Journal of the Neurological Sciences 368 (2016) 155–159

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

The levels of 7,8-dihydrodeoxyguanosine (8-oxoG) and 8-oxoguanine DNA glycosylase 1 (OGG1) – A potential diagnostic biomarkers of Alzheimer's disease☆

Agnieszka Sliwinska [b,1], Dominik Kwiatkowski [a,1], Piotr Czarny [a,e], Monika Toma [a], Paulina Wigner [a], Jozef Drzewoski [b], Krystyna Fabianowska-Majewska [d], Janusz Szemraj [e], Michael Maes [f,g,h], Piotr Galecki [c], Tomasz Sliwinski [a,*]

[a] University of Lodz, Department of Molecular Genetics, Lodz, Poland
[b] Department of Internal Disease, Diabetology and Clinical Pharmacology, Medical University of Lodz, Lodz, Poland
[c] Department of Adult Psychiatry, Medical University of Lodz, Lodz, Poland
[d] Department of Biomedical Chemistry, Medical University of Lodz, Lodz, Poland
[e] Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland
[f] Deakin University IMPACT Strategic Research Centre, Deakin University, School of Medicine, Barwon Health, Geelong, Australia
[g] Department of Psychiatry, Chulalongkorn University, Bangkok, Thailand
[h] Health Sciences Graduate Program, Health Sciences Center, State University of Londrina, Brazil

ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 2 June 2016
Accepted 7 July 2016
Available online 9 July 2016

Keywords:
Alzheimer's disease
Oxidative stress
Oxidative DNA damage
7,8-dihydrodeoxyguanosine (8-oxoG)
DNA base excision repair
8-oxoguanine DNA glycosylase 1 (OGG1)

ABSTRACT

Evidence indicates that oxidative stress contributes to neuronal cell death in Alzheimer's disease (AD). Increased oxidative DNA damage l, as measured with 8-oxoguanine (8-oxoG), and reduced capacity of proteins responsible for removing of DNA damage, including 8-oxoguanine DNA glycosylase 1 (OGG1), were detected in brains of AD patients. In the present study we assessed peripheral blood biomarkers of oxidative DNA damage, i.e. 8- oxoG and OGG1, in AD diagnosis, by comparing their levels between the patients and the controls. Our study was performed on DNA and serum isolated from peripheral blood taken from 100 AD patients and 110 controls. For 8-oxoG ELISA was employed. The OGG1 level was determined using ELISA and Western blot technique. Levels of 8-oxoG were significantly higher in DNA of AD patients. Both ELISA and Western blot showed decreased levels of OGG1 in serum of AD patients. Our results show that oxidative DNA damage biomarkers detected in peripheral tissue could reflect the changes occurring in the brain of patients with AD. These results also suggest that peripheral blood samples may be useful to measure oxidative stress biomarkers in AD.

1. Introduction

According to the WHO Report "Dementia: a public health priority" it is estimated that 35.6 million people suffer from dementia worldwide. Alzheimer disease (AD) is the most widespread cause of dementia in Western societies, especially in people aged 65 years or more [1]. The main feature of AD is a progressive decline in cognitive function, including memory, thinking, language and learning capacity. The diagnosis of AD is difficult due to a lack of specific markers. Currently, AD diagnosis involves a clinical assessment tools for checking signs of cognitive impairments with combination of brain imaging. However, definitive diagnosis can only be based on examining of patients brain tissue. The identification of effective biomarkers for evaluation of risk, diagnosis and monitoring progression of AD has currently been undertaken. Searching for tangible biomarkers should be associated with mechanisms that are involved in the development of AD.

Among numerous hypotheses explaining the pathogenesis for AD, the leading one is the neurodegeneration caused by the oxidative stress [2–7]. Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the efficiency of ROS detoxification. There are many potential sources of ROS which, when overproduced, may contribute to the development of AD. Excessive ROS may be generated by mitochondrial dysfunctions and/or aberrant accumulation of transition metals. Moreover, important source of ROS are amyloid beta, an oligopeptide which is increased in AD [8–10]. Neurons are thought to be prone to oxidative damage because they contain several hundred mitochondria due to their large energy demand. Moreover, neurons are postmitotic cells with limited capacity to regenerate in the adult central nervous system [3]. ROS are highly reactive and can immediately oxidize macromolecules in living cells, including lipids,

☆ The authors declare that there are no conflicts of interest.
* Corresponding author at: 141/143 Pomorska Street, 90-236 Lodz, Poland.
E-mail address: tomsliw@biol.uni.lodz.pl (T. Sliwinski).
[1] Both authors equally contributed to this work.

http://dx.doi.org/10.1016/j.jns.2016.07.008
0022-510X/

proteins, and nucleic acids, leading to various oxidative cellular damages, cell death or mutagenesis.

Numerous studies show increased lipid peroxidation, oxidation of proteins and oxidative DNA damage in brain of patients with AD [11–13]. Moreover, markers of oxidative damage to cellular macromolecules were detected in peripheral blood samples of AD patients [14,15].

Damage to nucleic acid is particularly hazardous for cells because highly altered genetic information present in genomic DNAs often causes cell death. ROS cause various base or sugar backbone modifications in DNA. One of them is 7,8-dihydrodeoxyguanosine (8-oxoG), an oxidized form of guanine, defined as a marker of oxidative stress. The most important DNA repair pathway dealing with oxidative DNA damage is the base excision repair (BER) system. One of BER enzymes responsible for 8-oxoG removing is 8-oxoguanine DNA glycosylase 1 (OGG1), a bifunctional enzyme with DNA glycosylase and apurinic/apyrimidinic (AP) lyase activities [16]. *OGG1* gene is located on the short arm of chromosome 3. This protein has two major isoforms resulting from alternative splicing of the C-terminal region: OGG1-1 located in nucleus and OGG1-2 present in mitochondria. Among various tissues, the expression of OGG1 gene was found to be the highest in the brain [16].

In the present study we aimed to evaluate the level of peripheral markers of oxidative stress, DNA level of 8-oxoG and serum OGG1, in AD patients in comparison to healthy volunteers.

2. Material and methods

2.1. Serum samples and protein preparation

In our study we involved 100 AD patients (44 man and 56 women, mean age 79.2 ± 4.9 years) and 110 healthy volunteers (46 man and 64 women, mean age 77.1 ± 7.3 years). Both AD patients and healthy volunteers (the control) came from Babinski Memorial Hospital. The study group consisted of persons diagnosed with Alzheimer's disease according to DSM-IV criteria. Exclusion criteria for controls were neurodegenerative disorders or a family history of AD. Due to possible false positive results in comet assays, we also excluded patients with dysfunction of kidney, liver and known inflammatory disorders which may increase the level of oxidative DNA damage. The marker of inflammation were elevated levels of C-reactive protein (CRP) and other acute phase proteins such as α2-macroglobulin, haptoglobin C3 and C4 in a clinical tests. Furthermore, in the control group we excluded patients with psychiatric diagnoses, axis I and II disorders (diagnosis based on Structured Clinical Interview for DSM-IV Axis I and II Disorders) and neurological illness. The study was approved by Committee of the Medical University of Lodz (No. RNN/70/14/KE). All AD and control patients gave their informed consent prior to enrollment in the study.

Venous blood samples were collected into 9 mL tubes containing EDTA. The serum was separated by the centrifugation at 5000 ×*g* for 5 min and stored at −20 °C until the protein preparation. For purification of serum from albumin, ProteoPrep Blue Albumin and IgG Depletion kit (Sigma Aldrich, Germany) were used. Specifically, 30 μL of serum sample were taken and purification was performed according to the manufacturer's instructions. To determine the protein concentration the Bradford protein assay was employed. Purified protein samples of known concentration were used to conduct the Western blotting and ELISA assays.

2.2. Detection of 8-oxoG

Genomic DNA was isolated from peripheral blood samples using Genomic Maxi AX Direct kit (AA Biotechnology, Poland). During the isolation, diethylenetriamine pentaacetic acid (0.1 mM) and ascorbic acid (2 mM) were used to prevent background oxidative DNA damage. For DNA strand digestion we used the nuclease P1 (NP1) and alkaline phosphatase (AP) enzymes [17]. Preparation of enzymes solution was as follow: NP1 from Penicillium citrinum (1 mg 1000 units of 3′-phosphomonoesterase activity) was dissolved in 100 μL 20 mM sodium acetate buffer (pH 5.2). NP1 was further diluted 10 times to a final concentration of 1 U/μL in the acetate buffer. AP from calf intestine (1 U/μL) was stored in 25 mM Tris HCl (pH 7.6), 1 mM $MgCl_2$, and 50% glycerol (w/v). Digestion was carried out in the following way: 20 μL 5 μg/μL of DNA sample were diluted to 100 μL with high purity water. After acidification with 1 μL 3 M acetate buffer (pH 5.2), DNA was first digested with 1 μL 1 U/μL NP1. After 1.5 h incubation, pH of the reaction mixture was adjusted to pH 7.4 by adding 10 μL 1 M Tris HCl buffer (pH 8), followed by 1 μL 1 U/μL AP for an additional 1 h. Digested DNA samples were used to determine the 8-oxoG level with Oxiselect oxidative DNA damage ELISA kit (Cell Biolabs, USA).

2.3. Western-blot and ELISA analysis of OGG1

For Western blot and ELISA we used protein fraction isolated from blood serum. We analyzed the OGG1 level with two methods: using enzyme-linked immunosorbent assay (ELISA) and immune blotting (Western blot, WB).

Western blot determination of OGG1 level was performed in 12% polyacrylamide gel. Equal amounts of protein (50 μg protein/lane, both protein sample and GAPDH as reference protein) were loaded into the wells. Electrophoresis was conducted in Tris/Glycine/SDS buffer (25 mM Tris, 190 mM glycine, 0.1% SDS, pH 8.3) for 1 h at 120 V in Mini-PROTEAN Tetra Cell apparatus (Bio-Rad, France). Gel-separated proteins were transferred to a Immobilon PVDF membrane (Millipore, USA). Nonspecific binding was blocked by 5% non-fat dry milk for 2 h at room temperature. To estimate the levels of the OGG1 protein, the membranes were incubated overnight with anti-OGG1/2 rabbit polyclonal antibody (ThermoFisher Scientific), and, in the case of GAPDH protein, anti-GAPDH rabbit polyclonal antibody (Santa Cruz Biotechnologies), diluted 1:1000. Next, nitrocellulose sheets were incubated with the second antibody – anti-rabbit conjugated with HRP (horseradish peroxidase) (Cell Signaling Technology, USA) at a dilution of 1:1000. For chemiluminescent reaction we incubated nitrocellulose sheets for 2 min in stable peroxide solution and an enhanced luminol solution in 1:1 proportion (Thermo Fisher Scientific, USA). To stain immunoreactive bands, peroxidase BMB was added (BM blue POD substrate precipitation; Roche, Germany). The surface area of the immunoreactive bands was measured and calculate in ImageJ software (Wayne Rasband, USA).

OGG1 level was also evaluated with enzyme linked immunosorbent assay. The samples were centrifuged at 1000 ×*g* for 20 min, then was performed an immunosorbent assay with ELISA kit for Oxoguanine Glycosylase 1 (Wuhan USCN Business Co., China) according to the manufacturer instruction.

2.4. Statistical analysis

The levels of 8-oxoG and OGG1 were expressed as mean ± SEM. Blinded replicate samples were used for quality control (QC). The obtained results of experiments were compared using the non-parametric Mann–Whitney's or t-student test for unlinked variables. Test selection was made after verifying normality of studied populations distribution. Form normality examination was used Shapiro–Wilk test, then if the distribution of the population was consistent with normal distribution we conducted paired *t*-test, otherwise Mann–Whitney's test was used. In addition, an area under the receiver operating characteristic (ROC) curve (AUC) analysis was performed to determine the overall accuracy of 8-oxoG and OGG1 as a marker of AD. AUC values was calculated with their 95% confidence intervals. Moreover, we performed a chi-square test to analyze the relationship between obtained results for 8-oxoG and OGG1 and age of patients. Statistical power of samples size was 91% at 5% confidence level.