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 LD50 (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 vitro15 and in vivo17 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 symptoms, 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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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. 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>
<thead>
<tr>
<th>Parameter</th>
<th>Basic therapy</th>
<th>Basic therapy + carnosine</th>
<th>% of decrease</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rigidity of hands</td>
<td>2.04 ± 0.2</td>
<td>1.38 ± 0.3</td>
<td>32</td>
<td>0.077</td>
</tr>
<tr>
<td>Rigidity of the legs</td>
<td>1.41 ± 0.2</td>
<td>0.88 ± 0.2</td>
<td>38</td>
<td>0.073</td>
</tr>
<tr>
<td>Hand movements</td>
<td>1.9 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>37</td>
<td>0.082</td>
</tr>
<tr>
<td>Rapid alternating movements of hands</td>
<td>1.9 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>47</td>
<td>0.015</td>
</tr>
<tr>
<td>Leg agility</td>
<td>1.8 ± 0.2</td>
<td>0.85 ± 0.20</td>
<td>53</td>
<td>0.005</td>
</tr>
<tr>
<td>Activities of daily living</td>
<td>11.4 ± 1.2</td>
<td>7.8 ± 0.8</td>
<td>32</td>
<td>0.009</td>
</tr>
<tr>
<td>Motor examination</td>
<td>22.5 ± 2.8</td>
<td>14.8 ± 1.7</td>
<td>34</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Statistically significant differences are in bold.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Improvement (% to initial value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic therapy</td>
<td>38.9 ± 2.5</td>
<td>32.5 ± 2.0</td>
<td>16.4 (p &lt; 0.05)</td>
</tr>
<tr>
<td>Basic therapy + carnosine</td>
<td>24.9 ± 2.1</td>
<td>24.9 ± 2.1</td>
<td>36.0 (p &lt; 0.01)</td>
</tr>
<tr>
<td>(p = 0.02)*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Compared to the group not treated with carnosine.
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 benzylamine as a substrate. 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).

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$_2$O, 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.

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$^{-1}$. cm$^{-1}$.

![Image](image_url)

**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 $\mu$L sample of blood serum was added to 2 mL of 0.28% CaCl$_2$ and 40 $\mu$L 1% heparin and centrifuged after 10 min incubation at room temperature for 15 min at 3000 rpm. The pellet was suspended into 900 $\mu$L phosphate buffer (60 mM KH$_2$PO$_4$ and 105 mM KCl [pH 7.45]). After addition to the mixture of 100 $\mu$L 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. 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 $\pm$ SE. Significance of the differences obtained was estimated by non-parametric criteria after Wilcoxon, Kruskal-Walles, and Dunnet. 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-
number 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,
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 oxidation16 or even to recover the oxidized proteins.28 This is exactly the case illustrated in Figure 3.

ChL test. Measuring the Fe2+ -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 Fe2+ -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 introduction of carnosine in the 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 activity13 and ability to protect cells and tissues against oxidative stress by protection from oxidation of SOD15,17 and other proteins16 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>
<thead>
<tr>
<th>Patients</th>
<th>Lipid hydroperoxides (Arb UI)</th>
<th>Lag period (s)</th>
<th>Rate of oxidation (Arb UI)</th>
<th>Maximal oxidation (Arb UI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy patients</td>
<td>111.0 ± 6.1</td>
<td>78.0 ± 4.3</td>
<td>2.2 ± 0.4</td>
<td>923 ± 100</td>
</tr>
<tr>
<td>Before treatment</td>
<td>107.4 ± 4.4</td>
<td>42.3 ± 2.7</td>
<td>2.8 ± 0.5</td>
<td>1078 ± 38</td>
</tr>
<tr>
<td>Basic therapy</td>
<td>107.6 ± 6.8</td>
<td>45.7 ± 3.2*(p = 0.05)</td>
<td>2.7 ± 0.6*(p = 0.02)</td>
<td>1093 ± 50</td>
</tr>
<tr>
<td>Basic therapy + carnosine</td>
<td>93.8 ± 4.4</td>
<td>56.6 ± 5.6**(p = 0.015)</td>
<td>2.3 ± 0.4**(p = 0.001)</td>
<td>1076 ± 51</td>
</tr>
</tbody>
</table>

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


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