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Platelets as a peripheral district where to study pathogenetic mechanisms of Alzheimer disease: the case of amyloid precursor protein

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Abstract

Alzheimer disease is a progressive neurodegenerative disease, characterised by a progressive cognitive and memory decline. From a neuropathological point of view, Alzheimer disease is defined by the presence of characteristic lesions, i.e. mature senile plaques, neurofibrillary tangles (NFTs) and amyloid angiopathy. In particular, accumulation of the amyloid β-peptide in the brain parenchyma and vasculature is an invariant event in the pathogenesis of both sporadic and familial Alzheimer cases. Amyloid β-peptide originates from a larger precursor, the amyloid precursor protein (APP) ubiquitously expressed. Among the different peripheral cells expressing APP forms, platelets are particularly interesting since they show concentrations of its isoforms equivalent to those found in brain. Moreover, a number of laboratories independently described alterations in APP metabolism/concentration in platelets of Alzheimer patients when compared to control subjects matched for demographic characteristics. These observations defined the frame of our work aimed to investigate if a correlation between levels of platelet APP forms and Alzheimer disease could be detected. We have reported that patients affected by Alzheimer disease show a differential level of platelet APP forms. This observation has several implications: APP processing abnormalities, believed to be a very early change in Alzheimer disease in neuronal compartment, do occur in extraneuronal tissues, such as platelets, thus, suggesting that Alzheimer disease is a systemic disorder; further, our data strongly indicate that a differential level of platelet APP isoforms can be considered a potential peripheral marker of Alzheimer disease allowing for discrimination between Alzheimer and other types of dementia.

Keywords: Alzheimer disease; Amyloid precursor protein (APP); Platelet; Diagnosis

1. Introduction

Alzheimer disease is a progressive neurodegenerative disorder characterized by a progressive cognitive and memory decline, increasingly frequent with advancing age until it affects as many as 50% of individuals 85 years of age and older. There is no remission in the progression of the disease nor are there any truly effective pharmacological interventions available at present, although new anticholinesterase inhibitors may represent a valid symptomatic treatment.

Prevention or delay of Alzheimer disease onset is among the most urgent moral, social, economic and scientific imperative in industrialised countries.

In the framework of an ageing society, in which many patients and families are devastated by the heavy burden of this irreversible disease, a better understanding of pathogenic mechanisms and identification of new pharmacological tools are key strategies.

From a neuropathological point of view, Alzheimer disease is defined by the presence of characteristic lesions i.e. mature senile plaques, neurofibrillary tangles (NFTs) and amyloid angiopathy.

In particular, accumulation of the amyloid β-peptide in the brain parenchyma and vasculature is an invariant event in the pathogenesis of both sporadic and familial Alzheimer disease.

Amyloid β-peptide originates from a larger precursor, the amyloid precursor protein (APP). Until now, nine APP transcripts have been found deriving from alternative splicing of a single gene localised on human chromosome 21,

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and encoding for different APP isoforms (ranging from 695 to 770 aminoacids).

2. The APP: structure and function

APP has been the centre of intense scrutiny in recent years due to its association with the pathogenesis of Alzheimer disease.

From a structural point of view, APP closely resembles a cell surface receptor comprising a signal peptide sequence, a large extramembranous N-terminal region, a single transmembrane domain and a small C-terminal tail (Fig. 1).

The general structure of the APP protein includes a number of folding and functional domains, each of them of putative relevance for the pathogenesis of the disease.

Starting from aminoterminal, a short 17 aa residue, the signal peptide, controls the correct topography of APP across cell membranes and secretion from the endoplasmic reticulum. A cystein-rich exon of 170 aa follows the signal peptide. This domain comprehends the first heparin binding site and a high affinity zinc-binding domain on exon 5. The zinc domain is responsible for the conformation of APP structure, thus, suggesting that environmental factors may act directly on APP protein processing. A 100 aa residue is then present in exons 5 and 6, forming the negatively charged region.

In two splice variants (APP 770–751), a Kunitz protease inhibitor (KPI) domain exists in exon 7. This domain is capable of regulating extracellular enzymes binding to and inactivating serine proteases. Another alternatively spliced exon is present in APP 770 following the KPI domain. This is the 19 aa product of an exon which is homologous to the OX-2 antigen and it is related to neurons and T cells (Kitaguchi et al., 1988; Weidemann et al., 1989). A second heparin binding domain is then present in exon 9, upon which interaction occurs with cell surface heparin sulphate proteoglycans, basal lamina and extracellular matrix. Nearer to the transmembrane region, two N-linked carbohydrate attachment sites are found on exons 13 and 14 (Pahlsson et al., 1992).

The amyloid β-peptide is finally found on exons 16 and 17 and it is followed by a short intracellular cytoplasmic domain, comprised by 46 aminoacid residues and possibly involved in signal transduction processes through association with Go proteins.

From the numerous functional domains described above, a number of physiological functions have been proposed for APP and its major metabolites. Indeed, the secreted form of APP (APPs, see below) can function as a growth factor and/or a neurotrophic factor, depending on the type of target cells (Van Nostrand et al., 1989). In addition, APP has been suggested to be crucial in regulating intracellular calcium concentrations (Mattson et al., 1992, 1993; Mattson, 1994). Furthermore, considering the finding that APP undergoes fast anterograde axonal transport, it has been postulated that APP is an important factor involved in neuronal cell homeostasis and in the maintenance or stability of synaptic structures and function (Koo et al., 1990).

APP can undergo endoproteolytic processing at three distinct sites, one at the aminoterminus of the amyloid β-domain (β-cleavage), one within the amyloid β-domain (α-cleavage), and one at the carboxyl-terminus of the β-domain (γ-cleavage).

Current evidence suggests the existence of diverse processing pathways for APP. One pool of the mature precursor is processed in a secretory pathway, with proteolytic α-cleavage occurring at or near the cell surface in a single site within the amyloid β-peptide sequence (Oltersdorf, 1990), in which α-secretase plays a key role. This pathway gives rise to a large NH_2-terminal ectodomain (secreted APP; APPs) secreted into extracellular fluids, as well as a carboxyl-terminal fragment, and precludes formation of amyloid deposits. Recently, two different candidates, members of the disintegrin and metalloproteases family (ADAM family) have been proposed for α-secretase activity: ADAM10 (Lammich et al., 1999) and tumor necrosis

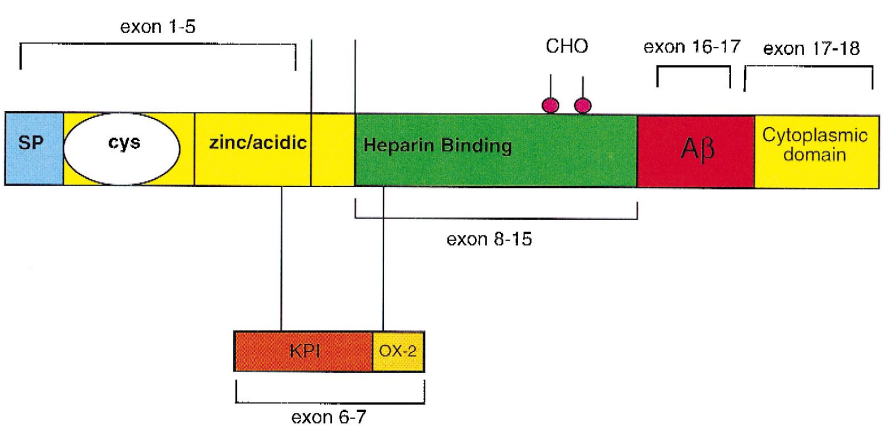

Fig. 1. Schematic representation of APP structure. SP: Signal peptide; Cys: cysteinerich domain; KPI: Kunitz protease inhibitor domain; CHO: carbohydrates binding.

factor α converting enzyme (TACE; Buxbaum et al., 1999). An alternative pathway involves cleavage of APP at the NH_2 terminal of the amyloid β-peptide sequence, which can ultimately lead to the release of amyloid β-peptide into the extracellular space (Seubert, 1993), and it is mediated by the action of β-secretase, a transmembrane aspartic peptidase recently cloned by Vassar et al. (1999) and known as beta site APP cleaving enzyme (BACE). These two proteolytic pathways are differentially compartmentalised within the cells. In fact, an α-secretase activity has been localised either in the trans Golgi network (TGN) or in the plasma membrane, whereas BACE activity is mainly confined to the endoplasmic reticulum and the endosomal/lysosomal system. Further differences between these two metabolic pathways derive from intracellular mechanisms that are able to modulate them. It is known that the metabolism of APP mediated by α-secretase is a highly regulated event influenced by both extracellular signals and intracellular second messengers. It has been previously reported that protein kinase C activation by phorbol esters, or by first messengers that act through protein kinase C, stimulates release of APPs from several mammalian cell lines (Buxbaum et al., 1990, 1992, 1994; Caporaso et al., 1992) and from slices of different brain areas (Farber et al., 1995; Caputi et al., 1997). Moreover, it is known that biochemical mechanisms able to regulate APP processing are further controlled by post-transductional modification of the protein, i.e. glycosylation. This biochemical process regulates in turn APP intracellular localisation, thus, preparing the protein to the differently compartmentalised amyloidogenic and non-amyloidogenic pathways. In the Golgi complex and endoplasmic reticulum, neuronal APP is subjected to glycosylation at asn and ser/thr residues (respectively N- and O-glycosylation); only the fully glycosylated form is able to reach and to be inserted into the plasma membrane, where it can be a substrate for α-secretase, thus, generating APPs, a fragment known to play many physiological roles (Mattson et al., 1993). Specific proteins are able to reinternalize the fully glycosylated APP into the cell and to store it into selected intracellular compartments, where the protein will undergo either α- or β-secretase cleavage. Moreover, although a great importance has been attributed to O-glycosylation processes of APP in regulating its functional role, the presence of N-glycosylation sites in APP suggests a possible functional role in the maturation processes of the protein (Yazaki et al., 1996; Tienari et al., 1996; Caporaso et al., 1992; Pahlsson and Spitalnik, 1996).

3. APP in platelets

APP is one of the most abundant proteins present in central nervous system, but it is also expressed in peripheral tissues, such as muscles cells, epithelial and circulating cells. Supporting the finding of ubiquitous expression of APP isoforms, large N-terminal fragments — product of secretase's activity — are found in cerebrospinal fluid (CSF), blood and urine.

Among the different peripheral cells expressing APP isoforms, platelets are particularly interesting since they show concentrations of its isoforms equivalent to those found in brain (Van Nostrand et al., 1990). Some differences between these two cellular populations are nevertheless present both at mRNA and at protein levels: the isoform 695, lacking the KPI domain, is by far the most abundant in neuronal tissue, whereas its expression is nearly undetectable in platelets where the major isoform is APP 770 (Tanzi et al., 1987; Kang et al., 1987). Further 50% of brain APP is full length, compared with only 10% in platelets.

Thus, platelets represent an important peripheral source of APP (Gardella et al., 1990, 1992; (Fig. 2). It has been demonstrated that three major APP isoforms with apparent molecular weight ranging from 106 to 130 kDa are inserted in the membrane of resting platelets and that both platelets and megakaryocytes express three transcripts encoding for the major isoforms: APP770, APP751, APP695 (Selkoe et al., 1988; Gardella et al., 1990, 1992). Following platelets' activation, full length APP is proteolytically processed (Li et al., 1995), surface APP detectability is increased threefold (Li et al., 1994) and α-granule stores of APP fragments are secreted (Bush et al., 1990; Cole et al., 1990). The processing and secretion of APP fragment imply fundamental role of APP in these circulating cells. Indeed, several hypotheses have been put forward on the possible physiological role of APP in blood (Cole et al., 1990; Smith et al., 1990; Van Nostrand et al., 1990): it is now known that APPs containing the KPI domain (sAPP-KPI +) is highly homologous to protease Nexin II, and it inhibits the activity of the blood coagulation factors IXa, Xa and XIa (Schmaier et al., 1993; Mahadi et al., 1995; Smith et al., 1990; Van Nostrand et al., 1990). The inhibitory activity of APPs on XIa factor is further increased by the presence of heparin and zinc (Smith et al., 1990; Van Nostrand, 1995). This may give a physiological rationale for the putative zinc and heparin-binding domains localised on APP sequence (see above, Fig. 1). It has been reported that APPs also inhibits the activation of human Hageman factor (factor XII) and prolongs the tromboplastin time (Niwano et al., 1995). The inhibition of factor XII is independent from the presence of the KPI domain, indicating that other regions of the APP structure are involved (Niwano et al., 1995).

It has been recently proposed (Li et al, 1998) that platelets contain all the enzymatic machinery to produce all APP metabolites from α- and β-secretase activities and that both APPs and the amyloid β-peptide can be stored in α-granules and released upon platelet activation.

Based on these observations, it is likely that the large majority of full length APP and its metabolites found in blood plasma may be derived from platelets and activated

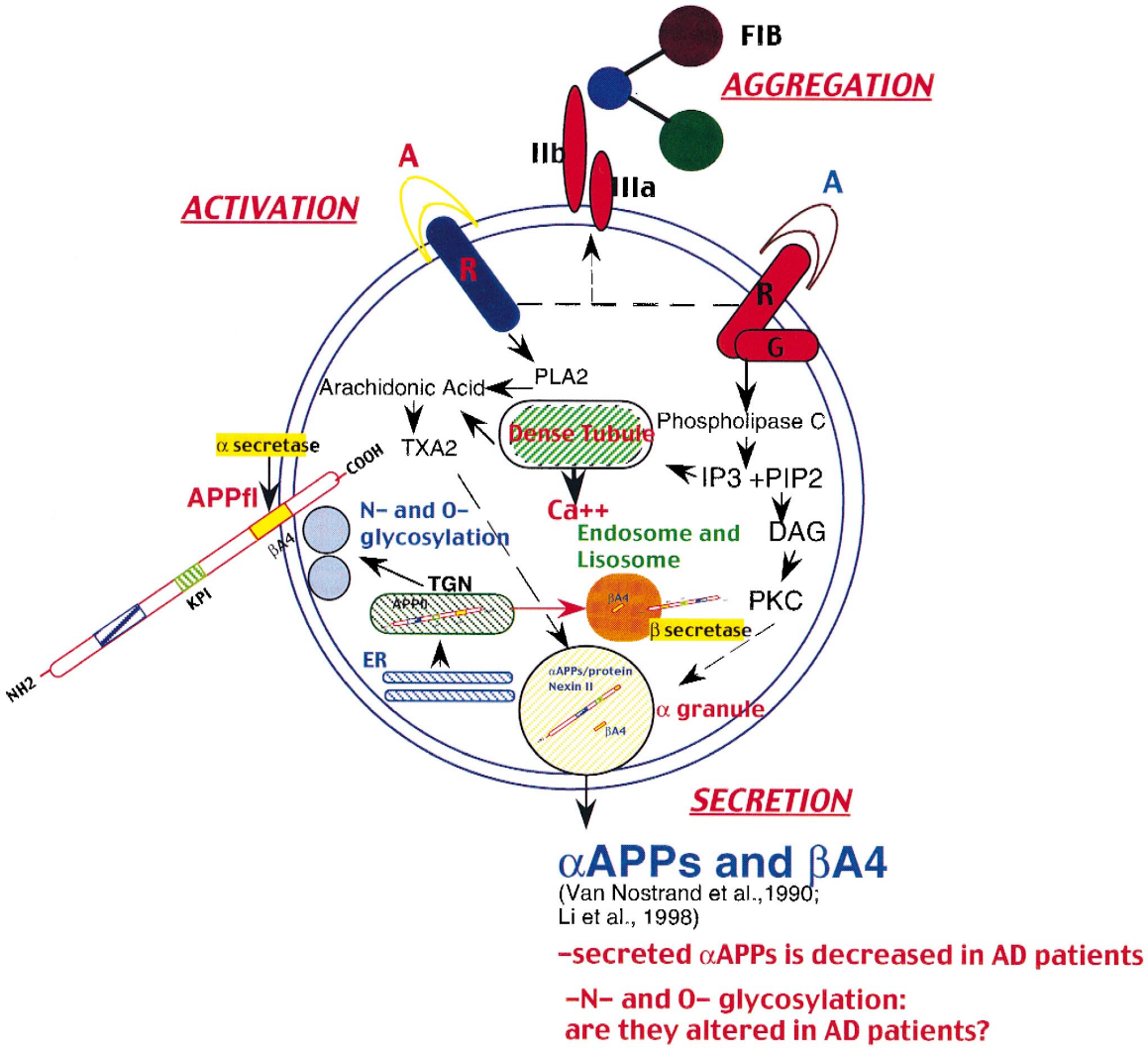

Fig. 2. Scheme of molecular mechanisms possibly interfering with APP processing in platelets.

lymphocytes/monocytes. Therefore, although unlikely to be the source or to contribute to cerebral amyloid deposition, platelet-associated APP forms provide an easily accessible source of human material to study APP biochemistry and metabolism both in physiological and pathological conditions. In addition, these studies may provide evidences for a parallelism between APP metabolism occurring in peripheral cells and in neuronal compartment.

The appropriateness to use platelets, as a cell mirroring some neurochemical processes, finds its rationale in the numerous similar features of platelets and neurons: platelets store and release neurotransmitters, express appropriate neurotransmitter transporters and some neuron-related proteins, such as NMDA receptors. These similarities, although provocative, define the framework for numerous studies describing some platelet's biochemical parameters as clinical markers for neurological disorders, such as depression and schizophrenia.

On this line, different authors reported abnormalities in platelets' physiology and function in Alzheimer disease. As first, Zubenko et al. (1987) described an alteration in platelet membrane fluidity in Alzheimer disease patients. Other studies performed on platelets, obtained from moderate to severe Alzheimer disease patients, reported cytoskeletal abnormalities, cytochrome oxidase deficiency, abnormal cytoplasmic calcium fluxes, abnormal Glutamate transporter activity, decreased phospholipase C activity and increased cytosolic protein kinase C levels (Davies et al., 1988; Sage and Ronk, 1987; Blass and Gibson, 1992; Matsushima et al., 1994, 1995).

Moreover, a number of laboratories independently described alterations in APP metabolism/concentration in platelets of Alzheimer patients when compared to control subjects matched for demographic characteristics (Di Luca et al., 1996; Davies et al., 1997a,b; Rosenberg et al., 1997).

These observations, taken together, identify platelets as an ideal cell where to study pathogenic mechanisms related to Alzheimer disease associated to the amyloid cascade, and raise the question whether platelets can be

considered an appropriate cell where to study peripheral markers of the disease of diagnostic relevance.

4. APP forms in platelets: a possible biochemical marker of Alzheimer disease?

It is well known that the diagnosis of Alzheimer disease is based on clinical assessments, confirmed post mortem by the presence of typical neuropathological lesions. In the last decade, different biochemical parameters, assessed in biological fluids, have been proposed as possible markers for Alzheimer disease (Gasparini et al., 1998); most of them have been evaluated in CSF (Vigo-Pelfrey et al., 1995; Motter et al., 1995). Nevertheless, the results obtained by different authors are controversial, and none of the described parameters seem to correlate to the progression of the clinical symptoms described for Alzheimer dementia.

These observations defined the frame of our work aimed to investigate if a correlation between levels of platelet APP forms and Alzheimer disease could be detected. To answer this question, we have evaluated a cohort of subjects including patients affected by sporadic Alzheimer disease, non-Alzheimer disease demented patients and control subjects matched with the other groups for demographic characteristics. Further, we have studied a population of Down syndrome affected patients (Di Luca et al., 1996). In all these subjects, we have evaluated the concentration of APP forms in whole platelet homogenate by means of western blot analysis with a monoclonal antibody recognising all APP forms present in the sample (Di Luca et al., 1996, 1998). This antibody recognised three different APP forms with apparent molecular weight in the range of 130 and 106–110 kDa. In platelets obtained from patients affected by sporadic Alzheimer diseases, a decrease of the level of 130 kDa band was present when compared to platelets prepared from control subjects and non-Alzheimer disease patients, thus, the ratio in optical density between the higher APP form and the lower APP forms is significantly decreased in Alzheimer patients when compared to both control subjects and patients affected by other kind of dementia (Fig. 3). Moreover, this APP altered level in platelet show a positive correlation to the progression of the disease, thus, suggesting that altered APP forms level in platelets may be strictly associated with the onset of Alzheimer disease. The mechanism by which the ratio in platelet APP forms is decreased in Alzheimer demented patients is under investigation. However, RT-PCR experiments demonstrated that Alzheimer patients showed the same levels of mRNAs encoding for the three major transcripts (APP770, APP751 and APP695) in platelets when compared to both control and non-Alzheimer demented patients groups. These findings suggest that the observed reduction in the ratio of platelet APP forms cannot be ascribed to a marked alteration in the

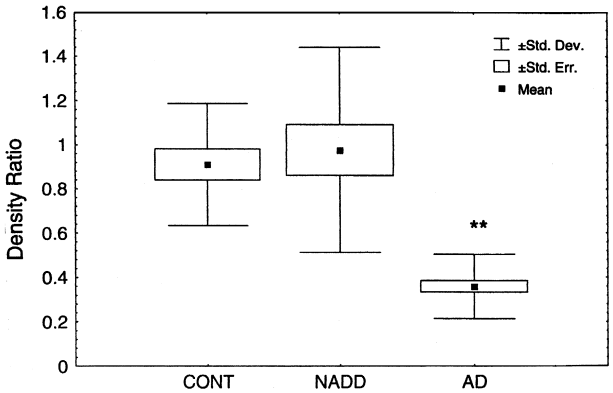

Fig. 3. Density ratio between the optical density of the upper band (130 kDa) and the lower bands (106–110 kDa) of platelet APP forms in control subjects (CONT), non-Alzheimer disease demented patients (NADD) and Alzheimer disease patients (AD).

expression of one of the three transcripts, since, although with a semiquantitative procedure, we were never able to observe any difference in the levels of these transcripts, even in the most severe cases of Alzheimer disease. Conversely, we can hypothesise that the observed decrease in platelet APP forms could be due to alteration in the processing of mature platelet APP in Alzheimer demented patients. The observation that Alzheimer patients show a differential level of platelet APP forms has several implications: APP processing abnormalities, believed to be a very early change in Alzheimer disease in neuronal compartment, do occur in extraneuronal tissues, such as platelets, thus, suggesting that Alzheimer disease is a systemic disorder; further, our data strongly indicate that a differential level of platelet APP forms can be considered a potential peripheral marker of Alzheimer allowing for discrimination between AD and other types of dementia.

Finally, platelets can be considered as a source of human biological material available for the study of the metabolic mechanisms mirroring, in the peripheral compartment, the evolution of the biochemical processes occurring in the central nervous system and related to Alzheimer disease.

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