Cerebrolysin administration counteracts elevated oxidative stress in blood of patients with Parkinson’s disease

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Effects of cerebrolysin (CBL) on prooxidant-antioxidant balance and oxidative stress (OS) developing in blood of patients with Parkinson’s disease (PD) were studied. Twenty patients with PD and 10 healthy persons (control) participated in this study. Clinical diagnosis of PD was established according UK Brain Bank Criteria; the grades of PD were II-III after Hoehn/Yahr. PD patients received therapy with CBL administration intravenously at dose 20 ml (61.5 mg/kg) per day along 10 days. As OS biomarkers, lipid peroxidation (from the formation of thiobarbituric acid – reactive substances, TBARS), H₂O₂ production, the activities of SOD, catalase, and GPxes, as well as glutathione pool indexes were measured in blood plasma and erythrocytes. It was established a significant rise in TBARS in plasma and H₂O₂ contents in erythrocytes from patients with PD compared to control. These events were accompanied by an increase in the SOD and catalase activities in plasma and a decrease in GSH content and GPx activity in erythrocytes. CBL administration counteracts the TBARS accumulation, reduces hyperactivation of SOD and catalase in plasma compared with the CBL-untreated patients. In erythrocytes of PD patients, CBL injection caused the limitation of H₂O₂ production as well as promotion the GSH pool recovery through an increase in GSH level and GPx activity.

Key words: oxidative stress; cerebrolysin; Parkinson’s disease.

INTRODUCTION

At present, mounting evidence shows that the excessive production and release of reactive oxygen species (ROS) of mitochondrial and nonmitochondrial origin have been linked with neurodegeneration as ROS can react with and oxidize molecules such as proteins, DNA and lipids resulting in oxidative stress (OS) [1].

Increased ROS generation and impaired antioxidant defense could both contribute to OS development in neurodegenerative disorders [2, 3]. Oxidative damage may contribute to the aggregation of protein into brain structures such as Lewy bodies which form the key histological hallmark of the second most prevalent neurodegenerative disease worldwide – Parkinson’s disease (PD) [2]. In PD, there is progressive selective loss of dopamine-producing neurons in the substantia nigra pars compacta (SNpc) – the most vulnerable brain region. It is well-established that OS may lower the threshold for neuronal apoptosis killing the energy-demanding dopaminergic neurons in the SNpc [4]. Elevation of virtually every established marker of OS has been documented in the postmortem brain tissue from patients with PD [1]. This is consistent with our previous data which showed that lipid peroxidation was significantly increased while reduced glutathione (GSH) and glutathione-dependent antioxidant enzymes activity markedly decreased in blood of patients with PD as well as in rat brain under modeling of PD [5, 6].

It comes as no surprise that PD therapy relies on the replacement of dopamine, accomplished with the L-dopa/carbidopa or the use of dopamine
agonists, catechol-0-methyltransferases or monoamine oxidase-B inhibitors. However, these drugs do not alter the course of the disease and no disease-modifying therapies are currently available. There is a need for disease-modifying drugs that would protect and repair nigrostriatal neurons, thereby halting the progression of the disease. The drugs with neurorestorative and/or neuroprotective properties might have therapeutic potential in PD. It is widely known that neurotrophic factors (NTFs) by binding to their specific receptors control metabolic maintenance of the neurons but also protect and repair injured neurons. NTFs are small secretory proteins, many NTFs have well-established neurorestorative properties in the nigrostriatal dopaminergic system in animal models of PD. It was earlier shown that the small peptides (10 kDa) mimic the effect of NTFs including ciliary neurotrophic factor, fibroblast growth factor 2, and insulin-like growth factor [7].

Many works have now demonstrated the beneficial clinical effects of neuroprotective drug cerebrolysin (CBL) in patients with various neurological disorders (stroke, epilepsy, traumatic brain injury, spinal cord injury, vascular dementia, etc). CBL is a porcine brain-derived small peptides (20%) and free amino acids (80%) preparation produced by a standardized enzymatic breakdown of purified brain proteins. In vitro and in vivo studies have demonstrated several beneficial effects of cerebrolysin, including decreased excitotoxicity, inhibiting free radical formation, microglial activation/neuroinflammation, and calpain activation/apoptosis [8]. The promising effects of cerebrolysin include inducing regeneration of the neural tissue along with improved clinical and functional outcome of patients with neurodegenerative disorders. The effects of CBL on OS developing in different tissues in PD might be of great importance. Indeed, results from the recent experimental study indicate that CBL protects against OS in different rat brain regions in a rotenone model of PD as well as CBL reduced the number of human apoptotic peripheral blood lymphocytes after OS induced by 2-deoxy-D-ribose [9, 10].

The role of increased oxidative damage and disorders in antioxidant ability in blood of PD patients remains unsolved. There is no sufficient information to date about the effects of CBL on prooxidant-antioxidant balance and OS developing in peripheral blood of patients with the moderate PD stages. This study was therefore designed to investigate such effects of CBL.

METHODS

Contingent and study design. Twenty patients with PD (12 men and 8 women) aged 52-75 years (mean age 63.5 ± 8.11 years) from the Department of Extrapyramidal Disorders of Chebotarev Institute of Gerontology NAMS of Ukraine participated in this study. Control group included 10 healthy sex-matched persons aged 55-75 years (mean age 65 ± 6.10 years) without neurodegenerative or psychiatric diseases and with similar dietary habits. Clinical diagnosis of PD was based on the presence of at least two cardinal symptoms of idiopathic PD according to UK Brain Bank Criteria, and a positive response to levodopa therapy. The stages of PD were II-III after Hoehn/Yahr rating scale (the moderate PD stages). All patients received the basal anti-parkinsonian therapy (L-dopa/carbidopa, dopamine agonists, MAO-B inhibitors, amantadine). This therapy was stable over a 28-30 day period before cerebrolysin administration. CBL (EVER Neuro Pharma, GmbH, Unterach, Austria) was provided in ampoules, in which each milliliter of CBL contains 215.2 mg of the active CBL concentrate in an aqueous solution. The appropriate dosage and duration of CBL administration were mainly based on the regimen that were given to the patients with Alzheimer’s disease and traumatic brain injury [11]. In our study, patients with PD received CBL injections intravenously dropwise daily at the dosage of 20 ml (61.5 mg/kg)/day for 10 days. None of the patients with PD and the con-
trol volunteers received any systemic or topical medication on admission at least 1 month prior to blood collection. Subjects presenting with chronic systemic diseases, chronic hepatopathy, chronic renal failure and infectious conditions (because oxidative stress markers in peripheral blood may be altered in such conditions) and those taking antioxidant drugs were excluded from all groups.

All investigations were performed in accordance with the policy statement of the Chebotarev Institute of Gerontology, NAMS of Ukraine and approved by the bioethics committee of this Institute (No. 2538, 25.07.2020). All subjects provided both verbal and written consent before participating in this study.

**Biochemical analyses.** Chemicals. Analytical grade chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) for use in the biochemical analyses. Blood samples were collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes from the patients and controls at the same time in the morning after overnight fasting. The samples were collected from a cubital vein into blood tubes, and immediately centrifuged at 3000g at 4°C for 10 min. After centrifugation, plasma and erythrocytes were separated. Plasma was frozen and stored at –80°C until analysis. The buffy coat on the erythrocyte sediment was carefully separated. The erythrocytes were subsequently washed twice with two volumes of 0.9% sodium chloride solution to remove the plasma remnants. Following this, the erythrocytes were hemolysed with two-fold volumes of ice-cold distilled water. After centrifugation (5000g, 10 min, 4°C), the supernatant was subdivided and transferred into polyethylene tubes. The hemoglobin (Hb) content was also measured (Hemoglobin Assay kit MAK115, Sigma-Aldrich, St. Louis, MO, USA). All assays were performed in duplicate and on first thaw.

**Oxidative stress biomarkers assays.** Lipid peroxidation in plasma was measured from the formation of thiobarbituric acid – reactive substances (TBARS) using the method [12]. H$_2$O$_2$ level in erythrocytes was measured by the FOX method, based on the peroxide-mediated oxidation of Fe$^{2+}$, followed by the reaction of Fe$^{3+}$ with xylene orange [13]. Absorbance of the Fe$^{3+}$-xylene orange complex (A 560) was detected after 45 min. Hydrogen peroxide content was determined against calibration plot and calculated per 1g of Hb.

**Enzymatic assays.** Total superoxide dismutase (SOD) (EC 1.15.1.1) activity was measured in plasma by the method [14], which is based on the inhibition of autooxidation of adrenaline to adrenochrome by SOD contained in the examined samples. The results were expressed as specific activity of the enzyme in units per ml of plasma. One unit of SOD activity being defined as the amount of protein causing 50% inhibition the conversion rate of adrenaline to adrenochrome under specified conditions. Catalase (EC 1.11.1.6) activity was measured in plasma by the decomposition of hydrogen peroxide, determined by a decrease in the absorbance at 240 nm [15]. Activity of selenium-dependent glutathione peroxidase (GPx) (EC 1.11.1.9) was determined in erythrocytes according to the method [16]. Briefly, the reaction mixtures consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM NaN$_3$, 0.2 mM NADPH, 1 mM GSH, 0.25 mM H$_2$O$_2$, 226 U/ml glutathione reductase, and rates of NADPH oxidation followed at 340 nm.

**Measurement of the reduced glutathione level.** The reduced glutathione (GSH) was determined as described [17]. The erythrocytes sample was mixed with sulphosalicylic acid (4%) and incubated at 4°C for 30 min. Thereafter, the mixture was centrifuged at 1200g for 15 min at 4°C, and 0.1 ml of this supernatant was added to phosphate buffer (0.1 M, pH 7.4) containing DTNB in abs. ethanol. The yellow color that developed was read immediately at 412 nm. The GSH content was calculated as μM GSH per 1g of Hb ($ε = 13.6 \times 10^3$ M$^{-1}$ cm$^{-1}$).

**Statistical analysis.** Data are expressed as mean ± SD. The differences among multiple experimental groups were detected by one-way
analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test. A P value of less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

In the present study, we registered the intensification of prooxidant processes in blood plasma and erythrocytes of patients with the moderate PD stages. It was established a significant rise (by 40%, P < 0.05) in the content of the secondary products of lipid peroxidation in blood plasma as well as a marked increase (by 50%, P < 0.05) of the H₂O₂ formation in erythrocytes of patients with PD compared to healthy persons (Figs. 1; 2).

The excessive production and release of ROS in PD occurs as a result of the combined action of mitochondrial dysfunction, dopamine autooxidation, and neuroinflammation [1, 2]. The metabolism of dopamine is accelerated in Parkinson’s disease by action of enzyme monoamine oxidase and excessive formation of hydrogen peroxide takes place. The polymerization of autooxidative products of dopamine may lead to the formation of characteristic pigmentation of the substantia nigra. It is well-established that the overproduction of ROS and their damaging effects on a multitude of cellular components have been linked to the loss of dopaminergic neurons in PD [2, 4]. Several lines of evidence have shown that LPO products are increased in the central nervous system (CNS) of PD patients that suggest the oxidative stress developing [2, 4, 11]. However, results in this regards in peripheral blood of PD patients remain to be contradictory. Some studies support our findings showing an increase in TBARS levels in cerebrospinal fluid, plasma and blood of PD patients [18, 19]. However, other studies have shown no differences in the serum or erythrocytes [20] TBARS contents between PD patients and controls.

We suggest that established activation of prooxidant processes causes the marked defensive response of antioxidant systems. It is

Fig. 1. Effect of cerebrolysin administration on oxidative and anti-oxidative status in plasma of PD patients. 1 - healthy control (n = 10); 2 - PD patients before cerebrolysin use (n = 20); 3 - PD patients after cerebrolysin use (n = 20). The data were analyzed for statistical significance using ANOVA followed by the Bonferroni post hoc test. *P < 0.05 vs group healthy control; #P < 0.05 vs group PD patients before cerebrolysin use
known that multiple systems are expressed in the cell for scavenging ROS and for repairing the damage to proteins, lipids, and DNA. Our assessment of the antioxidant protective effectiveness was largely based on the changes in activity of antioxidant enzymes – SOD and catalase which represent the first line of such defense providing with detoxification of reactive oxygen metabolites [21]. In patients with PD, we have found an increase in the activities of SOD and catalase by 21 and 69% (P < 0.05), respectively in comparison with healthy subjects. An increase in the activity of SOD can be explained as a compensatory reaction to the increased superoxide anion production, which is known serves as a substrate for SOD [21]. We also registered a concomitant increase in the catalase activity in response to the excess of H$_2$O$_2$ which is known serves as a substrate for this antiperoxide enzyme. It is known that superoxide anion readily reacts with iron-sulfur centers of nonheme proteins, such as respiratory complexes I–III and TCA cycle enzymes. This reaction releases free ferrous iron (Fe$^{2+}$) which generates the highly reactive and cytotoxic hydroxyl radical via Fenton chemistry, thereby initiating a chain of lipid peroxidation, protein modifications, and DNA damage [1].

The studies of antioxidant enzymes activity in blood and postmortem tissues from patients with PD have shown the conflicting results. Several studies have demonstrated a rise in the SOD activity in blood plasma, SNpc, and frontal cortex of patients with PD [19, 22], whereas Abraham et al. [23] reported a significant decrease in SOD activity in the blood of PD patients compared to controls. Furthermore, Sudha et al. [24] indicated no significant change in erythrocyte SOD activity of PD patients, while Cokal et al. [25] showed unaltered SOD activity in serum blood of PD patients compared to the control group. Peripheral blood studies of SOD activity in humans were discordant, but postmortem studies demonstrated significant increased activity of SOD in the substantia nigra of PD patients [22]. So, the cause and effects of

Fig. 2. Effect of cerebrolysin administration on oxidative and anti-oxidative status in erythrocytes of PD patients. 1 - healthy control (n = 10); 2 - PD patients before cerebrolysin use (n = 20); 3 - PD patients after cerebrolysin use (n = 20). The data were analyzed for statistical significance using ANOVA followed by the Bonferroni post hoc test. *P < 0.05 vs group healthy control.
activation or exhaustion of antioxidant defensive systems in PD are still heavily debated with the aim of understanding their role in neuronal death and dysfunction.

Glutathione system is one of the active components of antioxidant defense which acts to maintain the intracellular thiol redox balance and thus protects the cell against oxidative injury. Glutathione is an intracellular tripeptide (gamma-glutamyl-cysteinyl-glycine) which functions as a direct free-radical scavenger and as substrate for glutathione peroxidase and glutathione-S-transferases keeping sulphydryl groups of proteins in the reduced form [26]. A decrease in reduced glutathione would enhance OS, initiating oxidative stress-mediated neuronal death. Glutathione depletion increases cellular labile iron pool which plays an important role in OS developing in erythrocytes. In addition, the results from the recent clinical study indicate that a tight interrelation exists between reduced glutathione levels in CNS and blood of patients with PD, on the one hand, and PD severity, on the other hand [27]. We analyzed the glutathione cycle, which is one of the main intracellular mechanisms for maintaining redox homeostasis [26]. In our study, the concentration of GSH was significantly decreased (by 58%, P < 0.05) in erythrocytes of PD patients. Lower reduced glutathione level altered activities of GSH-related enzymes, such as glutathione peroxidase, glutathione reductase and glutathione-S-transferase. GPx is an important enzyme in the cellular antioxidant defense mechanisms because GPx directly catalyzes the reduction of $\text{H}_2\text{O}_2$, using glutathione as the electron donor. In the literature, there is wide variability in the reported GPx levels in PD. In general, the levels of GPx are decreased [23] or increased [25]. Our study, however, supports the finding of decreased GPx activity in peripheral blood of PD patients compared to healthy controls, though this change was not statistically significant (by 15%, P > 0.05). The lower GPx activity observed in these individuals may result in an accumulation of peroxides, which could contribute to causing oxidative damage in erythrocytes of PD patients.

In the present study, we found that CBL administration has moderate antioxidant effect in PD patients by decreasing the TBARS accumulation (by 17%, P > 0.05) in blood plasma as well as $\text{H}_2\text{O}_2$ content in erythrocytes (by 12%, P > 0.05) compared with the CBL-untreated patients. These effects of CBL were accompanied by a decrease in the activities of SOD (by 11%, P > 0.05) and catalase (by 20%, P > 0.05) in blood of patients with PD compared with the CBL-untreated ones. In CBL-treated patients, there was registered an increase in the GSH concentration (by 30%, P > 0.05) and GPx activity (by 15%, P > 0.05) in erythrocytes compared with PD patients (Figs. 1; 2). We can assume that such effects of CBL may be accompanied by reducing of impairments in intercellular redox state, restoring of balance in the pro/antioxidant system and modulating of an apoptotic signal [10]. So, an increase in GSH content and GPx activity following CBL treatment may be considered as an additional indicator of the oxidative stress intensity reducing. The potential mechanisms through which CBL suppress OS in PD are complex. Abdel-Salam et al. [10] have studied these mechanisms experimentally on a model of rotenone-induced OS and neurodegeneration in rat brain. These authors suggested that the protective effect of CBL on nigrostriatal damage could be mediated by upregulating paraoxonase 1 activity (PON1) in the face of increased OS. It is known that this enzyme associates with high-density lipoproteins, preventing their oxidation, and the increase in the enzyme activity reflects decreased OS. In support of this suggestion is a study showing decreased PON1 activity with elevated OS levels [28]. It was also established that administration of CBL prevented the decline in Bcl-2 protein concentration in rat striatum induced by rotenone [10]. It is known that expression of antiapoptotic protein Bcl-2 is modulated by oxidative stress: enhanced OS and altered level of antioxidants occurred in the brains of Bcl-2 – deficient mice [29] as well
as at acute 3-nitropropionic acid intoxication [3]. So, one of the mechanisms through which CBL counteracts elevated OS in PD could be regulation of Bcl-2 protein expression in neuronal cells.

In our opinion, one of the most promising effects of CBL on OS developing in blood and tissues in PD could be its ability to decrease intracellular α-synuclein aggregation in neurons, mimicking the action of classical NTFs and growth factors as well as of NTFs belonged to a novel family CDNF/MANF [7, 9]. It has long been known that α-synuclein can interact with complex I of the mitochondrial electron transport chain which in turn induces elevated ROS production leading to oxidative stress. Complex I inhibition by α-synuclein could serve a second signaling role by amplifying mitochondrial ROS generation and changing α-synuclein physical conformation to promote aggregation and Levy bodies formation [2, 9].

In conclusion, the results of the present study indicate an antioxidant action of cerebrolysin in patients with moderate stages of Parkinson’s disease. This action is mainly based on the decrease of the prooxidant events in peripheral blood of PD patients as well as on the prevention of thiol redox defects and reducing an imbalance in oxidant–antioxidant status. Our data support the suggestion that CBL could be potential therapeutic strategy for treatment in PD.

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The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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