Alzheimer’s disease treated patients showed different patterns for oxidative stress and inflammation markers

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ABSTRACT

Alzheimer’s disease (AD) is the most common type of dementia accounting for 60–80% of the reported cases. The aim of this study was to evaluate levels of certain parameters of oxidative stress and markers of endothelial dysfunction in the blood of 21 AD patients under standard treatment compared with 10 controls, in an attempt to elucidate the contribution of AD to the total oxidative stress status of the patients. Results indicate that IL-6, TNF–α, ADMA and homocysteine levels were significantly elevated in AD patients. Protein carbonyls levels were higher in AD group, while glutathione reductase and total antioxidant capacity were lower, depicting decreased defense ability against reactive oxygen species. Besides, a higher level of advanced glycation end-products was observed in AD patients. Depending on the treatment received, a distinct inflammatory and oxidative stress profile was observed: in Rivastigmine-treated group, IL6 levels were 47% lower than the average value of the remaining AD patients; homocysteine and glutathione reductase were statistically unchanged in the Rivastigmine and Donepezil–Memantine, respectively Donepezil group. Although the study is based on a limited population, the results could constitute the basis for further studies regarding the effect of medication and diet on AD patients.

KEYWORDS: Alzheimer’s disease; Biomarkers; Oxidative stress; Endothelial dysfunction; Inflammation

1. Introduction

Alzheimer’s disease (AD) is one of the most common progressive neurodegenerative disorders affecting the middle- to old-aged subjects. AD has multiple etiological factors including genetics or environmental conditions; people with an active lifestyle are more likely to slow down the progression of Alzheimer’s disease. The pathophysiological characteristics include extracellular formation of senile plaques (consisting of β-amyloid peptide), intracellular accumulation of aggregates of hyperphosphorylated tau protein, neuronal and synaptic loss, proliferation of astrocytes and activation of microglia (Walsh and Selkoe, 2007; Mao and Reddy, 2011; Feng and Wang, 2012).

The risk of AD increases significantly with age; among the hypotheses proposed to explain the causes of aging as well as AD, oxidative stress and mitochondrial oxidative damage play a central role.

Literature data suggests that brain tissues of AD patients are exposed to oxidative stress during the development of the disease. Oxidative stress – caused either by excess formation of reactive oxygen (ROS) or nitrogen species (NOS) or by reduction of endogenous antioxidant capacity – is associated with cellular damage, such as protein oxidation, lipid peroxidation, DNA oxidation, and glycoxidation. Several studies have identified many end-products of biomolecular peroxidation either in the brain tissue or in the blood circulation of AD patients – such as malondialdehyde (MDA), peroxynitrite, protein carbonyls (CRBNLs), advanced glycation end-products (AGEs) (Nunomura et al., 2006; Lovell and Markesbery, 2007; Kalaria et al., 2008; Mancuso et al., 2008;...
In addition, evidence was found that \( \beta \)-amyloid peptides (main components of amyloid plaques characteristic in AD) directly initiate free radical formation, mainly through activation of NADPH oxidase, thus leading to neuronal dysfunction and subsequent death (Hensley et al., 1995; Manzak et al., 2006; Lin and Beal, 2006; Hansson Petersen et al., 2008).

Although the classical theory states that \( \beta \)-amyloid peptide is deposited extracellularly, recent cellular and biochemical studies carried out in different models of AD and aging provided evidence that this peptide can also accumulate inside neurons, where it targets mitochondria, and contributes to the progression of the disease (Mao and Reddy, 2011).

Another mechanism involved in the induction of oxidative stress is mediated by N-methyl-D-aspartate (NMDA)-type glutamate receptors (NMDARs); under basal conditions, mild activation of synaptic NMDARs induces physiological ROS and RNS production, thus mediating normal signaling in neuronal function and survival. In AD brain tissue, the over activation of NMDARs causes excessive influx of \( \text{Ca}^{2+} \) ions, generating neurotoxic levels of ROS and RNS. Moreover, the \( \text{Ca}^{2+} \) ions activate neuronal and glial nitric-oxide synthase and the excess nitric oxide also contributes to the NMDARs over activation. NMDARs over activation, protein misfolding and mitochondrial dysfunction cumulatively induce neuronal damage and synaptic loss via excessive nitrosative and oxidative stress. (Brennan et al., 2009; Cho et al., 2009; Nakamura and Lipton, 2011).

Currently, there is no treatment to cure the AD patients; the existing treatments are designed mainly to alleviate the symptoms. Donepezil, one of the most commonly used chemical agents in the treatment of mild to moderate AD, which is a reversible acetylcholinesterase inhibitor, was shown to induce decreased oxidative stress in intra-cerebral streptozotocin-induced model of dementia in mice (Saxena et al., 2008). Memantine and derivatives, acting as blockers of NMDARs, could also improve the oxidative status of the patients (Lipton, 2006). Rivastigmine, acting as cholinesterase inhibitor, too, is used in the treatment of mild to moderate dementia of the Alzheimer's type and for mild to moderate dementia related to Parkinson's disease.

In this context, modern research is targeting the neuroprotective effects of antioxidants (such as tocopherols, ubiquinones and curcumin) in AD models (Dumont and Beal, 2011; Feng and Wang, 2012). Still, human studies provide inconsistent results; some studies report the delay of the disease progression upon administration of antioxidants, whereas in others no differences are observed between the patients who received or did not receive dietary supplements (Cummings, 2004; Chu, 2012). This could be due either to an individual mechanism of response to antioxidants (specific bioavailability and kinetics of the antioxidant studied), or to the multi-factorial and synergistic nature in the mode of action of antioxidants generally originating from vegetarian diet or plant dietary supplements (vitamin C, vitamin E, probucol, etc.) to the multi-factorial and synergistic nature in the mode of action of antioxidants (such as tocopherols, ubiquinones and curcumin). Treatment containing supplements (vitamin C, vitamin E, probucol, etc.) 2 weeks prior to the study.

The selected patients were divided into sub-groups depending on the therapy they received (Donepezil, Rivastigmine, Donepezil plus Memantine). All AD patients were under treatment for at least 24 months.

The study was approved by the Ethics Committee from the Carol Davila University of Medicine and Pharmacy and written informed consent was obtained from healthy subjects and legal caretakers of the patients.

Fasting venous blood samples were drawn from the subjects, for the evaluation of the general metabolic profile, as well as for the markers of oxidative stress, endothelial dysfunction and inflammation. Plasma samples were frozen and kept at –80°C for 1 month. All the samples (AD and controls) were analyzed at the same time for the markers of oxidative stress, endothelial dysfunction and inflammation.

2.2. Biochemical analysis

On serum samples separated from the total blood the general metabolic parameters were assessed using enzymatic commercial kits (Merck): fasting plasma glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, uric acid.

Serum samples were also analyzed for ADMA using an ADMA-ELISA kit (DLD), TNF-\( \alpha \) and IL-6 were measured using the IMMULITE® 1000TNF-\( \alpha \) and IMMULITE® 1000IL-6 assays (Siemens), respectively. Homocysteine was assessed using the FPIA IMX Homocysteine assay (Abbott).

The plasma level of AGES was evaluated using a spectrofluorimetric method, since AGES are fluorescent markers (Sebeková et al., 2001). Briefly, plasma samples were excited at 350 nm, fluorescent emission was registered between 350 nm and 550 nm, with the maximum at 450 nm. Fluorescence of PBS alone was subtracted from each data set.

GSH concentration, CRBNLs and TAC were determined as previously described (Veskuokis et al., 2008).

Table 1

Demographic and clinical characteristics of the study population (p values correspond to differences between AD patients and the control group).

<table>
<thead>
<tr>
<th>Demographic and clinical characteristics</th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (p = 0.408)</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Age (p = 0.095)</td>
<td>80.73 ± 5.70</td>
<td>78.07 ± 5.61</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donepezil (10 mg/day)</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>Rivastigmine (9.5 mg/day)</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Donepezil + memantine (10 mg + 20 mg/day)</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>Associated pathologies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes (p = 0.209)</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Type 1 diabetes (p = 0.483)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Cardiovascular disease (p = 0.525)</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Parkinson disease (p = 0.313)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hypertension (p = 0.853)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Osteoporosis (p = 0.717)</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>
Twenty microliters of plasma samples treated with 5% TCA were mixed with 0.8% (w/v) thiobarbituric acid (TBA) and then, the absorbance was read at 532 nm. The precipitation of the precipitate, an aliquot of the supernatant was reacted with an equal volumes of cold 5% (w/v) trichloroacetic acid to precipitate protein. After centrifugation of the supernatant, the absorbance was read at 520 nm.

Briefly, total antioxidant activity (TAC), expressed in mmol diphenyl-1-picrylhydrazyl (DPPH)/L, was determined by the DPPH spectrophotometric assay using stable DPPH radical as reagent. The plasma was mixed with PBS and DPPH, it was then incubated and centrifuged and the absorbance was read at 530 nm. Malondialdehyde level in plasma was determined based on the reaction with thiobarbituric acid (TBA) at 70 °C for 30 min. The sample was mixed with two volumes of cold 5% (w/v) trichloroacetic acid to precipitate protein. After centrifugation of the precipitate, an aliquot of the supernatant was reacted with an equal volume of 0.8% (w/v) TBA and then, the absorbance was read at 532 nm.

Protein carbonyls were determined based on the method of Patsoukis and Georgiou (2004). In this assay, 50 μL of 20% TCA (trichloroacetic acid) was added to 50 μL of plasma and this mixture was incubated on an ice bath for 15 min and centrifuged. The supernatant was discarded and 500 μL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2.5 N hydrochloric acid (HCl). The samples were incubated in the dark at room temperature for 1 h and were centrifuged. The supernatant was discarded and 1 mL of 10% TCA was added, vortexed and centrifuged. The supernatant was discarded and 1 mL of 1 mM 5,5-dithiobis-2 nitrobenzoate (DTNB). The samples were incubated in the dark at room temperature for 45 min and the absorbance was read at 412 nm. Each assay was performed in triplicate.

2.3. Statistical Analysis

Results are expressed as means ± standard deviation. For comparison among the groups we used Kolmogorov–Smirnov test followed by Mann–Whitney post hoc test. Bivariate correlation analysis (Spearman’s correlation coefficient) was performed to evaluate the interrelations between studied parameters using the Statistical Package for Social Sciences software (SPSS) version 15. Differences were considered significant for \( p < 0.05 \).

3. Results

3.1. Endothelial function parameters

Levels of ADMA, homocysteine, TNF-\( \alpha \) and IL6 are significantly increased in AD patients compared to controls (Fig. 1).

A positive significant correlation between the ADMA and homocysteine levels (\( \rho = 0.764, p = 0.017 \)) was found, with the two markers indicating the same type of cardiovascular disease (CVD) risk.

IL6 has been found 47% decreased in the Rivastigmine treated sub-group (\( p = 0.0001 \)) compared to the mean value for the remaining AD patients. In the Donepezil treated sub-group, TNF-\( \alpha \) values were 19% lower (\( p = 0.05 \)) compared to the mean value for the rest of the AD group. In the Donepezil + Memantine subgroup, homocysteine remained statistically unchanged compared with the controls, while in the two other medication sub-groups it was found statistically elevated (\( p < 0.05 \)) (Table 3).

3.2. Oxidative stress parameters for AD patients

TAC and GSH are statistically decreased by 28% and 21%, respectively, with respect to controls. Protein carbonyls were 38% increased, as well as AGE (33%), while MDA remained statistically unchanged (Fig. 2).

TAC was significantly decreased both in the Donepezil (\( p = 0.001 \)) and the Rivastigmine sub-group (\( p = 0.009 \)), GSH levels are statistically unchanged in the Donepezil treated patients compared to controls, while in the other two sub-groups, a significant reduction is observed (\( p = 0.012 \) for Rivastigmine and \( p = 0.047 \) for Donepezil + Memantine). Protein carbonyl increased significantly in all different sub-groups, compared to controls (\( p < 0.05 \)).

A positive correlation between TNF-\( \alpha \) and MDA levels (\( \rho = 0.831, p = 0.021 \)) within the control group, indicating that oxidative stress and inflammation are directly connected (see Table 4).

In the AD group a positive significant correlation was observed between TNF-\( \alpha \) and protein carbonyls level (\( \rho = 0.545, p = 0.044 \)), indicating that the impairment of the protein structure in oxidative stress conditions is associated with an increase of the inflammatory response.

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**Table 2**

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>AD patients (n = 21)</th>
<th>Control group (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donepezil treated-group</td>
<td>Rivastigmine treated-group</td>
</tr>
<tr>
<td>Fastin plasma glucose (mg/dl)</td>
<td>92.93 ± 15.73 (p = 0.226)</td>
<td>80.46 ± 5.94 (p = 0.925)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>192.75 ± 46.89 (p = 0.819)</td>
<td>213.00 ± 16.59 (p = 0.181)</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>122.30 ± 32.68 (p = 0.736)</td>
<td>137.60 ± 11.80 (p = 0.181)</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>41.35 ± 14.15 (p = 0.476)</td>
<td>46.60 ± 16.14 (p = 0.925)</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>4.54 ± 1.35 (p = 0.736)</td>
<td>3.38 ± 0.70 (p = 0.181)</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Parameters for the evaluation of the endothelial function and systemic inflammation in AD patients compared to controls.

Due to the small number of subjects enrolled in the groups, Rivastigmine group and 1 case in the Donepezil + Memantine groups – 2 cases of diabetes in the Donepezil group, 1 case in the vascular disease were found in both groups (AD and control). As for Parkinson, cases of hypertension, osteoporosis and cardiovascular disease that are generally known to modify the investigated parameters in the AD patients can be attributed to the disease/treatment. However, we consider that the interpretation of the results can be linked to AD and not to the associated pathologies.

Endothelial dysfunction and inflammation is the most frequent impairment characterizing aging patients (Puca et al., 2013; Vlassara et al., 2012; Bomboi et al., 2010). For the evaluation of endothelial dysfunction, ADMA and homocysteine were used, while IL6 and TNF-α levels depicted inflammation in the selected patients and controls. As for oxidative stress markers, we used MDA, CRBNLs and AGEs levels as main parameters to characterize the end-products of oxidative processes, and TAC and GSH levels to evaluate the individuals’ ability to control the oxidative processes.

ADMA antagonizes endothelium-dependent vasodilation and is recognized as a mediator molecule of the adverse vascular effects of many other factors, including oxidative stress. The effect of ADMA on cell metabolism is to reduce nitrogen oxide (NO) production through the competitive inhibition of nitric oxide synthase (NOS) and also to stimulate NOS-derived superoxide production by uncoupling of endothelial nitric oxide synthase (eNOS). ADMA can be catabolized to citruline and dimethylamine under the action of dimethylarginine dimethylaminohydrolases (DDAHs); the inhibition of this enzyme under the effect of TNF-α or oxidized LDL particles is associated with the increase of ADMA and a subsequent increase of CVD risk. Also, the increase in homocysteine can raise the risk of atherogenesis by direct inhibition of the activity of DDAH (Landim et al., 2009; Teerlink et al., 2009).

According to previous studies, the drugs currently used for the treatment of AD, besides their pharmacological effects, improve the biochemical changes associated with the development of the disease, including redox stress impairments. For example, Rivastigmine and Donepezil significantly reduced amyloid-β induced tyrosine nitration of hippocampal proteins, as marker of oxidative damage, in mice (Furukawa-Hibi et al., 2011). Donepezil has significant antioxidant activity (evaluated as MDA and GSH levels in brain tissue) in mouse streptozotocin-induced oxidative stress (Saxena et al., 2008). Memantine inhibits redox impairments (acting both at enzymatic and non-enzymatic levels) induced in rat brain tissue) in mouse streptozotocin-induced oxidative stress (Saxena et al., 2008). Memantine inhibits redox impairments (acting both at enzymatic and non-enzymatic levels) induced in rat models by methylmercury toxicants (Liu et al., 2013).

Fig. 2. Markers of oxidative stress for the AD patients compared to controls.

Table 3
Endothelial markers and systemic inflammation parameters in the different medication treatment-groups of AD patients.

<table>
<thead>
<tr>
<th>Endothelial function parameter</th>
<th>Groups</th>
<th>Donepezil (n = 8)</th>
<th>Rivastigmine (n = 5)</th>
<th>Donepezil + memantine (n = 8)</th>
<th>Control (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (μmol/L)</td>
<td></td>
<td>22.80 ± 7.61</td>
<td>21.67 ± 9.36</td>
<td>16.73 ± 2.92</td>
<td>14.42 ± 2.07</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td></td>
<td>34.33 ± 24.09</td>
<td>48.30 ± 23.49</td>
<td>47.90 ± 16.32</td>
<td>10.65 ± 4.56</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td></td>
<td>44.87 ± 19.41</td>
<td>22.80 ± 11.15</td>
<td>41.14 ± 28.91</td>
<td>10.7 ± 2.06</td>
</tr>
<tr>
<td>ADMA (μmol/ml)</td>
<td></td>
<td>1.25 ± 0.21</td>
<td>1.46 ± 0.33</td>
<td>1.51 ± 0.30</td>
<td>0.58 ± 0.27</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01, As compared to controls.

Table 4
Oxidative stress markers in the different medication treatment-groups of AD patients.

<table>
<thead>
<tr>
<th>Oxidative stress parameter</th>
<th>Groups</th>
<th>Donepezil (n = 8)</th>
<th>Rivastigmine (n = 5)</th>
<th>Donepezil + memantine (n = 8)</th>
<th>Control (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein carbonyls (nmol/mg protein)</td>
<td></td>
<td>0.54 ± 0.18</td>
<td>0.56 ± 0.16</td>
<td>0.56 ± 0.12</td>
<td>0.40 ± 0.08</td>
</tr>
<tr>
<td>TAC (mmol DPPH/L plasma)</td>
<td></td>
<td>0.57 ± 0.05</td>
<td>0.53 ± 0.10</td>
<td>0.62 ± 0.10</td>
<td>0.79 ± 0.18</td>
</tr>
<tr>
<td>GSH in plasma (μmol/L)</td>
<td></td>
<td>21.02 ± 3.48</td>
<td>16.65 ± 2.32</td>
<td>17.65 ± 1.94</td>
<td>23.34 ± 4.18</td>
</tr>
<tr>
<td>MDA (μg/mL)</td>
<td></td>
<td>0.52 ± 0.07</td>
<td>0.48 ± 0.09</td>
<td>0.46 ± 0.04</td>
<td>0.44 ± 0.13</td>
</tr>
<tr>
<td>AGE (fluorescence units)</td>
<td></td>
<td>333.28 ± 60.51</td>
<td>276.18 ± 40.27</td>
<td>331.72 ± 50.70</td>
<td>258.85 ± 29.97</td>
</tr>
</tbody>
</table>

* p < 0.05, As compared to controls.
endothelial function, through inflammation ignition, since the underlying pathologies were statistically matched between AD patients and controls. Different medication treatment had distinct inflammation triggering profiles: homocysteine and ADMA were moderately increased in the Donepezil subgroup, while the increase in IL-6 was pronounced. A different pattern is observed in the Rivastigmine treated patients (strong increase of the TNF-α and moderate increase of the IL6 levels), probably pointing to the fact that TNF-α might be a mediator of endothelial dysfunction in the Rivastigmine subgroup (Collins, 1993).

According to previously reported data, Donepezil and Rivastigmine have both anti-inflammatory effect, but in the case of Donepezil an anti-atherosclerotic outcome is described secondary to its known acetylcholinesterase inhibition effect (Inanaga et al., 2010; Nizri et al., 2008; Yoshiyama et al., 2010). These findings can be connected to our results, i.e. Donepezil treated patients are characterized by lower values for TC and LDL, respectively, compared to the Rivastigmine treated group (however not significantly different). Therefore, this could be an explanation for the fact that Donepezil treated patients have lower levels of TNF-α compared to Rivastigmine treated ones. For Rivastigmine, the anti-inflammatory effects is demonstrated in connection to the central nervous system; thus, Nizri et al. (2008) demonstrated that Rivastigmine decreased the reactivity of encephalitogenic T-cells and the production of pro-inflammatory cytokines (TNF-alpha, IFN-gamma and IL-17) without affecting IL-10 production. In cell line studies, Rivastigmine (1 μM), in combination with carbachol (10 μM), significantly decreased the release of nitric oxide, TNF-α, IL-1β and IL-6 from lipopolysaccharide-activated RAW 264.7 macrophages. Rivastigmine (1 and 2 mg) given rectally to rats with experimentally induced colitis also caused a dose-related reductino in the number of ulcers and area of ulceration, TNF-α levels and myeloperoxidase activity (Shifrin et al., 2013). Therefore Rivastigmine can act as an anti-inflammatory agent, but at different levels compared to Donepezil and its effects on the markers of atherosclerosis, if any, is more difficult to be pointed out.

Regarding the levels of IL6 in the two groups of AD patients, results could not be interpreted before since the coefficient of variation was very high for both groups. Due to the limited number of patients and the design of the study, we were not able to find out whether other mechanism of action is involved, leading to the obtained profile of inflammation in case of Donepezil and Rivastigmine groups.

According to literature reports (Selley, 2003; Arlt et al., 2008) the levels of ADMA and homocysteine are increased in plasma of AD patients. Our results confirmed these findings; we also pointed out that the Donepezil + Memantine treatment has the tendency to reduce the impairment of the homocysteine levels.

AGEs results from the nonenzymatic glycation and oxidation of proteins and lipids. Under normal physiological conditions AGEs are formed at a very slow rate in vivo. Their production, however, is accelerated in aging, in neurodegenerative disorders, inflammatory conditions, in cases of hyperglycemia and oxidative stress. Studies show that AGEs impair the vascular function by accumulating in the vessel wall and by quenching the nitric oxide released as endogenous vasodilatatory and anti-thrombotic molecule, thereby potentially impacting on vascular relaxation and function. AGE-bound to their specific receptors (RAGE) in the endothelium results in the production of reactive oxygen intermediates (Yan et al., 2003; Hörse et al., 2011). Therefore, AGE induce vascular pathologic changes through a direct and also through an indirect (oxidative stress) mechanism. Also, AGEs can be found in different types of food, especially in deep fried meat derivatives. Since the selected AD patients were on a diet that is not susceptible to have a high AGE content, we can exclude the exogenous intake of glycation products. Therefore, the measured AGE levels could give us information on the endogenous production. Results show that AGE is lower in the Rivastigmine treated patients.

Our study revealed the fact that Donepezil constitutes a type of AD treatment acting as GSH sparing, since the levels of this marker was higher in the Donepezil sub-group compared to the other two sub-groups. Also, Rivastigmine seems to inhibit the glycation, patients receiving this substance being characterized by lower levels of AGEs. All AD patients are characterized by the impairment of the endothelial function, thus being at risk of developing CVD disease. Interesting enough is the minimum effect of AD on MDA levels, in contrast all other oxidative stress parameters tested. One explanation could reside in the fact that the hydrosoluble antioxidant biomolecules are functionally impaired in AD patients, while the thio Barbarycin acid reactive species (TBARS), generally considered as MDA levels, are influenced to a lesser extent by the disease (Keller et al., 1997). Similar results were obtained by McGrath et al. (2001), who found in AD patients elevated levels of 4-hydroxy- nonenal, but not MDA or protein carbonyls. They considered this as an evidence of the importance of lipid peroxidation process in AD, the more so as the cognitive impairment correlates with the high level of 4-hydroxy- nonenal.

A limitation of the present study could be the small sample size. Based on that, the results of the present study could initiate future clinical trials evaluating the effect of different types of AD medication on endothelial dysfunction as well as redox stress markers in AD patients. A long-term periodical assessment of AD patients could elucidate the impact both of other underlying pathologies and therapy of treatment/diet on the inflammation and oxidative stress status of AD patients.

In conclusion, in AD patients oxidative stress, inflammation and endothelial dysfunction are elevated. Medical treatment administered to the patients, apart from alleviating the disease symptoms, seems to play also a distinct role in the inflammation and oxidative stress progression. Therefore, anti-oxidant supplementation and a special diet could additionally improve life quality in AD patients.

5. Conflict of Interest

The authors declare there are no conflicts of interest.

References

The amyloid beta-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. Proc. Natl. Acad. Sci. USA 105, 13145–13150.


