RESEARCH PAPER

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Serum levels of hepcidin and interleukin 6 in Parkinson's disease

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Neurodegeneration in Parkinson's disease (PD) includes processes of chronic inflammation and oxidative stress which are related to dysregulation in the homeostasis of iron metabolism. Hepcidin is a peptide hormone responsible for systemic iron homeostasis and simultaneously the inflammatory response protein, induced in response to interleukin 6 (IL-6). We assessed the serum concentration of hepcidin and IL-6 in the groups of patients with PD treated only pharmacologically with optimal individualized therapy (MT) and treated additionally with deep brain stimulation (DBS), compared to the control group. The serum concentrations of hepcidin and IL-6 in the group of all PD patients were significantly higher than in the control group. In the group of PD patients treated with DBS hepcidin and IL-6 concentrations were significantly higher compared to the control group. Additionally, the positive correlations between serum hepcidin and IL-6 were found in the PD (MT and DBS) and PD-DBS group. The obtained results may indicate the influence of immunological mechanisms on iron metabolism and oxidative stress, in particular when the inflammatory process is more active in the DBS-treated group. This effect can be protective as well as neurodegenerative.

Key words: Parkinson's disease, deep brain stimulation, neuroinflammation, hepcidin, interleukin 6

INTRODUCTION

The pathology of Parkinson's disease (PD) is considered to be multifactorial. In addition to causing alterations in protein transformation, genetic defects and mitochondrial disfunction, the disease also induces chronic neuroinflammation and elevated oxidative stress processes. Neurodegeneration in PD is associated with complex relationships between immune-inflammatory pathways and peripheral tissues. Evidence for peripheral and central chronic inflammation in patients with PD comes from studies showing elevated levels of pro-inflammatory cytokines in brain tissue (Nagatsu et al., 2005), cerebrospinal fluid (Blum-Degen et al., 1995) and serum (Brodacki et al., 2008), or from the data concerning activated microglia (Joe et al., 2018).

Published data also indicate the role of iron in inducing oxidative stress, due to its characteristic oxidation reduction properties and participation in the production of reactive oxygen species (Ke et al., 2003). Hepcidin is a peptide hormone that regulates systemic iron homeostasis (Ruchala et al., 2014). In addition, hepcidin is known to be widely expressed in the brain, involved in the regulation of the metabolism of other iron proteins, and responsible for brain iron homeostasis (Li et al., 2011; Du et al., 2011). Hepcidin decreases the levels of ferroportin 1 (FPN1), the cellular iron transporter, thus resulting in an increase of iron concentration inside the cells. Excess iron concentration in the neurons may result in oxidative stress and lead to neurodegeneration. On the other hand, emerging evidence suggests a neuroprotective role for hepcidin (Urrutia et al., 2017). In addition, hepcidin can be

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viewed as a type of mediator between the mechanism of systemic iron metabolism and immune response and inflammatory pathways, because it is primarily induced in response to interleukin 6 (IL-6) and is a type-II acute-phase inflammatory response protein (Nemeth et al., 2003).

Chronic neuroinflammation is closely associated with brain iron metabolism and homeostasis; however, many of its mechanisms, in particular related to neurodegeneration, are still unclear. A detailed understanding of the underlying mechanisms could be important for expanding the knowledge about pathogenesis of PD and the development of new therapeutic strategies.

In our previous study, higher concentration of pro-hepcidin (a 60-amino-acid pro-hormone) was observed in the serum of PD patients treated with DBS, which was statistically significant when compared to the concentration in the control group, as well as to the group of PD patients treated only pharmacologically (Kwiatek-Majkusiak et al., 2018). Higher concentration of pro-hepcidin in the PD group of patients undergoing surgical treatment may be reflective of an overlapping Parkinsonian pathology and immunomodulatory effect of DBS at the molecular level. These results, as well as reports on the possible neuroprotective role of hepcidin, were the basis of dividing PD patients into two groups (treated only pharmacologically and treated with DBS), as well as the premise for verifying the hypothesis that the concentration of active hepcidin hormone would be higher in the studied PD groups when compared to the control group, and further validate the obtained results.

As the purpose of the current study, we aimed to assess the concentration of active hepcidin hormone (25-amino-acid peptide) in the serum of PD patients undergoing only pharmacological treatment and in those treated with DBS in comparison to the control group. In addition, in the studied groups, we determined the serum concentration of IL-6 – a pro-inflammatory cytokine that is associated with the induction of hepcidin secretion.

METHODS

Subjects

The present study included 88 subjects. The PD group comprised 60 patients (27 female and 33 male) with PD. Among them, 47 patients were treated only pharmacologically, whereas 13 patients were additionally treated surgically with DBS. Two drugs are mainly used for providing pharmacological therapy: levodopa and dopamine agonist ropinirole. Levodopa Equivalent Dose Calculator (www.parkinsonsmeasurement.org) was used to determine the equivalent dosages of dopaminergic drugs. The target for DBS electrodes in the group of patients treated surgically was subthalamic nuclei (bilaterally). The mean time from implantation of DBS in the group of patients treated surgically was 30.28±44.16 months. Twenty-eight healthy volunteers who were age- and sex-matched to the group of patients with PD served as control subjects. The UK Parkinson's Disease Brain Bank criteria was used for the clinical diagnosis of idiopathic PD. A neurologist, specialized in movement disorders, performed the neurological examination. Unified Parkinson's Disease Rating Scale (part III motor score) was used to measure the parameters like patient's functional ability, mobility, and clinical staging of the disease. Subjects suffering from anemia and other coexisting diseases of possible inflammatory etiology were excluded from the study. We assessed clinical data, anti-Parkinsonian treatment, and medical history of the patients (Table I). The control group included healthy volunteers with no family history of anemia and other inflammatory and neurodegenerative disorders. Approval for the study was obtained from the Ethics Committee of the Medical University of Warsaw and all the study participants provided written informed consent.

Biochemical and statistical analyses

Peripheral blood samples were aseptically collected from PD patients and individuals of control group and were subjected to centrifugation. After separation, the sera were stored in 4 mL serum separation tubes with a clot activator at a temperature of -80°C for further use. All the blood samples were collected at the same time of the day, that is in the morning between 6 and 8 am, in order to avoid the possible influence of the circadian rhythm on the obtained results. Later, the physiological and biochemical properties of the collected blood samples were analyzed by processing at the laboratory. Enzyme-linked immunosorbent assay (ELISA) kits, Hepcidin 25 bioactive HS (DRG) and Human Il-6 ELISA Kit (PromoKine), were used for the quantitative measurements of the serum samples obtained from both the groups.

The R package (version 3.2.4) was used for the statistical analyses. Shapiro-Wilk test was used to analyze the compliance schedules showing normal distribution, whereas variables showing non-normal distribution, as observed in the MT group, were evaluated using non-parametric tests. The Mann-Whitney U test

Table I. Clinical features of PD patients divided into three groups: Total-PD group (all PD patients), MT-PD group (PD patients treated only pharmacologically), DBS-PD group (PD patients treated with deep brain stimulation) and control group.

Group	TOTAL-PD	MT-PD	DBS-PD	Control
Sex	27 females, 33 males	21 females, 26 males	6 females, 7 males	14 females, 14 males
Mean age ± SD	58.75±10.74 years	60.17±10.36 years	53.62±10.94 years	58.44±2.35 years
Mean age of onset (years) ± SD	49.88±11.27 years	51.74±11.55 years	43.08±7.06 years	–
Mean disease duration (years) ± SD	8.92±7.16 years	8.43±7.69 years	10.7±4.64 years	–
Mean score in Unifited Parkinson's Disease Rating Scale part III in ON phase ± SD	12.40±10.03	12.85±10.99	10.69±5.28	–
Mean score in Unifited Parkinson's Disease Rating Scale part III in OFF phase ± SD	39.07±14.52	35.78±13.15	50.92 ±13.37	–
Levodopa eqivalent daily dose (LEDD)	1,141	1,309	535	–
Levodopa treatment	58/60 patients = 96.67%	47/47 patients = 100%	11/13 patients = 84.62%	–
Dopamine agonist treatment	29/60 patients = 48.33%	20/47 patients = 42.55%	9/13 patients = 69.23%	–
Duration of DBS treatment	–	–	28.47±46.41 months	–
Dyskinesia	38/60 patients = 63.33%	26/47 patients = 55.32%	11/13 patients = 84.62%	–
Motor fluctuations	40/60 patients = 66.67 %	28/47 patients = 59.57%	10/13 patients = 76.92%	–

was used to compare the results of the PD group (MT and DBS combined) and the control group. Comparisons between more than two groups were done using Kruskal-Wallis' non-parametric test and Dunn's *post hoc* test. A p-value <0.05 was considered to indicate statistical significance. We also analyzed the correlation of the obtained results of hepcidin and IL-6 concentrations between the control group and groups of patients with PD using Spearman's rank correlation test.

RESULTS

The concentration of hepcidin (ng/mL) in the serum of the group including only PD patients (MT and DBS) was significantly higher than in the control group (p=0.006). On subgroup analysis by using Kruskal-Wallis test, significant differences were observed between DBS, MT and control groups. *Post hoc* tests revealed that hepcidin concentration was significantly higher in the DBS group when compared with the control group (p<0.00002). The concentration of hepcidin in the serum of PD patients undergoing only pharmacological treatment (MT) was higher compared to the control group, but the difference was not statistically significant (p=0.07).

The concentration of IL-6 (pg/mL) in the serum of the group including all PD patients (MT and DBS) was also significantly higher than in the control group (p=0.006). The subgroup analysis using Kruskal-Wallis test also revealed significant differences between DBS, MT and control groups. *Post hoc* tests indicated that the level of IL-6 was significantly higher in the group of patients treated with DBS in comparison to the control group (p=0.007). The concentration of IL-6 in the serum of PD patients treated only pharmacologically (MT) was higher when compared to the control group (p=0.04), but the difference was much smaller and not statistically significant. However, no statistically significant difference was observed between the groups of PD patients who underwent only pharmacological therapy and those who underwent surgical treatment with DBS (p=0.1). Furthermore, positive correlations between serum hepcidin and IL-6 levels were found in the PD (MT and DBS) and PD-DBS groups (p<0.0004, p=0.03, respectively).

We performed statistical analyzes regarding the dependence of hepcidin and interleukin 6 levels on the age and duration of Parkinson's disease in the studied groups, but no statistically significant correlations were obtained.

The statistical analysis results of hepcidin and IL-6 concentrations in PD patients and control group are summarized in Tables II and III, and Figs 1, 2 and 3.

Table II. Means (ng/mL) standard deviations (in the parentheses) and medians of hepcidin concentration in the serum of all PD patients studied (PD), PD patients treated only by pharmacological therapy (MT), PD patients treated surgically with DBS (DBS) and control group comprising healthy volunteers.

PD (MT+DBS)		PD-DBS		PD-MT		Controls	
Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
188.21 (43.18)	204.08	218.89 (24.56)	222.45	179.72 (43.52)	196.14	165.35 (42.26)	174.02

PD *vs.* Controls	Kruskal-Wallis test	*Post hoc* Dunn's test p-values		
p-value	p-value	DBS *vs.* Controls	MT *vs.* Controls	DBS *vs.* MT
0.006	0.0001	0.00002	0.07	0.0004

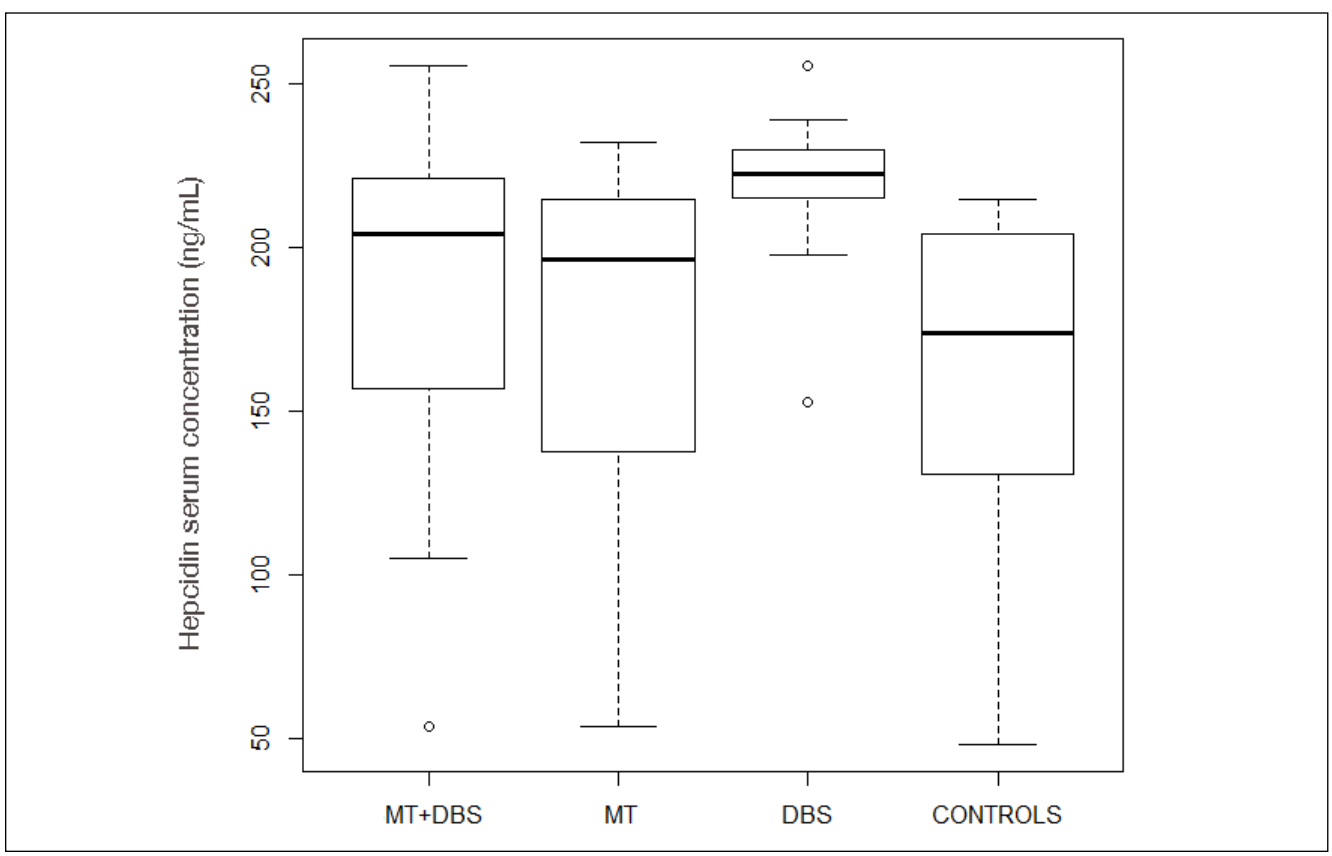

Fig. 1. Boxplots of hepcidin serum concentration (ng/mL) in all PD patients studied (PD), PD patients treated only by pharmacological therapy (MT), PD patients treated surgically with DBS (DBS) and control group comprising healthy volunteers.

Table III. Means (pg/mL) standard deviations (in the parentheses) and medians of IL-6 concentration in the serum of all PD patients studied (PD), PD patients treated only by pharmacological therapy (MT), PD patients treated surgically with DBS (DBS) and control group comprising healthy volunteers.

PD (MT+DBS)		DBS		MT		Controls	
Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
3.46 (2.89)	2.34	4.19 (3.41)	2.82	3.26 (2.74)	2.08	1.81 (0.87)	1.66

PD *vs.* Controls	Kruskal-Wallis test	*Post hoc* Dunn's test p-values		
p-value	p-value	DBS *vs.* Controls	MT *vs.* Controls	DBS *vs.* MT
0.006	0.006	0.007	0.04	0.1

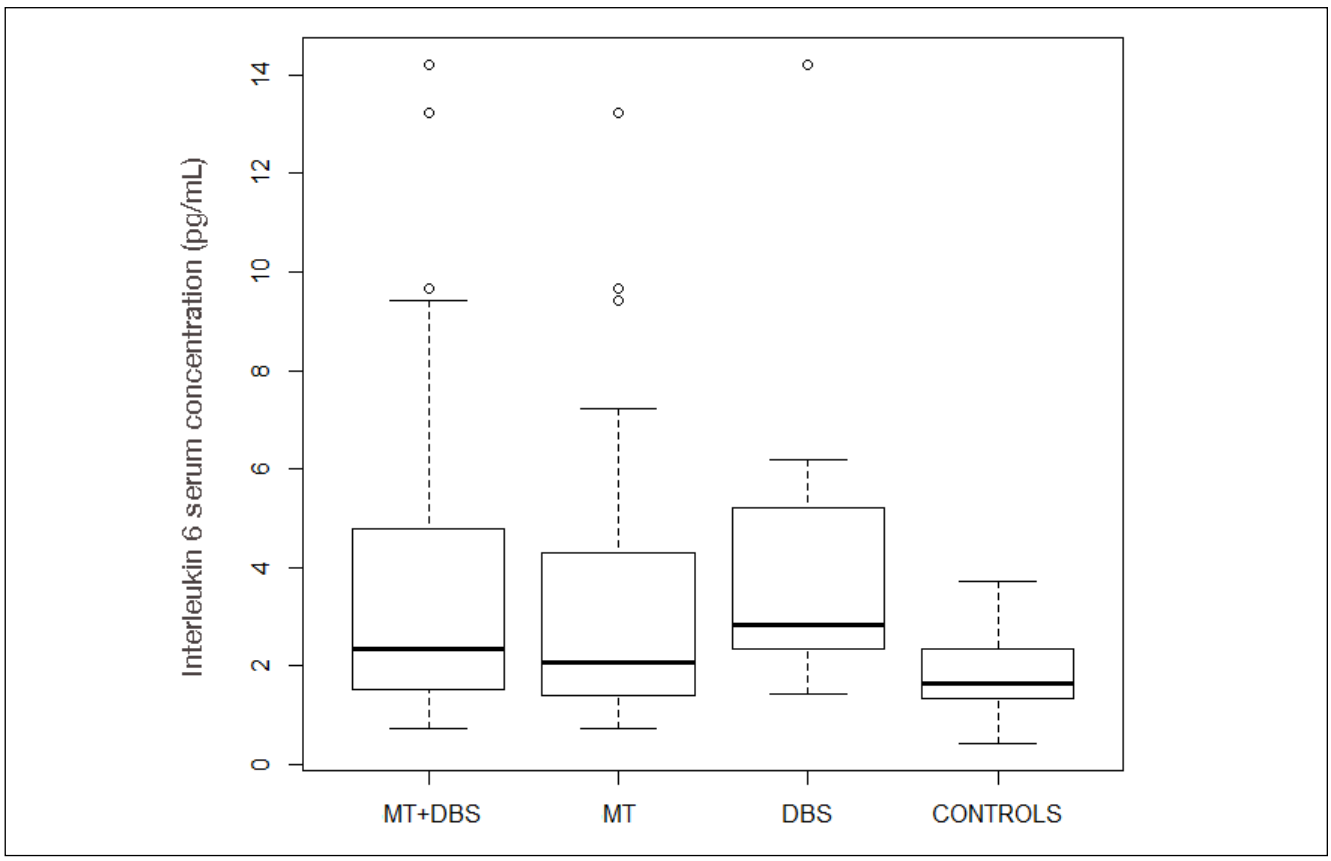

Fig. 2. Boxplots of interleukin 6 serum concentration (pg/mL) in all PD patients studied (PD), PD patients treated only by pharmacological therapy (MT), PD patients treated surgically with DBS (DBS) and control group comprising healthy volunteers.

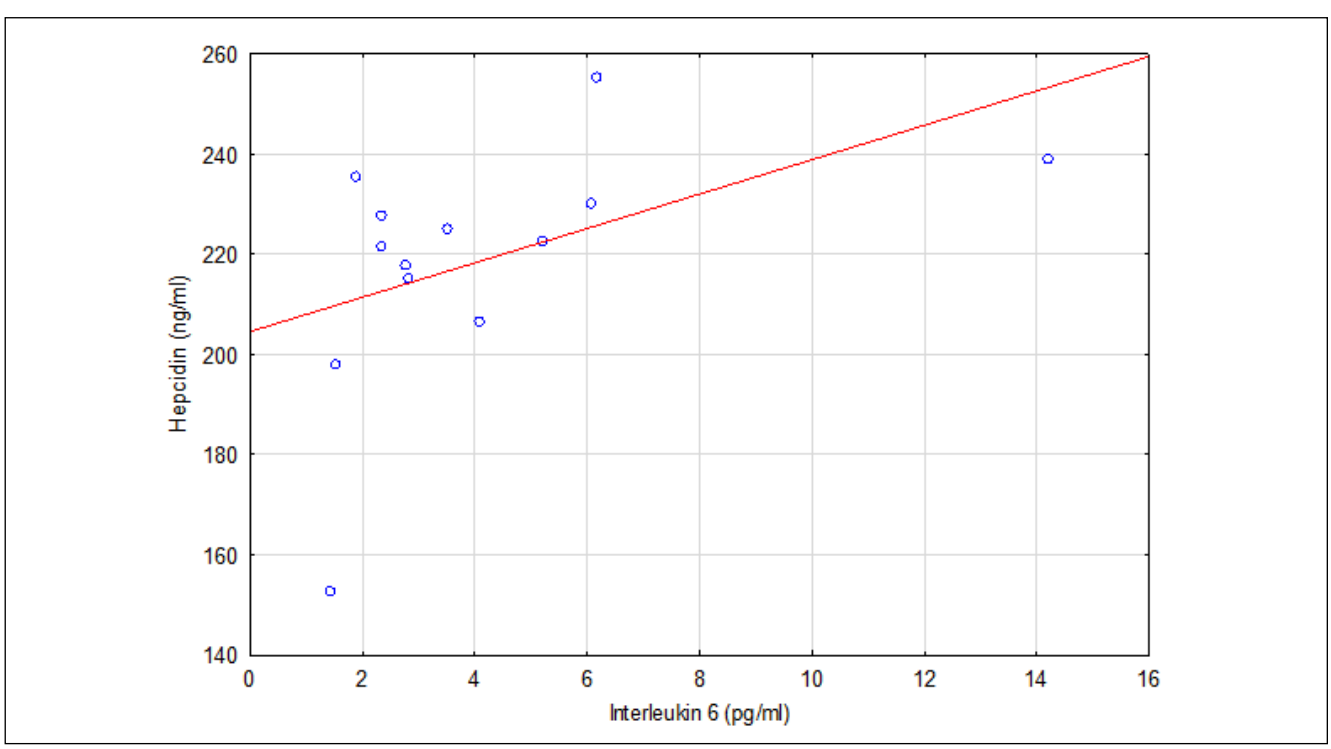

Fig. 3. Correlations between serum concentration of hepcidin and IL-6 in PD-DBS group.

DISCUSSION

The results of our study indicate a probable, interesting connection between the iron-modulation hormone hepcidin and chronic neuroinflammation. Both hepcidin and IL-6 are mediators that may potentially be involved in the neurodegeneration mechanisms of the PD, because they are associated with immune-mediated inflammatory processes and oxidative stress, in which iron plays a recognized role.

IL-6 is a pro-inflammatory cytokine classified as an acute phase protein and it is responsible for control of maturation and differentiation of immune cells, which also participate in the neurogenesis and maturation of neurons and glial cells. Data from the literature indicate that dysregulation in its synthesis and signaling pathways lead to various neurodegenerative disorders, including PD. Elevated concentrations of IL-6, beside other cytokines like tumor necrosis factor (TNF-α), were demonstrated to be present in both brain tissue and serum of PD patients (Mogi et al., 1996; Reale et al., 2009).

Many studies have indicated that during inflammation, IL-6 regulates the secretion of hepcidin. IL-6 was shown to induce the secretion of hepcidin in monocytes and macrophages (Nemeth et al., 2003, 2004). The inflammatory process is associated with the presence of activated microglia, which release numerous pro-inflammatory cytokines, including IL-6. This inflammatory cytokine regulates the hepcidin directly through phosphorylation and induction of cytoplasmic signal transducer and activator of transcription 3 (STAT3) (Wrighting et al., 2006; Verga Falzacappa el al. 2007; Pietrangelo et al., 2007). The upregulation of the hepcidin expression in both periphery and central nervous system requires the activation of IL-6 and STAT3 pathway (Qian et al., 2014). The inflammatory stimuli generated in the brain tissue induce the expression of hepcidin in astrocytes, microglia or epithelial cells (Marques et al., 2009; Wang et al., 2008, 2010).

In our study the concentration of IL-6 were found to be higher in the serum of PD patients when compared to healthy controls. The results are consistent with those reported by prior studies which pointed to elevated serum levels of IL-6 in PD patients (Reale et al., 2009; Qin et al., 2016). However, it is noteworthy that the difference in the IL-6 concentration, compared to the control, was more statistically significant in the group of PD patients treated surgically with DBS when compared to the group treated only pharmacologically. The differences suggest that dysregulations in the immune or inflammatory pathways may be involved in the neurodegeneration mechanisms characteristic of PD disease and also indicate the putative, additional effect of DBS therapy on these processes.

In the available literature we did not find any studies that evaluated the serum hepcidin concentration in PD patients, but the concentration of active hepcidin hormone obtained in our study conformed to the results of our previous studies that assessed the pro-hepcidin (prohormone) concentration in the serum of patients with PD. It seems intriguing that hepcidin concentration was significantly higher in the group of patients treated with DBS in comparison to the control group, as well as when compared to the control group of PD patients treated only pharmacologically.

An interesting issue is the possibility of induction of immunomodulatory effects following DBS therapy, which involves constant high-frequency stimulation (HFS) and also consists in the implantation of electrodes into the brain tissue (most often subthalamic nucleus is the target). The question pertaining to whether DBS treatment would lead to any kind of systemic effect seems to be potentially important. Data from animal studies indicate the presence of neuroinflammation in the brain tissue, following intracranial implantation of electrodes (Hirshler et al., 2010). Some studies also reported the presence of reactive astrogliosis and microgliosis around the electrodes and also in the distant brain regions like cortex, as well as in the early period after the DBS implantation and also many years later after the surgery (Kang et al., 1998; Griffith et al., 2006; DiLorenzo et al., 2010).

The duration of DBS therapy from the time of electrode implantation to STN till the time of hepcidin and interleukin 6 measurements was in the range between 28.47 and 46.41 months. In the available literature we have not found any studies on the potential immunomodulatory effects of DBS in humans, but some data from animal studies have indicated a complex brain tissue reaction to DBS neurostimulation: more severe in the first 6 months and less intense after 12 months after the implantation of the electrodes (Orlowski et al., 2017).

Our measurements were made after a period of more than 2 years after implantation of DBS, which reduces the likelihood of the effect of the reaction associated with the surgery itself on the results obtained.

We conducted statistical analyzes, but we did not find a relationship between the duration of DBS therapy and the concentration of hepcidin and interleukin 6. However positive correlations between serum hepcidin and IL-6 levels were found in the PD (MT+DBS) and PD-DBS groups. Simultaneously the limitation of our study is a small group of patients treated with DBS.

Hepcidin could play a multifunctional role in the brain (Su et al., 2010) and its effects in the brain tissue are complex. Consistent with our hypothesis and other reports, our study found significantly higher

concentrations of hepcidin in the group of patients treated with DBS, which indicates that hepcidin may also induce an anti-inflammatory effect. This effect of hepcidin, different from the previously proven pro-inflammatory role, was reported by Urrutia et al. (2017). The authors showed anti-inflammatory role of hepcidin in the inflammatory response induced by β-amyloid protein (Aβ), a histopathological hallmark of neurodegeneration in Alzheimer's disease. In the primary culture of microglia and astrocytes treated *in vivo* with Aβ with hepcidin, the expression and secretion of IL-6 and TNF-α were significantly reduced, when compared to analogous population of glial cells treated with Aβ without hepcidin. Additionally, stereotactic intracerebral injections of hepcidin in rats reduced astrocyte and microglial activation and IL-6 secretion triggered by Aβ, which according to the authors results in the protection of the surrounding neurons. Anti-inflammatory effects of hepcidin were also reported in macrophages in animal models, the binding of hepcidin to FPN1 resulted in a reduction of IL-6 and TNF-α secretion during the inflammatory response to lipopolysaccharide (De Domenico et al., 2010; Huang et al., 2012).

The established anti-inflammatory and neuroprotective effects of hepcidin can be considered to be consistent with the significantly higher hepcidin concentration obtained in our previous pro-hepcidin study conducted in a group of PD patients treated with DBS, compared to the group treated only pharmacologically. The results suggest that hepcidin may have a beneficial influence on attenuating iron-related processes of neurodegeneration. Simultaneously, the findings in relations to the effects of DBS on the pathogenesis of the PD clearly proved that performing DBS at STN improved the quality of life, reduced motor symptoms and allowed reduction of the daily dose of levodopa (Deuschl et al., 2006; Weaver et al., 2009; Williams et al., 2010). The statistically significant higher concentration of hepcidin, with a possible neuroprotective effect, found in the PD-DBS group remains in compliance with the clinical benefits achieved in PD patients treated with DBS.

The mechanism of DBS is very complex and the procedure involves insertion of microelectrodes in brain tissue, HFS and micro-inflammation. It is possible that the increase in hepcidin concentration in the DBS group following surgery may have a neuroprotective effect, analogous to the aforementioned studies with Aβ.

The limitation of the study is the lack of data regarding hepcidin and IL-6 concentrations before the DBS implantation, which may be more relevant to confirm their possible increase after surgery. Additionally, although hepcidin and IL-6 concentrations were statistically significantly higher, the weakness of the study is that only a small group of patients treated with DBS were included. Moreover, the levels of peripheral biomarkers may not reflect their levels on the brain tissue. However, according to some reports, cytokines produced in the brain tissue in response to an inflammation reaction may disseminate through the blood-brain barrier into the peripheral blood (Sawada et al., 2006), so the observed symptoms may be a result of the complex effects associated with dysregulation of immunological processes in the brain and peripheral tissues.

CONCLUSIONS

The results of our study revealed elevated serum concentrations of IL-6 and hepcidin and confirmed the presence of chronic inflammation and dysregulation in the homeostasis of iron metabolism in patients with PD. This study for the first time showed that a significantly higher concentration of hepcidin is found in the serum of PD patients treated with DBS, compared to control, which suggests its anti-inflammatory role in this method of treatment and also the neuromodulatory effect of DBS. The increase in hepcidin levels correlates with the elevated levels of IL-6 in the patients treated with DBS, but the underlying mechanism remains unclear and necessitates further investigation.

REFERENCES

Blum-Degen D, Müller T, Kuhn W, Gerlach M, Przuntek H, Riederer P (1995) Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci Lett 202: 17–20.

Brodacki B, Staszewski J, Toczyłowska B, Kozłowska E, Drela N, Chalimoniuk M, Stepien A (2008) Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFalpha, and INFgamma concentrations are elevated in patients with atypical and idiopathic parkinsonism. Neurosci Lett 441: 158–162.

De Domenico I, Zhang TY, Koening CL, Branch RW, London N, Lo E, Daynes RA, Kushner JP, Li D, Ward DM, Kaplan J (2010) Hepcidin mediates transcriptional changes that modulate acute cytokine-induced inflammatory responses in mice. J Clin Invest 120: 2395–2405.

Deuschl G, Schade-Brittinger C, Krack P (2006) A randomized trial of deep-brain stimulation for Parkinson's disease. N Engl J Med 355: 896–908.

DiLorenzo DJ, Jankovic J, Simpson R, Takei H, Powell SZ (2010) Long-term deep brain stimulation for essential tremor: 12-year clinicopathologic follow-up. Mov Disord 25: 232–238.

Du F, Qian C, Ming Qian Z, Wu XM, Xie H, Yung WH, Ke Y (2011) Hepcidin directly inhibits transferrin receptor 1 expression in astrocytes *via* a cyclic AMP-protein kinase a pathway. Glia 59: 936–945.

Griffith RW, Humphrey DR (2006) Long-term gliosis around chronically implanted platinum electrodes in the Rhesus macaque motor cortex. Neurosci Lett 406: 81–86.

Hirshler YK, Polat U, Biegon A (2010) Intracranial electrode implantation produces regional neuroinflammation and memory deficits in rats. Exp Neurol 222: 42–50.

Huang YH, Yang YL, Tiao MM, Kuo HC, Huang LT and Chuang JH (2012) Hepcidin protects against lipopolysaccharide-induced liver injury in a mouse model of obstructive jaundice. Peptides 35: 212–217.

Joe EH, Choi DJ, An J, Eun JH, Jou I, Park S (2018) Astrocytes, microglia, and Parkinson's disease. Exp Neurobiol 27: 77–87.

Kang J, Jiang L, Goldman SA, Nedergaard M (1998) Astrocyte-mediated potentiation of inhibitory synaptic transmission. Nat Neurosci 1: 683–692.

Ke Y, Ming Qian Z (2003) Iron misregulation in the brain: a primary cause of neurodegenerative disorders. Lancet Neurol 2: 246–253.

Kwiatek-Majkusiak J, Geremek M, Koziorowski D, Tomasiuk R, Szlufik S, Friedman A (2018) Higher serum levels of pro-hepcidin in patients with Parkinson's disease treated with deep brain stimulation. Neurosci Lett 684: 205–209.

Li L, Holscher C, Chen BB, Zhang ZF, Liu YZ (2011) Hepcidin treatment modulates the expression of divalent metal transporter-1, ceruloplasmin, and ferroportin-1 in the rat cerebral cortex and hippocampus. Biol Trace Elem Res 143: 1581–1593.

Marques F, Falcao AM, Sousa J C, Coppola G, Geschwind D, Sousa N, Correia-Neves M and Palha J A (2009) Altered iron metabolism is part of the choroid plexus response to peripheral inflammation. Endocrinology 150: 2822–2828.

Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T (1996) Interleukin (IL)-1, IL-2, IL-4, IL-4, IL-6 and trans forming growth factor-levels are elevated in ventricular cerebrospinal fluid in Juvenile parkinsonism and Parkinson's disease. Neurosci Lett 211: 13–16.

Nagatsu T, Sawada M (2005) Inflammatory process in Parkinson's disease: role for cytokines. Curr Pharm Des 11: 999–1016.

Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T (2004) IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 113: 1271–1276.

Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T (2003) Hepcidin, a putative mediator of anemia of inflammation, is a type II acute phase protein. Blood 101: 2461–2463.

Orlowski D, Michalis A, Glud AN, Korshøj AR, Fitting LM, Mikkelsen TW, Mercanzini A, Jordan A, Dransart A, Sørensen JCH (2017) Brain tissue reaction to deep brain stimulation – a longitudinal study of DBS in the Goettingen minipig. Neuromodulation 20: 417–423.

Pietrangelo A, Dierssen U, Valli L, Garuti C, Rump A, Corradini E, Ernst M, Klein C, Trautwein C (2007) STAT3 Is required for IL-6-gp130–dependent activation of hepcidin *in vivo*. Gastroenterology 132: 294–300 .

Qian ZM, He X, Liang T, Wu KC, Yan YC, Lu LN, Yang G, Luo QQ, Yung WH, Ke Y (2014) Lipopolysaccharides upregulate hepcidin in neuron *via* microglia and the IL-6/STAT3 signaling pathway. Mol Neurobiol 50: 811–820.

Qin XY, Zhang SP, Cao C, Loh YP, Cheng Y (2016) Aberrations in peripheral inflammatory cytokine levels in Parkinson disease: a systematic review and meta-analysis. JAMA Neurol 73: 1316–1324.

Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, Onofrj M (2009) Peripheral cytokines profile in Parkinson's disease. Brain Behav Immun 23: 55–63.

Ruchala P, Nemeth E (2014) The pathophysiology and pharmacology of hepcidin. Trends Pharmacol Sci 35: 155–161.

Sawada M, Imamura K, Nagatsu T (2006) Role of cytokines in inflammatory process in Parkinson's disease. J Neural Transm 70: 373–381.

Su Y, Zhang K, Schluesener HJ (2010) Antimicrobial peptides in the brain. Arch Immunol Ther Exp 58: 365–377.

Urrutia PJ, Hirsch EC, González-Billault C, Núñez MT (2017) Hepcidin attenuates amyloid beta-induced inflammatory and pro-oxidant responses in astrocytes and microglia. J Neurochem 142: 140–152.

Wang SM, Fu LJ, Duan XL, Crooks DR, Yu P, Qian ZM, Di XJ, Li J, Rouault TA, Chang YZ (2010) Role of hepcidin in murine brain iron metabolism. Cell Mol Life Sci 67: 123–133.

Wang Q, Du F, Qian ZM, Ge XH, Zhu L, Yung WH, Yang L, Ke Y (2008) Lipopolysaccharide induces a significant increase in expression of iron regulatory hormone hepcidin in the cortex and substantia nigra in rat brain. Endocrinology 149: 3920–3925.

Weaver FM, Follett K, Stern M (2009) Bilateral deep brain stimulation *vs.* best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial. JAMA 301: 63–73.

Williams A, Gill S, Varma T (2010) Deep brain stimulation plus best medical therapy *versus* best medical therapy alone for advanced Parkinson's disease (PD SURG trial): a randomised, open-label trial. Lancet Neurol 9: 581–591.

Wrighting DM, Andrews NC (2006) Interleukin-6 induces hepcidin expression through STAT3. Blood 108: 3204–3209.

Verga Falzacappa MV, Vujic Spasic M, Kessler R, Stolte J, Hentze MW, Muckenthaler MU (2007) STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. Blood 109: 353–358.