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Parkinson's disease and oxidative stress: evaluation by BAP and d-ROMs tests

V. Pizza, E. Iorio°, A. Capasso*
Neurophysiopatology Service, S. Luca Hospital, Vallo della Lucania, Salerno
°Biochemistry Department, Second University of Naples
*Department of Pharmacy, University of Salerno, Italy

Abstract

Parkinson's disease (PD) is a common adult-onset neurodegenerative disorder. Typically PD is a sporadic neurological disorder, and over time affected patients see their disability growing and their quality of life declining. Oxidative stress has been hypothesized to be linked to both the initiation and the progression of PD. Preclinical findings from both in vitro and in vivo experimental models of PD suggest that the neurodegenerative process starts with otherwise healthy neurons being hit by some etiological factors, which sets into motion a cascade of deleterious events. In these models initial molecular alterations in degenerating dopaminergic neurons include increased formation of reactive oxygen species, presumably originating from both inside and outside the mitochondria.

The aim of the present study is to evaluate the oxidative status in a sample of patients with PD by a specific serum tests: BAP and d-ROMs tests.

21 outpatients, (8 F, 13 M) mean age 70 years (SD 11.5), range 44-86 years, suffering PD (Hoehn & Yahr scale: 1-3) were enrolled. The mean duration of disease was 3.8 (SD 2.4) years, range 1-8 years. 2 patients was affected also of the dementia and in 8 patients concomitant vascular disease.

Serum total oxidant capacity was determined by performing the d-ROMs test (derived Reactive Oxygen Metabolites), whose chemical principle is based on the ability of a biological sample to oxidize N,N-diethylparaphenylenediamine (DPPD) and serum total antioxidant capacity was assessed by means the BAP tests (Biological Antioxidant Potential) which measures the ability of a serum sample to reduce iron from the ferric to the ferrous ionic form.

The results of our study indicates that the mean values of d-ROMs tests is 369,8 (SD 82.5) (range normal values is 250-300 U.CARR (Carratelli Unit; 1 U. CARR = 0.8 mg/L H_2O_2.) The mean value of BAP test is 1641,4 (SD 412.7) (range normal values is 2200-4000 microMol/L).

These data showed that signs of systemic oxidative stress, represented by high values of hydroperoxides serum, are present in subjects with Parkinson's disease and they can be quantified easily with a simple and inexpensive method as the D-ROMs test.

Key words: Parkinson's disease, oxidative stress, BAP test, dROMs test

Introduction

PD is one of most common neurodegenerative disease. Several studies indicate the presence of inflammatory mediators (including TNF-, IL-1â, IL-6, and interferon- (IFNã)) in the cerebrospinal fluid (CSF) of patients with PD as well as in the post-mortem substantia nigra pars compacta in PD patient brains [1-5]. The involvement of TNF- in inducing dopaminergic neuron death in chronic parkinsonism, was confrmed by high plasma TNF- levels detected in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated non-human primates one year after administration of the neurotoxin and in two endotoxin rat models [6-8]. TNF receptors are expressed in nigrostriatal dopamine (DA) neurons [9,10] which are selectively vulnerable to TNF--induced toxicity [11-15]. These early genetic studies and the more recent chronic inflammation models of PD strongly implicate TNF- and its downstream targets in neurotoxin- and endotoxin-induced loss of nigral DA neurons. Because the blood-brain barrier increase with age increases the likelihood of peripheral immune cell infiltration into the CNS, TNF- produced by brain-resident microglia may not be acting alone in mediating DA neuron cell death, but rather in concert with other circulating neurotoxic factors to increase the inflammatory susceptibility of nigral DA neurons and development of PD [16].

Several genetic mutations are associated with early-onset familial forms of PD and these have shed light on the potential mechanisms involved in disease pathogenesis.

Alpha synuclein (-syn) is an evolutionarily well-conserved phosphoprotein expressed in the brain which is believed to play an important role in synaptic plasticity. Mutations in the -syn gene lead to aggregation of the protein and Lewy body formation. In primary human embryonic cells derived from the mesencephalon, dopaminergic neurons are selectively vulnerable to -syn over-expression, while in a transgenic mouse expressing an -syn mutation, the animals developed adult-onset disease which closely mimicked the symptoms of PD [16]. Mutations in the parkin gene are also associated with early onset PD. This gene is mainly expressed in the CNS and codes for a family of proteins involved in ubiquitination and proteosomal degradation. Mutations in three other genes, DJ-1, ubiquitin carboxyl-terminal hydrolase-1, and PTEN induced kinase 1, have also been identified. These are involved, respectively, in oxidative stress response, the ubiquitin proteosomal pathway, and mitochondrial kinase activity [17]. These mutations imply that mitochondrial abnormalities, oxidative stress, and dysregulation of protein degradation are common mechanisms in the disease process. Since the concordance rate of PD occurrence between monozygotic twins is low, environmental factors may play an important role in the etiology of late-onset PD. Possible environmental agents that may be contributing factors for the disease are pesticides, such as rotenone, lipopolysaccharide (LPS), and a byproduct of synthetic heroin, MPTP.

In these settings, activation of microglia is found together with dopaminergic cell loss. In primary neuron-enriched or neuron-glia cultures derived from rat mesencephalon, rotenone-induced dopaminergic neurodegeneration was dependent on the presence of microglial cells [17]. This was mediated by the production of superoxide from activated microglia. The injection of LPS in the supranigral area of rat brains resulted in microglial and astroglial proliferation and these reactive glial cells were important mediators of the subsequent neuronal cell death. Furthermore, inhibition of glial cell activation in the mouse brain decreased MPTP-induced neurotoxicity by blocking the formation of inducible nitric oxide synthase (iNOS) and IL-1. Heightened innate immune responses are seen in the CNS of PD patients. There is an increase in the levels of proinflammatory cytokines in the CSF and nigrostriatal regions of PD brains. Furthermore, large numbers of reactive microglia are found in the substantia nigra of PD patients. These may chronically produce ROS, resulting in depletion of antioxidant stores that may jeopardize mitochondrial activity. Since aerobic respiration in mitochondria is responsible for most of the ROS produced in cells,

abnormalities in these organelles may exacerbate oxidative stress [17]

Given the above evidences, the aim of the present study is to evaluate the oxidative status in a sample of patients with PD by a specific serum tests: BAP and d-ROMs tests.

Patients and Methods

The study was performed and approved by Neurophysiopatology Service, Headache Centre, S. Luca Hospital, Vallo della Lucania (SA),Italy.

The study sample consists 21 outpatients, (8 F, 13 M) mean age 70 years (SD 11.5), range 44-86 years, suffering PD (Hoehn & Yahr scale: 1-3) were enrolled. The mean duration of disease was 3.8 (SD 2.4) years, range 1-8 years. 2 patients was affected also of the dementia and in 8 patients concomitant vascular disease.

Serum total oxidant capacity was determined by performing the d-ROMs test (derived Reactive Oxygen Metabolites) (18), whose chemical principle is based on the ability of a biological sample to oxidize N,N-diethylparaphenylenediamine (DPPD) and serum total antioxidant capacity was assessed by means the BAP tests (Biological Antioxidant Potential) which measures the ability of a serum sample to reduce iron from the ferric to the ferrous ionic form.

Results

The results of our study indicates that the mean values of d-ROMs tests is 369,8 (SD 82.5) (range normal values is 250-300 U.CARR (Carratelli Unit; 1 U. CARR = 0.8 mg/L H2O2.) The mean value of BAP test is 1641,4 (SD 412.7) (range normal values is 2200-4000 microMol/L) (See Table I).

Values in the normal range were noticed for 3 patients for the values of Droms and for 2 patients for the values of BAP.

Discussion

These results of the present study showed that high values of hydroperoxides serum are present in subjects with Parkinson's disease thus indicating a significative systemic oxidative stress in these patients.

Our study confirms and extends previous studies indicating that oxidative stress may induce both the initiation and the progression of PD [19-21].

Mitochondrial respiration produces reactive oxygen species (ROS) by leakage of intermediates from the electron transport chain [22]. These molecules are highly unstable because they have an unpaired electron; they therefore seek to achieve a stable state by appropriating electrons from nearby molecules. The latter, in turn, become unstable and so on, thus creating an instability chain reaction. Usually, the harmful activity of a small percentage of these free radicals is neutralized by cellular antioxidants. Antioxidants may be enzymatic (superoxide dismutase [SOD], catalase, glutathione peroxidase [GSHP]) and non-enzymatic (vitamins E, C, A). When, however, free radical levels increase (overeating, smoking, drug abuse, ultraviolet radiation, persistent chronic inflammation, etc.) the pool of antioxidants is saturated and the excess of free radicals damages biological structures. At first the damage is evident in mitochondria, which may affect DNA and RNA causing mutations, but may also act on proteins and lipids. Endothelial cells, fibroblasts, and tissue cells are the most affected by oxidative stress.

In order to understand how free radicals are involved in the neurodegenerative process it is worth reviewing the production and fate of ROS at the tissue level. Oxidases and mitochondria are the main ROS producers, which include highly reactive molecules such as superoxide anion. The fate of this molecule depends on the total oxidation-reduction equilibrium at the cellular level, which is controlled by SOD which produces H_2O_2 [22] followed by conversion into H_2O and O_2 through the action of catalase or GSHP. When the production of superoxide anion or H_2O_2 is such as to saturate the reduc-

tive capacity of the SODs or of GSHP, these molecules become substrates for the creation of highly reactive molecules such as hydroxyl (by means of a Fenton and/or Haber-Weiss reaction) which are responsible for cell and tissue damage [23]. The superoxide anion can react with nitric oxide in a diffusion limited reaction generating peroxynitrite, itself a powerful ROS. Most ROS are limited in their diffusion potential by their insolubility in lipids, and thus their effect is generally restricted to the intracellular compartment. Conversely, H_2O_2 can pass through cell membranes and thus react farther away from the production site. This theory posits that mitochondrial production of ROS regulates the ageing rate, since oxidative damage builds up over time. According to this hypothesis long-lived species produce fewer ROS by comparison to shorter-lived species, and mice subjected to calorie restriction live longer and produce fewer ROS than controls [22].

Currently, treatment for PD is focused merely on alleviating symptoms. As research progresses towards a better understanding of the molecular mechanisms underlying disease, hopefully a more effective therapy can ultimately be designed. Current trials to deliver compounds that can restore mitochondrial function and reduce oxidative burden will be informative and not only improve therapeutic treatment of PD but also provide vital results to guide future studies investigating the molecular mechanisms of neurodegeneration.

Obviously, our findings deserve confirmation on case studies and possibly more extensive correlation to the stages of disease with particular reference to the initial ones that could be useful therapeutic approach also addressed the correction of these parameters.

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Patients	Sex	Age	Years disease	BAP	dROMs
1	f	78	3	1252	589,6
2	m	80	2	1591	371
3	m	74	2	1692	367
4	m	68	8	1765	306,69
5	f	44	1	1623	332,41
6	M	73	2	2009	417
7	m	72	5	1341	389
8	m	63	3	1705	402,26
9	f	66	4	1626	462,4
10	m	67	1	1941	349
11	f	58	1	1081	411,11
12	f	62	2	2104	506
13	m	86	8	1349	373,37
14	m	86	8	1552	393,07
15	f	74	5	2449	280
16	m	50	2	2023	180
17	f	84	5	1359	345
18	m	78	3	1282	318,6
19	m	73	3	1208	452,6
20	m	81	3	2369	260
21	f	53	7	1148	412,6

Table I: Data of the sample and BAP-dROMs test