Williams et al. 2008). Once in the cytosol, Phe may follow any of three paths: it may be (1) incorporated into cellular proteins, (2) converted to phenylpyruvic acid, or (3) converted to tyrosine via a liver specific enzyme, L-phenylalanine-4-hydroxylase (PHA - EC 1.14.16.1) (Scriver and Kaufman 2001).

Deficiencies in this enzyme are responsible for the most common inborn error of amino acid metabolism: Phenylketonuria (PKU – OMIM 261600). The incidence of PKU in Caucasians is between 1 in 10,000 and 1 in 15,000

people (Vockley et al. 2014). The golden standard therapy is based on lifelong Phe-restricted diet by eliminating high-proteins foods (Scriver and Kaufman 2001). At the same time, newer treatments for PKU are emerging and some are current available such as administration of tetrahydrobiopterin, glycomacropeptide, large neutral amino acids (LNAA), phenylalanine ammonia lyase, etc. (Blau et al. 2010).

This autosomal recessive disorder in untreated patients is characterized biochemically by elevation and accumulation of Phe and its metabolites in biologic fluids and clinically by severe mental retardation and other neurological features (Scriver and Kaufman 2001; Casey 2013; Vockley et al. 2014). Multiple mechanisms by which Phe induces its deleterious effects on brain include impairment of LNAA uptake into brain, reduction of the availability for neurotransmitters synthesis, inhibition of key enzymatic activities, reduction of the activity of monoamine oxidase B as modifying gene and alteration on myelin metabolism (Ghozlan et al. 2004; Anderson et al. 2007; Blau et al. 2010).

In this scenario, evidences indicate that oxidative stress, which represents the imbalance between enhanced generation of reactive species and low cellular content of antioxidants, is involved in the pathophysiology of PKU possibly contributing for the neurological damage in this disorder (Sitta et al. 2009a, b; Sitta et al. 2011; Ribas et al. 2011; Rocha and Martins 2012). Such reactive species can cause oxidative damage by attacking cellular biomolecules like lipids, proteins and nucleic acids. Further, DNA is a particularly important object for oxidation generating several classes of products (single- and double-strand breaks) inter/intra-strand cross-links, DNA-proteins cross-links and sugar fragmentation products (Halliwell and Gutteridge 2007). Schulpis et al. (2005) reported incremented levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), a product of DNA oxidative damage, in serum of poorly controlled PKU patients (with high levels of Phe), which were positively correlated with plasma Phe concentrations. More recently, in vitro and in vivo experiments with PKU animal models have provided the effect of hyperphenylalaninemia in DNA damage (Simon et al. 2013). In addition, it has been demonstrated that DNA damage from peripheral blood leukocytes is elevated in PKU patients, possibly due to the oxidative damage mediated by high Phe levels or its metabolites (Sitta et al. 2009b).

L-Carnitine (LC) plays an important physiological role in the mammalian cellular energy and lipid metabolism, (Vaz and Wanders 2002). Previous studies have shown that LC has antioxidant and anti-inflammatory effects on various pathophysiological conditions, including inherited disorders (Izgut-Uysal et al. 2003; Ferrari et al. 2004; Gülcin 2006; Onem et al. 2006; Ribas et al. 2012; Ribas et al. 2014). It was demonstrated that LC supplementation reduces oxidative damage to lipids and increases antioxidant defenses in PKU patients (Sitta et al. 2011). In MSUD patients, it was observed that lipid peroxidation was reduced by LC-supplementation (Mescka et al. 2011). In addition, LC was able to prevent the in vitro DNA damage induced by propionic and methylmalonic acids in human leukocytes (Ribas et al. 2010). In this context, an important question arises: LC could has a protective effect on Phe-induced DNA damage? In the present study, the in vitro effect of different concentrations of LC on DNA damage-induced by high Phe levels was examined in white blood cells from normal individuals using the comet assay. Besides, urinary 8-hydroxydeoguanosine (8-OHdG) levels, a biomarker of oxidative DNA damage, were measured in PKU patients under therapy with protein restriction and supplemented with a special formula containing LC. Plasmatic sulfhydryl content was investigated as well, since its oxidation may reflect protein oxidation or otherwise a diminution of antioxidant defense. We also correlated blood Phe and free LC levels with these oxidative stress parameters examined.

Materials and methods

Subjects

All participants or their legal guardians gave informed written consent for the present study which was approved by the Ethics in Research Committee of Hospital de Clínicas de Porto Alegre, RS, Brazil (projects n.° 04–080 and 14–0180). A total of six healthy volunteers individuals (mean age 20.3± 1.358) were recruited for the in vitro study.

For urinary 8-OHdG measurement and plasmatic sulfhydryl content, eight classical PKU patients (mean age 16.6± 3.85) under diet treatment at least for more than 6 months were analyzed. The treatment consisted in a Phe-restricted diet with low consumption of protein supplemented with a semi synthetic amino acids formula (according to age: PKU 2 Secunda for individuals above 8 years old and PKU 3 Advanta for individuals above 15 years old – Support®) that contains LC (LC: 105 to 98 mg/Day, respectively). The blood Phe and free LC levels from PKU patients were, respectively, 641± 36.37 μmol/L and 37±2.04 μmol/L. Eleven healthy subjects sex and age matched (mean age 12.4±8.7) were selected for the control group.

Samples collection and preparation

Venous blood was collected under sterile conditions in heparinized vials. Immediately, whole blood cells were submitted to the in vitro study protocol. To PKU patients and controls, 50 μL of the whole blood was spotted onto specialized paper cards for posterior measurement of phenylalanine and free L-carnitine concentrations. Whole blood was centrifuged at 3000 rpm, plasma was rapidly removed by aspiration and

frozen at –80 °C until analysis of the plasmatic sulfhydryl content. Occasional urine samples were collected in sterile flasks, aliquoted and frozen at – 80 °C until analysis.

In vitro studies

Whole blood cells from each subject were incubated without Phe and LC (control group), in the presence of Phe at two concentrations (1000 and 2500 μM), and in a co-treatment with Phe (1000 and 2500 μM) and LC at different concentrations (15, 30, 120, and 150 μM). Whole blood cells were incubated for 6 h at 37 °C with Phe and/or LC following comet assay protocol (Tice et al. 2000; Hartmann et al. 2003).

Comet assay (single cell gel electrophoresis)

The alkaline Comet assay, that measures single and double DNA strand breaks, was performed following to the method described by Singh et al. (1988) and was performed according to general comet assay guidelines (Tice et al. 2000; Hartmann et al. 2003). Aliquots of 100 μL from whole blood were suspended in agarose and spread into a glass microscope slide pre-coated with agarose. Slides were placed in lyses buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris; 10 % DMSO; pH 10.0–10.5) to remove cell proteins, leaving DNA as "nucleoids". After treatment with lyses buffer and alkaline buffer solution (200 mM NaOH and 1 mM EDTA; pH >13), the slides were submitted to a horizontal electrophoresis. This technique was performed for 20 min (25 V; 300 mA; 0.9 V/cm). After electrophoresis, the slides were neutralized with 0.4 M Tris (pH 7.5), washed in bi-distilled water, and the DNA fixed and stained using silver nitrate staining protocol (Nadin et al. 2001). After drying at room temperature overnight, gels were analyzed using an optical microscope. One hundred cells (50 cells from each of the two replicate slides) were selected, and analyzed. Cells were visually scored according to tail length and receive scores from 0 (no migration) to 4 (maximal migration) according to tail intensity (Fig. 1). Therefore, the damage index (DI) for cells ranged from 0 (all cells with no migration) to 400 (all cells with maximal migration). The slides were analyzed under blind conditions at least by two different individuals.

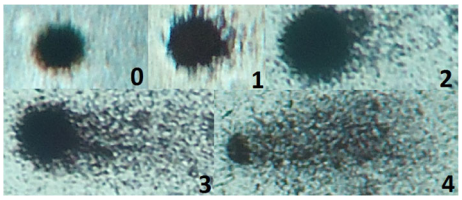

Fig. 1 Evaluation of DNA damage using comet assay (single cell gel electrophoresis) (400×). The cells are assessed visually and received scores from 0 (undamaged) to 4 (maximally damaged), according to the size and shape of the tail

Urinary 8OHdG analysis

Urinary 8-OHdG levels were determined by Highly Sensitive 8-OHdG Check ELISA kit (JaICA, Fukuroi, Japan). The 8-OHdG Check ELISA kit is a competitive in vitro enzyme-linked immunosorbent assay for quantative measurement of the oxidative DNA adduct 8-hydroxy-2′-deoxyguanosine. This assay employs a microplate with 96 wells pre-coated with 8-OHdG, a monoclonal antibody specific for 8-OHdG (primary antibody), an HPR-conjugated antibody (secondary antibody) and colorimetric detection substrate whose absorbance was measured in an ELISA microwell reader at 450 nm. The sample 8-OHdG concentrations (ng/mL) were calculated using a polynomial equation from the relative absorbance of standard curve. After creatinine correction, the results were expressed as ng/mg creatinine.

Creatinine determination

Urinary creatinine (Cr) was determined by picric acid method — Creatinine K kit of Labtest® (Labtest Diagnóstica, Lagoa Santa, MG, Brazil). Urinary creatinine reacts with picric acid under alkaline conditions producing an orange color whose absorbance was determined in a spectrophotometer at 492 nm. The results were expressed in mg/ml.

Phe and free LC determination

Phe and free LC 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). Results were reported in μmol/L.

Plasmatic sulfhydryl content

This assay is based on the reduction of 5.5-dithio-bis (2-nitrobenzoic acid) (DTNB) by thiols, which in turn become oxidized (disulfide), generating a yellow derivative (2-nitro-5-thiobenzoate - TNB) whose absorption is measured spectrophotometrically at 412 nm, according to the method described by Aksenov and Markesbery (2001). The sulfhydryl content is inversely correlated to oxidative damage to proteins. Results were reported as nmol TNB/mg protein.

Protein determination

Plasma protein concentrations were measured by the Biuret method (Gornall et al. 1949) from Labtest kit (Labtest

Diagnóstica, MG, Brazil) using bovine serum albumin as standard

Statistical analysis

All statistical analyses were performed using SPSS software (15.0 version for Windows). Data were expressed as mean ± standard error of mean (SEM). In order to evaluate the in vitro effect of different concentrations of LC on DNA damage induced by high Phe levels in white blood cells from normal individuals using the comet assay, comparisons between means were analyzed by one-way ANOVA followed by Duncan test when the F value was significant, expressed as [F(degree of freedom between groups, degree of freedom within groups) = F value, significance]. Comparisons between means of controls and PKU patients were analyzed by unpaired Student's *t* test. Correlations between urinary 8-OHdg and others parameters were performed by Pearson's correlation test. Differences were considered statistically significant when *p* value was lower than 0.05.

Results

L-carnitine decreases in vitro the Phe-induced DNA damage

In this work, we evaluated the in vitro effect of LC at different concentrations (15, 30, 120 and 150 μM) on DNA damage Phe-induced in white blood cells from healthy individuals. Both tested Phe concentrations (1000 and 2500 μM) have resulted in DNA damage index (DI) significantly higher than the control group ($p<0.001$) (Figs. 1 and 2).

Figure 2a shows the in vitro effect of LC at various concentrations on DNA damaged induced by Phe (1000 μM) [$F(5,30)=6.541$, $p<0.01$], which means that significant differences exist between groups or at least one group differs from other. It was verified that the co-treatment with LC at the concentrations 15, 30, 120 and 150 μM reduced significantly DI (47.05, 56.35, 64.94 and 68.47 %, respectively) when compared to Phe (1000 μM) group. Representative Comet assay images of the in vitro effect of LC (15, 30, 120 and 150 μM) on DNA damage induced by Phe (1000 μM) in leukocytes from whole blood of normal individuals are presented in Fig. 2b. As denoted in this figure, there is gradual decrease in the tail intensity (comet tail length, as well as proportion) in cells, with increasing concentration of LC.

It was also verified the in vitro effect of LC at different concentrations on DNA damaged induced by Phe (2500 μM) [$F(5,29)=8.298$, $p<0.01$], which means that significant differences exist between groups or at least one group differs from other (Fig. 3a). Once again, it was observed that the co-treatment with LC (15, 30, 120 and 150 μM) diminished significantly (59.25, 72.06, 77.59 and 82.15 %, respectively) Phe-induced DNA damage (2500 μM). Representative Comet assay images of the in vitro effect of LC (15, 30, 120 and 150 μM) on DNA damage induced by Phe (2500 μM) in leukocytes from whole blood of normal individuals are presented in Fig. 3b. As denoted in this figure, there is gradual decrease in the tail intensity (comet tail length, as well as proportion) in cells, with increasing concentration of LC.

Urinary 8-OHdG and plasmatic sulfhydryl content in PKU treated patients

The urinary excretion of 8-OHdG, which reflects oxidative DNA damage, was similar between controls and treated PKU patients (Table 1) ($p>0.05$). The plasmatic sulfhydryl content, which represents oxidative damage to plasma protein sulfhydryl groups, did not show significant difference in PKU treated patients when compared to control group ($p>0.05$) (Table 1). In PKU treated patients, urinary 8-OHdG levels were positively correlated with blood Phe levels ($r=0.712$; $p<0.05$) (Fig. 4) and negatively correlated with free LC ($r=-0.677$, $p<0.05$) (Fig. 5) and plasmatic sulfhydryl content ($r=-0.747$, $p<0.05$) (Fig. 6). Plasma sulfhydryl content was positively correlated with free LC ($r=0.944$, $p<0.001$) (Fig. 7).

Discussion

Numerous studies have shown that oxidative stress plays an important role in the pathogenesis and progression of PKU. These works have reported that oxidative stress in PKU may be the result of restricted diets on the antioxidant status and/or excessive production of reactive species, due to the high Phe levels and/or its metabolites (Rocha and Martins 2012; Ribas et al. 2011). It is well known that an excessive production of reactive species lead to cell damage, including inactivation of cellular enzymes, structural DNA damage, and cells apoptosis (Höhn et al. 2014). It should be emphasized that a variety of lesions in DNA can occur by induction of reactive species including oxidized bases, abasic sites, DNA strand breaks, and cross-links between DNA and proteins (Halliwell and Gutteridge 2007). These products may elicits mutations, microsatellite instability, loss of heterozygosity, chromosomal aberration, cytotoxicity and neoplastic growth (Cooke et al. 2003). Considering that DNA damage occurs in PKU patients and the supplementation with LC was capable to correct lipid and protein oxidation in these patients (Sitta et al. 2011), our goal in this study was evaluated the in vitro effect of LC at different concentrations on DNA damage Phe-induced in white blood cells from healthy individuals using the comet

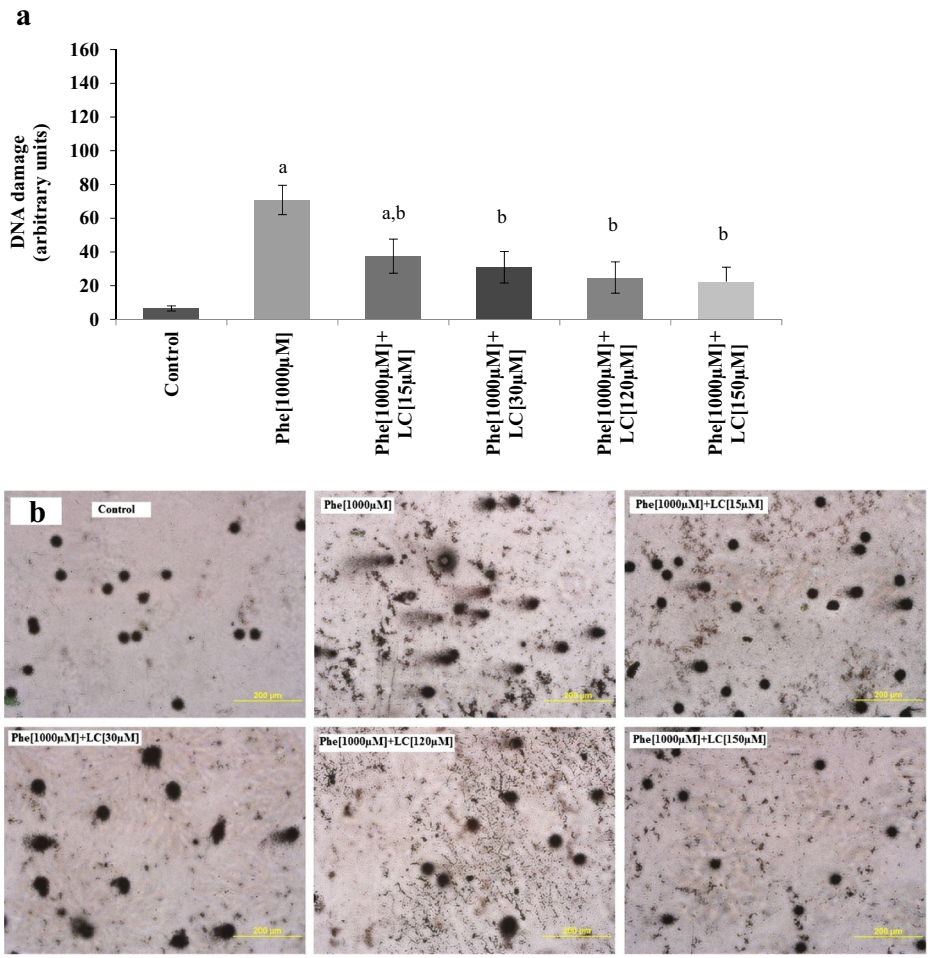

Fig. 2 **a** In vitro effect of LC (15, 30, 120 and 150 μM) on DNA damage induced by Phe (1000 μM) in leukocytes from whole blood of healthy subjects. Data represent mean ± SEM. Compared to the control group (negative control), a $p<0.05$; compared to the Phe (1000 μM/L), b $p<0.05$ (ANOVA, followed by Duncan test). **b** Representative comet assay images of the in vitro effect of LC (15, 30, 120 and 150 μM) on DNA damage induced by Phe (1000 μM) in leukocytes from whole blood of normal individuals

assay. Besides, urinary 8-hydroxydeoguanosine (8-OHdG) levels and sulfhydryl content were measured in PKU patients under therapy with protein restriction and supplemented with a special formula containing LC.

We determined DNA damage in peripheral leukocytes by using the alkaline comet assay that measures DNA strand breaks in single cells developed by Singh et al (1988). In this method (single-cell gel electrophoresis), increased DNA migration can be associated with incomplete excision repair sites (Tice et al. 2000), which are generated as an intermediate step during the action of different DNA-repair systems. The single-cell electrophoresis assay is a widely used assay, extremely sensitive for detecting low levels of DNA damage, cheap and quick method (Liao et al. 2009).

First, whole blood cells from healthy individuals were exposed to different concentrations of Phe (100, 250, 500, 1000 and 2500 μM) for 6 h at 37 °C and DNA damage was determined using comet assay (data not shown). Since Phe at the concentrations of 1000 and 2500 μM induced significant DNA damage ($p<0.01$), these doses were used in the subsequent experiments. The representative Comet assay images of the in vitro DNA damage induced by Phe (1000 and 2500 μM) in leukocytes from whole blood of normal individuals demonstrated an increment in the tail intensity and length in these cells in comparison with controls. These results are in agreement with previous in vitro findings in human and in rat blood showing that DNA damage index had increased with increasing Phe concentrations added to medium (Sitta et al 2009b and Simon et al 2013). Yet, it is important to emphasize that Phe concentrations (1000 μM and 2500 μM) used in this study are similar to those found in blood from PKU patients at diagnosis or in relaxed diet and/or no-treated (Williams et al. 2008; Demirkol et al. 2011; Trefz et al. 2011).

In the next step, we tested the in vitro effect of LC on Phe-induced DNA damage. After 6 h of incubation, Phe at 1000 μM induced DNA damage in whole blood cells from normal individuals (Fig. 2a and b). Interestingly, the co-treatment with LC (15, 30, 120 and 150 μM) reduced significantly DNA damage (47.05, 56.35, 64.94 and 68.47 %, respectively) when compared to Phe alone condition. Similarly, Phe at 2500 μM also induced DNA damage (Fig. 3a and b). Most notably, co-treatment with LC resulted in decreased DI, with a particularly large inhibitory effect on Phe 2500 μM/L, since LC's concentrations ranging from 15, 30, 120 and 150 μM diminished Phe-induced DNA damage in 59.25,

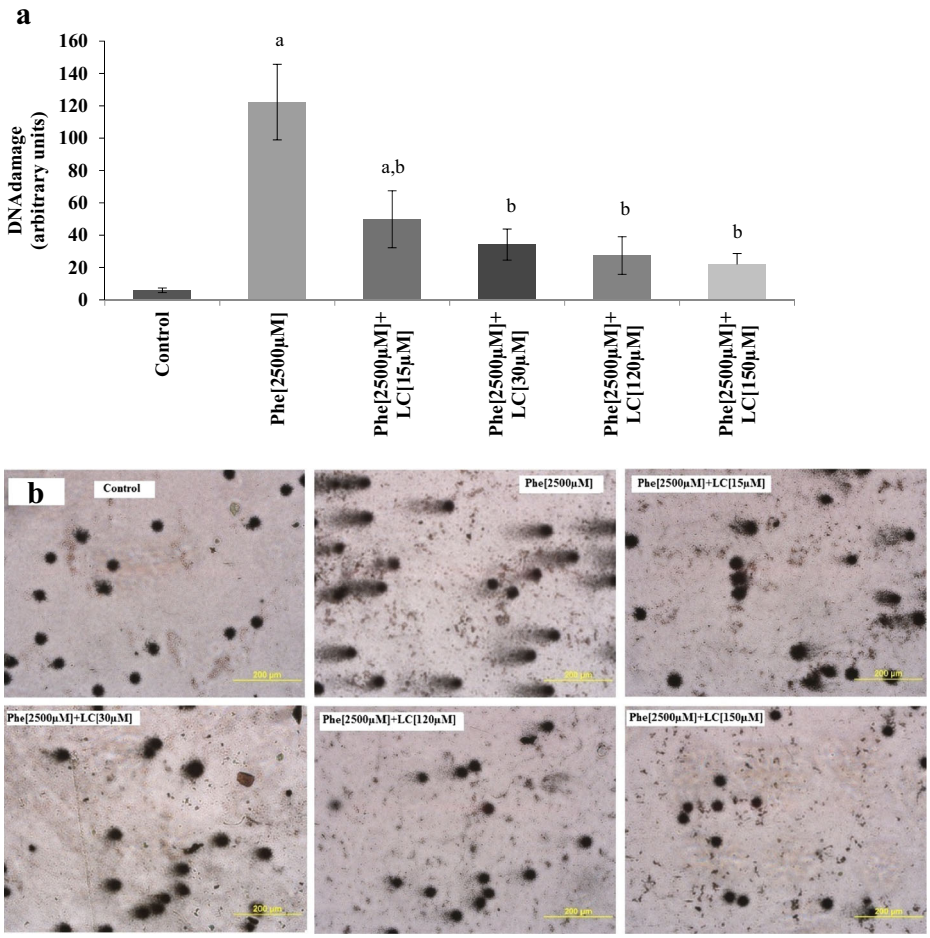

Fig. 3 **a** In vitro effect of LC (15, 30, 120 and 150 μM) on DNA damage induced by Phe (2500 μM) in leukocytes from whole blood of healthy subjects. Data represent mean ± SEM. Compared to the control group (negative control), a $p<0.05$; compared to the Phe (2500 μM) group, b $p<0.05$ (ANOVA, followed by Duncan test). **b** Representative comet assay images of the in vitro effect of LC (15, 30, 120 and 150 μM) on DNA damage induced by Phe (2500 μM) in leukocytes from whole blood of normal individuals

72.06, 77.59 and 82.15 %, respectively. Ribas and co-workers (2011) have conducted a similar in vitro study in which it was observed that LC at concentrations over 60 μM was able to reduce DNA damage induced by organic acids (propionic and L-methylmalonic acids). Therefore, in present work, we observed that high Phe levels induced DNA damage in whole blood cells from healthy individuals and that co-treatment with L-carnitine reduced this damage.

Oxidative DNA damage is a result of the attack by reactive oxygen species both in nuclear and mitochondrial DNA, producing predominantly an oxidized form of guanosine, 8-OHdG (Halliwell and Gutteridge 2007). The oxidized DNA is continuously repaired and the oxidized nucleoside is excreted into the bloodstream and into urine (Cooke et al. 2003). Serum and urinary 8-OHdG reflect the equilibrium between its production and repair in both DNA and the nucleotide pool

Table 1 Biochemical findings in PKU treated patients vs controls

	Control (n=11–8)	PKU (n=8)	P values
8-OHdG (ng/mg creatinine)	6.81±0.81	5.30±1.42	NS
Sulfhydryl content (nmol TNB/mg protein)	5.41±0.35	5.22±0.49	NS
Phe (μmol/L)	26.69±1.61	641.37±36.37**	$P<0.001$
Free LC (μmol/L)	34.12±2.29	37.00±2.04	NS

Data represent the mean ± SEM. Difference from control, **$p<0.001$ (Non paired Student t test)

NS not statistically significant

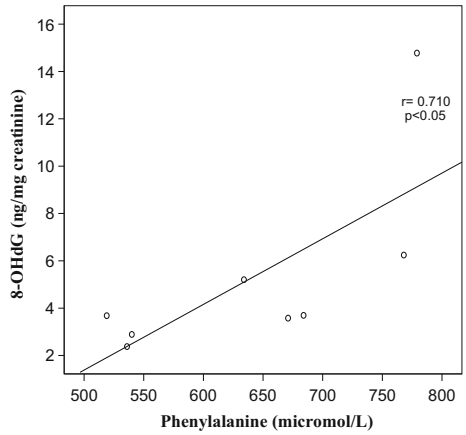

Fig. 4 Correlation between urinary 8-OHdG levels and blood Phenylalanine concentration in PKU patients (Pearson's correlation)

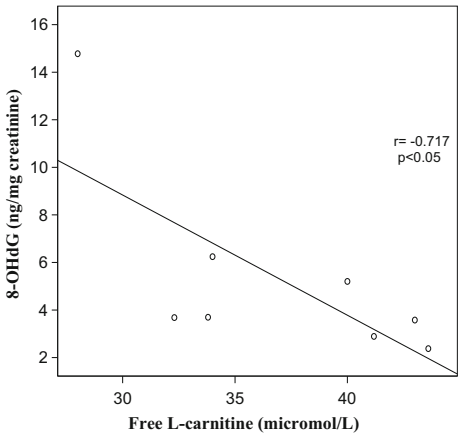

Fig. 5 Correlation between urinary 8-OHdG levels and blood Free L-carnitine concentration in PKU patients (Pearson's correlation)

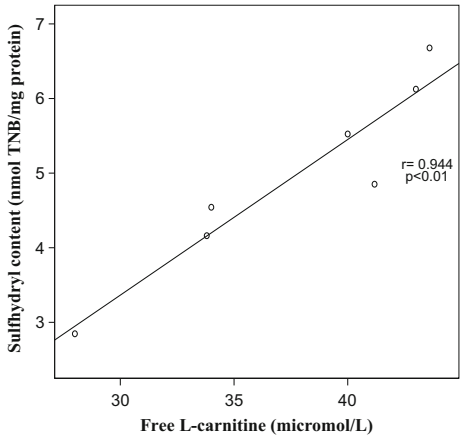

Fig. 7 Correlation between plasmatic sulfhydryl content and blood Free L-carnitine concentration in PKU patients (Pearson's correlation)

(Cooke et al. 2003). Higher amount of serum 8-OHdG of poorly controlled PKU patients (with high levels of Phe) was reported, which were positively correlated with plasma Phe concentrations (Schulpis et al. 2005).

Taking into consideration that DNA damage occurs in PKU patients and that the in vitro concomitant treatment with LC was capable to reduce Phe-induced DNA damage, our next goal in this study was to evaluate urinary 8-OHdG levels in PKU patients under diet therapy with protein restriction and supplemented with a special amino acid formula (without Phe) containing LC. It was observed a similar urinary excretion of 8-OHdG when comparing control individuals with PKU treated patients. This finding is in agreement with the results observed in our study about the in vitro effect of LC on reducing Phe-induced DNA damage and somewhat expected since these PKU patients were supplemented with a semi synthetic amino acids formula according to patients' age that contains LC. Next, we examined plasmatic sulfhydryl content, which is a measure of protein oxidative damage, in PKU treated patients and control individuals. Thiol/ sulfhydryl group is

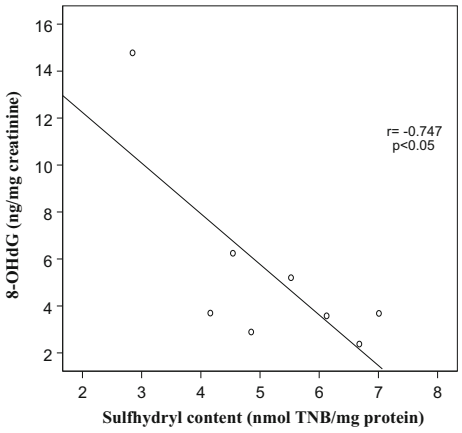

Fig. 6 Correlation between urinary 8-OHdG levels and plasmatic sulfhydryl content in PKU patients (Pearson's correlation)

a physiological free radical scavenger in cells and in biological fluids and may scavenge oxidants (Halliwell and Gutteridge 2007). The decrease in sulfhydryl content reflects the existence of oxidative damage to proteins because thiol groups are easily oxidized and reduced pending on the redox status of the cell (Aksenov and Markesbery 2001). These groups can be oxidized by reactive species, especially at protein cysteine residues that may mediate regulatory processes of protein, potentially leading to alterations of protein function (Halliwell and Gutteridge 2007). The plasmatic sulfhydryl content was similar in PKU treated patients in comparison with control group. In addition, a strong positive correlation was found between blood LC concentration and plasmatic sulfhydryl content. It is important to emphasize that a previous study had demonstrated that the treatment with PKU's formula that contains LC reversed lipid peroxidation and oxidation of thiol groups in plasma from PKU patients (Sitta et al. 2011).

In this study, a negative correlation between urinary 8-OHdG concentration and plasmatic sulfhydryl content was observed, suggesting that LC treatment provokes beneficial effect upon oxidative protein and DNA damage. Besides, urinary 8-OHdG levels in PKU patients were positively correlated with blood Phe level (raging from 519 to 779 μmol/L) and negatively correlated with blood free LC concentration (raging from 28 to 44 μmol/L), reinforcing that Phe level is correlated with DNA damage (Schulpis et al. 2005; Sitta et al. 2009b) as well as the antioxidant role of LC (Sitta et al. 2009a; Sitta et al. 2011).

In addition, there are evidence that LC improves oxidative stress in some inherited neurometabolic disorders and decreases the hypercoagulation state in Diabetes Mellitus (Elgendy and Abbas 2014; Ribas et al. 2014). In the present work, we verified that lower LC concentrations (15 and 30 μM) already have protective in vitro effect on Phe-induced DNA damage. Thus, the results obtained in this study and those found in the literature indicate that LC can be a novel adjuvant therapeutic agent.

One question remains to be answered: what is the underlying mechanism by which LC reduces DNA damage induced by Phe? LC is classically known as a key regulator of lipid metabolism (Hatamkhani et al. 2013). Diverse human and animal studies have suggested an antioxidant and a free radical scavenger role for LC (Gülcin 2006; Ribas et al. 2014). Then, it is plausible to propose that LC may diminish Phe-induced DNA damage by reduction of reactive species. In agreement, it has been shown that lipid peroxidation and protein oxidative damage was decreased in PKU patients after the LC supplementation (Sitta et al. 2011). It is worthy to mention that LC concentrations between 20 and 60 μM are often observed in blood of PKU patients on diet and LC supplementation (Sitta et al. 2011).

LC has also been related to increase DNA repair enzyme, which could to explain its protective effect on DNA damage induced by Phe (Moretti et al. 2002). Other authors demonstrated that LC accelerates the disappearance of DNA single-strand breaks induced by oxygen radicals and alkylating agents in human peripheral blood lymphocytes (Boerrigter et al. 1993). Recently, an in vitro study has observed that LC at concentrations over 60 μM was able to reduce DNA damage induced by organic acids (Ribas et al. 2011).

In conclusion, the present work yields experimental evidence that LC can reduce the in vitro DNA injury induced by high concentrations of Phe, as well as, allow us to hypothesize that LC protect against DNA damage in PKU patients. However, our present in vitro results should be interpreted with caution and further in vivo experiments with a higher number of patients are necessary to clarify this issue since we used specimens from only a few patients.

Acknowledgments We are indebted to all study participants (PKU patients and healthy volunteers) and their families for their fundamental collaboration. We gratefully acknowledge the Brazilian Foundation *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for financial support of our study (grant n.° 007481/2011-13). This study was also supported in part by grants from *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and *Fundo de Incentivo à Pesquisa e Eventos* (FIPE/HCPA). The authors also thank the staff from Serviço de Genética Médica/Hospital de Clínicas de Porto Alegre.

Conflict of interest The authors declare that there is no financial interests and/or no conflict of interest disclosure associated with this manuscript.

Ethical standards All human studies presented in this paper have been approved by the appropriate ethics committees and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This included approval from the ethics in research committee of Hospital de Clínicas de Porto Alegre for data acquired at Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil (projects n.° 04 –080 and 14 –0180).

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