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Analysis of neurotrophic and antioxidant factors related to midbrain dopamine neuronal loss and brain inflammation in the cerebrospinal fluid of the elderly

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ABSTRACT

Midbrain dopamine neuronal loss and neuroinflammation are two phenomena that are associated with brain senescence. Neurotrophic factor changes and oxidative stress could subserve these phenomena. Aging-related brain changes can be well monitored through the cerebrospinal fluid (CSF). The objective was to analyze neurotrophic and oxidative parameters that could be related to midbrain dopamine neuronal loss or brain inflammation in the CSF of elderly subjects: 1) levels of the dopaminotrophic factors BDNF, GDNF, persephin, and neurturin, 2) levels of the proinflammatory factors TGFβ$_1$ and TGFβ$_2$; 3) activity of main antioxidant enzymes (catalases, glutathione-peroxidase, glutathione-reductase, glutathione-S-transferases, peroxirredoxins, and superoxide-dismutases), 4) ferritin content, antioxidant protein which reduces reactive free iron, and 5) antioxidant potential of the cerebrospinal fluid. ELISA and PAO tests were used. Subjects were also evaluated clinically, and the group of old subjects with mild cognitive impairment was studied separately. The findings indicate that normal elderly CSF is devoid of changes in either dopaminotrophic or proinflammatory factors. The antioxidant efficacy is slightly reduced with normal aging, through a reduction of glutathione-S-transferase activity in people older than 74 years ($p < 0.05$). However old people with mild cognitive impairment show reduced BDNF levels, and stronger signs of oxidative stress such as low antioxidant potential and glutathione-S-transferase activity ($p < 0.05$). To sum up, the present study demonstrates that, in CSF of normal senescence, dopaminotrophic factors and proinflammatory TGF-family ligands are not affected, and antioxidant efficacy is slightly reduced. CSF of elderly subjects with mild cognitive impairment shows more oxidative and trophic changes that are characterized by reduction of BDNF content, glutathione-S-transferase activity, and antioxidant potential.

1. Introduction

Midbrain dopamine neuronal loss and inflammation are two phenomena that are associated with brain senescence. Thus, midbrain dopamine cell death is enhanced with aging, and this cellular decline has been estimated to affect 4–7% of dopamine substantia nigra neurons per decade (Scherman et al., 1989; Fearnley and Lees, 1991; Budni et al., 2015). Brain inflammation seems to be associated to activation of microglia due to senescence (Godbout and Johnson, 2004; Pertusa et al., 2007; Ritzel et al., 2015). Neurotrophic factor deficit and oxidative stress could subserve these aging-associated phenomena (Barja, 2014; Budni et al., 2015).

Regarding neurotrophic factors that are related to midbrain dopamine neurons, the survival of this neuronal population is critically dependent on the "dopaminotrophic" factors of the glial cell line-related ligands family (GFL family), and brain-derived neurotrophic factor (BDNF). The GFL family includes ligands such as glial cell line-derived neurotrophic factor (GDNF), neurturin, and persephin (Lin et al., 1993;

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BMI, body mass index; BMJ, British Medical Journal; CSF, cerebrospinal fluid; ELISA, Enzyme-Linked ImmunoSorbent Assay; GDNF, glial cell line-derived neurotrophic factor; GFL, glial cell line-related family ligands; GPX, glutathione-peroxidase; GR, glutathione-reductase; GSH, glutathione; GST, glutathione-S-transferase; IADL, Instrumental activities of daily living; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination test; PAO, antioxidant potential; PRDX, peroxirredoxins; RAVLT, Rey verbal learning test; SOD, superoxide dismutase; TGF, transforming-growth factor; TGFβ$_1$, transforming-growth factor beta 1; TGFβ$_2$, transforming-growth factor beta 2; WHO, World Health Organization

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Krieglstein et al., 1998; Rosenblad et al., 1999; Airaksinen and Saarma, 2002; Fjord-Larsen et al., 2005). BDNF is important for the development and survival of dopaminergic substantia nigra neurons (Mogi et al., 1999). BDNF, apart from its dopaminotrophic effect, is also critical for the maintenance of other populations of neurons which mediate cognitive processes such as learning and memory (Korhonen et al., 1998; Albrecht et al., 2006; Silhol et al., 2008).

Brain inflammation could be exacerbated, at least in part, by trophic factors of the transforming-growth factor family (TGF family), such as TGFβ_1 and TGFβ_2. These ligands are released by activated microglia, and they are considered as proinflammatory factors (Unsicker et al., 1991; Wyss-Coray et al., 2001; Hirsch and Hunot, 2009; Hirsch et al., 2012). In addition, BDNF is also related to neuroinflammation. This factor can be released by activated microglia, and the release of BDNF can, in turn, activate microglia and inflammation (Zhang et al., 2014). Hence BDNF acts as a powerful pro-inflammatory cytokine (Zhang et al., 2014). Accordingly, BDNF seems to be a dual compound: BDNF excess is proinflammatory, but BDNF deficit reduces its neurotrophic efficacy. However, this role of BDNF in neuroinflammation has been put into question, because recently some authors have attributed anti-inflammatory effects to excess amounts of BDNF (Lai et al., 2018). They propose that BDNF is released by activated microglia, but it participates in endogenous cross-talks between astrocytes and microglia to regulate anti-inflammatory actions rather than pro-inflammation.

Neuronal death and inflammation are tightly linked to oxidative stress (Navarro-Yepes et al., 2014; Barja, 2014), and it is widely accepted that brain aging could be accelerated by the damaging action of reactive oxygen species (Harman, 1956; Gerschman et al., 1964; Barja, 2014). The body possesses antioxidant defenses which scavenge free radicals and minimize their effects, but it seems that these defenses reduce their efficacy with aging (Jenner, 2003; García-Moreno et al., 2013; Navarro-Yepes et al., 2014). As a consequence, reactive species could participate in the development of cell senescence (Afanas'ev, 2010; Barja, 2014). In the brain, many antioxidant molecules are known to be affected with senescence. Thus, nocturnal secretion of melatonin along with levels of coenzyme Q and ascorbate are reduced (Spindler et al., 2009; Escames et al., 2010; Phillipson, 2014). In addition, melatonin hypoactivity down-regulates physiological activity of antioxidant molecules such as superoxide-dismutases (SOD), catalases and glutathione (GSH) (Martin et al., 2000; Phillipson, 2014). In this context, decreased GSH has already been found in old people (Phillipson, 2014), although changes in SOD and catalase activity are far from clear (Carrillo et al., 1992; Okabe et al., 1996). Oxidative stress can be also caused by accumulation of reactive metals such as iron or cupper. The iron storage protein ferritin, which exerts an anti-oxidant protective role reducing reactive free iron, could be affected in the brain with aging (Dexter et al., 1990; Kuiper et al., 1994; Bradbury, 1997).

Aging-related changes in the central nervous system can be well monitored through the cerebrospinal fluid (CSF), a fluid in close contact with nerve tissue and considered as a good witness of brain processes (Kroksveen et al., 2011). The objective of this study was to analyze neurotrophic and oxidative parameters that could be related to midbrain dopamine neuron loss or neuroinflammation in the CSF of elderly subjects: 1) levels of the dopaminotrophic factors BDNF, GDNF, persephin, and neurturin, 2) levels of the proinflammatory factors TGFβ_1 and TGFβ_2; 3) activity of main antioxidant enzymes (catalases, glutathione-peroxidase, glutathione-reductase, glutathione-S-transferases, peroxirredoxins, and superoxide-dismutases), 4) levels of ferritin, iron storage protein, and 5) antioxidant potential of the fluid (PAO test).

2. Material and methods

2.1. Study participants

The cross-sectional study was carried out in Hospital Macarena, Sevilla, Spain. Subjects were recruited from volunteers subjected to intradural anesthesia in the Service of Surgery of Hospital Macarena. All of them were operated from hip fracture, umbilical hernia or anal surgery. Anesthesia and the surgery themselves had no impact on the CSF parameters to be analyzed. Clinical information was gathered from each patient: age, sex, body weight, height, body mass index (BMI), hypertension, hyperlipidemia, coffee drinking, smoking, alcohol use, taking of vitamins A/E/C supplement, and thyroid dysfunction (hypo- or hyper-thyroidism). Hypertension was diagnosed when blood pressure repeatedly (two measures fifteen days apart) exceeded 140 mm Hg (systolic) and/or 90 mm Hg (diastolic) or when a subject was taking antihypertensive medication to control hypertension. Hyperlipidemia was diagnosed when blood cholesterol levels were higher than 200 mg/dl, triglycerides content was over 250 mg/dl and HDL levels were lower than 35 mg/dl, or subject were taking drugs for lowering lipidemia. Use of vitamins was defined as daily intake of either Vitamin A or Vitamin E or Vitamin C. Drinking coffee was defined as daily intake of at least 300 ml of coffee. Smoking was defined as current smoker who consumed cigarettes on a daily basis. Alcohol use was defined as drinking > 210 g of alcohol per week.

Mild cognitive impairment (MCI) was also evaluated by a psychiatrist of the Service of Psychiatry of the hospital. Cognition was modeled using the Rey verbal learning test (RAVLT, Spanish version), which is very sensitive in distinguishing control and MCI patients through the evaluation of verbal cognition (Ryan and Geisser, 1986; Estéves-González et al., 2003), and the Spanish version of the Mini-Mental State Examination test (MMSE), which allows evaluating different cognitive aspects (Folstein et al., 1975; Lobo et al., 1979; Manubens et al., 1998). The cutoff for the Spanish version of the MMSE test was 23/24 (range, 0–35). In addition, activities of daily living (ADL) were assessed, because many studies point out that people in the preclinical stage of dementia and mild cognitive impairment patients have instrumental ADL disabilities (Perneczky et al., 2006; Jefferson et al., 2008). The Instrumental activities of daily living (IADL) were evaluated with the Spanish versions of the Lawton-Brody and Pfeffer Scales, which investigate the level of independence in daily living tasks (Lawton and Brody, 1969; Pfeffer et al., 1982; Olazarán et al., 2005; Montejo-Carrasco et al., 2012). The cutoff for the Lawton-Brody scale was 19/20 (range, 0–25), and that for the Pfeffer scale, which allows a more accurate discrimination of dementia, was 6/7 (range, 0–33). RAVLT and MMSE tests and Lawton-Brody and Pfeffer scales were administered to all included participants.

Individuals suffering from liver, renal and cardiac dysfunctions, malabsorption, morbid obesity, autoimmune diseases, rheumatoid arthritis, AIDS, dementia or Alzheimer disease (AD), and infectious conditions were excluded from the study. Oxidative stress markers in the CSF may be altered in such conditions (Martín de Pablos et al., 2015). Rheumatoid arthritis was defined based on WHO criteria. Morbid obesity was diagnosed when BMI was higher than 35 kg/m^2.

2.2. CSF collection and analysis

CSF was collected through lumbar puncture. Three ml of CSF were collected and stored in polypropylene tubes (Eurotube, Spain) protected by the light, and rapidly aliquoted, coded and frozen at −80 °C for further studies. A half ml collection in a glass tube was employed to observe the absence of traumatic puncture and to quantify red cells before storing. CSF with excess of red cells was discarded (> 500 red cells/microL). Fresh, never before thawed, 0.5 ml aliquots for each subject were analyzed on 96-well plates in the same analytical run for this study to minimize run to run and reagent kit lot sources of variation. One fresh CSF aliquot was used for measuring routine parameters in the Hospital laboratory settings. These parameters included total protein content, glucose, pH, density, and number of leukocytes per microliter. Density was measured at 37 °C in a Density Meter that displayed density to the fourth decimal point, and was accurate to