Platelet serotonin concentration and monoamine oxidase type B activity in female patients in early, middle and late phase of Alzheimer's disease

Mück-Šeler, Dorotea; Presečki, Paola; Mimica, Ninoslav; Mustapić, Maja; Pivac, Nela; Babić, Ana; Nedić, Gordana; Folnegović-Šmalc, Vera

Source / Izvornik: **Progress in Neuro-Psychopharmacology & Biological Psychiatry, 2009, 33, 1226 - 1231**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/10.1016/j.pnpbp.2009.07.004

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:454983

Mück-Šeler D., Presečki P., Mimica N., Mustapić M., Pivac N., Babić A., Nedić G., Folnegović-Šmalc V. (2009) ***Platelet serotonin concentration and monoamine oxidase type B activity in female patients in early, middle and late phase of Alzheimer's disease*****. Progress in neuro-psychopharmacology & biological psychiatry, 33 (7). pp. 1226-1231. ISSN 0278-5846**

http://www.elsevier.com/locate/issn/02785846

http://www.sciencedirect.com/science/journal/02785846

http://dx.doi.org/10.1016/j.pnpbp.2009.07.004

Platelet serotonin concentration and monoamine oxidase type B activity in female patients in early, middle and late phase of Alzheimer's disease

Dorotea Muck-Seler[a*#], Paola Presecki[b#], Ninoslav Mimica[c,d], Maja Mustapic[a], Nela Pivac[a], Ana Babic[a], Gordana Nedic[a], Vera Folnegovic-Smalc[c,d]

[a]Ruder Boskovic Institute, Division of Molecular Medicine, Bijenicka cesta 54, HR-10000 Zagreb, Croatia

[b]Psychiatric Hospital Sveti Ivan, Jankomir 11, HR-10090 Zagreb, Croatia
[c]Psychiatric Hospital Vrapce, University Department of Psychiatry, Bolnicka cesta 32, HR-10090 Zagreb, Croatia

[4]School of Medicine, University of Zagreb, Salata 3b, HR-10000 Zagreb, Croatia

[#] D Muck-Seler and [#]P Presecki equally contributed to this work.

Running title: Peripheral serotonergic markers in Alzheimer's disease

Corresponding author:

*Dorotea Muck-Seler, PhD, senior scientist

Laboratory of Molecular Neuropsychiatry, Division of Molecular Medicine

Rudjer Boskovic Institute, POBox 180 , HR-10002 Zagreb, Croatia

Tel: 385-1- 4571 207; Fax- 385-1- 4561 010; E-mail: seler@irb.hr

Conflict of Interest: There are no conflicts of interest for all authors.

Abstract

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder with unclear etiology. Cognitive impairment in AD might be associated with altered serotonergic system. The aim of the study was to determine platelet serotonin (5-HT) concentrations and platelet monoamine oxidase type B (MAO-B) activity in patients with different severity of AD. Platelet 5-HT concentrations and MAO-B activity were determined spectrofluorimetrically in 74 female patients with AD (NINCDS-ADRDA, DSM-IV-TR criteria), subdivided according to the Mini Mental State Examination (MMSE) scores in three groups with a) 23 patients in early (MMSE scores 19-24), b) 23 patients in middle (MMSE 10-18), and c) 28 patients in late (MMSE 0-9) phase of AD, and in 49 age-matched healthy women. Platelet 5-HT concentrations and MAO-B activity were similar between all patients with AD and healthy subjects, but were significantly lower in patients in the late phase of AD than in other phases of AD, and in healthy controls. The significant correlations were found between MMSE scores and platelet 5-HT concentrations, MAO-B activity and age. Lower platelet 5-HT concentration and MAO-B activity in the late phase of AD suggested that these markers might indicate severity and/or clinical progress of AD.

Key words: Alzheimer's disease, blood platelets, monoamine oxidase type B, phase of Alzheimer's disease, serotonin

Abbreviations: AD, Alzheimer's disease; ANOVA, One Way Analysis of Variance; HPLC, high pressure liquid chromatography; MAO, monoamine oxidase; MMSE, Mini Mental State Examination; NINDS-ADRDA, National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; PRP, Platelet rich plasma; 5-HT, serotonin; TPH, tryptophan hydroxylase

1. Introduction

Alzheimer's disease (AD) is a complex and progressive neurodegenerative disorder, with still unclear aetiology (Forsyth and Ritzline, 1998). The neurobiological background of AD includes the accumulation of amyloid plaques and neurofibrillary tangles in the brain, and dysfunction in the cholinergic function (Pakaski and Kalman, 2008). The alterations in the other neurotransmitter systems, especially serotonergic and dopaminergic, are also thought to be responsible for the cognitive deficits and the behavioral disturbances observed in patients with AD (Garcia-Alloza et al. 2005; Terry et al. 2008).

Serotonin (5-hydroxytryptamine, 5-HT) has an important role in the regulation of the synaptic function, neurite outgrowth, synaptogenesis and cell survival (Terry et al. 2008). It regulates different physiological functions (circadian rhythm, food intake, locomotion, thermoregulation, nociception, sexual activity), behaviors (Lucki, 1998), and cognitive functions such as learning and memory (Mattson et al. 2004). The decrease in the brain concentration of 5-HT and its main metabolites 5-hydroxyindoleacetic acid, the loss of serotonergic 5-HT1A receptors in the hippocampus and nuclei raphe (Kepe et al. 2006), and 5-HT2 receptors in cerebral cortex (Blin et al. 1993) were found in patients with AD. Genetic investigations suggested the role of serotonergic 5-HT2A and 5-HT2C receptors polymorphisms in the development of behavioral and psychological symptoms of AD (Pritchard et al. 2008). Recently, a beneficial effect of the additional treatment with selective 5-HT reuptake inhibitor, fluoxetine, on daily living and functioning was found in AD patients treated with rivastigmine (Mowla et al. 2007).

Blood platelets have been proposed as an easy obtainable limited peripheral model for some processes in the presynaptic (5-HT reuptake, monoamine oxidase type B /MAO-B/ activity) and postsynaptic (5-HT2A receptors binding) parts of the central serotonergic

neurons (Camacho and Dimsdale, 2000; Mendelson, 2000), and for the expression of the brain amyloid precursor protein (Cattabeni et al. 2004). The studies on platelet 5-HT concentration and platelet MAO-B activity in AD yielded inconsistent results. The increased (Meszaros et al. 1998), decreased Kumar et al. 1995) or unaltered (Mimica et al. 2005) platelet 5-HT concentrations, as well as increased (Adolfsson et al. 1980) or unaltered (Ahlskog et al. 1996) platelet MAO-B activity were observed in AD. Our recent studies showed that platelet MAO-B activity might be used as a biomarker for the presence of psychotic features in AD (Mimica et al. 2008), and for the early or late onset AD (Mimica et al. 2005).

Platelet 5-HT concentration (Mueller-Orlinhausen et al. 2004) and platelet MAO-B activity (Oreland, 2004) were found to be altered in different psychopathologies, behaviors, personality traits and particular neuro-psychiatric symptoms. In addition, platelet MAO-B activity is assumed to represent a biological marker for central 5-HT activity (Fahlke et al. 2002; Oreland, 2004). Therefore, the hypothesis of the present study was that these platelet biochemical markers would correlate with the cognitive indices of severity in AD. As both markers, platelet 5-HT concentration (Muck-Seler et al, 1996, 1999) and platelet MAO-B activity (Mimica et al. 2005; Pivac et al. 2005) are under influence of sex, and platelet MAO-B activity is affected by smoking (Oreland, 2004; Pivac et al. 2006a), the study included only female non-smoking subjects. The aim of the study was to determine platelet 5-HT concentrations and platelet MAO-B activity in patients with probable AD and control subjects, and in patients subdivided according to the severity of dementia into groups of patients in early, middle and late phase of AD.

2. Methods

2.1. Subjects

The study included 74 female patients with AD (mean age ± SD, 76.9 ± 9.9 years, range 53-96 years). All AD patients met the diagnostic criteria of probable AD according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (APA, 2000) and the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer‘s Disease and Related Disorders Association (NINDS-ADRDA; McKhann et al. 1984). The exclusion criteria for all patients were diagnoses of severe organic disease (cancer, heart disease, epilepsy, brain trauma), major functional psychiatric disorders (depression schizophrenia, mania), smoking, alcoholism and treatment with any medication known to influence platelet 5-HT concentration (selective serotonin reuptake inhibitors, tricyclic antidepressants) or platelet MAO-B activity (selegiline, iproniazid, tranylcypromine, vitamine B12). Patients were treated with acetylcholinesterase inhibitors like donepezil and memantine, but were medication-free for at least 1 week before blood sampling. The severity of dementia was assessed by Mini Mental Status Examination (MMSE; Folstein et al. 1975). The mean MMSE scores in all patients with AD were 11.7 ± 8.1. Patients were subdivided according to the MMSE scores into three groups: a) 23 patients in the early (MMSE 19-24) phase of AD, b) 23 patients in the middle (MMSE 10-18) phase of AD and c) 28 patients in the late (MMSE 0-9) phase of AD. MMSE was translated and validated to Croatian population. The permission and licensing are available on www.parinc.com.

Control group consisted of 49 carefully age- and sex- matched healthy older adults (mean age ± SD, 73.7 ± 8.8 years, range 56-89 years), recruited from the local senior centers. They were evaluated with a clinical interview to rule out for Axis I disorders, current and past medical status, and MMSE. Exclusion criteria were psychotropic medication mentioned above, MMSE less than 26 (indicating possible dementia) or any Axis I disorder.

Participants or their guardians gave informed consent. The study design was approved by the local Ethics Committee. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.2. Biochemical analysis

Blood samples (8 ml) were taken during routine laboratory tests from a jugular vein, after an overnight fasting, in a plastic syringe with 2 ml of acid citrate dextrose anticoagulant. Platelet rich plasma (PRP) was obtained after centrifugation of whole blood, and platelets were sedimented by further centrifugation of PRP. Platelet 5-HT concentration was determined by the spectrofluorimetric method, as previously described Muck-Seler et al. (1999), using Varian Cary Eclipse spectrofluorimeter. Platelet MAO-B activity was determined using kynuramine as a substrate by modification of the method of Krajl (1965), as previously described (Muck-Seler et al. 2002). Platelet protein levels were measured by the spectrofotometric method (Lowry et al. 1951). All biochemical determinations were performed by laboratory personnel who were blind to diagnosis.

2.3. Statistical analysis

The results were expressed as means ± standard deviations (SD). Due to the failed normality of the data, statistical evaluation was done using Kruskal–Wallis one-way analysis of variance (ANOVA) by ranks, followed by Mann-Whitney rank sum test for pair-wise comparisons. The Spearman Rank Order Correlation was used to assess the coefficient of correlation between age or MMSE scores and/or biochemical parameters. The significance was set to p values less than 0.05. The statistical analysis of the data was conducted with SigmaStat 3.1. (Jandell Scientific Corp., San Raphael, California, USA).

3. Results

Age of the patients with AD and control group did not differ significantly (*P=0.066*, Mann-Whitney t-test). There was a significant difference (H=16.0, df=2, P<0.001, Kruskal-Wallis ANOVA) in age between patients with AD subdivided according to the different phases of the disease. Mann-Whitney test showed that patients with AD in the late phase of AD were significantly (P<0.001) older than patients in the early and middle phase of disease, while patients in the middle phase of AD were significantly (P<0.001) older than patients in early phase of AD (Table 1).

As expected, MMSE scores (mean age ± SD) were significantly different (P<0.001, Mann-Whitney test) between control women (28.56 ± 1.94, range 26-30) and patients with AD (11.69 ± 8.14, range 1-24). The expected, significant (P<0.001, Mann-Whitney test) decline in the severity of dementia in AD was found in patients in different phases of AD, with the highest MMSE scores in early phase of AD and the lowest in the late phase of AD (Table 1).

Platelet 5-HT concentration (nmol/mg protein) was similar (P=0.697, Mann-Whitney test) in control women (0.81 ± 0.34) and all patients with AD (0.84 ± 0.43). When patients with AD were subdivided according to the different phases of AD, their platelet 5-HT concentration was significantly different (H=10.5, df=2; P=0.005, Kruskal-Wallis ANOVA). Mann-Whitney test revealed that patients in the late phase of AD had significantly lower platelet 5-HT concentration than healthy controls (P=0.015), and than patients in the early (P<0.011), or middle (P=0.012) phase of AD (Fig 1).

Platelet MAO-B activity (nmol 4OHQ/mg protein/h) did not differ significantly (P=0.568) between control women (30.64 ± 12.18) and all patients with AD (29.11 ± 11.69). Between patients in the early, middle and late phase of AD, platelet MAO-B activity was significantly different (H=9.08, df=2, P=0.011, Kruskal-Wallis ANOVA), as patients in the late

phase of AD had significantly (Mann-Whitney test) lower platelet MAO-B activity than healthy controls (P=0.038), than patients in the early phase of AD (P=0.003), and than patients in middle (P=0.033) phase of AD (Fig 2).

A significant positive correlation (Spearman's correlation) was found between MMSE scores and platelet 5-HT concentration (r=0.299, P=0.009), or MMSE scores and platelet MAO-B activity (r=0.327, P=0.005) in patients with AD. There was a significant (r= -0.460, P=0.000) negative correlation between age and MMSE scores in patients with AD, or between MMSE scores and age in control subjects (r=-0.568, P=0.000).

No significant correlation was observed between age and platelet 5-HT concentration (r=0.008, P=0.945), or age and platelet MAO-B activity (r=-0.162, P=0.168) in patients with AD, or between age and platelet 5-HT concentration (r=0.012, P=0.941), age and platelet MAO-B activity (r=-0.012, P=0.943), MMSE scores and platelet 5-HT concentration (r=-0.192, P=0.241), or MMSE scores and platelet MAO-B activity (r=0.153, P=0.351) in control subjects.

4. Discussion

The most important findings of the present study were 1) similar values of platelet 5-HT concentration or platelet MAO-B activity between all patients with AD and sex- and age-matched control subjects; and 2) significant relationship between the severity of dementia in AD and platelet 5-HT concentration and platelet MAO-B activity in female patients in the early, middle and late phase of AD. To the best of our knowledge, this is the first report of the significantly lower platelet 5-HT concentration and reduced platelet MAO-B activity in patients in the late phase of AD compared to patients in the early phase of AD.

In the present study we have found similar platelet 5-HT concentrations between patients with AD as a group and control subjects. This finding is in agreement with alike platelet 5-HT concentrations in patients with early or late onset AD (Mimica et al. 2005), but in contrast with decreased platelet 5-HT concentration in patients with AD (Kumar et al. 1995), and increased platelet 5-HT concentrations in demented patients with delusions (Meszaros et al. 1998) compared to 5-HT values in healthy controls. The explanation for the discrepancies between studies should be sought in different diagnoses (AD vs. dementia) and presence of delusions ((Meszaros et al. 1998), which were reported to increase platelet 5-HT concentration (Pivac et al. 2006b). In addition, previous studies (Kumar et al. 1995; Meszaros et al. 1998) included smaller number of patients and control subjects, the severity of AD was not specified, and different methods i.e. fluorimetric (Mimica et al. 2005) or HPLC (Kumar et al. 1995; Meszaros et al. 1998) were used for the determination of platelet 5-HT concentrations.

The most important finding of the presents study is a significantly different platelet 5-HT concentration in patients with AD subdivided according to cognitive impairment. A pronounced decrease in platelet 5-HT concentrations was found in the late stage of AD compared to platelet 5-HT values in the other phases of disease and to values in healthy controls. It is noteworthy that in agreement with our previous study (Mimica et al. 2005) platelet 5-HT concentrations in patients with early and middle phase did not differ from the 5-HT values in healthy controls.

The reasons leading to the alterations of platelet 5-HT in different phases of AD are still unknown. The reduced 5-HT concentration in the present and previous (Mimica et al. 2008) study in platelets, CSF (Tohgi et al. 1992) and brain (Garcia-Alloza et al. 2005) of patients in the late phase of AD would suggest the decrease in 5-HT synthesis. The main factors that influence 5-HT synthesis are plasma availability of its precursor tryptophan, and

the activity of the rate-limiting enzyme tryptophan hydroxylase (TPH). Since tryptophan is an essential amino acid, its plasma levels depend on the dietary intake and eating pattern that might occasionally be altered in AD (Ikeda et al. 2007). The literature data on tryptophan concentrations in body fluids and brain of patients with AD are inconsistent. The majority of studies have found low tryptophan concentrations in serum (Widner et al. 2000), plasma (Fekkes et al. 1998) and CSF (Tohgi et al. 1992) while other studies showed no alterations in tryptophan concentrations in plasma (Fonteh et al. 2007) and brain (Storga et al. 1996) in patients with AD. Low plasma tryptophan concentration might be also a consequence of the enhanced tryptophan degradation via the kynuramine pathway (Widner et al. 2000), since a dysregulation of both serotonergic and kynuramine pathways of tryptophan metabolism have been associated with pathophysiology of AD (Ruddick et al. 2006). In addition, the decline in the cognitive performance was observed after tryptophan depletion in healthy volunteers (Park et al. 1994), patients with AD (Porter et al. 2000) and patients with mild to moderate AD (Newhouse et al. 2002), confirming the role of 5-HT system in behavioral and cognitive changes in AD.

The TPH is a specific iron-containing enzyme involved in the biosynthesis of 5-HT. Reduced TPH activity was found in some brain areas of patients with AD (Gottfries, 1990) that might be associated with a decrease in 5-HT synthesis (Garcia-Alloza et al. 2005). It has been shown that tetrahydrobiopterin and folic acid are two cofactors necessary for TPH activity. The lack of the tetrahydrobiopterin in AD patients could alter THP activity and consequently decrease 5-HT synthesis. As a cofactor for nitric oxide synthase (Foxton et al. 2007), tetrahydrobiopterin is also involved in the production of free radicals within the cell. Since TPH activity is sensitive to reactive oxygen species, the tetrahydrobiopterin deficiency could also impair 5-HT synthesis through oxidative damage of TPH (Cash, 1998).

Since platelets do not synthesize 5-HT, their 5-HT concentrations depend on the 5-HT synthesis in the enterocromaffine cells of the gut, and on 5-HT active transport throughout the platelet membrane. Our results showing a reduced platelet 5-HT concentration in patients with AD, with most severe symptoms, compared to platelet 5-HT concentration in patients with the mild or moderate symptoms, might be a consequence of the different 5-HT uptake among groups. Although in the present study we did not determine platelet 5-HT uptake, several studies (Arora et al. 1991; Koren et al. 1993) have found reduced 5-HT uptake, expressed as a decrease in maximum number (Vmax) of 5-HT uptake sites in patients with AD compared to values in healthy control subjects. Since significantly lower 5-HT uptake or reduced Vmax were found in severely ill patients with AD compared to both, patients with mild AD, and to healthy controls (Arora et al. 1991), decreased platelet 5-HT concentration observed in our patients in the late phase of AD might be related to a reduced 5-HT uptake. Platelet 5-HT uptake depends on 5-HT transporter and 5-HT transporter (5-*HTT*) gene. However, a relationship between the changes in 5-HT uptake and 5-*HTT* gene is not clear, since no difference in the allelic distribution on the deletion/insertion polymorphism of the 5-*HTT* gene between patients with AD and non-demented controls (Zill et al. 2000), or a relationship between long allele of the 5-*HTT* gene and development of aggressive behavior in AD (Sukonick et al. 2001), was reported.

To avoid the complex gender-related differences observed in the clinical presentation (Grossi et al. 2005) and cognitive deficits (McPherson et al. 1999) in AD, and gender related difference in platelet 5-HT concentrations (Muck-Seler et al. 1996, 1999), in platelet MAO-B activity (Muck.Seler et al. 1991; Pivac et al. 2005), and in the rate of 5-HT synthesis in the brain (Nishizawa et al. 1997), the present study included only female patients with AD and control women.

It has been shown that CSF biomarkers of AD (beta-amyloid 1–42, total tau and tau phosphorylated at threonine 181) differ according to age, suggesting that AD pathology might differ between young and old patients (Bouwman et al. 2008, Meltzer et al. 1998). In our study patients and controls were matched for age and the age range was similar for all groups. Although the patients in the late stage of disease were the oldest, there was no significant correlation between age and platelet 5-HT concentrations, suggesting no influence of aging on platelet 5-HT values. In contrast to our results, a positive correlation between age and platelet 5-HT concentration, and increased platelet 5-HT concentration in older patients with dementia was found (Meszaros et al. 1998).

We have found similar platelet MAO-B activity between all patients with AD and corresponding control subjects. Previously it has been reported that platelet MAO-B activity was increased (Adolfsson et al. 1980; Mimica et al. 2005, 2008) or unaltered (Ahlskog et al. 1996; Meszaros et al. 1998) in patients with AD or dementia compared to values in healthy controls. These discrepancies might be explained by the different diagnostic entities across studies i.e. AD vs. dementia (Meszaros et al. 1998), and in the fact that our earlier studies (Mimice et al. 2005, 2008) included small number of both male and female AD patients in early and middle phase of AD, and that platelet MAO-B activity was compared with enzyme activity in control subjects who were considerably younger than patients with AD.

The results of the present study have shown significantly lower platelet MAO-B activity in patients in the late stage of AD compared to values in patients in the early and middle phase of AD and healthy controls, indicating a decline in platelet MAO-B activity associated to cognitive impairment in AD. The reduced platelet MAO-B activity in severely demented patients with AD agrees with the “MAO-B vulnerability hypothesis”, suggesting that platelet MAO-B activity is a genetic marker for the capacity of central 5-HT system (Oreland, 2004). The reason for the altered platelet MAO-B activity during the progress of

AD is at present unknown. There are several factors that might influence platelet MAO-B activity such as aging, sex, alcohol abuse, smoking, different medication and ethnicity (Oreland, 2004). Sex related difference in platelet MAO-B activity, i.e. higher enzyme activity in female than in male subjects, observed in healthy controls (Muck-Seler st al. 1991; Oreland, 2004; Pivac et al. 2005) were excluded since the present study included only female patients with AD and female control subjects. To avoid the possible effect of ethnicity or race (Sobell et al. 1997) on platelet MAO-B activity, the subjects in our study were ethnically uniform Caucasian of the Croatian origin. Psychotropic drugs like haloperidol (Meszaros et al. 1998), lithium (Oreland, 2004) or lamotrigine (Muck-Seler et al. 2008) were reported to increase platelet MAO activity, while antidepressant drug sertraline (Pivac et al. 2003), or vitamin B12 (Reglan et al. 1991) decreased platelet MAO-B activity. As our patients or control subjects did not receive haloperidol, lithium, lamotrigine, sertraline or vitamin B12, the possible effect of medication on platelet MAO-B activity could be excluded.

The data on the effect of older age on platelet MAO-B activity are inconsistent. Increased (Meszaros et al. 1998; Nicotra et al. 2004) or unchanged (Pivac et al. 2006a) platelet MAO-B activity was found in healthy controls during aging. Since MAO is a mitochondrial enzyme, it is possible that altered MAO-B activity in AD might be a consequence of the changes in the structure of the mitochondrial membrane occurring during aging (Nicotra et al. 2004). As no significant correlation between age and platelet MAO-B activity was found in our patients with AD or control subjects, and our patients with AD had lower and not higher platelet MAO-B activity, age of the patients presumably did not influence reduced platelet MAO-B activity found in severely ill AD patients.

It has been reported that lower platelet MAO-B activity might be induced by smoking (Oreland, 2004; Pivac et al. 2006a, 2007) and chronic alcohol abuse (Oreland, 2004). To exclude the effect of smoking, all female subjects included in the study were nonsmokers.

Regarding alcoholism, our (Pivac et al. 2005), as well as other (Anthenelli et al. 1998) data failed to find the lowering effect of chronic alcohol abuse on platelet MAO-B activity. In addition, platelet MAO-B activity did not differ between patients with dementia and patients with alcoholic dementia (Meszaros et al. 1998). Since our patients with the lowest platelet MAO-B activity were hospitalized and did not abuse alcohol in the controlled hospital environment, the possible lowering effect of alcohol on platelet MAO-B might be excluded.

The reason for the alterations in platelet MAO-B during the progress of AD may be related to transcriptional changes in MAO-B protein and/or to changes in the cell milieu causing manipulation of the enzyme activity (Oreland, 2004). Platelet MAO-B activity might be affected by genetic factors (Oreland 2004). Polymorphism in intron 13 of *MAO-B* gene does not alter platelet MAO-B activity (Jansson et al. 2005; Pivac et al. 2005, 2007). However, two *MAOA* gene haplotypes in the *MAOA* gene (Jansson et al. 2005), as well as polymorphism on the promoter region of the 5-*HTT* gene (Paaver et al. 2007) were shown to be associated with lower platelet MAO activity.

We have found a positive correlation between MMSE scores and platelet 5-HT concentration, and MMSE scores and platelet MAO-B activity in AD patients. The results indicate that more severe AD symptoms are associated with reduced platelet 5-HT concentration and lower platelet MAO-B activity. The mild but significant positive correlation between MMSE scores and platelet MAO-B activity is in line with the association between enzyme activity and severity of AD (Bongioanni et al. 1996). MMSE scores, indicating severity of dementia, were significantly negatively correlated with age in both control subjects and patients with AD, indicating decline in cognitive functions associated with aging.

The limitations of the present study were that the results are restricted to changes of platelet serotonergic variables, and that dietary habits of patients were not controlled. Namely,

plasma levels of tryptophan are under influence of diet, and reduced intake of tryptophan would result in reduced platelet 5-HT concentration. Platelet MAO-B activity might be reduced after B12 therapy in patients with dementia of AD type (Reglan et al. 1991). In contrast to the uncontrolled dietary habits of the patients in early and middle stage of AD, patients in the late stage of AD, who had the lowest platelet 5-HT concentration, and reduced platelet MAO-B activity, were hospitalized, and fed with the balanced hospital diet, and therefore the influence of the diet might be excluded. The advantages of the study were in ethnically uniform Caucasian sample, matched patients in terms of race, age, gender, phase of AD, in detailed clinical assessment, in the inclusion of sex- and age-matched control group, and in determination of two biological markers.

5. Conclusions

We have found a positive correlation between MMSE scores and platelet 5-HT concentration, and MMSE scores and platelet MAO-B activity in AD patients. The results indicate that more severe AD symptoms are associated with reduced platelet 5-HT concentration and lower platelet MAO-B activity. The mild but significant positive correlation between MMSE scores and platelet MAO-B activity is in line with the association between enzyme activity and severity of AD (Bongioanni et al. 1996). MMSE scores, indicating severity of dementia, were significantly negatively correlated with age in both control subjects and patients with AD, indicating decline in cognitive functions associated with aging.

6. Acknowledgements

The authors are indebted to the staff of the Psychiatric Hospital Vrapce. The study was supported by Croatian Ministry of Science, Education and Sport, grants numbers 098-0982522-2455, 098-0982522-2457 and 108-1081870-2418.

References

Adolfsson R, Gottfnes CG, Oreland L, et al. Increased activity of brain and platelet monoamine oxidase in dementia of Alzheimer type. Life Sci 1980; 7:1029-34.

Ahlskog JE, Uitti RJ, Tyce, et al. Plasma catechols and monoamine oxidase metabolites in untreated Parkinson's and Alzheimer's diseases. J Neurol Sci 1996; 136: 162-68.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. IV Edition. American Psychiatric Press, Washington, DC 2000.

Anthenelli RM, Tipp J, Li T-K, et al. Platelet monoamine oxidase activity in subgroups of alcoholics and controls: Results from the collaborative study on the genetics of alcoholism. Alcohol Clin Exp Res 1998; 22: 598-604.

Arora RC, Emery OB, Meltzer HY. Serotonin uptake in the blood platelets of Alzheimer's disease patients. Neurology 1991; 41: 1307-09.

Blin J, Baron JC, Dubois B, et al. Loss of brain 5-HT_2 receptors in Alzheimer's disease. Brain 1993; 116: 497-510.

Bongioanni P, Donato M, Castagna M, et al. Platelet phenolsulphotransferase activity, monoamine oxidase activity and peripheral-type benzodiazepine binding in demented patients. J Neural Transm 1996; 103: 491-501.

Bouwman FH, Schoonenboom NSM, Verwey NA, et al. CSF biomarker levels in early and late onset Alzheimer's disease. Neurobiol Aging doi:10.1016/j.neurobiolaging.2008.02.007.

Camacho A, Dimsdale JE. Platelets and psychiatry: lessons learned from old and new studies. Psychosom Med 2000; 62: 326-36.

Cash CD. Why tryptophan hydroxylase is difficult to purify: A reactive oxygen-derived species-mediated phenomenon that may be implicated in human pathology. Gen Pharmacol 1998; 30: 569-74.

Cattabeni F, Colciaghi F, Di Luca M. Platelets provide human tissue to unravel pathogenic mechanisms of Alzheimer disease. Prog Neuro-Psychopharmacol Biol Psychiatry 2004; 28: 763-70.

Fahlke C, Garpenstrand H, Oreland L, et al. Platelet monoamine oxidase activity in a nonhuman primate model of type two excessive alcohol consumption. Am J Psychiatry 2002; 159: 2107-09.

Fekkes D, Van Der Cammon TJM, Van Loon CMP, et al. Abnormal amino acid metabolism in patients with early stage Alzheimer dementia. J Neural Transm 1998; 105: 287-94.

Folstein M, Folstein SE, McHugh PR. "Mini-Mental State", a Practical Method for Grading the Cognitive State of Patients for the Clinician. J Psychiatr Res 1975; 12: 189-98.

Fonteh AN, Harrington RJ, Tsai A, et al. Free amino acid and peptide changes in the body fluids from Alzheimer's disease subjects. Amino Acids 2007; 32: 213-24.

Forsyth E, Ritzline PD. An overview of the etiology, diagnosis and treatment of Alzheimer Disease. Phys Ther 1998; 78: 1325-31.

Foxton RH, Land JM, Heales SJR. Tetrahydrobiopterin availability in Parkinson's and Alzheimer's disease; Potential pathogenic mechanisms. Neurochem Res 2007; 32: 751-56.

Garcia-Alloza M, Gil-Bea FJ, Diez-Ariza M. et al. Cholinergic -serotonergic imbalance contributes to cognitive and behavioural symptoms in Alzheimer's disease. Neuropsychologia 2005; 43: 442-49.

Gottfries CG. Disturbance of the 5-hydroxytryptamine metabolism in brains from patients with Alzheimer's dementia. J Neural Transm 1990; 30(Suppl): 33-43.

Grossi E, Massini G, Buscema M, et al. Two different Alzheimer diseases in men and women: Clues from advanced neural networks and artificial intelligence. Gender Med 2005; 2: 106-17.

Ikeda M, Brown J, Holland AJ, et al. Changes in appetite, food preference, and eating habits in frontotemporal dementia and Alzheimer's disease. J Neurol Neurosurg Psychiatry 2007; 73: 371-76.

Jansson M, McCarthy S, Sullivan PF, et al. MAOA haplotypes associated with thrombocyte-MAO activity. BMC Genetics 2005; 6: 46-54.

Kepe V, Barrio JR, Huang S-C, et al. Serotonin 1A receptors in the living brain of

Koren P, Diver-Haber A, Adunsky A, et al. Uptake of serotonin into platelets of senile dementia of the Alzheimer type patients. J Gereontol 1993, 48: B93-B96.

Krajl M. A rapid microfluorimetric determination of monoamine oxidase. Biochem Pharmacol 1965; 14: 1683-85.

Kumar AM, Sevush S, Kumar M, et al. Peripheral serotonin in Alzheimer's Disease. Neuropsychobiology 1995; 32: 9-12.

Lowry OH, Rosenbrough NS, Farr AC, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-75.

Lucki I. The spectrum of behaviors influenced by serotonin. Biol Psychiatry 1998; 44: 151-62.

Mattson MP, Maudsley S, Martin B. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci 2004; 27: 589-94.

McKhann G, Drachmann D, Folstein M, et al. Clinical diagnosis of alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 1984; 34:939-44.

McPherson S, Back C, Buckwalter JG, et al. Gender-related cognitive deficits in Alzheimer' disease. Int Psychogeriatr 1999; 11:117-22.

Meltzer CC, Smith G, DeKosky ST, et al. Serotonin in aging, late-life depression, and Alzheimer's disease: The emerging role of functional imaging. Neuropsychopharmacology 1998; 18: 407-30.

Mendelson SD. The current status of the platelet 5-HT2A receptors in depression. J Affect Disord 2000; 57: 13-24.

Meszaros Z, Borsiczky D, Mate M, Tarcali J, Szombathy T, Tekes K, et al. Platelet MAO-B activity and serotonin content in patients with dementia: effect of age, medication and disease. Neurochem Res 1998; 23: 863-8.

Mimica N, Muck-Seler D, Pivac N, et al. Platelet serotonin and monoamine oxidase activity in patients with early-onset and late-onset of Alzheimer's disease. Period Biol 2005; 107: 211-15.

Mimica N, Muck-Šeler D, Pivac N, et al. Platelet serotonin and monoamine oxidase in Alzheimer's disease with psychotic features. Coll Antropol 2008; 32 (Suppl 1): 119-22.

Mowla A, Mosavinasab M, Haghshenas H, et al. Does serotonin augmentation have any effect on cognition and activities of daily living in Alzheimer's dementia? A double-blind,

Muck-Seler D, Jakovljevic M, Deanovic Z. Platelet serotonin in subtypes of schizophrenia and unipolar depression. Psychiatry Res 1991; 38: 105-13.

Muck-Seler, D, Jakovljevic M, Pivac N. Platelet 5-HT concentrations and suicidal behavior in recurrent major depression. J Affec Disord 1996; 39: 73-80.

Muck-Seler D, Pivac N, Jakovljevic M. Sex differences, season of birth and platelet 5-HT levels in schizophrenic patients. J Neural Transm 1999; 106:337-47.

Muck-Seler D, Pivac N, Sagud M, et al. The effects of paroxetine and tianeptine on peripheral biochemical markers in major depression. Prog Neuro-Psychopharmacol Biol Psychiatry 2002; 26:1235-43.

Muck-Seler D, Sagud M, Mustapic M, et al. The effect of lamotrigine on platelet monoamine oxidase type B activity in patients with bipolar depression. Prog Neuro-Psychopharmacol Biol Psychiatry 2008; 32, 1195-8.

Mueller-Oerlinghausen B, Roggenbach J. Franke L. Serotonergic platelet markers of suicidal behavior – do they really exist? J Affect Disord 2004; 79, 13-24.

Newhouse P, Tatro A, Naylor M, et al. Alzheimer's disease, serotonin systems and tryptophan depletion. Am J Geriatr Psychiatry 2002; 10:483-84.

Nicotra A, Pierucci F, Parvez H et al. Monoamine oxidase expression during development and aging. Neurotoxicology 2004; 25: 155-65.

Nishizawa S, Benkelfat C, Young SN, et al. Difference between males and females in rates of serotonin synthesis in human brain. Proc Natl Acad Sci USA 1997; 94: 5308-13.

Oreland L. Platelet monoamine oxidase, personality and alcoholism: The rise, fall and resurrection. Neurotoxicology 2004; 25: 79-89.

Paaver M, Nordquist N, Parik J, et al. Platelet MAO activity and the 5-HTT gene promoter polymorphism are associated with impulsivity and cognitive style in visual information processing. Psychopharmacology 2007; 194: 545–54.

Pakaski M, Kalman J. Interactions between amyloid and chiolinergic mechanisms in Alzheimer's disease. Neurochem Int 2008; 53:103-11.

Park SB, Coull JT, McShane RH, et al. Tryptophan depeletion in normal volunteers produces selective impairments in learning and memory. Neuropharmacology 1994; 33: 575-88.

Pivac N, Muck-Seler D, Sagud M, et al. Long-term sertraline treatment and peripheral biochemical markers in female depressed patients. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2003; 27: 759-765.

Pivac N, Muck-Seler D, Kozaric-Kovacic D, et al. Platelet monoamine oxidase in alcoholism. Psychopharmacology 2005; 182: 194-6.

Pivac N, Kozaric-Kovacic D, Mustapic M, et al. Platelet serotonin in combat related posttraumatic stress disorder with psychotic symptoms. J Affect Disord 2006a; 93, 223-7.

Pivac N, Knezevic J, Mustapic M, et al. The lack of association between monoamine oxidase (MAO) intron 13 polymorphism and platelet MAO activity among men. Life Sci. 2006b; 79: 45-9.

Pivac N, Knezevic J, Kozaric-Kovacic D, et al. Monoamine oxidase (MAO) intron 13 polymorphism and platelet MAO-B activity in combat related posttraumatic stress disorder. J Affect Disord 2007; 103: 131-8.

Porter RJ, Lunn BS, Walker LL, et al. Cognitive deficit induced by acute tryptophan depletion in patients with Alzheimer's disease. Am J Psychiatry 2000; 157:638-40.

Pritchard AL, Harris J, Pritchard CW, et al. Role of 5-HT_{2A} and 5-HT_{2C} polymorphisms in behavioural and psychological symptoms of Alzheimer's disease. Neurobiol Aging 2008; 29:341-47.

Reglan B, Gottfries C-G, Oreland L. Vitamin B12- induced reduction of platelet monoamine oxidase activity in patients with dementia and pernicious anaemia. Eur Arch Psych Clin Neurosci 1991; 240: 288-91.

Ruddick JP, Evans AK, Nutt DJ, et al. Tryptophan metabolism in the central nervous system: medical implications. Expert Rev Mol Med 2006; 8: 1-27.

Sobell JL, Lind TJ, Hebrink DD, et al. Screening the monoamine oxidase B gene in 100 male patients with schizophrenia: a cluster of polymorphisms in African-American but lack of functionally significant sequence changes. Am J Med Genet B 1997; 74: 44-9.

Storga D, Vrecko K, Birkmayer JGD, et al. Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients. Neurosci Lett 1996; 203:29-32.

Sukonick DL, Pollock BG, Sweet RA, et al. The 5-HTTPR*S/*L polymorphism and aggressive hebavior in Alzheimer's disease. Arch Neurol 2001; 58:1425-28.

Terry AV, Buccafuscoa JJ, Wilsona C. Cognitive dysfunction in neuropsychiatric disorders: Selected serotonin receptor subtypes as therapeutic targets. Behav Brain Res 2008*;* 195: 30-8.

Tohgi H, Abe T, Takahashi S, et al. Concentrations of serotonin and its related substances in the cerebrospinal fluid in patients with Alzheimer type dementia. Neurosci Lett 1992; 141: 9-12.

Widner B, Leblhuber F, Walli J, et al. Tryptophan degradation and immune activatop in Alzheimer's disease. J Neural Transm 2000; 107: 343-53.

Zill P, Padberg F, de Jonge S, et al. Serotonin transporter (5-HTT) gene polymorphism in psychogeriatric patients. Neurosci Lett 2000; 284:113-5.

Legend to Figures

Fig. 1. Platelet serotonin (5-HT) concentrations in female patients with early, middle and late phase of the Alzheimer's disease (AD) and in healthy controls. Each column represents mean ± SD. Number of subjects is given in parenthesis.

* P <0.015 vs. healthy controls; ** P =0.012 vs. middle phase; *** P=0.011 vs. early phase of AD (Kruskal Wallis ANOVA on ranks and Mann-Whitney t-test)

Fig. 2. Platelet monoamine oxidase type B (MAO-B) activity in female patients with early, middle and late phase of the Alzheimer's disease (AD) and in healthy controls. Each column represents mean ± SD. Number of subjects is given in parenthesis.

* P =0.038 vs.healthy controls; ** P =0.033 vs. middle phase; *** P=0.003 vs. early stage of AD (Kruskal Wallis ANOVA on ranks and Mann-Whitney t-test)

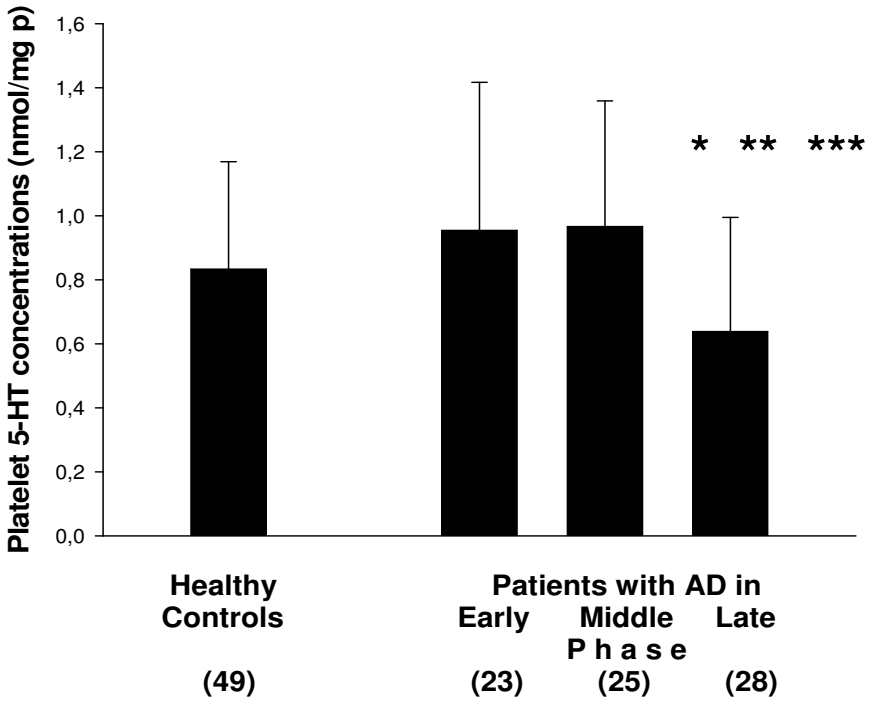
Platelet 5-HT concentrations (nmol/mg p)
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
* ** ***
Healthy Controls
(49)
Patients with AD in
Early
Middle
Late
P h a s e
(23)
(25)
(28)

Figure 1.

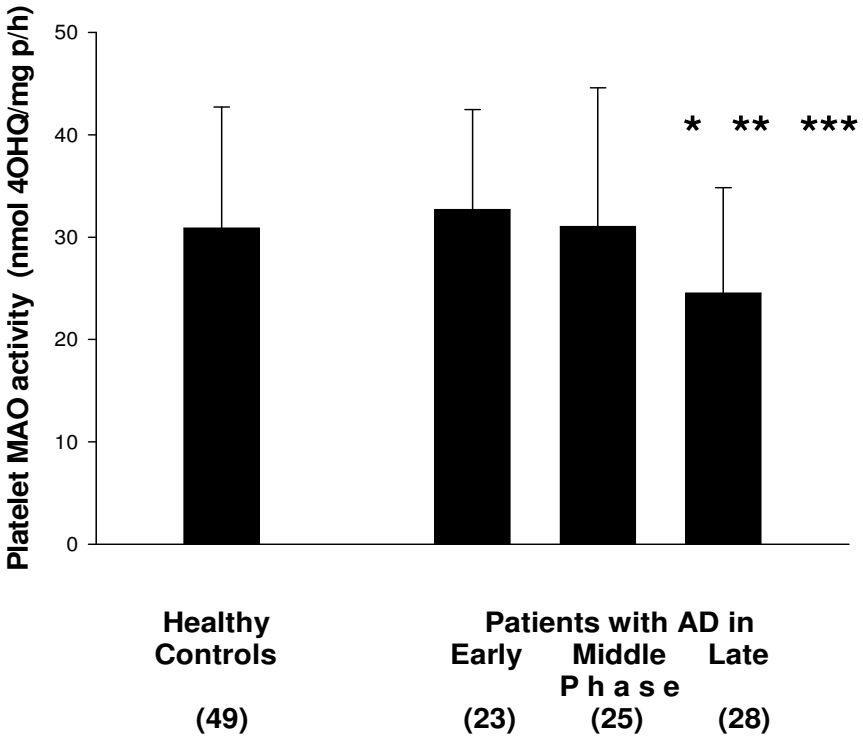
Platelet MAO activity (nmol 4OHQ/mg p/h)
50
40
30
20
10
0
* ** ***
Healthy Controls
(49)
Patients with AD in Early Middle Late Phase
(23)
(25)
(28)

Figure 2.

Table 1. Demographic data of patients with Alzheimer's disease (AD) in different stage of disease. Age and Mini Mental Status Examination (MMSE) are represent as mean ± SD.

	Patients with AD		
	Early phase	Middle phase	Late phase
Number of patients	23	23	28
Age (years)	70.9 ± 10.2	77.0 ± 8.8*	82.0 ± 8.1** ***
range	59-91	56-91	57-96
MMSE (scores)	20.8 ± 1.3	14.2 ± 2.3#	2.2 ± 1.9 #
range	19-24	10-18	0-9

*P<0.037 vs. early phase; **P<0.015 vs. middle phase; ***P<0.001 vs. early phase;

# P<0.001 vs. all other phases (Kruskal Wallis ANOVA on ranks and Mann-Whitney t-test)