Betaine as a methyl donor and an antioxidant agent in levodopa-induced hyperhomocysteinemia and oxidative stress in rat's kidney

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Abstract:

BACKGROUND: Betaine has been shown to be antioxidant and methyl donor effects in our recent studies. **OBJECTIVES:** In the present study, the antioxidant and methyl donor properties of betaine in levodopa/benserazide-mediated hyperhomocysteinemia and levodopa-induced oxidative stress in rat's kidney were examined. **METHODS:** Sprague-Dawley male rats were divided into levodopa (LD), Betaine (Bet.), levodopa plus betaine (LD/Bet.), levodopa plus benserazide (LD/Ben.), levodopa plus betaine-benserazide (LD/Bet.-Ben.), and control groups. The experimental groups received LD (3 × 100 mg/kg), Bet. (1.5% w/w of the total diet), Ben. (3 × 25 mg/kg), and distilled water was given to controls for 10 consecutive days, orally by gavage. **RESULTS:** Plasma total homocysteine (tHcy) concentration decreased significantly in Bet.-, LD/Bet.-, and LD/Bet.-Ben.-treated rats compared to LD/Ben. group. Thiobarbituric acid reactive substances concentration (as a lipid peroxidation marker) in renal tissue reduced statistically in betaine group in comparison with LD and LD/Ben. groups. Renal catalase activity increased significantly in LD-treated rats when compared to controls. Renal superoxide dismutase activity significantly decreased in LD-treated group when compared to LD/Ben. group. However, there was not any significant difference in renal glutathione peroxidase (GPx) activity among the groups. **CONCLUSIONS:** These findings indicate that LD and LD/Ben. have side effects in kidney due to induction of hyperhomocysteinemia and oxidative stress. In contrast, betaine acts as a promising antioxidant and methyl donor agent versus LD-induced complications.

Introduction

Oxidative stress is a hypothesis for the association of reactive oxygen species (ROS) with neurodegenerative and inflammatory diseases such as Parkinson's disease (PD), chronic renal failure, and end-stage renal disease (ESRD) (Alirezaei et al., 2011; Neamati et al., 2011). ROS such as superoxide anion, hydrogen peroxide and hydroxyl radical are generated during oxidative metabolism and can inflict damage on all classes of macromolecules, eventually leading to cell death (Bergamini et al., 2004; Smith et al., 2005; Alirezaei et al., 2011). Regarding oxidative stress, betaine has recently been shown antioxidant and methyl donor effects in our previous reports (Alirezaei et al., 2011, 2012b,c,d, 2014a,b; Kheradmand et al., 2013). Betaine (trimethylglycine) transfers a methyl group via the enzyme betaine homocysteine methyl transferase (BHMT) to become dimethylglycine (Alirezaei et al.,

2011, 2012b,c). BHMT is the only known enzyme that uses betaine as a substrate, mediates the transfer of a methyl group from betaine to homocysteine (Hcy), forming methionine and dimethylglycine (Alirezaei et al., 2011; Fridman et al., 2012). BHMT activity in rat kidney in several orders of magnitude lower than that in rat's liver and only restricted to the renal cortex (Fridman et al., 2012). Betaine also acts as osmolyte in kidney medulla, protecting cell from high extracellular osmolarity (Fridman et al., 2012).

It is interesting to note that chronic and acute repeated intake of levodopa (LD) elevates total homocysteine (tHcy) concentration, and hyperhomocysteinemia is known as a side effect of LD consumption (Muller et al., 2011). LD is the most effective dopaminergic agent for PD, it is available in immediate and controlled release forms and is routinely combined with a dopa-decarboxylase inhibitor (DDI) such as benserazide to reduce its peripheral metabolism so as to avoid peripheral toxicity and to enhance its brain penetration (Muller et al., 2011; Schapira et al., 2008). Although chronic intake of LD/DDI improves motor complications in PD patients according to the concept of continuous dopaminergic stimulation, PD patients receive several grams of LD/DDI daily (Schapira et al., 2008). This regimen elevates tHcy as a risk factor for cardiovascular and neurodegenerative diseases, as well as renal diseases (McGregor et al., 2002; Alirezaei et al., 2011). Plasma tHcy increases as renal function declines and more than 80% of people with ESRD are hyperhomocysteinemic. Another concern regarding LD is the potential to induce ROS-mediate damage and thereby induce and or accelerate renal cell dysfunction and death (McGregor et al., 2002).

Taking the above-mentioned issues into consideration, we decided to determine whether oral administration of betaine concomitant with LD and LD/DDI could act as an antioxidant agent to prevent oxidative stress in kidney of rats. We also examined possible protective effects of betaine in a LD-induced hyperhomocysteinemia model by measuring tHcy in plasma of rats.

Materials and Methods

Materials: MADOPAR® (Levodopa 100 mg+ Benserazide [as a dopa-decarboxylase inhibitor] 25 mg) was obtained from Roche Company, New Zealand. Betaine (Betafin® 96%) was prepared from Biochem Company (Brinkstrasse 55, D-49393 Lohne, Germany). GPx and SOD kit were obtained via Randox® Company (Randox, UK). The homocysteine enzymatic kit was prepared by Axis® Homocysteine (Axis-Shield AS, UK). Levodopa kindly provided by JALINOOS Pharmacy (Karaj, Alborz province, Iran). The other chemicals used were of analytical grade.

Animals: Sprague-Dawley rats (weighing 150-170 g, obtained from Shiraz University of Medical Sciences, Animal House Center, Iran) were housed in temperature-controlled conditions under a 12:12-h light/dark with food and tap water supplied ad libitum. All rats were treated in compliance with the recommendations of Animal Care Committee for Lorestan University of Medical Sciences (Khorramabad, Iran). All experimental procedures were carried out between 08.00 am and 17.00 pm for prevention of circadian rhythm changes among days.

Experimental design: The rats were divided into six equal groups (n=7 rats per group), weight gain and food consumption were determined at 5-day intervals and treated for 10 consecutive days orally by gavage in the following order: control group received 1 mL distilled water, the levodopa (LD) group were treated with LD (3 × 100 mg/kg p.o. at 8.5 am, 12.5 am, and 16.5 pm.), the betaine (Bet.) group received betaine (1.5% w/w of the total diet dissolved in distilled water was given orally by gavage at 8.00 am), the levodopa plus betaine (LD/Bet.) group were treated by LD (3 × 100 mg/kg p.o. at 4 hour intervals) plus betaine (1.5% w/w of the total diet at 8.00 am), the levodopa plus benserazide (LD/Ben.) group received MADOPAR® (3 × (LD 100 mg + Ben. 25 mg) at 4 hour intervals), and the final group, levodopa plus betaine-benserazide (LD/Bet.-Ben.), was treated via MADOPAR® (3 ×(LD 100 mg + Ben. 25 mg) at 4 hour intervals) and betaine (1.5% w/w of the total diet as betaine group at 8.00 am). Betaine, LD, and LD/Ben. were dissolved in distilled water before administration, daily. Doses of levodopa and benserazide were determined according to a previous report (Nissinen et al., 2005), and betaine was found in our previous works (Alirezaei et al., 2011, 2012b,c). 2 hours after the last gavage, the rats were sacrificed upon light diethyl ether anesthesia (Dagenham, UK). Blood

samples were collected via cardiac puncture, whole blood containing EDTA was centrifuged at 3,000 RPM for 10 min and plasma was removed in aliquot microtubes. The right kidneys were removed and carefully cleaned of fat and adhering, and then plasma and kidney samples were stored at -70°C until biochemical analysis.

Tissue preparation for protein measurement, TBARS detection and enzyme assay: Rat kidneys were thawed and manually homogenized in cold phosphate buffer (0.1 M, pH 7.4), containing 5 mM EDTA, and debris was removed by centrifugation at 2000× g for 10 min (Centrifuge 5415 R; Rotofix 32A, Germany). Supernatants were recovered and used for antioxidant enzyme activities, TBARS concentrations and protein measurement. Protein content of tissue homogenates was determined using a colorimetric method of Lowry with bovine serum albumin as a standard (Lowry et al., 1951).

Measurement of tHcy concentration: Total homocysteine of plasma which refers to the sum of protein-bound, free-oxidized, and reduced species of homocysteine was determined by the Axis® Homocysteine enzymatic kit (Golbahar et al., 2005; Karthikeyan et al., 2007; Alirezaei et al., 2011). tHcy concentration was expressed as micromoles per liter (μmol/L) of plasma.

Measurement of lipid peroxidation: The amount of lipid peroxidation was indicated by the content of thiobarbituric acid reactive substances (TBARS) in the kidney. Tissue TBARS was determined by following the production of thiobarbituric acid reactive substances as described previously (Subarao et al., 1990); it was reported previously in our laboratory (Alirezaei et al., 2011, 2012a, b). In short, 40 μL of homogenate was added to 40 μL of 0.9% NaCl and 40 μL of deionized H2O, resulting in a total reaction volume of 120 μL. The reaction was incubated at 37º C for 20 min and stopped by the addition of 600 μL of cold 0.8 M hydrochloride acid, containing 12.5% trichloroacetic acid. Following the addition of 780 μL of 1% TBA, the reaction was boiled for 20 min and then cooled at 4º C for 1 h. In order to measure the amount of TBARS produced by the homogenate, the cooled reaction was spun at 1500× g in a microcentrifuge for 20 min and the absorbance of the supernatant was read spectrophotometrically (S2000 UV model; WPA, Cambridge, UK) at 532 nm, using an extinction coefficient of 1.56×10^5/M Cm. The blanks for all of the TBARS assays contained an additional 40 μL of 0.9% NaCl instead of homogenate as just described. TBARS results were expressed as nanomoles per milligram of tissue protein (nmol/mg protein).

Measurement of CAT activity: Tissue catalase activity was assayed using the method described previously (Claiborne, 1986). The reaction mixture (1 mL) consisted of 50 mM potassium phosphate (pH 7.0), 19 mM H2O2, and a 20-50 μL sample. The reaction was initiated by the addition of H2O2, and absorbance changes were measured at 240 nm (25 °C) for 30 s. The molar extinction coefficient for H2O2 is 43.6/M Cm. The CAT activity was expressed as the unit that is defined as μ mol of H2O2 consumed per min per milligram of tissue protein (U/mg protein).

Measurement of SOD activity: The activity of superoxide dismutase (SOD) was evaluated with Randox® SOD detection kit according to the manufacturer's instructions, was reported previously in our laboratory (Alirezaei et al., 2011, 2012a,b). The role of SOD is to accelerate the dismutation of the toxic superoxide (O2-) produced during oxidative phosphorylation in electron transport chain to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5- henyltetrazolium chloride (I.N.T.) to form a red formazan dye. The SOD activity is then measured by degree of inhibition of this reaction. One unit of SOD is that which causes 50% inhibition of the rate of reduction of INT under the conditions of the assay. SOD levels were recorded at 505 nm and through a standard curve and expressed as unit per milligram of tissue protein (U/mg protein).

Measurement of GPx activity: The activity of glutathione peroxidase (GPx) was evaluated with Randox® GPx detection kit according to the manufacturer's instructions, was reported previously in our laboratory (Alirezaei et al., 2011, 2012a,b). GPx catalyze the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidised glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance was

measured spectrophotometrically against blank at 340 nm. One unit (U) of GPx was defined as l µmol of oxidized NADPH per min per milligram of tissue protein. The GPx activity was expressed as unit per milligram of tissue protein (U/mg protein).

Statistical analysis: Statistical analysis was performed using the statistical package GraphPad PRISM version 5 (GraphPad Software Inc., San Diego, CA, USA). All variables were tested for normal and homogeneous variances by Leven's statistic test. All results are presented as mean± (S.E.M.). The statistical differences were applied among the all groups by one-way analysis of variance (ANOVA) with Tukey's post hoc analysis. A calculated P value of less than 0.05 was considered statistically significant.

Results

Treatment of rats with MADOPAR® (levodopa plus dopa-decarboxylase inhibitor) significantly increased tHcy in plasma of the LD/Ben. group compared to the other groups, while administration of betaine to the Bet. and LD/Bet. groups could suppress tHcy increasing ($p<0.05$; Figure 1).

Regarding lipid peroxidation, treatment of rats with levodopa, and levodopa plus benserazide significantly increased TBARS concentration in the LD- and LD/Ben.-treated rats when compared to the control and betaine groups ($p<0.05$). However, the concentration of TBARS in the LD/Bet.-Ben. group was slightly lower compared with the LD/Ben.-treated rats ($p>0.05$; Figure 2).

The mean values (±SEM) of the catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities of the kidney tissue from experimental groups are presented in figures 3, 4, and 5. CAT activity was significantly higher in the LD group compared to the control, and there was not any significant difference among the other groups for CAT activity ($p>0.05$). SOD activity was significantly higher in the LD/Ben. group compared to the LD-treated rats ($p<0.05$). However, SOD activity was insignificantly higher in the betaine group compared to the LD and LD/ Bet. groups ($p>0.05$). Although the activity of the GPx in the LD group was slightly lower compared to the other groups, these reductions were not statistically significant ($p>0.05$). Indeed, when betaine was administered with LD and LD/Ben., it could increase the level of GPx activity near to the control group (Figure 5).

Discussion

This report is, to the best of our knowledge, the first to have evaluated the effects of betaine on levodopa/ benserazide (LD/Ben.)-mediated hyperhomocysteinemia and levodopa (LD)- induced oxidative stress in kidney tissue. Our data show that LD/Ben. treatment, as a new drug for Parkinson's disease (PD), induces hyperhomocysteinemia and this effect is more noticeable than is the LD treatment. Based on the present results, betaine is not only a methyl donor, but also it is as an antioxidant agent versus oxidative stress mediated by LD and LD/Ben. Treatment in the rat kidney. Although the primary role of betaine in the kidney is likely to be as an osmoprotectant, the methyl donor and antioxidant properties of betaine are promising, particularly in management of hyperhomocysteinemia and oxidative stress in kidney tissue. Indeed, it seems that combination of betaine with LD or LD/Ben. is promising to reduce the drug side effects.

In recent years, there has been increasingly more evidence to show LD-induced hyperhomocysteinemia (Nissinen et al., 2005; Muller et al., 2011; Schapira et al., 2008). LD, the most effective drug known in the treatment of PD, has been observed to induce elevations in plasma tHcy concentrations (Nissinen et al., 2005). LD is metabolized by four major metabolic pathways including: decarboxylation, O-methylation, transamination, and oxidation. The principal path is decarboxylation, whereby dopamine is formed by aromatic amino acid decarboxylase (Muzzi et al., 2008; Schapira et al., 2008). Methoxylation of LD to 3-O-methyldopa (3-O-MD) by catechol-O-methyltransferase (COMT) is a second less prominent metabolic pathway. Transamination and oxidation are additional metabolic pathways (Muzzi et al., 2008). In the present study, LD is administered with a peripheral-acting dopa decarboxylase inhibitor (DDI), benserazide, in order to prevent its metabolism to dopamine so as to enhance brain penetration. The administration of benserazide with LD results in increased metabolism of LD to 3-O-MD via the enzyme COMT in peripheral tissues

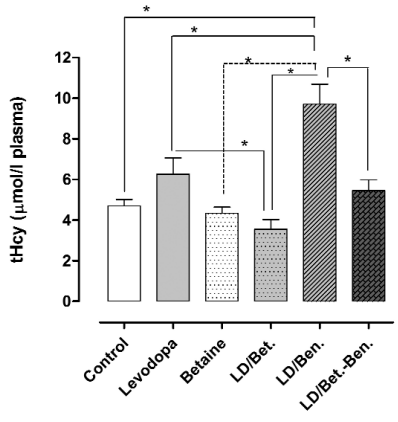

Figure 1. Comparison of plasma total homocysteine (tHcy) concentration among the control and treated groups. Values represent mean± SEM of tHcy (µmol/L of plasma). Asterisk indicates statistically difference among the groups ($p<0.05$). LD; levodopa, Bet.; betaine, Ben.; benserazide.

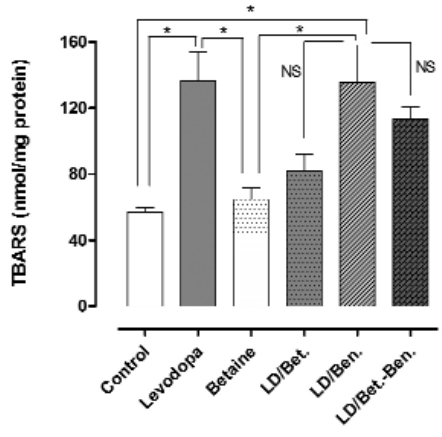

Figure 2. Comparison of thiobarbituric acid reactive substances (TBARS) concentration among the control and treated groups. Values represent mean±SEM of TBARS (nanomoles per milligram protein of kidney tissue). Asterisk indicates statistically difference among the groups ($p<0.05$). There is no significant difference between levodopa and LD/Bet. groups ($p>0.05$). NS; not significant. LD; levodopa, Bet.; betaine, Ben.; benserazide.

(Nissinen et al., 2005; Muller et al., 2011). It is known that COMT activity requires S-adenosyl-L-methionine (SAM) as the methyl donor and this leads to the formation of Hcy (Nissinen et al., 2005). Thus, in the present study tHcy increased in LD/Ben.-treated rats according to the above-mentioned processes. However, tHcy increased slightly in LD-treated rats in comparison with controls and betaine could suppress tHcy increasing in LD/Bet.-Ben. group when compared to the LD/Ben.-treated rats (Figure 1).

As LD is used chronically in the treatment of PD, we studied the effect of LD concomitant with betaine. It has been shown that the elevated plasma Hcy levels found in PD patients treated with LD are associated with a nearly two-fold increased prevalence of coronary artery diseases (Rogers et al., 2003; Nissinen et al., 2005). Previous reports also suggest that elevated plasma Hcy levels may be a risk factor for neuropsychiatric disorders such as dementia, depression and PD (Nissinen et al., 2005; Alirezaei et al., 2011). The high prevalence of hyperhomocysteinemia in the population and its easy treatment make Hcy an interesting amino acid for studies in the prevention of degenerative brain disorders such as PD (Sachdev et al., 2005; Alirezaei et al., 2011). In the present study, betaine, a methyl donor that continuously generates SAM, is shown to lead to long-term lowering of tHcy during supplementation in the dietary intake range of 1.5% (w/w) of total diet (Alirezaei et al., 2011). BHMT has been shown to be expressed at high levels in the livers of all vertebrate species tested. In mammalian liver, BHMT represents 1% or more of the total soluble protein (Barak et al., 2002; Fridman et al., 2012). BHMT activity also exists in the renal cortex and BHMT activity was found about 1-2% of the BHMT specific activity as that found in liver (Fridman et al., 2012). Betaine can also be endogenously produced in the liver and kidney cortex from its metabolic precursor choline via choline oxidase (Fridman et al., 2012). Betaine regulates cell volume by counteracting changes in extracellular tonicity and stabilizing macromolecules against a variety of physiological perturbations (Alirezaei et al., 2011; Fridman et al., 2012). Based on our results, tHcy concentration decreased moderately in betaine group in comparison with LD-treated rats, while tHcy concentration in the plasma of LD/Ben.-treated rats due to lack of SAM elevated significantly. LD is predominantly metabolized to 3-OMD in the presence of a DDI, like benserazide, by COMT. COMT is the essential enzyme for this O-methylation of LD, which demands for a methyl group transfer from the donor SAM (Muzzi et al., 2008; Muller et al., 2011). As one consequence, SAM is transformed into the short living S-adenosyl-homocysteine and then to Hcy. Thus, we assume that a certain balance between LD and betaine is developed during this treatment protocol. Because Hcy synthesis represents a

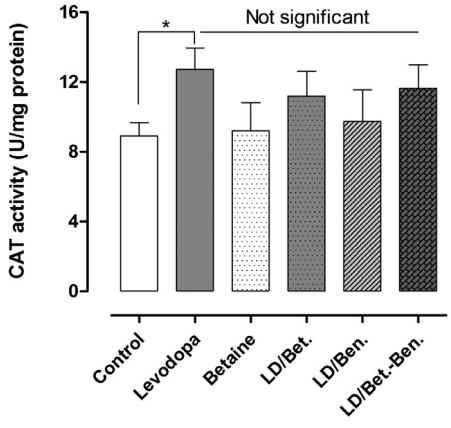

Figure 3. Comparison of CAT activity among the control and treated groups. Values represent mean±SEM of enzyme activity (unit/mg protein of kidney tissue). Asterisk indicates statistically difference between control and levodopa groups ($p<0.05$) while, there is not any significant difference among the other groups ($p>0.05$). LD; levodopa, Bet.; betaine, Ben.; benserazide.

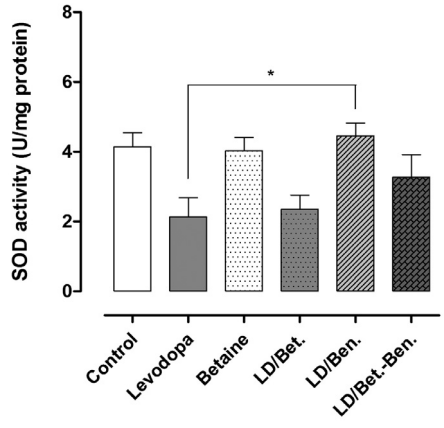

Figure 4. Comparison of SOD activity among the control and treated groups. Values represent mean±SEM of enzyme activity (unit/mg protein of kidney tissue). Asterisk indicates statistically difference between levodopa and LD/Ben. groups ($p<0.05$) while, there is not any significant difference among the other groups ($p>0.05$). LD; levodopa, Bet.; betaine, Ben.; benserazide.

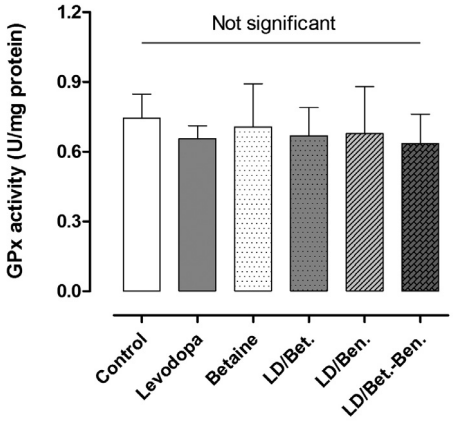

Figure 5. Comparison of GPx activity among the control and treated groups. Values represent mean±SEM of enzyme activity (unit/mg protein of kidney tissue). There is not any significant difference among the groups ($p>0.05$). LD; levodopa, Bet.; betaine, Ben.; benserazide.

secondary reaction product of O-methylation of LD to 3-OMD, one may hypothesize that betaine/or methyl donors supplementation may exert a certain preventive effect on the onset of hyperhomocysteinemia during LD/Ben. administration.

There is abundant evidence for oxidative stress in substantia nigra area of brain in PD patients (Nissinen et al., 2005; Muller et al., 2011; Schapira et al., 2008). Nevertheless, it remains unknown whether an increased oxidative load produced by LD would induce oxidative stress in kidney tissue of PD patients. It is known that LD administration produces dopamine in peripheral tissues and in the dopaminergic cells of the substantia nigra pars compact it converts to dopamine (Schapira et al., 2008). Dopamine may undergo autooxidation, semiquinone formation, and polymerization with the production of ROS (Tse et al., 1976; Fahan et al., 2005; Schapira et al., 2008). Dopamine can also be metabolized by monoamine oxidase-B to produce hydrogen peroxide (H2O2) which can then, in the presence of iron, be converted by the Fenton reaction to produce the highly reactive hydroxyl radical (Schapira et al., 2008). The potential pro-oxidant actions of LD have added to the debate over the role of oxidative stress in PD and its role in disease progression (Fahan et al., 2005; Shulman et al., 2000, Weiner et al., 2000). Hyperhomocysteinemia also inhibits the expression of antioxidant enzymes which might potentiate the toxic effects of ROS (Bleich et al., 2004; Alirezaei et al., 2011). In addition, auto-oxidation of homocysteine is known to generate ROS, whereby the prevention of LD-induced toxicity by catalase suggests that hydrogen peroxide acted as a mediator of oxidative injury, leading to oxidative stress in the rat kidney (Austin et al., 1998; D'Emilia and Lipton, 1999). In this regard, only catalase activity was significantly higher in the LD group compared to the controls (Figure 3). It seems that the elevation of CAT activity is a compensatory mechanism. It is known that LD can act both as a pro-

oxidant and anti-oxidant molecule depending on circumstances. "For example, low concentrations of LD can induce an up-regulation in glutathione (GSH) molecule possibly because the drug acts as a minimal stressor that enhances the production of protective molecules" (Schapira et al., 2008). Indeed, catalase prevented H2O2 accumulation and elevated its activity (in order compensatory) to suppress oxidative stress in the LD group; however, this elevation was not able to prevent lipid peroxidation in the rat kidney.

LD has the potential to increase free radical generation through oxidative pathways and thereby could theoretically contribute to the lipid peroxidation and potentially accelerate oxidative stress in kidney. Betaine is believed to play a significant role in maintaining the structural and functional integrity of cell membranes. Previous studies have demonstrated that betaine, through its participation in sequential methylation within the cellular membranes, maintains a proper balance between phosphotidyl ethanolamine and phosphotidyl choline, thus sustaining proper membranes (Kharbanda et al., 2007; Ganesan et al., 2010; Alirezaei et al., 2011). In our study, LD and LD/Ben. consumption caused significantly increased TBARS concentration (as a lipid peroxidation marker) in the LD- and LD/Ben.-treated rats and betaine treatment restored this elevated TBARS concentration in the LD/Bet. group near the control group (Figure 2). On the other hand, there were significant differences among the control and betaine with LD and LD/Ben. groups, indicating the occurrence of oxidative stress in LD- and LD/Ben.-treated rats.

All cells are able to defend themselves from damaging effects of oxygen radicals by their own antioxidant mechanisms, including enzymatic and non-enzymatic antioxidant systems (Alirezaei et al., 2011; Neamati et al., 2011). GPx and CAT are two key antioxidant enzymes that can decompose hydrogen peroxide to water (Alirezaei et al., 2011; Kheradmand et al., 2013). SOD, another antioxidant enzyme in cells, rapidly converts superoxide anion (O2-) to less dangerous H2O2 then GPx, and CAT can decompose H2O2 to water. Although H2O2 is not a reactive product, it can be reduced to the highly reactive metabolites hydroxyl radicals (Alirezaei et al., 2012c; Neamati et al., 2011). The increase in SOD activity in our investigation for LD/Ben.-treated rats (Figure 4) correlates well with the increase of lipid peroxidation in renal tissue. SOD shifts highly reactive O2- to H2O2 and thus prevents the renal cell membrane damage caused by this highly toxic anion. It seems that elevation of SOD activity in LD/Ben. group is a compensatory mechanism that was not able to prevent lipid peroxidation in the kidney. In this context, Masella et al. (2004) have expressed that "antioxidant responsive elements (AREs) are present in the promoter regions of many of the genes inducible by oxidative and chemical stresses". Thus, it appears that in the present study the consumption of LD and LD/Ben. (both oxidative agents) can increase the activity of the antioxidant enzymes such as CAT and SOD by a compensatory mechanism via AREs.

Although the mean activity of GPx in our study was slightly lower in the LD and LD/Ben. groups than betaine group, these differences were not statistically significant (Figure 5). Possibly, prolonged treatments by LD or higher doses are needed to induce greater activity of GPx for suppression of oxidative stress in the kidney. On the other hand, as indicated in Figure 5, GPx seems to be less affected by LD and LD/Ben., and these drugs could not influence renal GPx activity markedly.

As mentioned above, this is the first in vivo study to show that betaine treatment results in an overall decrease in the lipid peroxidation in rat's kidney. Betaine is a methyl donor agent like SAM, and it also stabilizes SAM levels via BHMT pathway (Alirezaei et al., 2011; 2012b,c,d, 2014a,b). In addition, it reduces Hcy concentration (as an oxidative agent) via BHMT. With regard to BHMT, which is abundant in primates, The beneficial properties of betaine are promising and reduce the elevated plasma Hcy concentrations via the BHMT pathway (Alirezaei et al., 2011; 2012b,c). Although betaine demonstrated as a potential methyl donor and antioxidant agent for prevention of hyperhomocysteinemia and oxidative stress, further studies including evaluation of kidney function and histochemical techniques are needed to clarify protective effects of betaine in the kidney tissue.

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Conflict of interest: The author declares that there is no conflict of interest in the present study.

References

1. Alirezaei, M., Dezfoulian, O., Kheradmand, A., Neamati, S., Khonsari, A., Pirzadeh, A. (2012a) Hepatoprotective effects of purified oleuropein from olive leaf extract against ethanol-induced damages in the rat. Iran J Vet Res. 13: 218-226.
2. Alirezaei, M., Jelodar, G., Ghayemi, Z. (2012b) Antioxidant defense of betaine against oxidative stress induced by ethanol in the rat testes. Int J Pept Res Ther. 18: 239-247.
3. Alirezaei, M., Jelodar, G., Ghayemi, Z., Khordad Mehr, M. (2014a) Antioxidant and methyl donor effects of betaine versus ethanol-induced oxidative stress in the rat liver. Comp Clin Pathol. 23: 161-168.
4. Alirezaei, M., Jelodar, G., Niknam P., Ghayemi Z., Nazifi, S. (2011) Betaine prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. J Physiol Biochem. 67: 605-612.
5. Alirezaei, M., Jelodar G., Niknam, P., Khoshdel, Z., Yavari, M. (2014b) Nutritional effects of betaine on weight and length of rat offspring. Comp Clin Pathol 1-6. DOI 10.1007/s00580-012-1650-z
6. Alirezaei, M., Niknam, P., Jelodar, G. (2012c) Betaine elevates ovarian antioxidant enzyme activities and demonstrates methyl donor effect in non-pregnant rats. Int J Pept Res Ther. 18: 281-290.
7. Alirezaei, M., Saeb, M., Javidnia, K., Nazifi, S., Saeb, S. (2012d) Hyperhomocysteinemia reduction in ethanol-fed rabbits by oral betaine. Comp Clin Pathol. 21: 421-427.
8. Austin, R.C., Sood, S.K., Dorward, A.M., Singh, G., Shaughnessy, S.G., Pamidi, S., Outinen, P.A., Weitz, J.I. (1998) Homocysteine-dependent Alterations in Mitochondrial Gene Expression, Function and Structure. Homocysteine and H2O2 act synergistically to enhance mitochondrial damage. J Biol Chem. 273: 30808-30817.
9. Barak, A.J., Beckenhauer, H.C., Tuma, D.J. (2002) Methionine synthase a possible prime site of the ethanolic lesion in liver. Alcohol. 26: 65-67.
10. Bergamini, C.M., Gambetti, S., Dondi, A., Cervellati, C. (2004) Oxygen, reactive oxygen species and tissue damage. Curr Pharm Des. 10: 1611-1626.
11. Bleich, S., Degner, D., Sperling, W., Bönsch, D., Thürauf, N., Kornhuber, J. (2004) Homocysteine as a neurotoxin in chronic alcoholism. Prog Neuropsychopharmacol Biol Psychiat. 28: 453-464.
12. Claiborne A (1986) Catalase activity. In: CRC Handbook of Methods for Oxygen Radical Research. Greenwald, R.A. (ed.).Vol 1.CRCPress, Boca Raton, Florida, USA. p. 283-284.23.
13. D'Emilia, D.M., Lipton, S.A. (1999) Ratio of S-nitrosohomocyst (e) ine to homocyst (e) ine or other thiols determines neurotoxicity in rat cerebrocortical cultures. Neurosci Lett. 265: 103-106.
14. Fahn, S. (2005) Does levodopa slow or hasten the rate of progression of Parkinson´s disease? J Neurol. 252: iv37-iv42.
15. Fridman, O., Morales, A.V., Bortoni, L.E., Turk-Noceto, P.C., Prieto, E.A. (2012) Corticoadrenal activity regulates in rat betaine-homocysteine S-methyltransferase expression with opposites effects in liver and kidney. J Biosci. 37: 115-123.
16. Ganesan, B., Buddhan, S., Anandan, R., Sivakumar, R., Anbinezhilan, R. (2010) Antioxidant defense of betaine against isoprenaline-induced myocardial infarction in rats. Mol Biol Rep. 37: 1319-1327.
17. Golbahar, J., Aminzadeh, M.A., Hamidi, S.A., Omrani, G.R. (2005) Association of red blood cell 5-methyltetrahydrfoate folate with bone mineral density in postmenopausal Iranian women. Osteop Int. 16: 1894-1898.
18. Karthikeyan, G., Thachil, A., Sharma, S., Kalaivani, M., Ramakrishnan, L. (2007) Elevated high sensitivity CRP levels in patients with mitral stenosis and left atrial thrombus. Int J Cardiol. 122: 252-254.
19. Kharbanda, K.K., Mailliard, M.E., Baldwin, C.R., Beckenhauer, H.C., Sorrell, M.F., Tuma, D.J. (2007) Betaine attenuates alcoholic steatosis by restoring phosphatidylcholine generation via the phosphatidylethanolamine methyltransferase pathway. J Hepatol. 46: 314-321.
20. Kheradmand, A., Alirezaei, M., Dezfoulian, O. (2013) Cadmium-Induced Oxidative Stress in the Rat Testes: Protective Effects of Betaine. Int J Pept Res Ther. 19: 337-344.
21. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randal, R.J. (1951) Protein measurement with the Folin phenol reagent. J Biol Chem. 193: 265-275.

22. Masella, R., Vari, R., D'Archivio, M., Di Benedetto, R., Matarrese, P., Malorni, W., Scazzocchio, B., Giovannini, C. (2004) Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. J Nutr. 134: 785-791.
23. McGregor, D.O., Dellow, W.J., Robson, R.A., Lever, M., George, P.M., Chambers, S.T. (2002) Betaine supplementation decreases post-methionine hyperhomocysteinemia in chronic renal failure. Kidney Int. 61: 1040-1046.
24. Muller, T., Jugel, C., Ehret, R., Ebersbach, G., Bengel, G., Muhlack, S., Klostermann, F. (2011) Elevation of total homocysteine levels in patients with Parkinson´s disease treated with duodenal levodopa/carbidopa gel. J Neural Transm. 118: 1329-1333.
25. Muzzi, C., Bertocci, E., Terzuoli, L., Porcelli, B., Ciari, I., Pagani, R., Guerranti, R. (2008) Simultaneous determination of serum concentrations of levodopa., dopamine., 3-O-methyldopa and O-methyldopa by HPLC. Biomed Pharmacotherap. 62: 253-258.
26. Neamati, S., Alirezaei, M., Kheradmand, A. (2011) Ghrelin acts as an antioxidant agent in the rat kidney. Int J Pept Res Ther. 17: 239-245.
27. Nissinen, E., Nissinen, H., Larjonmaa, H., Vaananen, A., Helkamaa, T., Reenila, I., Rauhala, P. (2005) The COMT inhibitor, entacapone, reduces levodopa-induced elevations in plasma homocysteine in healthy adult rats. J Neural Transm. 112: 1213-1221.
28. Rogers, J.D., Sanchez-Saffon, A., Frol, A.B., Diaz-Arrastia, R. (2003) Elevated plasma homocysteine levels in patients treated with levodopa: association with vascular disease. Arch Neurol. 60: 59-64.
29. Sachdev, P.S. (2009) Homocysteine and brain atrophy. Prog Neuropsychopharmacol Biol Psychiat. 29: 1152-1161.
30. Schapira, A.H.V. (2008) The clinical relevance of levodopa toxicity in the treatment of Parkinson's disease. Mov Disord. 23: S515-S520.
31. Shulman, L.M. (2000) Levodopa Toxicity in Parkinson Disease: Reality or Myth?: Reality--Practice Patterns Should Change. Arch Neurol. 57: 406-407.
32. Smith, A.M., Zeve, D.R., Grisel, J.J., Chen, W.J.A. (2005) Neonatal alcohol exposure increases malondialdehyde (MDA) and glutathione (GSH) levels in the developing cerebellum. Dev Brain Res. 160: 231-238.
33. Subbarao, K.V., Richardson, J.S., Ang, L.C. (1990) Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro. J Neurochem. 55: 342-345.
34. Tse, D.C.S., McCreery, R.L., Adams, R.N. (1976) Potential oxidative pathways of brain catecholamines. J Med Chem. 19: 37-40.
35. Weiner, W.J. (2000) Is levodopa toxic? Arch Neurol. 57: 408.

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بتائین به‌عنوان یک دهنده گروه متیل و آنتی کسیدان در برابر افزایش هموسیستئین خون و استرس اکسیداتیو ناشی از لوودوپا در کلیه موش صحرایی

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چکیده

زمینه مطالعه: بتائین اثرات آنتی اکسیدانی و دهندگی گروه متیل در مطالعات اخیر ما نشان داده است. **هدف:** مطالعه ویژگی‌های آنتی اکسیدانی و دهندگی گروه متیل بتائین در افزایش هموسیستئین خون بواسطه ترکیب لوودوپا-بنسرازید و استرس اکسیداتیو ناشی از لوودوپا در کلیه موش‌های صحرایی. **روش کار:** موش‌های نر از نژاد اسپراگ-داولی به گروه‌های لوودوپا (LD)، بتائین (Bet.)، لوودوپا+بتائین (LD/Bet.)، لوودوپا+بنسرازید (LD/Ben.)، لوودوپا+بتائین+بنسرازید (LD/Bet.-Ben.) و کنترل تقسیم شدند. گروه‌های مورد آزمایش لوودوپا (۱۰۰mg/kg ×۳)، بتائین (۱/۵٪ وزنی/وزنی جیره کل)، بنسرازید (۲۵mg/kg ×۳) و آب مقطر برای گروه کنترل به‌مدت ۱۰ روز پیوسته به‌صورت خوراکی با گاواژ دریافت کردند. **نتایج:** غلظت هموسیستئین کل (tHcy) پلاسما به‌طور معنی‌داری در موش‌های درمان‌شده با بتائین، لوودوپا+بتائین و لوودوپا+بتائین+بنسرازید در مقایسه با گروه LD/Ben. کاهش یافت. در بافت کلیه غلظت مواد واکنش دهنده با اسید تیوباربیتوریک (به‌عنوان شاخص پراکسیداسیون لیپید) به‌طور معنی‌داری در گروه بتائین در مقایسه با گروه‌های LD و LD/Ben. کاهش یافت. فعالیت آنزیم کاتالاز کلیوی به‌طور معنی داری در موش‌های درمان شده با لوودوپا در مقایسه با گروه کنترل افزایش یافت. فعالیت آنزیم سوپراکسید دیسموتاز در کلیه به‌طور معنی‌داری در گروه درمان‌شده با لوودوپا در مقایسه با گروه LD/Ben. کاهش یافت. اما، هیچ تفاوت معنی‌داری در فعالیت آنزیم گلوتاتیون پراکسیداز کلیوی (GPx) در بین گروه‌ها وجود نداشت. **نتیجه‌گیری نهایی:** این یافته‌ها نشان می‌دهند که لوودوپا و لوودوپا+بنسرازید بخاطر القاء استرس اکسیداتیو در کلیه و افزایش هموسیستئین خون اثرات جانبی دارند. در مقابل، بتائین به‌عنوان یک ماده امید بخش آنتی اکسیدان و دهنده گروه متیل در برابر مشکلات ناشی از لوودوپا عمل می‌کند.

واژه‌های کلیدی: بنسرازید، بتائین، هموسیستئین، کلیه، لوودوپا

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