Clinical Biochemistry 47 (2014) 51–53

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Alzheimer and platelets: Low-density platelet populations reveal increased serotonin content in Alzheimer type dementia

Micha Milovanovic [a,b,*], Kristoffer Eriksson [a], Bengt Winblad [c], Staffan Nilsson [d], Tomas L. Lindahl [e], Claes Post [e], Petter Järemo [a]

[a] Department of Internal Medicine, Vrinnevi Hospital, Norrköping, Sweden
[b] Department of Social and Welfare, Linköping University, Linköping, Sweden
[c] Department of Neurobiology, Care Sciences and Society (NVS), KI Alzheimer Disease Research Center, Karolinska Institutet, Huddinge, Sweden
[d] Department of Medical and Health Sciences, Division of Community Medicine/General Practice, Linköping University, Linköping, Sweden
[e] Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form 5 July 2014
Accepted 8 July 2014
Available online 17 July 2014

Keywords:
Alzheimer's disease
Fibrinogen
Platelets
Platelet activity
Platelet density
Platelet heterogeneity
Serotonin

ABSTRACT

Introduction: Alzheimer's disease (AD) is a progressive form of dementia characterized by an increase in the toxic substance β-amyloid in the brain. Platelets display a substantial heterogeneity with respect to density. They further contain a substantial amount of β-amyloid precursor protein. Platelets take up and store serotonin (5-HT) that plays an important role in the pathogenesis of severe depression. The current study aims to investigate platelet serotonin content in different platelet density populations.

Material and methods: The study involved 8 patients (age 70 ± 8 (SD) years) (3 females/5 males) with moderate AD. 6 healthy elderly subjects (age 66 ± 9 (SD) years) (3 females/3 males) served as controls. The platelet population was divided into 17 subpopulations according to density, using a linear Percoll™ gradient. Platelets were counted in all fractions. After cell lysis an ELISA technique was employed to determine the 5-HT content in each platelet subfraction.

Results: The two study groups did not differ significantly regarding platelet distribution in the gradients, but AD sufferers have a significantly higher 5-HT content ($p < 0.05$) in the lighter platelet populations.

Discussion: AD-type dementia proved to be associated with lighter platelets containing more 5-HT. It is possible that platelets from AD patients release less 5-HT. It is speculated that AD synapses are affected in a manner comparable to platelets, which could explain why 5-HT reuptake inhibitors are less effective in AD dementia.

Introduction

Alzheimer's disease (AD) is a progressive form of dementia having increasing prevalence due to an aging population. The condition is characterized by an increase of β-amyloid plaques in the brain [1]. Platelets are important providers of both β-amyloid and β-amyloid precursor protein [2] suggesting that they are involved in AD pathophysiology [3,4]. Malfunctioning platelets are a feature of progressive AD [5] and, in dementia, low-density platelet populations display low *in vivo* activity [6]. Platelets do not synthesize serotonin (5-HT) [7] but they do accumulate the molecule in a manner comparable to serotonergic neurons [8,9]. 5-HT is stored in platelet dense granules and is released upon activation [10]. Sporadic reports have described how advanced AD is characterized by less circulating 5-HT derived from human platelets [11]. A previous report determined intracellular 5-HT in AD platelets showing reduced platelet 5-HT content [12]. In contrast, other scientists have published evidence indicating that platelet 5-HT content is increased in AD [13]. A common feature among AD patients is depression, that constitutes a major clinical problem. It occurs more frequently in early AD [14,15]. Contemporary medication of depressive symptoms in AD most commonly makes use of 5-HT re-uptake inhibitors. These drugs increase 5-HT concentration in the synaptic cleft. The treatment has little effect on depression in AD dementia, however [16]. The current work examines aspects of platelet features in AD *versus* control individuals with respect to density subpopulations and 5-HT [17–19]. Platelets were divided into density fractions using a linear Percoll™ (a density medium) gradient. Each subfraction was characterized further. We have shown previously that high density platelets circulate "more activated", as estimated from surface bound fibrinogen [19]. In health they also contain less P-selectin, as a sign of *ex vitro* platelet α-granule release. Low-density platelets circulate as "more activated" as well. In contrast, compared to high-density platelets, they contain more P-selectin and less dense bodies [18]. Platelets are

* Corresponding author at: Department of Internal Medicine, The Vrinnevi Hospital, SE-601 82 Norrköping, Sweden. Tel./fax: +46 700896320.
E-mail address: micha.milovanovic@liu.se (M. Milovanovic).

http://dx.doi.org/10.1016/j.clinbiochem.2014.07.007
0009-9120/

believed to be involved both in AD pathogenesis and in 5-HT metabolism. Depressive symptoms are a serious problem in AD-type dementia. In consequence, this work has been devoted to platelet 5-HT in dementia. We did in particular investigate subjects having moderate AD with respect to dissimilarities of platelet 5-HT content of platelet density subfractions.

Methods

Research subjects

The Institutional Review Board of Linköping University Hospital permitted the current study. All participants have signed an informed consent. 8 patients (age 70 ± 8 (SD) years) (3 females/5 males) with moderate AD were engaged. Geriatricians diagnosed AD based on DSM-IV criteria. Computerized axial tomography excluded brain tumors and/or previous major cerebral infarctions. No other exclusion criteria were applied. 6 healthy elderly subjects (age 66 ± 9 (SD) years, 3 females/3 males) free from obvious memory problems served as controls. As expected AD subjects to a higher extent were on acetylcholinesterase treatment. Otherwise, the groups were similar with respect to clinical and demographic characteristics (data not shown). The unpaired two-tailed Student's *t*-test was used for statistical evaluation. *P*-values < 0.05 were considered statistically significant difference.

Laboratory investigations

Venous blood (7.5 mL) was anticoagulated with 2.5 mL 0.129 M disodium citrate. A linear Percoll™ (GE Healthcare Bio-Sciences AB, Sweden) gradient was used to separate platelets according to density [17–19]. The following substances were mixed in order to form the two Percoll™ solutions (1.09 and 1.04 kg/L) employed to manufacture the gradient:

Percoll™ solutions	1.09 (kg/L)	1.04 (kg/L)
Percoll™	32.84 (g)	8.88 (g)
H_2O	11.42 (g)	19.14 (g)
Platelet-inhibitory solution	4.50 (g)	3.00 (g)

The platelet inhibitory solution was made by mixing equal amounts of the following:

1. 0.15 mol/L Na_2 citrate* and 0.13 mol/L Na_3 EDTA* (pH 7.4 at 25 °C).
2. 0.001 g/L prostaglandin E_1* and 1 mL 95% ethanol in H_2O.
3. 2.7 mmol/L theophylline* dissolved in 0.15 mol/L TRIS chloride buffer (pH 7.4 at 25 °C).

*The substance was purchased from Sigma-Aldrich, Missouri, U.S.A.

With the aid of a two-chamber gradient maker, linear gradients covering a density span of 1.09 to 1.04 kg/L were constructed in 50 mL test tubes. 7.63 g of the 1.09 kg/L Percoll™ mixture was layered in the bottom of the test tube. Then, 13.08 g of the 1.09 kg/L and 12.48 g of the 1.04 kg/L Percoll™ solutions were poured into the two-chamber gradient maker to form the gradient. Subsequently, 10 mL citrate anticoagulated whole blood was cautiously layered on top of the gradient. The tube was centrifugated at 2767 *g* for 1 1/2 hours. Thereafter, the bottom of the test tube was pierced and allowed the gradient to be separated by gravity into 17 different density fractions [19]. In this way, each fraction holds about 2 mL of the test tube content. In all fractions, platelet counts were determined electronically. Spontaneous platelet bound fibrinogen was measured with a Beckman Coulter EPICS XL-MCL Flow Cytometer (Beckman Coulter, Inc., California, U.S.A.) [18–20]. In short, a FITC-conjugated chicken antihuman fibrinogen polyclonal antibody (Diapensia, Linköping, Sweden) distinguished membrane-bound fibrinogen. Platelets were identified with a PE-conjugated antibody against GPIb (Dako AS, Copenhagen, Denmark). As no agonist was used platelet-bound fibrinogen in this setting reflects platelet activity *in vivo*. Subsequently, all density subfractions were lysed with a detergent (Triton X-100 final concentration 1%) (Sigma-Aldrich, Missouri, U.S.). Cell fragments were detached by centrifugation at 2000 *g* for 10 min. Finally, in all fractions an ELISA kit (R&D, UK) was employed to determine platelet 5-HT content. In the ELISA all samples were analyzed in duplicate.

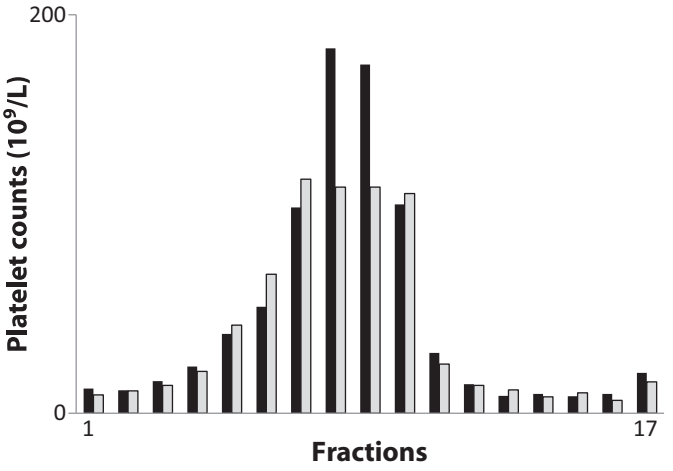

Fig. 1. The distribution of platelets ($\times 10^9$/L) (mean values) in the gradient for Alzheimer patients ($n = 8$) and controls ($n = 6$). The black bars denote Alzheimer patients. Fraction no. 1 holds platelets having the highest density.

Results

Figs. 1–3 compare the characteristics of platelet subpopulations of the eight AD subjects *versus* the corresponding measurements of the six controls. Fig. 1 shows the platelet distribution in the 17 density fractions. There were no statistical differences between AD individuals and controls. Fig. 2 displays 5-HT content in all density fractions, showing that lighter platelets (fractions nos. 8/10-13) contained significantly more 5-HT ($P < 0.05$) in the AD group. Fig. 3 compares for all subfractions platelet *in vivo* activity, *i.e.* spontaneous platelet fibrinogen binding. There was a tendency that lighter platelets of AD sufferers express less platelet-bound fibrinogen. In this setting, however, the differences were statistically insignificant.

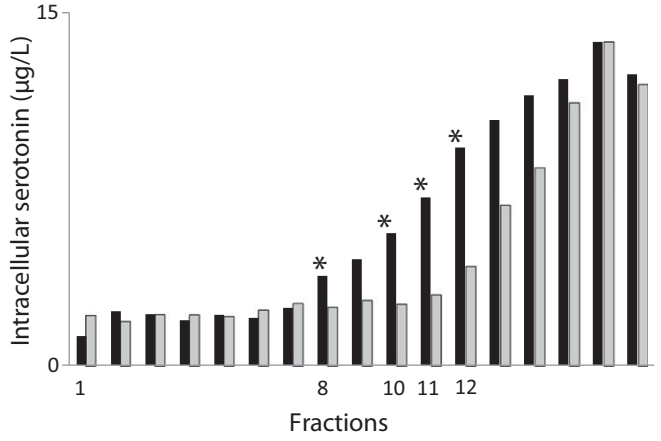

Fig. 2. Serotonin (µg/L) (mean values) content of the different density fractions for Alzheimer's dementia (black bars) ($n = 8$) and controls ($n = 6$). Fraction no. 1 contains the heaviest platelets. * denotes statistical significance ($p < 0.05$).

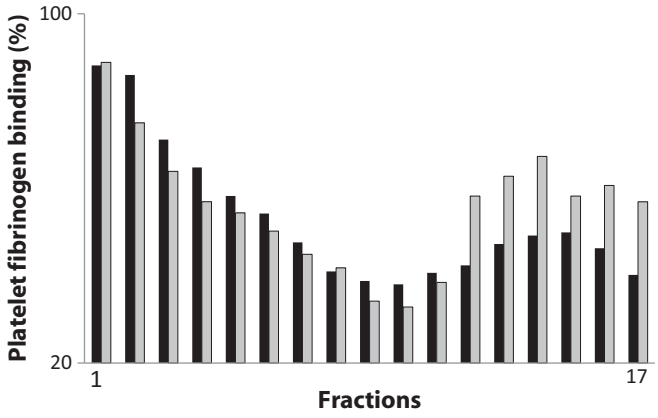

Fig. 3. Platelet fibrinogen binding (%) (mean values) in subjects with moderate Alzheimer's disease ($n = 8$) and controls ($n = 6$). The black bars illustrate results for Alzheimer demented subjects. Platelets of fraction no. 1 have the highest density.

Discussion

The present study shows that AD-type dementia is characterized by substantial modification of platelet 5-HT content, in that lighter AD platelets (fractions nos. 8/10-13) contain significantly more 5-HT (Fig. 2). Previous studies report less 5-HT in AD platelets [11,12], while another study takes the contrary view [13]. Obviously, the current data are in keeping with the latter work. It is conceivable that increased intracellular 5-HT reflects depressed *in vivo* activation in AD. The current study convinces us that platelet fractions (nos. 8/10-13) release less 5-HT in disease. Earlier work at our laboratory indicated that low-density platelets contain fewer dense granules [18]. That disagrees with the present findings. One possible explanation is that each dense granule contains more 5-HT in AD dementia. It may also be hypothesized that 5-HT has other storage locations in human platelets. Our research group has studied red cells in AD and found them to be damaged [21]. AD also modifies circulating granulocytes [22]. Platelet alterations are a feature of the disease [23], which is characterized by low-density platelets circulating less activated [6]. That is in keeping with this work. One might postulate that elevated platelet 5-HT content is a sign of less spontaneous platelet activity with less 5-HT release in AD dementia. It may also indicate cell damage. Of necessity sample size in this work was low. It is a major caveat but all subjects have been evaluated extensively with respect to platelet characteristics. Some confounders exist, in particular were AD sufferers frequently treated with acetyl- cholinesterase inhibitors. It is generally agreed that depression in AD represents a challenge [16]. Today's drugs that prevent 5-HT uptake in the synapses are frequently used as a therapeutic option in AD dementia. In analogy to platelet behavior it can be postulated that neurons release less 5-HT in AD. This may reduce the amount of 5-HT in the synaptic cleft, which would explain the lower effectiveness of anti-depressive drugs in dementia. In consequence, platelet 5-HT behavior may be important in AD as a potential biomarker both AD disease progression and SSRI response indicator. This requires further research.

Conflict of interest

Professor T.L. Lindahl owes Diapensia HB. The other authors declare no conflict of interest.

Acknowledgements

Funding from the Ahlen's Foundation and from the Gun and Bertil Stohne's Foundation made this work possible. The Magnus Bergvall's Foundation, the “Stiftelsen för Gamla Tjänarinnor”, and the Swedish Alzheimer Foundation also made valuable contributions to the research funding. Grants from the Hultman's foundation are greatly appreciated. The study was also supported by the Swedish Board for Health and Welfare and Pfizer AB, Sweden.

References

[1] Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001;81:741–66.
[2] Baskin F, Rosenberg RN, Iyer L, Hynan L, Cullum CM. Platelet APP isoform ratios correlate with declining cognition in AD. Neurology 2000;54:1907–9.
[3] Padovani A, Pastorino L, Borroni B, Colciaghi F, Rozzini L, et al. Amyloid precursor protein in platelets — a peripheral marker for the diagnosis of sporadic AD. Neurology 2001;57:2243–8.
[4] Padovani A, Borroni B, Colciaghi F, Pettenati C, Cottini E, et al. Abnormalities in the pattern of platelet amyloid precursor protein forms in patients with mild cognitive impairment and Alzheimer disease. Arch Neurol 2002;59:71–5.
[5] Wanga RT, Jina D, Lia Y, Liangc QC. Decreased mean platelet volume and platelet distribution width are associated with mild cognitive impairment and Alzheimer's disease. J Psychiatr Res 2013;47:644–9.
[6] Järemo P, Milovanovic M, Buller C, Nilsson S, Winblad B. Platelet heterogeneity and Alzheimer's disease: low density platelets populations demonstrate low *in vivo* activity. Platelets 2012;23:116–20.
[7] McNicol A, Israels SJ. Platelet dense granules: structure, function and implications for haemostasis. Thromb Res 1999;95:1–18.
[8] Paasonen MK. Platelet 5-hydroxytryptamine as a model in pharmacology. Ann Med Exp Biol Fenn 1968;46:416–22.
[9] Sneddon JM. Blood platelets as a model for monoamine-containing neurones. Prog Neurobiol 1973;1:151–98.
[10] De Clerck F, Xhonneux B, Leysen J, Janssen PA. Evidence for functional 5–HT2 receptor sites on human blood platelets. Biochem Pharmacol 1984;33:2807–11.
[11] Muck-Šeler D, Presecki P, Mimica N, Mustapic M, Pivac N, et al. Platelet serotonin concentration and monoamine oxidase type B activity in female patients in early, middle and late phase of Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 2009;33:1226–31.
[12] Kumar AM, Sevush S, Kumar M, Ruiz J, Eisdorfer C. Peripheral serotonin in Alzheimer's disease. Neuropsychobiology 1995;32:9–12.
[13] Meszaros Z, Borsiczky D, Mate M, Tarcali J, Szombathy T, 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.
[14] Fisher P, Simanyi M, Danielczyk W. Depression in dementia of the Alzheimer type and in multi-infarct dementia. Am J Psychiatry 1990;147:1484–7.
[15] Komahashi T, Ohmori K, Nakana T, Fujinuma H, Higashimoto T, et al. Epidemiological survey of dementia and depression among the aged living in the community in Japan. Jpn J Psychiatry Neurol 1994;48:517–26.
[16] Banerjee S, Hellier J, Dewey M, Romeo R, Ballard C, et al. Sertraline or mirtazapine for depression in dementia (HTA-SADD): a randomised, multicentre, doubleblind, placebo-controlled trial. Lancet 2011;378:403–11.
[17] Järemo P. Computerised method for recording platelet density distribution. Eur J Haematol 1995;54:304–9.
[18] Milovanovic M, Lysen J, Ramström S, Lindahl TL, Richter A, et al. Identification of low-density platelet populations with increased reactivity and elevated alpha granule content. Thromb Res 2003;111:75–80.
[19] Milovanovic M, Lotfi K, Lindahl T, Hallert C, Järemo P. Platelet density distribution in essential thrombocythemia. Pathophysiol Haemost Thromb 2010;37:35–42.
[20] Järemo P, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. J Int Med 2002;252:233–8.
[21] Järemo P, Milovanovic M, Nilsson S, Buller C, Post C, et al. Alzheimer's disease is characterized by more low-density erythrocytes with increased volume and enhanced β amyloid x-40 content. J Intern Med 2011;270:489–92.
[22] Järemo P, Milovanovic M, Buller C, Nilsson S, Winblad W. Alzheimer's disease and granulocyte density diversity. Eur J Clin Invest 2013;43:545–8.
[23] Järemo P, Milovanovic M, Buller C, Nilsson S, Winblad B. P-selectin paradox and dementia of the Alzheimer type: circulating P-selectin is increased but platelet-bound P-selectin after agonist provocation is compromised. Scand J Clin Lab Invest 2013;73:170–4.