

changes in membrane phospholipids and alterations in signal transduction mechanisms (6-19). However, little is known about AD platelets in terms of activation responses such as their ability to adhere, undergo shape change, develop stickiness, bind adhesive proteins, aggregate and secrete granule contents.

In this manuscript we report findings from an ongoing study on platelets from patients with AD. Results demonstrate that alterations in membrane fluidity, biochemical constituents, and signal transduction mechanisms in AD platelets are not sufficiently different from those of cells from normal donors to use as diagnostic tools to monitor the onset or severity of AD. Compromised response of AD platelets to the action of agonists can be detected, however, and may be related to a factor present in plasma of patients with AD.

METHODS

Fura-2 acetoxymethylester (Fura-2), Fura-free acid, 1,6-diphenyl-1,3,5-hexatriene (DPH) and 2,7-bis-(carboxyethyl)-5(6) carboxyfluorescein-acetoxymethylester (BCECF) were obtained from Molecular Probes, Inc. (Eugene OR). ^{14}C -arachidonic acid and ^{14}C -5-hydroxytryptamine (5-HT, serotonin) were purchased from NEN (Boston MA). Arachidonic acid was from NuChek Prep (Elysian MN). Injectable adrenaline (epineprine) and thrombin were from Parke-Davis (Detroit MI). Other chemicals used in this study were obtained from Sigma Chemical Co (St. Louis MO).

Preparation of platelets: Blood was obtained from human volunteers (30-50 years of age, male and female) who had abstained from anti-inflammatory drugs, such as aspirin, for at least two weeks prior to the study. The blood was drawn into sterile plastic tubes containing a 9:1 ratio of blood to citrate-citric acid-dextrose (CCD, 0.1 M citrate, 7 mM citric acid, 0.14 M dextrose, pH 6.5). Blood also was obtained from Alzheimer's patients (60 years of age or older, male and female). All patients were diagnosed as having probable dementia of the Alzheimer type on the basis of clinical evaluation by the

physicians in the Department of Neurology, University of Minnesota Hospitals and Clinics. Citrated blood was centrifuged at 100 g for 20 min at room temperature to obtain platelet-rich plasma (PRP). To obtain washed platelets PRP was diluted 1:1 with CCD. Platelets were sedimented by centrifugation at 200 g for 20 min at room temperature. The supernatant was removed and the platelet pellet resuspended in 9 parts Hanks Balanced Salt Solution (HBSS) to 1 part CCD supplemented with adenosine (5 mM) and theophylline (3 mM). The resuspended cells were centrifuged again at 200 g for 15 min, and the platelet pellets were resuspended in appropriate buffers for different experimental procedure (20).

Studies of platelet function: Platelet aggregation in response to agonists such as epinephrine (5 μM), adenosine diphosphate (ADP, 3 μM), arachidonate (0.45 μM), thrombin (0.2 ml) was monitored on a Chronolog lumiaggregometer (Chronolog Corp., Haverton PA). Release of ATP was monitored by using the luciferin-luciferase system (20). All studies were repeated a minimum of three times with blood obtained from different patients. Aggregation profiles presented as figures are typical representations of actual studies.

Serotonin and adenine nucleotides: Platelet serotonin levels were measured by monitoring the fluorescence of endogenous amine by spectrofluorometry (21). Adenine nucleotides were quantitated by high performance liquid chromatography as described from our laboratory (22).

Serotonin uptake studies: Platelet samples were incubated for 3 min at 37°C with radiolabelled serotonin. Serotonin uptake was terminated by adding 1 $\mu\text{g}/\text{ml}$ of prostaglandin E_1 and subsequent rapid filtration over Whatman GF/C filters, followed by washing with cold saline. Filters were counted in a toluene-triton based scintillation fluid. Active serotonin uptake was computed by subtracting the passive uptake, at 0°C, from the uptake at 37°C (16, 17).

Platelet membrane fluidity: To measure membrane fluidity, washed platelets and membrane preparations were incubated with 1 μ M DPH for 60 min. To prepare membrane, washed platelets were subjected to a freeze-thaw cycle, and then sonicated. Membranes were pelleted by centrifuging at 20,000 g for 30 min. The pellet was resuspended in HEPES buffer and incubated with 1 μ M DPH for 60 min. Fluorescence measurements were made in a Perkin-Elmer LS5B spectrofluorometer with polarization accessory according to the methods described by other laboratories (6-13). DPH fluorescence was excited at 360 nm, the emission at 430 nm and the light intensity was measured with all four possible excitation/emission polarizing filter combinations.

Intracellular ionized calcium: For measurement of cytosolic free calcium, washed platelets suspended in appropriate physiologic buffers were used. Ionized calcium was monitored by using Fura-2 AM as the calcium fluorophore. Platelets were incubated with Fura-2 AM (2 mM) for 10 minutes at 37°C, then washed and resuspended in HEPES buffer (145 mM NaCl, 1 mM MgSO₄, 0.5 mM Na₂HPO₄, 10 mM HEPES, 5 mM glucose, and 1 mM CaCl₂, pH 7.4). Fluorescence measurements of Fura-2 calcium complex were made in Perkin-Elmer LS5B spectrofluorometer as described in our laboratory (23).

Release of arachidonic acid: Platelets obtained from normal donors and from AD patients, were washed, resuspended in HBSS, and incubated at 37°C with radiolabelled arachidonic acid. After 60 min excess radioactive material were removed by washing and resuspending washed platelets in HBSS containing calcium (1 mM) and 4% bovine serum albumin. Washed platelets in suspension exposed to indomethacin (10 μ M) were incubated with 0.2 U/ml thrombin at 37°C for 3 minutes. Reaction was stopped with the addition of 100 μ l of 0.34 M EDTA. Samples were spun and pellets and supernatant were separated and radioactivity analyzed in a scintillation counter.

Arachidonic acid metabolism: To measure ¹⁴C-arachidonic acid conversion of thromboxane by intact platelets, each reaction mixture containing 1 x 10⁹ cells suspended in 1 ml HBSS was stirred on an aggregometer for five minutes with 1 μ g of labelled arachidonic acid (24). At the end of this period, 1 ml of ethyl acetate was added to each reaction mixture and acidified to pH 3.5 with 0.5 M citric acid. After thorough mixing, the organic solvent layer was separated, and the samples were re-extracted with an equal volume of ethyl acetate. Pooled ethyl acetate fractions were concentrated with nitrogen bubbling and spotted on a thin layer plate (silica gel G). The solvent system used for the separation of thromboxane was ether: methanol:acetic acid (135:2:2 vol/vol). Radioactive metabolites were monitored with a Berthold radiolabel scanner, and quantitation was achieved by scraping radioactive spots off the thin layer plate and scintillation counting.

RESULTS

Platelet function studies: A total of 13 AD patients were studied for determining relative response of AD platelets to the action of various physiological agonists. Less than 25% of patients with AD were free of any medication. Normal control platelets aggregated irreversibly and secreted ATP when stirred with epinephrine (5 μ M), arachidonate (0.45 mM) and thrombin (0.2 U/ml). The response of AD platelets to these agonists was considerably compromised. Only 4 of 13 patients tested responded normally to arachidonic acid and only 3 of 13 aggregated irreversibly on stirring with thrombin. AD platelets failed to aggregate irreversibly in response to weak agonists such as epinephrine and ADP. Threshold concentrations of arachidonate (0.45 mM) failed to cause aggregation of AD platelets (9/13). At high concentrations (0.9 mM) the platelets of some patients responded normally to arachidonate. In some cases, platelets of AD patients who had taken no medication failed to aggregate in response to arachidonate, although when stirred with radiolabelled arachidonate they synthesized normal amounts of thromboxanes (Fig.1).

Influence of Arachidonate on Normal Control Platelets and that of Platelets with Alzheimer's Dementia

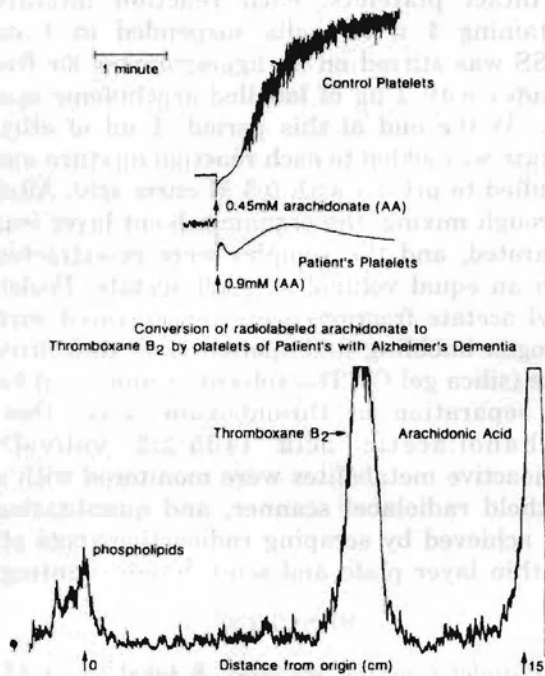


Fig. 1 : Platelets of normal individuals aggregated irreversibly when stirred with arachidonate. Platelets of patients with AD failed to respond to the action of arachidonate, although they converted radiolabelled arachidonate to thromboxane.

Platelets of patients with AD that failed to aggregate in response to epinephrine alone or arachidonate, aggregated irreversibly when these agonists were tested together (Fig. 2). Normal control platelets aggregated irreversibly in response to thrombin (0.2 U/ml) and released ATP. However, even at more than twice the thrombin concentration (0.5 U/ml) platelets of AD patients failed to aggregate (10/13) (Fig. 3).

To explore the presence of any inhibitory factors that could compromise the effect of thrombin on platelets of patients with AD, further studies were conducted in plasma-free suspension. Platelets of patients with AD washed free of plasma and suspended in calcium containing buffer developed irreversible aggregation in response to normal concen-

Influence of Epinephrine and Arachidonate on Platelets of Patients with Alzheimer's Dementia

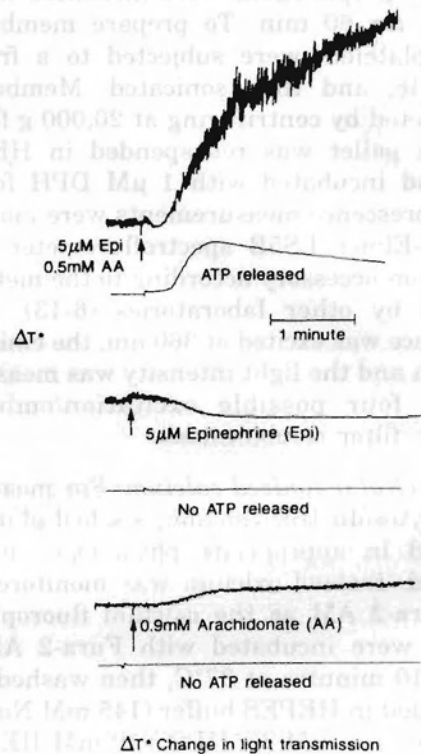


Fig. 2 : Platelets of patients with AD failed to respond with aggregation when stirred with epinephrine or arachidonate. Epinephrine exposure of AD platelets restored the sensitivity of refractory cells to the action of arachidonate and caused irreversible aggregation.

trations of thrombin (0.2 U/ml). Furthermore, platelets of normal donors that responded to threshold concentrations of thrombin, when suspended in platelet-poor plasma of patients with AD, failed to respond to the action of thrombin (Fig. 4).

Serotonin, adenine nucleotide levels and serotonin uptake: Normal control platelets had 793 ± 310 nanograms of serotonin per 10^9 cells, whereas AD platelets had 798 ± 446 ngs/ 10^9 cells. Control platelets had 0.8 ± 0.2 AMP, 3.6 ± 0.6 ADP, and 5.3 ± 1.0 ATP per 10^{11} cells. Platelets of AD patients had 0.9 ± 0.2 , 3.1 ± 0.5 ,

5.0 ± 1.2 AMP, ADP, ATP, respectively per 10¹¹ cells (Table I), (Fig. 5). Percent uptake of radiolabelled serotonin in normal control platelets incubated for 3 minutes at 37°C was 18.6 ± 2.8%, whereas AD platelets had an uptake of 19.2 ± 3.5% (Table II).

Response of Normal Control Platelets and That of Patients with Alzheimer's Dementia to the Action of Thrombin

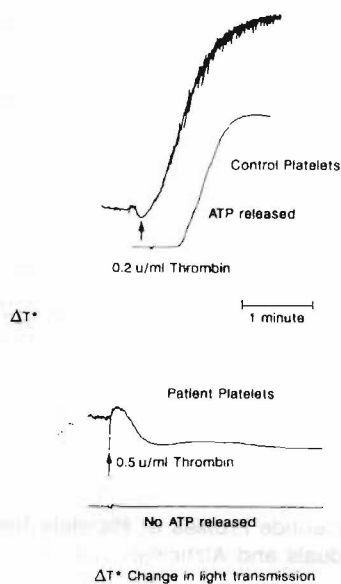


Fig. 3 : Normal control platelets aggregated irreversibly when stimulated with thrombin, and secreted their granule contents. Thrombin, even at twice the threshold concentration, failed to cause aggregation and mediate secretion.

TABLE I : Serotonin and adenine nucleotides of platelets of Alzheimer type patients.

	ngs/10 ⁹ cells ¹	μmols/10 ¹¹ cells ²		
		AMP	ADP	ATP
Control	793±310	0.8±0.2	3.6±0.6	5.3±1.0
Patient ³ platelets	798±446	0.9±0.2	3.1±0.5	5.0 ±1.2

¹ Mean and the standard deviation (n = 12).
² Mean and the standard deviation (n = 6).
³ No significant difference in dense body constituents (P >.05).

TABLE II : Serotonin uptake into platelets of the Alzheimer type patients.

	Percent uptake of radiolabelled serotonin
Control	18.6 ± 2.8*
Patient platelets ¹	19.2 ± 3.5

*Mean and the standard deviation (n = 6).
¹No significant difference in uptake (P>.05).

Influence of a Plasma Factor on Thrombin-Mediated Platelet Aggregation

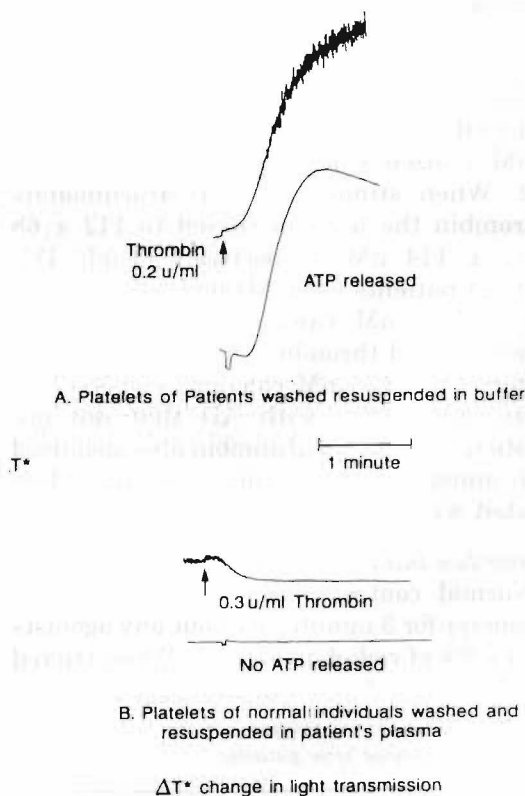


Fig. 4 : Platelets of patients with AD that were non-responsive to thrombin upon washing and resuspension in buffer containing calcium (1 mM) aggregated irreversibly when stimulated with thrombin. Platelets of normal donors that responded to thrombin, when suspended in platelet-poor plasma of patients with AD, failed to aggregate in response to thrombin stimulation.

Measurements of membrane fluidity: Fluorescence anisotropy values for intact normal control platelets were 0.225 ± 0.12 and for the membranes 0.260 ± 0.11 (Table III). Platelets of patients with AD had 0.251 ± 0.14 and their membranes 0.269 ± 0.12 .

TABLE III : Platelet membrane fluidity of Alzheimer type patients.

	Intact platelets	Membranes
Control	0.225 ± 0.12	$0.260 \pm 0.11^*$
Patient platelets ¹	0.251 ± 0.14	0.269 ± 0.12

*Mean and the standard deviation (n = 6).

¹No significant difference in fluidity (P>.05).

Agonist-mediated calcium mobilization: Normal control platelets had a basal level of 86 ± 14 nM ionized calcium as monitored with Fura 2. When stimulated with arachidonate and thrombin the level increased to 112 ± 68 and 742 ± 114 nM, respectively (Table IV). Platelets of patients with AD had a basal level of 122 ± 19.0 nM calcium. In response to arachidonate and thrombin they mobilized 168 ± 77 and 819 ± 125 nM calcium, respectively. Platelets of patients with AD that did not aggregate in response to thrombin also mobilized normal amounts of cytosolic calcium when stimulated with thrombin (data not shown).

Thrombin-induced release of arachidonic acid: Normal control platelets stirred on an aggregometer for 3 minutes without any agonists released 2.9% of radiolabelled AA. When stirred

TABLE IV : Calcium mobilization by platelets of Alzheimer type patients.

	Basal	Thrombin	AA
	CALCIUM (nM)		
Control	86 ± 24	742 ± 114	$112 \pm 68^*$
Patient platelets ¹	122 ± 19.0	819 ± 125	168 ± 77

*Mean and the standard deviation (n = 6).

¹No significant difference in any comparison (P>.05).

with 0.2 U/ml of thrombin they released 4.1% of arachidonic acid (Table V). AD platelets stirred on an aggregometer with buffer released 2.1% of arachidonic acid, and when stirred with thrombin released 8.8% of the radiolabel.

Conversion of arachidonic acid to thromboxane: Normal control platelets stirred with radiolabelled arachidonate converted 25.1% of the substrate to thromboxane (Table VI). Platelets of AD patients stirred with radiolabelled substrate converted only 12.6% of

TABLE V : Thrombin (TH) induced release of arachidonic acid.

	% Release*	
	Control	Patients
Basal	2.9 ± 0.6	2.1 ± 0.9
Stimulated ¹ (0.2 U/ml, TH)	4.1 ± 1.6	8.8 ± 0.8

*Mean and the standard deviation (n = 3).

¹Significant difference between controls and patients. (P>.05).

Adenine Nucleotide Profiles of Platelets from Normal Individuals and Alzheimer Type Patients

Column: Radial-Pak™
Packing: μ BONDAPAK C₁₈
Solvent: Acetonitrile-water-Pic A

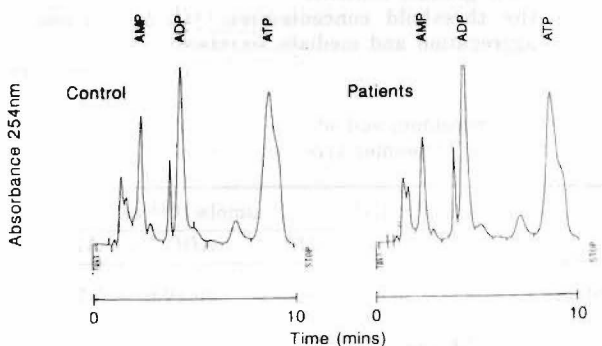


Fig. 5 : Adenine nucleotide profiles of platelets in normal control donors and those of patients with AD. No significant difference in the levels of AMP, ADP and ATP could be demonstrated in the platelets of AD patients compared to those of cells from normal donors.

the arachidonic acid to thromboxane. Platelets of patients with AD that did not aggregate irreversibly in response to arachidonate when stirred with radiolabelled arachidonate synthesized normal amounts of thromboxane B_2 .

TABLE VI : Conversion of radiolabelled arachidonic acid (AA) to thromboxane B_2 .

	Percent conversion of AA
Normal control	25.1 ± 3.4*
Patient platelets ¹	12.6 ± 6.8

* Mean and the standard deviation (n = 4).

¹ Significant difference between controls and patients. (P < .05).

DISCUSSION

A large number of studies have been done on platelets of patients with Alzheimer-type dementia, to identify a platelet lesion that can be used as a biological marker for the onset and severity of AD. Some of the alterations reported include increased monoamine oxidase, serotonin uptake, α_2 adrenergic receptor density, increased internal membranes, membrane fluidity, cholesterol/phospholipid ratio, and ionized calcium (1-19). However, little is known about the functional response of platelets to the action of various physiological agonists. Since protease nexins have been demonstrated in platelets, there is considerable interest in their ability to antagonize the action of thrombin and coagulation factor XIa. In this study we have evaluated the biochemistry, physiology and function of platelets of patients with clinical manifestations believed to be characteristic of Alzheimer type dementia.

Biochemical events associated with agonist-mediated activation leads to formation of second messengers, mobilization of cytosolic calcium and synthesis and release of bioactive molecules capable of causing irreversible aggregation. Platelets of patients with AD responded poorly to the action of weak agonists such as epinephrine and ADP. Even though they

responded to thrombin and arachidonate, their ability to release granule contents seemed to be significantly compromised. Some of the AD patients had platelets that did not aggregate in response to high concentrations of arachidonate. However, they converted radiolabel arachidonic acid to thromboxane. This observation is similar to results obtained in studies of canine platelets (25). In those investigations arachidonate refractory canine platelets regained their sensitivity to arachidonate when exposed to epinephrine. Similarly, platelets of patients with AD that were refractory to arachidonate, aggregated irreversibly when exposed to epinephrine before a further challenge by arachidonate.

Platelets of patients that had compromised cyclooxygenase activity required high concentrations of arachidonate to aggregate. In an earlier study we demonstrated that partial inhibition of cyclooxygenase with low dose aspirin (80 mgs) renders normal platelets refractory to the action of a threshold concentration of arachidonate (26). However, they do aggregate irreversibly when challenged with high doses of arachidonate (0.9 mM).

A second wave of aggregation in response to weak agonists is mediated by newly synthesized thromboxanes and secreted ADP. Exposure of platelets to cyclooxygenase inhibitors, such as aspirin and indomethacin, abolishes the second wave response as well as their ability to release granule contents. A potent agonist such as thrombin can cause secretion of granule contents and mediate aggregation of drug-induced refractory platelets. In view of this observation, it was surprising to find donors (10/13) whose platelets failed to aggregate or secrete granule contents in response to high concentrations of thrombin (1.0 U/ml). Platelets nonresponsive to thrombin were capable of normal calcium mobilization as monitored with Fura-2 loaded cells.

Since the calcium measurements are made with platelets devoid of plasma, we speculated that a plasma factor might be inhibiting the

action of thrombin. Aggregation studies made with washed platelets of patients with AD proved our suspicion to be correct. Platelets suspended in buffer aggregated irreversibly and secreted normal amounts of ATP in response to thrombin. In addition, suspension of normal control platelets in platelet-poor plasma of AD patients blocked thrombin's action, suggesting the presence of an inhibitory factor (3). Anxin 1 seems to be a potent inhibitor of thrombin. However, earlier studies on platelet annexins showed that these proteins are not releasable by platelet activation (27). It is not known whether platelet destruction or lysis annexins release their proteins into the external milieu. Characterization of the plasmatic thrombin inhibitory factor needs further in depth study.

In view of the fact that the majority of patients with AD had platelets that were relatively refractory to the action of agonists, we studied the biochemistry and activation signalling mechanisms to characterize the nature of the defect that underlies compromised platelet function. Studies on platelet biochemistry included analysis of serotonin, adenine nucleotides, serotonin uptake, membrane fluidity measurements, agonist-mediated release of arachidonic acid and conversion of free arachidonic acid to thromboxane. Serotonin and adenine nucleotide levels in platelets of patients with AD were normal compared to levels found in platelets of control subjects. Radiolabelled serotonin uptake was also normal. Earlier studies by Koren et al demonstrated that serotonin uptake into platelets decreased with age, and the reduction in uptake of amine was more prominent in platelets of patients with AD (17). Anderson et al used radiolabelled paroxetine and found that platelet serotonin uptake studies cannot be used as a peripheral marker of AD (16).

Several earlier studies have reported significant reduction in the fluorescence anisotropy of DPH in platelet membranes suggesting increased membrane fluidity (6-13). In addition, measurements of enzyme activities and enzyme marker associated with intracellular

and plasma membranes also have supported such observations (10). In this study, using fluorescence anisotropy of DPH, we were unable to show significant alterations in membrane fluidity of intact platelets or in isolated membranes. Kakkola et al observed increased platelet membrane fluidity in patients with dementia, but did not find it specific for AD (11). Furthermore, Kukull et al reported an extensive study of 95 clinically diagnosed AD patients and concluded that increased membrane fluidity is not a diagnostic marker for AD (6).

Little is known about how amyloid β protein causes neuronal dysfunction. There is considerable speculation that this protein may disrupt calcium homeostasis and increased intracellular calcium concentrations (28). The presence of annexins I and V have been demonstrated in platelets (27). These proteins seem to play a role in signal transduction. They may serve as substrates for protein kinase C, phosphotyrosine kinases and as inhibitors of phospholipase C and phospholipase A_2 . Bothmer et al studied platelet phosphatidylinositol kinase and found no alteration in the activity of this enzyme in platelets of patients with AD (18). In patients with bipolar disorders increased calcium mobilization in response to thrombin has been reported (29). In our study no significant difference in thrombin-mediated cytosolic calcium elevation could be demonstrated in platelets of patients with AD. Davies et al monitored thrombin-mediated signal transduction in AD platelets, and did not find any difference in their ability to mobilize calcium (19).

Agonist-mediated signal transduction, formation of second messengers, and elevation of cytosolic calcium facilitate the activation of phospholipase A_2 and liberation of arachidonic acid. Thrombin-induced release of arachidonic acid was significantly enhanced in AD platelets. However, ability to convert arachidonic acid to thromboxane seemed to be compromised. In view of the fact that the majority of patients with AD are on multiple drugs, it is difficult to

conclude that the compromised activity of cyclooxygenase was real or drug-induced. Further work with patients free of any medication will provide definitive information regarding the status of cyclooxygenase in platelets of patients with Alzheimer's dementia.

In conclusion, our studies demonstrate that platelets of patients with AD have compromised response to the action of agonists. Reduced response of these cells is not related to any significant defects in the platelet biochemistry or alterations in the signalling mechanisms. In view of the fact that thrombin elicits normal signal transduction events in platelets of patients with AD, the compromised aggregation and secretion response observed in response to thrombin appears to be due to some inhibitory

factor in the plasma. Significant alterations in serotonin uptake, membrane fluidity, calcium mobilization, and liberation of arachidonic acid could not be demonstrated in platelets of patients with AD. Therefore, these biochemical parameters cannot be used as biological markers to measure the onset or the progress of AD.

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REFERENCES

- Gardella JE, Ghiso J, Gorgone GA, Maratta D, Kaplan AP, Frangione B, Gorevic PD. Intact Alzheimer amyloid precursor protein (APP) is present in platelet membranes and is encoded by platelet mRNA. *Biochem Biophys Res Comm* 1990; 173: 1292-1298.
- Van Nostrand WE. Protease nexin-11 (amyloid beta protein precursor): A platelet alpha-granule protein. *Science* 1990; 248: 745-748.
- Wagner SL, Geddes JW, Cotman CW, Lau AL, Gurwitz D, Isackson PJ, Cunningham DD. Protease nexin-1, an antithrombin with neurite outgrowth activity, is reduced in Alzheimer's disease. *Proc Nat Acad Sci USA* 1989; 86: 8284-8288.
- Smith RP, Higuchi DA, Broze GJ. Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. *Science* 1990; 248: 1126-1128.
- Katzman R. Alzheimer's disease. *N Engl J Med* 1986; 314: 964-973.
- Kukull WA, Hinds TR, Schellenberg GD, Van Belle, Larson EB. Increased platelet membrane fluidity as a diagnostic marker for Alzheimer's disease: A test in population-based cases and controls. *Neurol* 1992; 42: 607-614.
- Zubenko GS, Brenner RP, Teply I. Electroencephalographic correlates of increased platelet membrane fluidity in Alzheimer's disease. *Arch Neurol* 1988; 45: 1009-1013.
- Zubenko GS, Wusylko M, Cohen BM, Boller F, Teply, I. Family study of platelet membrane fluidity in Alzheimer disease. *Science* 1987; 238: 539-542.
- Hicks N, Bramer MJ, Hymas N, Levy R. Platelet membrane properties in Alzheimer and multi-infarct dementia. *Alz Dis Assoc Disord* 1987; 1: 90-97.
- Haji Mohammadreza I, Brammer MJ, Eagger S, Burns A, Levy R. Platelet and erythrocyte membrane changes in Alzheimer's disease. *Biochim Biophys Acta* 1990; 1025: 208-214.
- Kakkola S, Rosenberg PH, Alila A, Ekinjuntti T, Sulkava R, Palo, J. Platelet membrane fluidity in Alzheimer's disease and multi-infarct dementia: a spin label study. *Acta Neurol Scand* 1991; 84: 18-21.
- Piletz JE, Sarasua M, Whitehouse P, Chotani M. Intracellular membranes are more fluid in platelets of Alzheimer's disease patients. *Neurobiol Aging* 1991; 12: 401-406.
- Van Rensburg SJ, Carstens ME, Potocnik CV, Aucamp AK, Taljaard JJF, Koch KR. Membrane fluidity of platelets and erythrocytes in patients with Alzheimer's disease and the effect of small amounts of aluminium on platelet and erythrocyte membranes. *Neurochem Res* 1992; 17: 825-829.
- Zubenko GS, Cohen BM, Reynolds CF, Boller F, Malinakova I, Keefe N. Platelet membrane fluidity in Alzheimer's disease and major depression. *Am J Psychiat* 1987; 144: 860-868.
- Adolfson R, Gottfries GC, Orelund L, Wilberg A, Winblad B. Increased activity of brain and platelet monoamine oxidase in dementia of the Alzheimer's type. *Life Sci* 1980; 27: 1029-1034.

16. Andersson A, Adolfsson R, Ericksson K, Marcusson J. Platelet [³H] paroxetine binding to 5-HT uptake sites in Alzheimer's disease. *Neurobiol Aging* 1991; 12: 531-534.
17. Koren P, Driver-Haber A, Adunsky A, Rabinowitz M, Heshkowitz M. Uptake of serotonin into platelets of senile dementia of the Alzheimer type patients. *J Gerontol* 1993; 48: B93-B96.
18. Bothmer J, Markerink M, Coppens R, Jolle SJ. Platelet phosphatidylinositol kinase activity is not altered in Alzheimer disease. *Molec Chem Neurobiol* 1993; 19: 249-257.
19. Davies TA, Fine RE, Johnson RJ, Levesane CA, Rathbun WH, Seetoo KF, Smith SJ, Strohmeier G, Volicer L, Delva L, Simons ER. Non-age related differences in activation in platelets from Alzheimer's disease patients. *Biochem Biophys Res Comm* 1993; 194: 537-543.
20. Rao GHR, Escolar G, Smith CM II and White JG. Influence of heat on platelet biochemistry, physiology and function. *J Lab Clin Med* 1993; 122: 455-465.
21. Rao GHR, White JG, Jachimowicz AA, Witkop CJ Jr. An improved method for the extraction of endogenous platelet secretion. *J Lab Clin Med* 1976; 87: 129-137.
22. Rao GHR, White JG, Jachimowicz AA, Witkop CJ Jr. Nucleotide profiles of normal and abnormal platelets by high pressure liquid chromatography. *J Lab Clin Med* 1974; 84: 839-850.
23. Rao GHR. Measurement of ionized calcium in normal human blood platelets. *Anal Biochem* 1988; 169: 400-404.
24. Rao GHR, Johnson GJ, Reddy KR, White JG. Rapid return of cyclooxygenase active platelets in dogs after a single oral dose of aspirin. *Prostaglandins* 1981; 22: 761-772.
25. Hardy JA, Higgins GA. Alzheimer's disease: The amyloid cascade hypothesis. *Science* 1992; 256: 184-185.
26. Murphy CJ, Peers SH, Forder RA, Flower RJ, Carey F, Westwick J. Evidence for the presence and location of aneoxins in human platelets. *Biochim Biophys Res Comm* 1992; 189: 1739-1746.
27. Kusumi I, Koyama T, Yamashita I. Thrombin-induced platelet calcium mobilization is enhanced in bipolar disorders. *Biol Psychiat* 1992; 32: 731-734.
28. Johnson GJ, Leis LA, Rao GHR, White JG. Arachidonate-induced platelet aggregation in the dog. *Thromb Res* 1979; 14: 147-154.
29. Rao GHR, Reddy KR, White JG. Low dose aspirin, platelet function and prostaglandin in synthesis: Influence of epinephrine and alpha adrenergic blockage. *Prost Med* 1981; 6: 485-494.