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Chemical characterization of pro-inflammatory amyloid-beta peptides in human atherosclerotic lesions and platelets

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ABSTRACT

Amyloid-β (Aβ) peptides are intimately involved in the inflammatory pathology of atherosclerotic vascular disease (AVD) and Alzheimer's disease (AD). Although substantial amounts of these peptides are produced in the periphery, their role and significance to vascular disease outside the brain requires further investigation. Amyloid-β peptides present in the walls of human aorta atherosclerotic lesions as well as activated and non-activated human platelets were isolated using sequential size-exclusion columns and HPLC reverse-phase methods. The Aβ peptide isolates were quantified by ELISA and structurally analyzed using MALDI-TOF mass spectrometry procedures. Our experiments revealed that both aorta and platelets contained Aβ peptides, predominately Aβ40. The source of the Aβ pool in aortic atherosclerosis lesions is probably the activated platelets and/or vascular wall cells expressing APP/PN2. Significant levels of Aβ42 are present in the plasma, suggesting that this reservoir makes a minor contribution to atherosclerotic plaques. Our data reveal that although aortic atherosclerosis and AD cerebrovascular amyloidosis exhibit clearly divergent end-stage manifestations, both vascular diseases share some key pathophysiological promoting elements and pathways. Whether they happen to be deposited in vessels of the central nervous system or atherosclerotic plaques in the periphery, Aβ peptides may promote and perhaps synergize chronic inflammatory processes which culminate in the degeneration, malfunction and ultimate destruction of arterial walls.

1. Introduction

Numerous studies have demonstrated that amyloid-beta (Aβ) peptides have a central role in the pathogenesis of Alzheimer's disease (AD) and recent evidence derived from epidemiologic, correlative and experimental data has strongly linked intracranial atherosclerotic vascular disease (AVD) with the pathogenesis of AD [1–3]. The Baltimore longitudinal study on aging suggests that the presence of intracranial AVD significantly increases the odds of dementia, independent of cerebral infarction [4]. Multiple established risk factors for AVD have also been recognized to be risk factors for the development of AD (reviewed in [5,6]). Clinical investigations have reported that coronary artery disease, myocardial infarction, carotid artery disease, peripheral artery disease, hypertension, diabetes mellitus, cigarette smoking and obesity are all more common in subjects with AD (reviewed in [2]). Longitudinal and twin studies have reinforced the tenet of cardiovascular failure and AVD, in the absence of stroke, as risk factors for AD in carriers of the apolipoprotein E (ApoE) ε4 allele [7]. Moreover, the association between cardiovascular disease and dementia is not explained by genetic or early life environmental factors common in both disorders [7]. Individuals with midlife hyperlipidemia have an elevated risk of developing AD, and individuals with clinically or neuropathologically diagnosed AD have higher blood cholesterol levels compared to non-demented controls [8–10]. Considerable evidence from experimental studies links cholesterol to Aβ metabolism [11,12].

Both AD and AVD evolve in an intensely inflammatory milieu [13–19] that ultimately destroys brain and vascular tissues. In AD, Aβ accumulation induces microvascular inflammation mediated by

Abbreviations: Aβ, amyloid-beta; AD, Alzheimer's disease; ApoE, apolipoprotein E; APP, amyloid precursor protein; AVD, atherosclerotic vascular disease; BBB, blood–brain barrier; CAA, cerebral amyloid angiopathy; FPLC, fast protein liquid chromatography; GDFA, glass-distilled formic acid; HPLC, high performance liquid chromatography; KPI, Kunitz (serine) protease inhibitory; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; PN2, protease nexin-2; RT, room temperature; TFA, trifluoroacetic acid

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cytokines and chemokines that act on neurons, glia, endothelium, myocytes and pericytes [20–23]. Under normal and pathological conditions, the Aβ peptides play an important role in the modulation of inflammation [24,25]. In addition, pathological interactions between molecules of the coagulation cascade and Aβ have been shown to alter thrombosis and fibrinolysis thus establishing associations among atherosclerosis, thrombosis, cerebral amyloid angiopathy (CAA), brain hypoperfusion and AD [26]. The amyloidogenic 40/42 amino acid-long Aβ peptides are derived, by the proteolytic action of the β- and γ-secretases, from larger amyloid-beta precursor proteins that are 695, 751 and 770 amino acids in length (APP_{695}, APP_{751} and APP_{770}). Large amounts of Aβ peptides deposited in the brain microvascular walls result in considerable pathophysiological alterations, and the severity of cognitive decline is associated with the degree of CAA and arteriosclerosis/lipohyalinosis [27]. In particular, the capillary deposition of Aβ42 is highly correlated with AD pathology [28], blood–brain barrier (BBB) disruptions and severe alterations in brain perfusion [13,29–31].

The Aβ peptides are generated in appreciable quantities outside of the central nervous system in places such as the neuromuscular junctions of skeletal muscle fibers [32] and in the α-granules of platelets [33] that contribute to the pool of Aβ in the circulation. Other potential sources of APP/Aβ are the endothelial cells of the *vasa vasorum* that invade and feed the walls of the atherosclerotic lesion [34]. In platelets, the Aβ peptides are mainly derived from the APP_{751} and APP_{770}, known as protease nexin-2 (PN2), which carries a Kunitz (serine) protease inhibitory (KPI) domain. The APP/PN2 molecule has important functions in the coagulation cascade as it acts as an inhibitory component of factors, IXa, Xa, XIa and tissue factor VIIa complex [35–38], thus modulating hemostasis following vascular injury by limiting thrombosis [39]. In a strain of transgenic mice (Tg-rPF4/βAPP) that over-express the APP/PN2, the thrombotic-capacity is diminished and the extent of cerebral hemorrhage is increased accordingly [39].

Activated platelets participate in the pathogenesis and evolution of atherosclerosis due to a strong contribution in the development of inflammatory responses in leukocytes, endothelial cells and vascular myocytes [40–42]. The prothrombotic interactions between platelets and vascular wall cells contribute to the establishment of the atheromatous lesions from their early stages to the final plaque rupture and thrombosis (reviewed in [43]). The elegant immunocytochemical and electron microscopic studies of De Meyer and colleagues clearly demonstrated the presence of APP and Aβ within platelets that were phagocytized by macrophages in areas of neovascularization in advanced atherosclerotic lesions [34]. The biochemical characterization of Aβ peptides presented in this study is complementary to these meticulous histological investigations. Although platelets and circulating Aβ may be a significant source of these peptides in the atherosclerotic lesions, it is possible that the Aβ peptides also originate, under pathological conditions, from the arterial tunica media myocytes and endothelial cells that also express APP [44].

In the present investigation we quantified, chromatographically isolated and characterized by mass spectrometry the Aβ peptides present in the walls of human atherosclerotic aortas. We also investigated the chemical characteristics of the Aβ peptides by mass spectrometry in freshly isolated inactivated and activated platelets as a possible source of the Aβ found in atherosclerotic lesions. In addition, we discuss the possible source of atherosclerotic Aβ as well as the potential relevance of Aβ pro-inflammatory activity to vascular pathology in the context of both AVD and AD.

2. Materials and methods

2.1. Thoracic aortas

Thoracic aortic sections (2.5 g each) exhibiting severe atherosclerotic lesions were obtained in the immediate postmortem from 6 Caucasian individuals with a mean age of 84 years. There were 3 female and 3 male subjects, 4 of whom were demented with AD and one with Parkinsonian dementia. One subject, a 92 year-old male, was non-demented. The aortas exhibited severe regions of calcification complicated by ulcerations, intramural hemorrhages, rupture of fibrous caps and thrombosis as shown in Fig. 1.

2.2. Extraction of Aβ peptides from aortas

The aortic sections were thoroughly rinsed with cold deionized water to remove all traces of blood, and the adventitial connective tissue was detached and discarded. The intima/media of aortic sections were pooled and finely minced and ground in liquid nitrogen. Half of the pulverized tissue was placed in a beaker with 20 ml of 98% glass-distilled formic acid (GDFA), stirred overnight at 4 °C and centrifuged at 250,000×*g* for 1 h at 4 °C. The acid-soluble phase between the floating fatty layer and the bottom pellet was collected and remaining solids were removed by gravimetric filtration using Whatman #1 filter paper. The filtrate was centrifuged a second time and the acid-soluble fraction divided into 500 μl aliquots which were submitted to fast protein liquid chromatography (FPLC). The second half of the pulverized aortic tissue was suspended in 400 ml of 0.1 M ammonium bicarbonate and 0.01% sodium azide and stirred overnight at 4 °C. The suspension was centrifuged at 30,000×*g* for 1 h at 4 °C. The supernatant (water-soluble fraction) was filtered through a 45 μm nylon mesh, freeze-dried and lyophilized. Fifty mg aliquots of the lyophilized water-soluble fraction were then dissolved in 500 μl of 80% GDFA and submitted to FPLC.

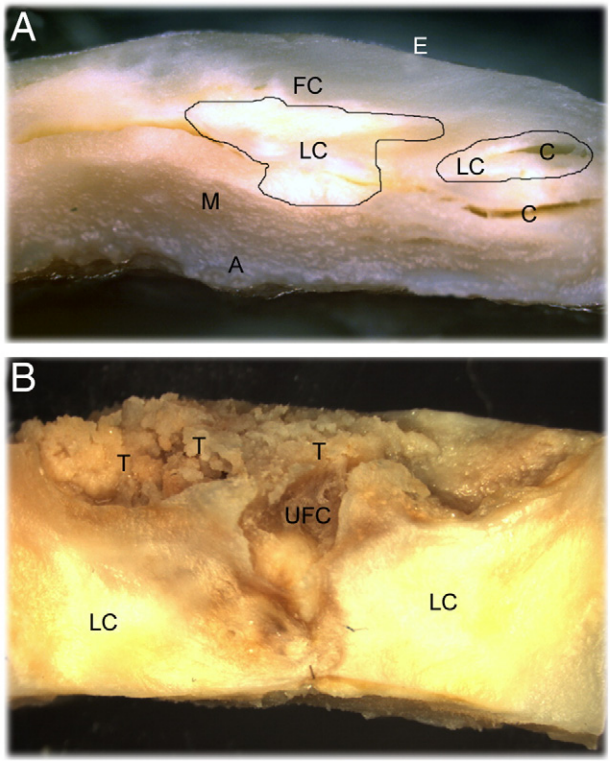

Fig. 1. Representative human aorta atherosclerotic lesions. A) An example of the advanced aortic atherosclerotic lesions used in this study demonstrating lipid cores with areas of calcification underlying the fibrous cap. B) Complicated atherosclerotic lesions with ulceration and rupture of the fibrous caps showing areas of thrombosis. E = endothelium; FC = fibrous cap; LC = lipid cores; C = areas of calcification; M = tunica media; A = adventitia; T = thrombosis; UFC = ulcerated (crater-like) fibrous cap.

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2.3. Chromatographic separation of total aortic Aβ peptides

Both acid-soluble fractions and water-soluble fractions were separated by size-exclusion FPLC on a pre-calibrated 1 cm × 30 cm Superose 12 column (Pharmacia-Amersham-GE, Uppsala, Sweden). The column was equilibrated and developed with 80% GDFA at a flow rate of 15 ml/h at room temperature (RT) and absorbance monitored at 280 nm. The fractions containing 2–10 kDa molecules were collected and the volume reduced by vacuum centrifugation (Savant, Farmingdale, NY). To enrich the Aβ peptides and eliminate most of the flanking contaminations, 3 runs of the 2–10 kDa chromatographic eluants were pooled and re-chromatographed under the same conditions as described above.

To further purify the Aβ peptides, both the acid-soluble and water-soluble fractions obtained by size-exclusion FPLC were reduced to 100 μl and submitted to high performance liquid chromatography (HPLC, Thermo Separation Products, Waltham, MA) on a Synchropak GPC-peptide size-exclusion 7.8 cm × 90 cm column (Synchrom Inc. Lafayette, IN). The chromatographies were conducted at RT using 80% GDFA at a flow rate of 0.2 ml/min and the eluate absorbance was monitored at 280 nm. Western blots, using antibodies against Aβ40 and Aβ42, were employed to localize the Aβ peptide-containing fractions. The Aβ fraction from three column runs was pooled, the volume reduced to 500 μl and injected into an HPLC reverse-phase C8 semi-preparative 9.4 cm × 25 cm column (Zorbax 300 SB/5 μm beads; Wilmington, DE). The chromatography was developed using an elution gradient from 20% to 60% acetonitrile in water containing 0.1% trifluoroacetic acid (TFA) with a flow rate of 1.5 ml/min, at 80 °C. Eluate absorbance was monitored at 214 nm. For identification of the Aβ peptide-containing fractions, the specimens were reduced in volume and submitted to Western blot analysis.

2.4. Analysis of Aβ peptides from inactivated and activated human platelets

Fresh platelets were obtained from 5 separate American Red Cross volunteer donors by platelet-pheresis and immediately processed following the procedures published by Li et al. [33,45] with modifications. A complete description of the technical details has been previously published by our group [46]. Both inactivated and thrombin-collagen activated platelets were submitted to FPLC (Superose 12), HPLC (Synchropak GPC-peptide) and reverse-phase HPLC (Zorbax 300 SB) as described above for the aortic tissue.

2.5. Western blots analysis of Aβ peptides

Samples from the HPLC, representing the total fractionation runs, were assessed for the presence of Aβ40 and Aβ42 peptides (Invitrogen, Carlsbad CA) using Western blots as described before [47].

2.6. Identification of Aβ peptides by MALDI-TOF mass spectrometry

A matrix assisted laser desorption ionization-time of flight (MALDI-TOF) Voyager DE STR mass spectrometer (Applied Biosystems, Foster City, CA) was employed to obtain data from the HPLC fractions. The applied Biosystems CalMix-2 was used as calibration mixture. The detailed protocol and composition of the calibration markers was previously published [48].

3. Results

3.1. Atherosclerotic aorta analyses

Our specific experimental goal was to establish the chemical characteristics of Aβ peptides in atherosclerotic aortic walls and in inactivated and activated platelets. Aortic vessels of elderly individuals with severe atherosclerotic lesions showed that the Aβ40 peptide was on the average 100 times more abundant than Aβ42: 75 ng/g of tissue and 0.7 ng/g of tissue, respectively [46]. MALDI-TOF mass spectrometric analysis of both the water-soluble and acid-soluble aortic fractions, that were submitted to sequential steps of FPLC and HPLC reverse-phase column separations (Fig. 2A–D), revealed the presence of Aβ peptides with N-termini beginning at residues 1 through 5 and with C-termini ending at residues 39, 40, 41, 42 and 43 (Table 1). In addition, elongated Aβ peptides, extending from residues 1 to 48 (Aβ sequence numbering), were detected in peak 7 of the HPLC C3 reverse-phase column water-soluble fraction (Fig. 2D). This peptide could result from γ-secretase cleavage close to APP ε-site, which corresponds in the Aβ sequence to residue 49. Western blot analyses of the reverse-phase column fractions did not show the presence of significant amounts of Aβ42 possibly due to the comparatively low values of this peptide as detected by immunoassay.

3.2. Platelet analyses

Immunoassay analysis revealed that the inactivated platelets had an average of 84 ng/g tissue of Aβ40 and activated platelets contained an average of 57 ng/g tissue, while in both cases there were very low levels of Aβ42: 1.6 ng/g tissue and 1.7 ng/g tissue, respectively [46]. The Aβ42 in the platelets, whether activated or inactivated, represents only 2–3% of the Aβ40 values, closely mimicking the values observed for the atherosclerotic aorta [46]. In agreement with these low Aβ42 values, we did not observe Aβ42 in the chromatographic fractions of the aorta (Fig. 2D) or inactivated platelets by Western blotting (Fig. 3A), but this peptide was found in the activated platelets (Fig. 3B). Using size-exclusion FPLC and reverse-phase HPLC, the inactivated (Fig. 3A) and (Fig. 3B) activated platelet Aβ peptides were independently enriched and subsequently characterized by MALDI-TOF mass spectrometry (Table 2). Mass spectrometry of inactivated platelets chromatographic fractions confirmed the absence of Aβ42, while in the activated platelets, this peptide species was present, theoretically corresponding to peptide Aβ6-42 (Table 2). Intriguingly, the total Aβ value in the inactivated platelets is 30% higher than in the activated platelets [46]. A possible explanation is that aggregated Aβ becomes undetectable by the immunoassay or alternatively that upon degranulation of platelets, the Aβ was immunologically hindered by interaction with platelet-released fibrinogen [26]. Alternatively, any excess of free Aβ in the circulation will be complexed with albumin for which Aβ has a very high affinity [49–51]. Mass spectrometry showed that activated platelet Aβ peptides included species starting at residues 1, 2, 3, 4, 5 and 6, with C-termini at residues 37 through 42 (Table 2). The degraded N-terminal Aβ sequences in platelets and in atherosclerotic vessels are very similar to those observed in the brains of AD subjects [52,53].

4. Discussion

The atherosclerotic aortas contained Aβ peptides composed primarily of the Aβ40 species [46]. The Aβ peptides may have been produced at the site of the lesions by myocytes, endothelial cells [54,55] or platelets [33,34]. The preponderance of Aβ40 species in the vascular lesions may simply reflect the greater solubility and mobility of this peptide. Immunoassay studies demonstrate that significant levels of Aβ42 are found in plasma [46,49], while in the aorta this peptide species is less represented [46], suggesting that the plasma Aβ42 is not the dominant contributor to atherosclerotic plaques. Little is known about the catabolic fate of the Aβ peptides generated in skeletal muscle, a tissue composing about one third of the total body mass. The post-synaptic domain of the skeletal muscle cholinergic neuromuscular junctions contains APP and Aβ peptides [56]. The Aβ peptides may be degraded mainly in the muscle itself, but it is possible

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that some of the intact Aβ peptides are cleared into the circulation. The skeletal muscles contain a higher ratio of Aβ42 [57] and analogous proportions of this molecule are not observed in aortic walls. Based on the tissue ratios of Aβ40:Aβ42, the experimental observations suggest that the Aβ contributions by plasma, brain or muscle to the atherosclerotic lesions may be minimal. Measurements of Aβ40 and Aβ42 in activated and inactivated platelets revealed that Aβ40 was the dominant peptide, similar to the values observed for the atherosclerotic aorta [46]. Therefore, judging from the relative amounts of Aβ40 and Aβ42 it could be suggested that the Aβ peptides present in the atherosclerotic lesions mainly originate from activated platelets.

Table 1
Mass spectrometric analyses of the water-soluble and formic acid-soluble aortic Aβ peptides.

Peak	Mass observed	Mass calculated	Peptide fragment	Modification
Aorta acid soluble				
5	4287.89	4287.77	1–39	2 F
5	4330.24	4330.91	1–40	–
5	4399.66	4400.06	2–42	–
5	4529.57	4529.17	2–43	F
6	4514.41	4515.15	1–42	–
6	3981.14	3981.6	5–41	–
Aorta water soluble				
4	4259.37	4259.77	1–39	F
4	4374.36	4374.91	1–40	F + O
4	4487.59	4488.07	1–41	F + O
4	4259.37	4259.82	2–40	F + O
5	4531.37	4531.14	1–42	O
5	4547.38	4547.15	1–42	2 O
5	4643.42	4644.26	1–43	F
5	4243.06	4243.82	2–40	F
5	4516.30	4517.17	2–43	O
5	4243.06	4243.86	4–42	F + O
5	4332.14	4332.97	4–43	2 O
6	4444.67	4444.07	1–41	–
6	4516.29	4515.15	1–42	–
6	4356.53	4356.98	2–41	F
6	4444.67	4444.06	2–42	F + O
6	4311.59	4310.98	3–42	PG
6	4356.53	4356.98	3–42	F
6	4356.53	4356.97	4–43	2 F
7	4516.23	4515.15	1–42	–
7	5158.29	5157.96	1–48	O

The M_r deviation between the calculated and observed Aβ related peptides in all instances is less than ±1 Da. Post-translational modifications: O = oxidation; F = formylation; PG = pyroglutamyl.

The α-granules of platelets are considered the major source of circulating APP/PN2 and of Aβ [33,45]. The relentless vascular wall damage that accompanies atherosclerosis and concomitant reduced ability to repair are phenomena inherent to aging-associated vascular deterioration. These events favor increased platelet activation that is mostly mediated by local secretion of tissue factor VIIa and exposure to the von Willebrand factor [58,59]. Thus, platelets carrying APP/PN-2 are abundantly available in areas of vascular injury as they participate in the coagulation cascade. In the atherosclerotic lesions, activated macrophages contain endocytic vesicles loaded with platelets and Aβ peptides [34]. A body of evidence reveals a pathological correlation between AVD, AD and CAA [3,5,13,14,60] probably mediated by sustained arterial wall injury that elicits, in all of these conditions, chronic vascular and brain inflammation. In addition to the putative platelet-atherosclerotic-linked Aβ source, the Aβ peptides in the atherosclerotic plaques may also be directly derived from the arterial

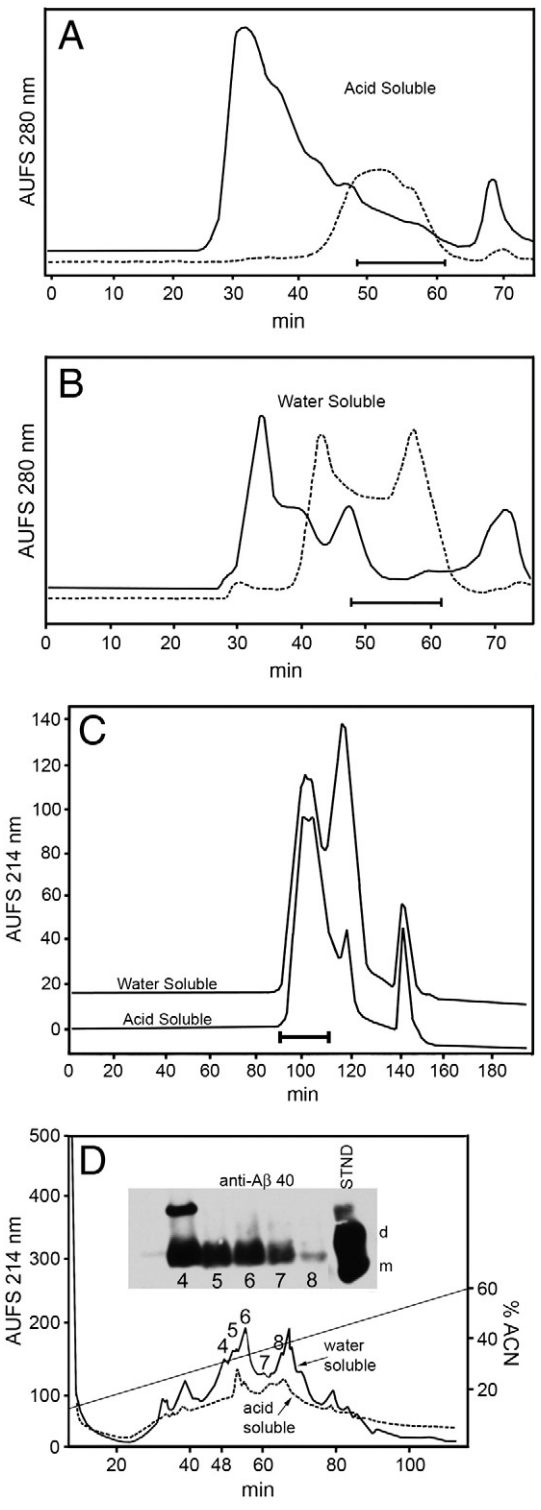

Fig. 2. Chromatographic profiles of aortic atherosclerotic wall. A) FPLC Superose 12 size-exclusion profile (solid line trace) of the whole aorta pulverized in liquid nitrogen and extracted with ammonium bicarbonate. The specimens containing the 2–10 kDa molecules (48–62 min, solid bar) were collected and re-chromatographed under the same conditions to enrich the Aβ-containing fractions (hyphened trace). B) FPLC Superose 12 size-exclusion chromatographic traces of the whole aorta pulverized in liquid nitrogen and extracted with GDFA. The continuous line represents the profile of the initial GDFA-soluble fraction. The eluants, collected from 2 of these chromatographies containing the 2–10 kDa, were pooled, concentrated and re-chromatographed under the same conditions (hyphened line trace). C) Size-exclusion HPLC (GPC-Protein column) profiles of both water and GDFA fractions collected in A and B, respectively. Western blot (data not shown) demonstrated the Aβ peptides were contained in the first peak (indicated by bar). D) Reverse-phase HPLC profiles of the Aβ-containing peaks shown in C. The fractions containing the Aβ peptides were detected by Western blot (insert). Only the Aβ peptides ending at residue 40 were detected in both the water-soluble and acid-soluble fractions. The standards correspond to monomeric (m) and dimeric (d) Aβ1-40.

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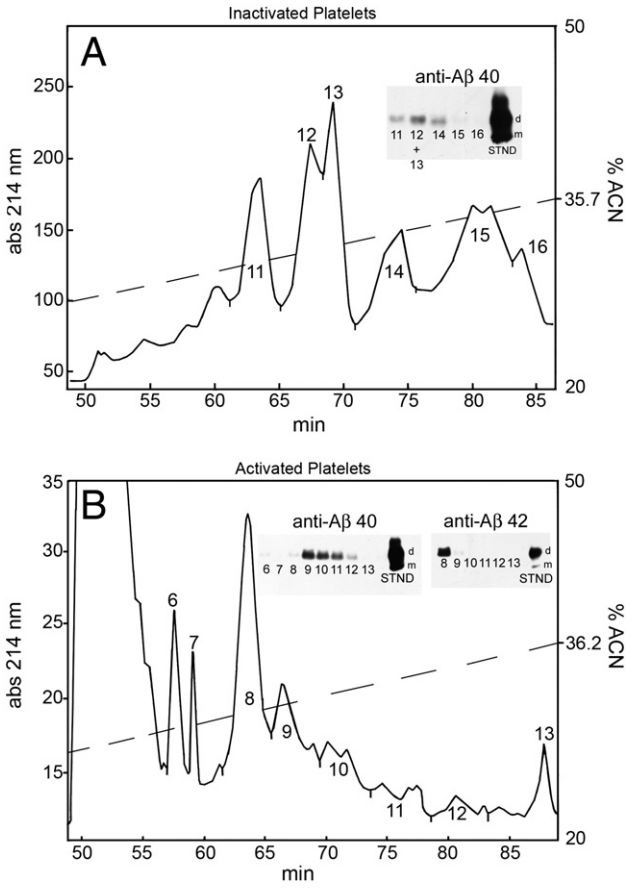

Fig. 3. Reverse-phase HPLC profiles and Aβ Western blots of human platelets. A) Resting platelets were lysed in GDFA and the acid-soluble fraction submitted to Superose 12 FPLC and Synchropak-GPC peptide HPLC fractionation. The fractions containing the Aβ peptides were further purified by C8 reverse-phase. Fractions 11–14 contained Aβ ending in residue 40. B) Amyloid-β peptides in activated platelets (see Methods section) were chromatographically enriched as described in A. The trace shows the HPLC C8 reverse-phase elution profile. Fractions 9–12 contain the Aβ peptides ending in residue 40 (see insert). Aβ42 was also observed by Western blot in peak 8 and confirmed by mass spectrometry. In both A and B, the standards correspond to monomeric (m) and dimeric (d) Aβ1-40/42 and the diagonal line indicates the acetonitrile gradient concentration.

Table 2
Mass spectrometric analyses of the water-soluble and formic acid-soluble activated and inactivated platelets.

Peak	Mass observed	Mass calculated	Peptide fragment	Modification
Inactivated platelets				
11	3915.98	3915.43	4–39	–
12	3943.05	3943.43	4–39	F
12	4030.02	4030.57	4–40	O
12	3882.05	3883.39	5–40	O
13	4043.96	4044.5	2–38	F
13	4043.96	4044.55	3–39	–
14	4028.95	4030.57	4–40	O
15	4070.55	4070.57	4–40	2 F
15	3925.36	3923.39	5–40	2 F
Activated platelets				
Peak	Mass observed	Mass calculated	Peptide fragment	Modification
8	4498.48	4499.01	1–41	2 F
8	4070.45	4070.57	4–40	2 F
8	3895.73	3895.39	5–40	F
8	3895.73	3895.44	6–42	–
9	4269.94	4270.76	2–40	2 F
9	3976.77	3977.42	3–38	O
9	4099.60	4100.55	3–39	2 F
9	4071.24	4070.57	4–40	2 F
9	3883.26	3883.39	5–40	O
10	4016.56	4016.50	2–38	–
10	4072.13	4072.55	3–39	F
10	3883.69	3883.39	5–40	O
11	4072.07	4072.50	2–38	2 F
11	4072.07	4072.55	3–39	F
11	3883.96	3883.39	5–40	O
12	3975.80	3975.44	2–37	O
12	4071.03	4070.57	4–40	2 F
12	3882.80	3883.39	5–40	O

The M_r deviation between the calculated and observed Aβ related peptides in all instances is less than ±1 Da. Post-translational modifications: O = oxidation; F = formylation.

walls proper, since vascular cells, like platelets, express the APP/PN2 with a KPI domain [61].

The presence of Aβ peptides in atherosclerotic plaques may synergistically increase the chronic inflammatory processes that sustain the degeneration and destruction of the arterial walls. Addition of human platelets to human and rodent macrophages in culture results in phagocytosis with induction of iNOS and foam cell formation [34]. Furthermore, in the atherosclerotic lesions, macrophage activation is triggered by, among other eliciting mechanisms, Aβ peptides that stimulate the production of iNOS and COX-2 [34]. A focal vascular inflammatory response in the vulnerable region of atheroma plaques may contribute to plaque instability and rupture [34]. A potent pro-inflammatory capacity of the Aβ peptides has been mechanistically demonstrated. The amino acid sequence of Aβ His-His-Gln-Lys, corresponding to residues 13–16, binds to heparan sulfate proteoglycans on the surface of macrophage microglia in the brain, inducing these cells to kill neurons [24,25]. In *in vivo* competition experiments, administration of this synthetic peptide suppressed Aβ-induced inflammation in the rat cerebral cortex [25]. Likewise, suppression of microglial synthesis of glycosaminoglycans or degradation of glycosaminoglycans also blocked activation of microglia and prevented neuronal demise [25]. We have previously shown that Aβ elicits the differentiation of monocytes into macrophages and induces the secretion of inflammation-related cytokines and chemokines [62]. Moreover, in a model of the BBB when Aβ is present on the brain side, it promotes monocyte/macrophage migration across the blood–brain barrier [62]. Vascular inflammation in AVD has traditionally been thought to be limited to large and medium-sized arteries, but recent work indicates that the systemic microvasculature is also affected [63–67] probably due to increased circulating concentrations of inflammatory mediators. In elderly humans, elevated serum C-reactive protein concentrations correlate with vascular inflammation and atherosclerosis, and with markers of brain microvascular inflammation in AD [68,69].

Hemostasis abnormalities such as increased activated factor VIIa and von Willebrand factor have been reported in AD [59]. Elevated platelet membrane fluidity [70], disturbances in protein kinase C [71] and in phospholipases C [72], and abnormal platelet activation [58] as well as increased serum levels of thrombomodulin and E-selectin [73] have also been observed in AD. In addition to the macrophage-mediated vascular inflammatory reactions, a second mechanism appears to link hemostasis with cerebrovascular amyloidosis. With advancing age the incidence of endothelial injury increases and the ability to repair decreases. This relentless emerging situation ultimately enhances APP/PN-2 production in platelets and vascular walls thereby contributing to a local reduction of hemostatic activity that favors production of cerebrovascular microhemorrhages [74,75].

5. Conclusions

Atherosclerotic vascular disease and AD cerebrovascular amyloidosis, despite their different end-stage manifestations, share pathophysiological pathways. Our studies demonstrate that the atherosclerotic lesions, common in elderly individuals, contain a heterogeneous

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mixture of Aβ peptides. Immunoassays suggest a preponderance of the shorter Aβ40 over Aβ42. Most likely the sources of these Aβ peptides are the cells of the vascular walls that express APP/PN2/Aβ and the platelets that participate in the atherosclerotic inflammatory and disturbed coagulation cascades inherent to chronic arterial wall degeneration. The increased and persistent synthesis of proinflammatory-related molecules by activated macrophages/microglia and activated platelets are, in part, mediated by the presence of Aβ peptides. In both AVD and AD, the Aβ peptides appear to contribute to the inflammatory reactions that ultimately culminate in vascular wall destruction.

Author disclosures

The authors stipulate that they have no competing interests.

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