Neuroscience Letters 469 (2010) 6–10

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease

Manuela Padurariu[a], Alin Ciobica[b,c,*], Lucian Hritcu[c], Bogdan Stoica[a], Walther Bild[a], Cristinel Stefanescu[a]

[a] "Gr. T. Popa" University of Medicine and Pharmacy, 700115 Iasi, Romania
[b] Laboratory for Experimental and Applied Physiology, Romanian Academy, 700505 Iasi, Romania
[c] Laboratory of Animal Physiology, "Alexandru Ioan Cuza" University, 700506 Iasi, Romania

ARTICLE INFO

Article history:
Received 11 October 2009
Received in revised form 4 November 2009
Accepted 9 November 2009

Keywords:
Mild cognitive impairment
Alzheimer's disease
Oxidative stress

ABSTRACT

Mild cognitive impairment (MCI) is a nosological entity proposed as an intermediate state between normal aging and dementia. MCI seems to represent an early stage of Alzheimer's disease (AD) and there is a great interest in the relationship between MCI and the progression to AD. Some studies have demonstrated an accumulation of products of free radical damage in the central nervous system and in the peripheral tissues of subjects with AD or mild cognitive impairment. The aim of the present work was to evaluate the serum levels of some enzymatic antioxidant defences like superoxide dismutase (SOD) and glutathione peroxidase (GPX), as well as lipid peroxidation markers like MDA (malondialdehyde), in MCI and AD patients, compared with age-matched healthy controls. The subjects of this study (45 patients) consisted of 15 individuals with mild cognitive impairment (MCI), 15 with Alzheimer's disease (AD) and 15 healthy age-matched controls. Biochemical analyses showed a similar decrease of the main enzymatic antioxidant defences (SOD and GPX) and increased production of lipid peroxidation marker (MDA) in the serum of the MCI and AD patients, compared to age-matched control group. This study clearly demonstrates that oxidative stress damage occurs in patients with MCI and AD. Moreover, some enzymatic markers of oxidative stress are similar in MCI and AD patients, suggesting that oxidative damage could be one important aspect for the onset of AD.

Mild cognitive impairment (MCI) is a nosological entity proposed as an intermediate state between normal aging and dementia. MCI seems to represent an early stage of Alzheimer's disease (AD) and there is a great interest in the relationship between MCI and the progression to AD [26]. It is generally estimated that the rate of progression is almost 50% within 4 years [2,3,10], approximately 12% per year, supporting the concept that MCI represents a prodromal stage of AD.

According to the free radical theory, aging can be considered as a progressive, inevitable process partially related to the accumulation of oxidative damage into biomolecules (nucleic acids, lipids, proteins or carbohydrates) due to an imbalance between pro-oxidants and antioxidants in favor of the former [25]. A large body of evidence implicates oxidative damage in AD pathogenesis [3,39]. It is believed that oxidative damage to critical molecules occurs early in the pathogenesis of AD and precedes pronounced neuropathological alterations [2,22,32]. Because oxidative damage begins early in the progress of the disease, it represents a potential therapeutic target for slowing the onset and progression of AD [22].

Oxidative stress is the condition that could occur from the imbalance between reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals and antioxidant systems. Also, brain is particularly vulnerable to oxidative stress as a result of the relatively low levels of antioxidants, high levels of polyunsaturated fatty acids and increased need of oxygen [40].

Post-mortem and *in vivo* studies have demonstrated an accumulation of products of free radical damage in the central nervous system and in the peripheral tissues of subjects with AD or mild cognitive impairment [6,7,24]. Also, this situation is combined with a reduction of most enzymatic antioxidant defences, like glutathione peroxidase (GPX), glutathione S-transferase and superoxide dismutase (SOD) [13]. Furthermore, markers of lipid peroxidation, like malondialdehyde (MDA), have been found elevated in AD [2,15,21,27].

The aim of the present work was to evaluate the specific activity of some peripheral antioxidant defences (SOD and GPX) and the level of MDA (lipid peroxidation maker), in MCI and AD patients, compared with age-matched healthy subjects. We found

* Corresponding author at: "Alexandru Ioan Cuza" University, Department of Biology, B dul Carol I, 11, 700506 Iasi, Romania. Tel.: +40 751218264; fax: +40 232201472.
E-mail address: alin.ciobica@uaic.ro (A. Ciobica).

0304-3940/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2009.11.033

that antioxidant enzymatic damage occurs in the patients with MCI and AD in a similar way, suggesting that oxidative stress might represent a signal of the AD pathology.

The subjects of this study (45 patients) consisted of 15 individuals with MCI (5 females and 10 males; age: 63.2 years ± 4.2), 15 with AD (6 females and 9 males; age: 65.8 years ± 3.9) and 15 healthy age-matched controls (7 females and 8 males; age: 62.5 years ± 3.4). Patients were recruited from the Psychiatry University Hospital, Iasi, Romania.

Cognitive and functional status was assed by Mini-Mental State Examination (Controls 26 ± 0.5; MCI 22.2 ± 0.3; AD 18.5 ± 0.3) and Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog) (Controls 7 ± 0.2; MCI 14 ± 0.4; AD 18.5 ± 0.3) [30,36]. AD patients fulfilled the NINCDS ADRDA criteria [28], whereas MCI diagnosis followed the criteria of Petersen et al. when there was evidence of memory impairment, preservation of general cognitive and functional abilities and absence of diagnosed dementia [15,33].

None of the subjects studied was taking antioxidant supplements. Also, subjects with acute comorbidities were excluded from the study.

The study was conducted according to provisions of the Helsinki Declaration and the local ethics committee approved the study. All the patients or their families signed the consent for the participation in this study.

Blood samples were collected in the morning, before breakfast, allowed to clot and centrifuged immediately. Sera were aliquoted into Eppendorf tubes and stored at −80 °C until measurement.

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (Fluka, 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37 °C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma Chemicals). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity.

MDA levels were determined by thiobarbituric acid-reactive substances (TBARs) assay. 200 μL serum was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.9 mL of TRIS–HCl (pH 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100 °C for 20 min. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant read at 532 nm. The signal was read against an MDA standard curve and the results were expressed as nmol [12,20].

The results for antioxidant enzymes activity and MDA level were analyzed using one-way analysis of variance (one-way ANOVA). All results are expressed as mean ± S.E.M.; *Post hoc* analyses were performed using Tukey's honestly significant difference test. *F* values for which $p < 0.05$ were regarded as statistically significant. Pearson's correlation coefficient and regression analysis were used to evaluate the connection between the antioxidant defence and lipid peroxidation.

Biochemical analyses showed a similar decrease of the main enzymatic antioxidant defences (SOD and GPX) and increased production of lipid peroxidation marker (MDA) in the serum of the MCI and AD patients, compared to age-matched control group.

Fig. 1 shows a significant decrease of the SOD specific activity ($F(2,42) = 21$, $p < 0.0004$) in the serum of MCI ($n = 15$) and AD ($n = 15$) patients compared to the age-matched control group ($n = 15$). *Post hoc* analyses revealed that MCI and AD groups showed significant decrease of SOD serum activity ($p < 0.0002$) compared to age-matched control group, but did not differ from each other.

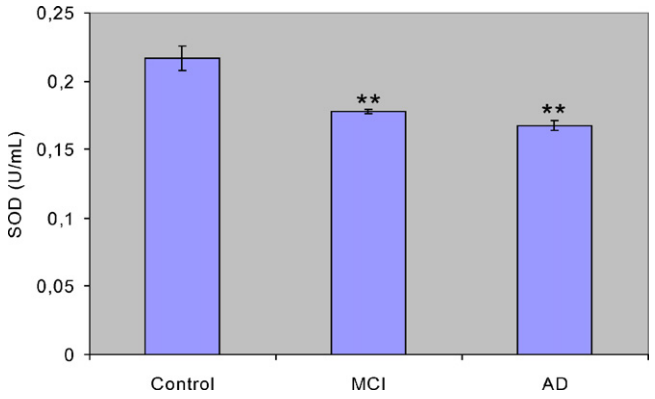

Fig. 1. Superoxide dismutase specific activity in Control, MCI and AD patients serum. The values are mean ± S.E.M. ($n = 15$ per group), $^{**}p < 0.0004$ vs. control.

We also observed a significant decrease of the GPX specific activity ($F(2,42) = 54$, $p < 0.0001$) in the serum of MCI ($n = 15$) and AD ($n = 15$) patients compared to the age-matched control group ($n = 15$) (Fig. 2). *Post hoc* analyses revealed that MCI and AD groups showed significant decrease of GPX serum activity ($p < 0.0001$) compared to age-matched control group, but did not differ from each other.

In addition, a significant increase of the MDA level ($F(2,42) = 90$, $p < 0.0005$) was found in the serum of MCI ($n = 15$) and AD ($n = 15$) patients compared to the age-matched control group ($n = 15$) (Fig. 3). Also, *post hoc* analyses revealed that MCI and AD groups showed a significant increase of the MDA serum level ($p < 0.0002$) compared to age-matched control group and significantly statistical differences were found between MCI and AD groups ($p < 0.0005$).

When we determined the linear regression between GPX vs. SOD, GPX vs. MDA and SOD vs. MDA we found significant positive correlations, especially in the case of GPX vs. MDA ($n = 45$, $r = 0.814$, $p = 10^{-8}$) (Fig. 4A), but also for SOD vs. MDA ($n = 45$, $r = 0.642$, $p = 10^{-5}$) and GPX vs. SOD ($n = 45$, $r = 0.697$, $p = 10^{-6}$) (Fig. 4B and C). However, separate linear regression analysis for each group resulted in no significant correlations.

No significant differences between genders in the activity or level of any determined oxidative stress marker were observed (data not shown).

In the present study we measured the specific activity of some enzymatic antioxidant systems (SOD and GPX) and the MDA levels, as a lipid peroxidation marker, in the peripheral blood of MCI and

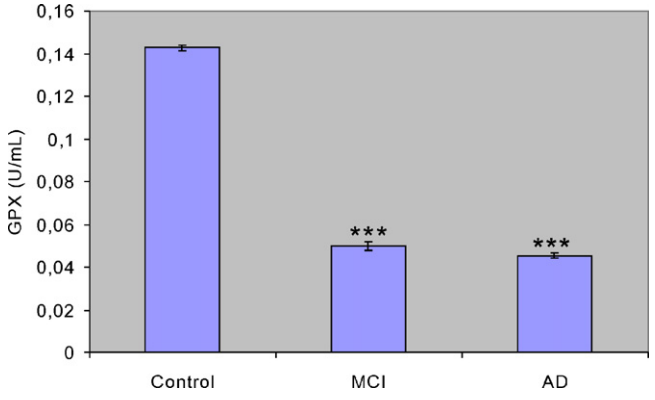

Fig. 2. Glutathione peroxidase specific activity in Control, MCI and AD patients serum. The values are mean ± S.E.M. ($n = 15$ per group), $^{***}p < 0.0001$ vs. control.