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Carnisone Increases Efficiency of DOPA Therapy of Parkinson's Disease: A Pilot Study

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Abstract

The addition of the neuropeptide carnosine (β-alanyl-L-histidine) as a food additive to the basic protocol of Parkinson's disease treatment results in significant improvement of neurological symptoms, along with increase in red blood cell Cu/Zn-SOD and decrease in blood plasma protein carbonyls and lipid hydroperoxides, with no noticeable change in platelets MAO B activity. The combination of carnosine with basic therapy may be a useful way to increase efficiency of PD treatment.

Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease characterized by progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNPC). The development of PD in patients becomes clinically apparent as severe motor symptoms, including uncontrolled resting tremor, bradykinesia, rigidity, and postural imbalance.[1,2] In most cases, these symptoms appear after 70-80% of SNPC dopaminergic neurons are lost.[3] The exact etiology of PD remains unclear, but what we clearly know is that the disease is of multi-factorial nature, including both environmental and genetic factors, which result in development of oxidative stress in specific areas of the brain.[4]

Initial stages of PD are tightly connected with mitochondrial dysfunction,[5,6] accumulation of reactive oxygen species (ROS),[7] and appearance of apoptotic signals in the SNPC area of the brain.[8] Death of dopaminergic neurons results in release of a large amount of free dopamine, the spontaneous oxidation of which is accompanied by ROS generation and aggravates oxidative stress in SNPC.[9] Development of PD proceeds along with progressive deficit in the antioxidant system, being reflected in decrease in red blood cell Cu/Zn-superoxide dismutase (SOD), catalase, and glutathione peroxidase.[10]

The traditional protocol of PD treatment consists of replacing lost dopamine with its agonists, including L-DOPA therapy, administration of MAO B or catechol-*o*-methyltransferase inhibitors, and other factors directed to facilitation of symptomatic improvement, but not alteration of the course of disease. For this reason, treatment of PD patients is accompanied by several side effects that make the treatment nearly ineffective.[11] For these reasons it is important to develop a complex treatment of PD including antioxidants and neuroprotectors in the protocol.[12]

In this respect the use of the neuropeptide carnosine (β-alanyl-L-histidine) as an additional therapy for Parkinsonism may be reasonable. Carnosine is a natural component of human brain possessing a number of useful properties.[13] Among these is a neuroprotection described in several experimental models of brain ischemia.[14] Carnosine has been predicted to be useful for treatment of brain injury in Parkinson's and Alzheimer's diseases.[15,16] It is a highly hydrophilic compound that easily penetrates the blood–brain barrier; its content in excitable tissues (brain, heart, skeletal muscle) varies from 1.5 to 40(!) mmoles/kg of raw tissue, and LD_{50} (determined for rodents) is about 20 g/kg of body mass.[13] Also, carnosine has been found to protect SOD from oxidative damage under both *in vitro*[15] and *in vivo*[17] conditions.

Recently, carnosine was found to efficiently protect senescence accelerated mice from MPTP-induced Parkinsonism.[18] In these experiments, carnosine was taken in a daily dose of 100 mg/kg body mass; the dipeptide was found to improve neurological symptomatics, to prevent activation of MAO B, and to decrease oxidation of proteins and lipids. In this report, we describe positive effect of carnosine used as an additive for treatment of PD patients simultaneously with basic therapy. A preliminary report of this study was made recently.[19]

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Table 1. Neurological State of PD Patients on the UPDRS Scale

PD patients	Total amount of points (UPDRS scale)		
	Before treatment	*After treatment*	*Improvement (% to initial value)*
Basic therapy	38.9 ± 2.5	32.5 ± 2.0	16.4 ($p < 0.05$)
Basic therapy + carnosine		24.9 ± 2.1 ($p = 0.02$)*	36.0 ($p < 0.01$)

*Compared to the group not treated with carnosine.

Materials and Methods

PD patients and neurological examination

Thirty-six patients with trembling-rigid and trembling manifestations of PD (20 males and 16 females), ages ranging between 46 and 68 years (mean age 53.7 ± 15.2), at several stages (from 1 to 2.5) of disease, took part in the trial. Among them were no patients with chronic systemic diseases such as diabetes type I or II, brain chronic ischemia, arterial hypertension, epilepsy, or oncology, as well as pregnant or breast-feeding patients. The average age at the beginning of disease was 46.0 ± 14.7 years, and the registered period of the disease was from 2.5 to 16 years. Twenty essentially healthy donors were in the control group presenting data for comparison; their average age was 42.0 ± 6.7 years. Each patient signed a letter of informed consent, a document confirming voluntary participation in the trial. The neurological state of the patients was estimated using the Unified Parkinson's Disease Rating Scale (the UPDRS approach). This rating scale contains more than 40 positions characterizing mentality and behavior, activity of daily living, motor examination, and complication of therapy.[20] Since its introduction in 1987, the UPDRS has been extensively used by many researchers to quantify the PD development. In our study, all UPDRS parameters were used to evaluate the state of the patients, which were expressed in points (see Table 1). The most important parameters (extremity rigidity, agility of legs, activity of daily living, motor examination, etc.) were analyzed separately (Table 2). The neurological characterization of the patients was made by a physician who was not informed about the treatment protocol to avoid an opportunity of biased conclusion.

Protocol of treatment

The treatment was performed as a pilot comparative clinical trial. All PD patients were distributed into two groups comparable in age, duration of disease, and neurological symptomatics. The group receiving basic therapy only consisted of 16 patients, and the other group—basic therapy in combination with carnosine—consisted of 20 patients. The treatment lasted for 30 days. Medical examination was made twice: before treatment (at first visit) and after the end of the treatment (second visit). The basic therapy was identical for both groups of patients, including DOPA-containing drugs (average dose of levodopa was in the range of 200 to 800 mg/day), agonists of dopamine receptors Piribedil (150–300 mg/day) or Pramipexol (1.5-3.0 mg/day), and amantadines (100-300 mg/day) in the individually selected doses, depending on the state and the severity of clinical findings. Simultaneously, for patients in the second group, carnosine was prescribed in a daily dose of 1.5 g. In the trial, Sevitine (carnosine-containing tablets covered with an acid-stable envelope) was used as a source of carnosine. Sevitine is a commercially available food additive produced by Medtekhnika Ltd. (Tbilisi, Russia). Each Sevitine tablet contained 0.25 g of carnosine; thus Sevitine was used in a course of two tablets, three times a day for 30 days.

Table 2. Effect of Carnosine Containing Treatment on Several Parameters of PD Symptomatic on the UPDRS Scale

Parameter	*Basic therapy*	*Basic therapy + carnosine*	*% of decrease*	P *value*
Rigidity of hands	2.04 ± 0.2	1.38 ± 0.3	32	0.077
Rigidity of the legs	1.41 ± 0.2	0.88 ± 0.2	38	0.073
Hand movements	1.9 ± 0.3	1.2 ± 0.3	37	0.082
Rapid alternating movements of hands	1.9 ± 0.3	**1.0 ± 0.2**	**47**	**0.015**
Leg agility	1.8 ± 0.2	**0.85 ± 0.20**	**53**	**0.005**
Activities of daily living	11.4 ± 1.2	**7.8 ± 0.8**	**32**	**0.009**
Motor examination	22.5 ± 2.8	**14.8 ± 1.7**	**34**	**0.039**

Statistically significant differences are in bold.

Biochemical measurements

Using blood from the patients, several biochemical parameters were estimated before and after the treatment: MAO B and Cu/Zn-SOD were measured in platelets and in red blood cells, respectively; protein carbonyls were estimated in blood plasma; and Fe^{2+}-induced lipoprotein oxidation in blood lipoproteins was tested.

A portion of blood (35 mL) was taken during fasting. Blood was collected from the cubital vein of patients early in the morning (before breakfast) in a heparinized tube (50 U/40 mL blood). Platelets and red blood cells isolated separately from this portion of blood were used for measuring MAO B and Cu/Zn-SOD, respectively.

MAO B determination. MAO B was measured using benzyl amine as a substrate.[21] Samples of 30 mL volume were centrifuged 15 min at 350 *g*. The upper layer enriched with platelets was collected and centrifuged 15 min at 1500 *g*. The supernatant was discarded and the pellet was re-suspended in 15 mL 0.01 M Na/K-phosphate buffer (pH 7.4) and centrifuged in the same manner. The pellet was homogenized in 4 mL of the same buffer and centrifuged 20 min at 14000 *g*. The final pellet was suspended in 1.5 mL of 0.2 M of phosphate buffer and kept at −80°C until measuring (no more than 1 month).[14]

Cu/Zn-SOD determination. One mL of blood was centrifuged at 500 *g* at room temperature; the pellet was suspended in 1 mL distilled water and kept at −80°C until determination (no more than 1 month). Before measuring, the hemolysate was diluted 1/5-fold with distilled water, and suspended; 0.75 mL sample was placed in medium containing 0.15 mL H_2O, 0.35 mL ethanol/chloroform mixture (3:4, v:v) and shaken for 5 min using a Super-Mixer (Lab-Line Instruments, Melrose Park, IL). Then samples were centrifuged 20 min at 1200 *g*. Cu/Zn-SOD was monitored by the reduction of nitro blue tetrazolium in the presence of xanthine/xanthine oxidase mixture.[22]

Protein carbonyls. Protein carbonyls were estimated at the tenth and thirtieth days of the trial in reaction with 10 mM dinitrophenyl hydrazine in 2 N HCl. A 200 μL portion of blood plasma was taken in 1 mL of the solution and incubated 45 min at room temperature. Then protein was precipitated with 40% trichloroacetic acid, washed three times with 2.5 mL ethanol/ethyl acetate (1:1), and dissolved in 6 M guanidine chloride. Extinction of the samples was measured at 375 nm, taking the molar extinction coefficient as 22000 $M^{-1} \cdot cm^{-1}$.[23]

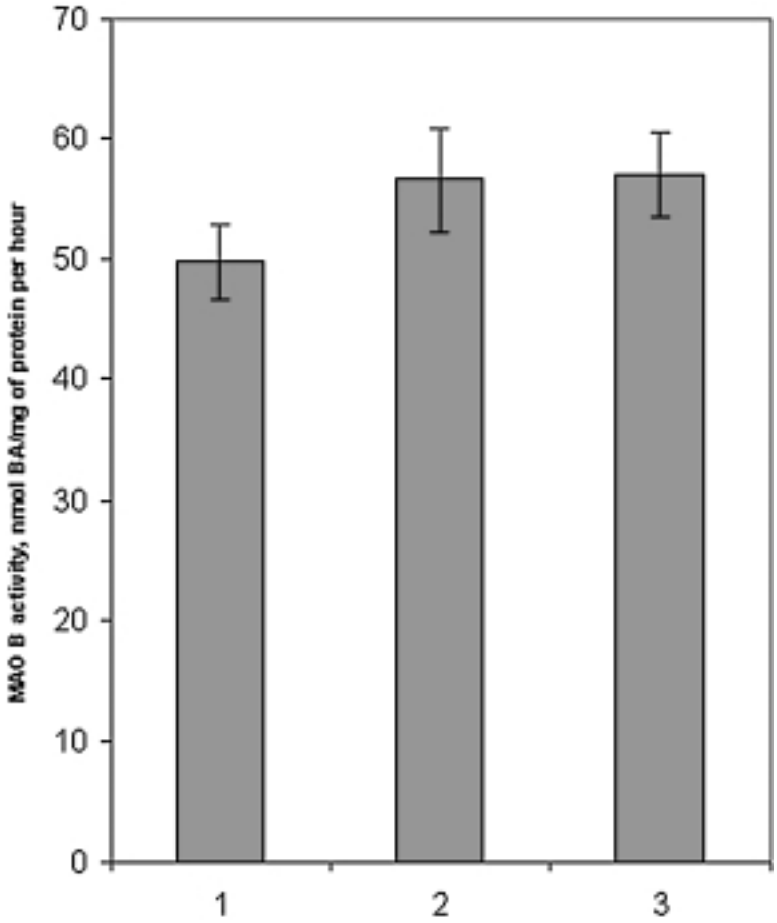

FIG. 1. MAO B activity in PD patients with different protocols of treatment. 1, All patients before treatment; 2, patients after 30 days of basic therapy; 3, same as 2 with addition of carnosine in a daily dose of 1.5 g. The data presented are M ± SE.

Fe^{2+}-induced lipoprotein oxidation. This measurement was analyzed using chemiluminescence signal derived from addition of ferrous ions to lipoprotein suspension (ChL test). Lipoproteins of low and very low density were obtained using the following procedure: 200 μL sample of blood serum was added to 2 mL of 0.28% $CaCl_2$ and 40 μL 1% heparin and centrifuged after 10 min incubation at room temperature for 15 min at 3000 rpm. The pellet was suspended into 900 μL phosphate buffer (60 mM KH_2PO_4 and 105 mM KCl [pH 7.45]). After addition to the mixture of 100 μL of 25 mM $FeSO_4$, a fast flash appeared with amplitude proportional to the amount of pre-formed lipid hydroperoxides. Further lag period characterized the stability of the sample to the Fe^{2+}-induced oxidation (the longer the lag period, the more stable resistance of the biological material to oxidation), and the following slow rising chemiluminescence signal reflected the rate of lipid oxidation.[24] All three parameters were used to characterize the antioxidant state of the patients' blood before and after treatment.

Statistical analysis

Analysis was done using the standard computing program Statistica 5.0, and the data presented were expressed as mean ± SE. Significance of the differences obtained was estimated by non-parametric criteria after Wilcoxon, Kruskal-Walles, and Dunnet.[25] Significant difference was considered at $p < 0.05$.

Results and Discussion

Clinical data

The initial level of neurological symptoms of patients corresponded to 38.9 points on the UPDRS scale (Table 1). After 30 days this level decreased to 32.5 points in the basic therapy group and to 24.9 points in the group with basic therapy combined with carnosine. Efficiency of treatment consisted of 16.5% ($p < 0.05$), and 36.0% ($p < 0.01$), respectively. Thus, including carnosine as an additional treatment significantly improved the neurological state of the patients.

The effect of carnosine on several parameters of the neurological state of the patients was expressed in a different manner (Table 2). In the carnosine-treated group, improvement of locomotion system (rigidity of extremities and hand movements) consisted of 32-38% compared to the group with basic therapy. This correlated well with improvement of one of the most important clinical signs of Parkinsonism—hypokinesia. The difference between these parameters was sufficient while not statistically significant because of the num-

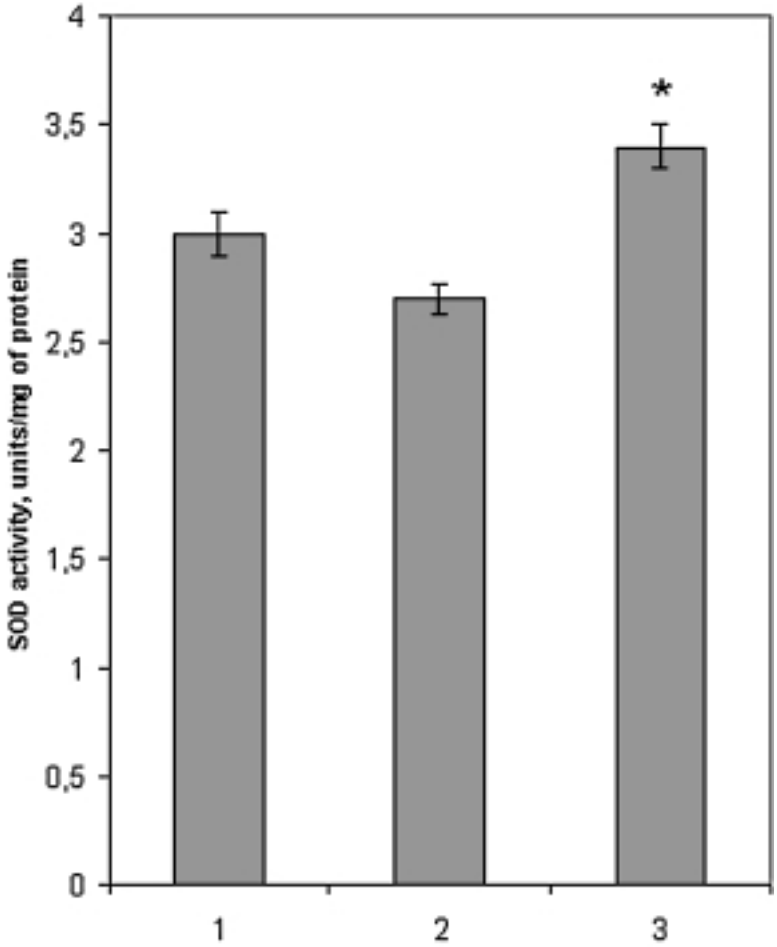

FIG. 2. Cu/Zn-SOD activity in red blood cells of PD patients with different protocols of treatment. 1, All patients before treatment; 2, patients after 30 days of basic therapy; 3, same as 2 with addition of carnosine in a daily dose of 1.5 g. *Statistically significant difference from control.

ber of patients treated. However, based on the p values (see Table 2), the tendency to improve these parameters by including carnosine in the protocol of treatment is clearly seen.

Symptoms of so-called pronation-supination and easiness of leg movements (rapid, alternating movements of hands and leg agilities, see Table 2) were twice as low in the carnosine-treated patients compared with the basic therapy group. At the same time, the typical "finger taps" test (the patient taps thumb with index finger in rapid succession), as well as rigidity of both extremities and trembling, demonstrated similar improvement in both groups of patients.

It is important to note that so-called everyday activity was also improved significantly more in the carnosine-treated patients, which enabled more independent self-service (daily life activity is increased in the carnosine-treated group by 32%). Motor examination showed a 34% improvement because of carnosine treatment. It is important to note that carnosine intake was accompanied by neither negative side effects nor incompatibility with basic DOPA therapy.

Estimation of oxidative state of the patients

In order to characterize the oxidative state of the patients, we measured MAO B, Cu/Zn-SOD, and the level of protein carbonyls and Fe-induced oxidation of blood lipoproteins. MAO B is known to regulate biogenic amine metabolism, whose activation is accompanied with ROS accumulation. It was demonstrated that MAO B is an inducible enzyme whose activation usually takes place during treatment with DOPA-containing drugs.[26] Stable activation of MAO B is one of the features of oxidative stress that can stimulate oxidation of both lipids and proteins.[13] SOD is one of the natural regulators restricting ROS accumulation; thus its activity is obviously used to estimate antioxidant capacity of cells and tissues.

MAO B activity. Figure 1 illustrates the changes in MAO B activity in platelets of PD patients before and after treatment. It is seen that basic therapy alone and that combined with carnosine result in increased MAO B activity. It is noteworthy that this fact appears along with improvement of neurological symptoms; one can conclude that the rise in MAO B activity is still controlled by the intrinsic antioxidant defense system.

Cu/Zn-SOD activity. Figure 2 demonstrates how Cu/Zn-SOD is changed in the two groups of PD patients after basic treatment and that combined with carnosine. After 30 days of treatment in the group receiving basic therapy a noticeable decrease in SOD activity was detected, whereas in the patients treated with carnosine this parameter was significantly increased above the initial level; thus the difference between SOD activities reached 26% ($p = 0.035$). Decrease in SOD activity in patients receiving the basic therapy was not statistically significant from that before treatment.

The importance of SOD as a key enzyme of antioxidant defense is illustrated by an observation of positive correlation between its activation and decrease in neurological symptoms. This correlation was independent of protocol of treatment, and the correlation coefficient was $r = 0.52$. The relatively implicit level of correlation apparently correlates with the multifactorial nature of the disease. Nevertheless,

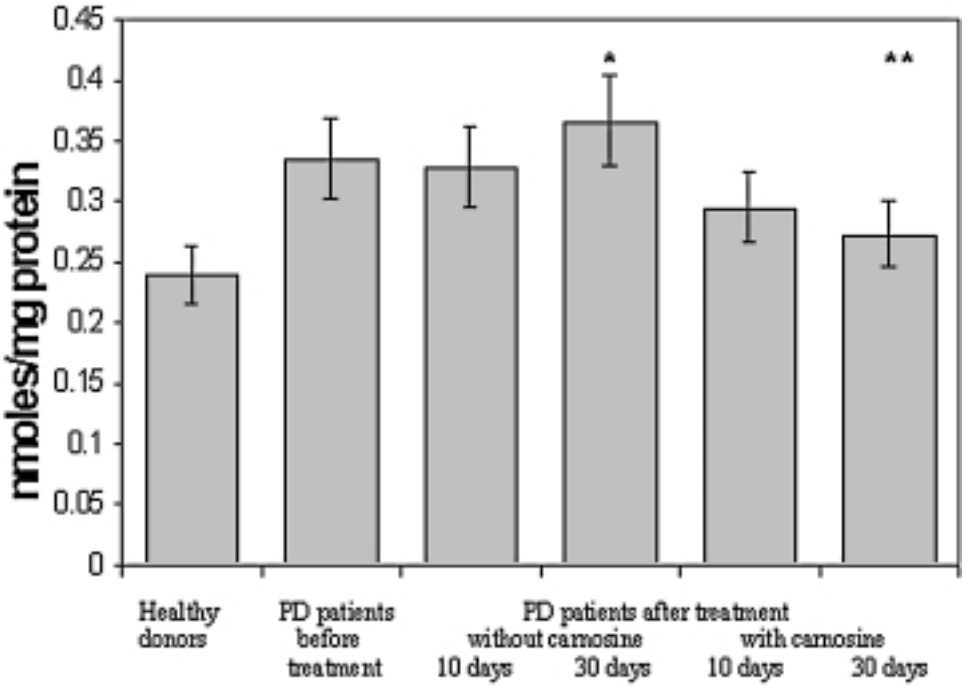

FIG. 3. Level of protein carbonyls in blood plasma of PD patients before and 30 days after treatment using basic therapy or that in combination with carnosine (as indicated) compared with protein carbonyl level of healthy donors. *Significant difference between group 1 and healthy donors. **The same between patients from groups 2 and 3.

Cu/Zn-SOD can be suggested to be one of the important directions of therapeutic effect of carnosine in the organism, which correlates well with recently described protection of SOD by carnosine under hypobaric hypoxia in rats.[17]

There are contradicting data in the literature about SOD activity in PD patients. Some reports showed decrease,[10] but another showed increase of SOD activity compared to healthy donors.[27] Comparison of our data from Table 1 and Figure 2 suggests that some success in the treatment can be achieved without effect on SOD, whereas therapy resulting in restoration of SOD may be more efficient.

Protein carbonyls. Figure 3 illustrates the level of protein carbonyls in blood plasma of healthy donors and the different groups of PD patients. The amount of oxidized protein molecules is seen to be increased in PD patients and not to be changed during basic therapy. However, introduction of carnosine in the protocol of treatment results in noticeable decrease in this parameter, which is in good correlation with the ability of carnosine to protect cellular proteins against oxidation described in the literature.[13,16] Moreover, in some models of oxidative protein damage, carnosine was able to prevent protein oxidation[16] or even to recover the oxidized proteins.[28] This is exactly the case illustrated in Figure 3.

ChL test. Measuring the Fe^{2+}-induced chemiluminescence in blood of PD patients showed that the initial level of hydroperoxides is not changed by basic therapy, whereas when carnosine is used this level is somewhat decreased (by 15%, not statistically significant; see Table 3). Lag period of the oxidation, which reflects the resistance of samples to Fe^{2+}-induced oxidation, is nearly twice shorter than in the control (healthy donors) and extended much further in the group of carnosine-treated patients (by 21%, $p < 0.05$). Similar dependence was observed for the rate of oxidation; it was increased in PD patients compared to control values by 20-32% independently of basic therapy and decreased nearly to the control level with the carnosine protocol (Table 3). All the data indicated that the carnosine effect is not restricted to SOD protection, but also relates to other important macromolecules prone to oxidative stress.

Conclusion

Thus our data taken together demonstrate efficiency of carnosine when it is used as a food additive to increase efficiency of basic therapy of PD patients. Positive changes in neurological symptoms are manifested with no change of MAO B but with pronounced restoration of SOD, which is suppressed in PD patients (Fig. 2). This indicates that carnosine demonstrates pronounced neuroprotective action on PD, increasing efficiency of basic therapy.

Carnosine is known to be a natural component of excitable tissues where its high level is supported by two mechanisms, the major being provided by carnosine synthase and the minor by dietary resources (different kinds of meat and fish). Carnosine demonstrates multifactorial protecting effects on cells and tissues. It is an important factor possessing both direct antioxidant activity[13] and ability to protect cells and tissues against oxidative stress by protection from oxidation of SOD[15,17] and other proteins[16] as well as lipids.[14] It was found recently that carnosine is able to prevent aldehyde modification of proteins,[16] to disaggregate proteins damaged by oxidative modification,[28] and to prevent protein carbohydrate cross-linking.[29]

Under stress conditions, exhausting physical exercise, or accumulation of senile or neurodegenerative features, carnosine level in tissues is decreased, which apparently diminished resistance of tissues against oxidative stress.[13] For this reason, in many animal models of oxidative stress, carnosine demonstrates efficient protection of the brain against oxidative injury.[14,17,30] It is very probable that similar mechanisms are working in the human body. Our data first demonstrate efficiency of carnosine as a neuroprotector for human beings. The combination of carnosine with basic therapy for PD patients (at least of those forms of PD tested in this study) may be a reasonable way to improve the protocol of PD treatment and to decrease the possible toxic effects of over-loading of DOPA-containing drugs.

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TABLE 3. Fe^{2+}-INDUCED CHEMILUMINESCENCE OF BLOOD LIPOPROTEINS IN PD PATIENTS

	Parameters of Fe^{2+}-induced chemiluminescence			
Patients	*Lipid hydroperoxides (Arb U)*	*Lag period (s)*	*Rate of oxidation (Arb U)*	*Maximal oxidation (Arb U)*
Healthy patients	111.0 ± 6.1	78.0 ± 4.3	2.2 ± 0.4	923 ± 100
Before treatment	107.4 ± 4.4	42.3 ± 2.7	2.8 ± 0.5	1078 ± 38
Basic therapy	107.6 ± 6.8	45.7 ± 3.2* ($p = 0.05$)	2.7 ± 0.6* ($p = 0.02$)	1093 ± 50
Basic therapy + carnosine	93.8 ± 4.4	56.6 ± 5.6** ($p = 0.015$)	2.3 ± 0.4** ($p = 0.001$)	1076 ± 51

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*P value compared to control.
**P value compared to the data before treatment.

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References

1. Forno LS. Neuropathology of Parkinson's disease. J Neuropathol Exp Neurol 1996;55:259–272.
2. Samii A, Nut JG, Ransom BR. Parkinson's disease. Lancet 2004;363:1783–1793.
3. Cookson M. The biochemistry of Parkinson's disease. Annu Rev Biochem 2005;74:29–52.
4. Wolters ECh, van Laar T, Berendse HW. Parkinsonism and Related Disorders. VU University Press, Amsterdam, 2007.
5. Dawson TM, Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. Science 2003;302:819–822.
6. Illarioshkin SN. The conformation diseases of the brain M [in Russian]. 2003.
7. Olanov CW. A radical hypothesis of neurodegeneration. Trends Neurosci 1993;16:439–444.
8. Mouradian MM. Recent advances in the genetics and pathogenesis Parkinson's disease. Neurology 2002;58:179–185.
9. Jenner P, Olanow CW. Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 1996;47(suppl3): S161–170.
10. Abraham S, Soundararajan CC, Vivekanandhan S, Behari M. Erythrocyte antioxidant enzymes in Parkinson's disease. Ind J Med Res 2005;121:111–115.
11. Youdim M, Geldenhuys W, van der Schyf C. Why should we use multifunctional neuroprotective and neurorestorative drugs for Parkinson's disease? Parkinsonism Rel Disor 2007;13:S281–291.
12. Hirsch EC. How to improve neuroprotection in Parkinson's disease? Parkinsonism Rel Disor 2007;13:S332–335.
13. Boldyrev A. Carnosine and oxidative stress in cells and tissues. Nova Science, New York, 2006.
14. Dobrota D, Fedorova T, Stvolinsky S, Babusikova E, Likavcanova K, Drgova A, Strapkova A, Boldyrev A. Carnosine protects the brain of rats and Mongolian gerbils against ischemic injury: after-stroke-effect. Neurochem Res 2005;30: 1283–1288.
15. Choi SY, Kwon HY, Kwon OB, Kang JH. Hydrogen peroxide-mediated Cu,Zn-superoxide dismutase fragmentation: protection by carnosine, homocarnosine and anserine. Biochim Biophys Acta 1999;1472:651–657.
16. Hipkiss A. Could carnosine or related structures suppress Alzheimer's disease? J Alz Dis 2007;11:229–240.
17. Stvolinsky SL, Fedorova TN, Yuneva MO, Boldyrev AA. Protective effect of carnosine on Cu,Zn-superoxide dismutase during impaired oxidative metabolism in the brain in vivo. Bull Exp Biol Med 2003;135(2):151–154.
18. Boldyrev AA, Fedorova TN, Stvolinsky SL, Borras C, Sastre J, Vina J. Chemical intervention in senescence-accelerated mice metabolism for modeling neurodegenerative diseases: an overview. In: Nomura Y. (Ed.), Senescence-Accelerated Mouse (SAM): An Animal Model of Senescence. Elsevier, Amsterdam, 2004.
19. Boldyrev A, Fedorova T, Stvolinsky S, Stepanova M, Dobrotvorskaya I, Kozlova E, Bagyeva G, Ivanova-Smolenskaya I, Markova E, Illarioshkin S. Carnosine increases efficiency of L-DOPA therapy of parkinsonics. Parkinsonism Rel Disor 2007;13:S99.
20. Fahn S, Elton R. Members of the UPDRS Development Committee. Unified Parkinson's disease rating scale. Recent developments in Parkinson's disease. In: S. Fahn, C. Marsden, D. Calne, M. Goldstein (Eds.), McMillan Healthcare Information. 1987;2:153–163: http://www.mdvu.org/library/ratingscales/pd/
21. Vetreshchak TV, Poleshchuk VV, Ivanova-Smolenskaya IA, Markova ED, Stvolinsky SL, Kramarenko GG. Monoamine oxidase B and Cu/Zn-superoxide dismutase of peripheral blood at Parkinson disease. Neurochemistry (Russian) 2002;19(3):213–218.
22. Misra HP, Fridovich J. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. Biochemistry 1972;247:3170–3175.
23. Winterbourn CC, Buss IH, Chan TP, Plank LD, Clark MA, Windsor JA. Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients. Crit Care Med 2000; 28(1):143–149.
24. Fedorova TN, Boldyrev AA, Gannushkina IV. Lipid peroxidation in experimental ischemia of the brain. Biochemistry (Moscow) 1999;64:75–79.
25. Glantz S. Primer of Biostatistics. McGraw-Hill, New York, 1994.
26. Götz ME, Freyberger A, Riederer P. Oxidative stress: a role in the pathogenesis of Parkinson's disease. J Neural Transm 1990;29:241–249.
27. Serra JA, Dominguez RO, de Lustig ES, Guareschi EM, Famulari AL, Bartolome EL, Marschoff ER. Parkinson's disease is associated with oxidative stress: comparison of peripheral antioxidant profiles in living Parkinson's, Alzheimer's and vascular dementia patients. J Neural Transm 2001;108(10): 1135–1148.
28. Seidler NV, Yeargans GS, Morgan TG. Carnosine disaggregates glycated α-crystallin: an in vitro study. Arch Biochem Biophys 2004;427:110–115.
29. Yan H, Harding JJ. Carnosine protects against the inactivation of esterase induced by glycation and a steroid. Biochim Biophys Acta 2005;1741:120–126.
30. Yasuhara T, Hara K, Maki M, Masuda T, Sanberg CD, Sanberg PR, Bickford PC, Borlongan CV. Dietary supplementation exerts neuroprotective effects in ischemic stroke model. Rejuvenation Res 2008;11(1):201–214.

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