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REVIEW

Beta amyloid peptide: from different aggregation forms to the activation of different biochemical pathways

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Abstract Amyloid beta peptide (Aβ) is the major component of amyloid plaques in the brain of individuals affected by Alzheimer's disease (AD). The formation of the plaques is due to an overproduction of Aβ by APP processing, its precursor, and to its ability to convert under specific conditions from its soluble form into highly ordered fibrillar aggregates. Although neuronal degeneration occurs near the amyloid plaques, some studies have suggested that intermediates such as protofibrils or simple oligomers are also involved in AD pathogenesis and even appear to be the more dangerous species in the onset of the pathology. Further, toxic properties of aggregates of different size have been investigated and the obtained results support the hypothesis that different aggregate sizes can induce different degeneration pathways. In the present review some of the knowledge about the biochemical routes of Aβ processing and production and the relationship among Aβ and oxidative stress, metal homeostasis, inflammatory process, and cell death are summarized. Moreover, current strategies addressing both fibrillogenesis process and different Aβ altered biochemical pathways utilized for therapies are described.

Keywords Beta amyloid · Fibrillogenesis · Apoptosis · Oxidative stress · Inflammation

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Introduction

Alzheimer's disease (AD), the most common form of dementia in the elderly, is characterized by neuronal cell loss and progressive accumulation of neurofibrillary tangles (NFT) in neurons, and amyloid fibers in neuritic (senile) plaques and in the walls of blood vessels (Wisniewski et al. 1997). The vast majority of people with AD have the late-onset form of the disease, in which symptoms of memory loss become evident at the age of 60 years or more. Less than five percent are diagnosed with the inherited form of the disease, sometimes as early as their 30 or 40 s. The major constituent of the amyloid deposits is an amphiphilic peptide (Aβ) derived from proteolysis of a large membrane spanning precursor protein, the amyloid precursor protein (APP) (Glenner and Wong 1984). This peptide, under some specific conditions, is converted from its native conformational state into a structure that differs from its native state. This phenomenon is known as misfolding of protein and it is a characteristic feature of different neurodegenerative diseases (Chiti and Dobson 2006). The intermolecular association, prompted by instability, strongly correlates with the increase of ordered structures rich in β-sheets, typical of amyloid assemblies. In vitro studies have demonstrated that Aβ forms easily fibrils, which can also further associate to build large fibrillar aggregates as mature fibrils or bundles of fibrils, similar to the constituents of neuritic plaques seen in vivo. These plaques are correlated with the extent of cognitive loss (Cummings et al. 1996). Filamentous tangles are, instead, composed by neurofilaments and hyperphosphorylated *tau* protein, a microtubule associated polipeptide. Although debated until now, according to the so-called amyloid hypothesis, tangles are considered only a secondary event in the disease progression, being a consequence of the formation of β-amyloid plaques (Verdile et al. 2004).

While the etiology of AD remains unclear, there is an increase of the genetic evidence that altered cellular processing of APP is a causative factor in many cases (Selkoe 1999). However, citoxicity of Aβ is not only due to the ability to form fibrillar aggregates in the extracellular environment, but also to the presence of soluble Aβ oligomers into the intracellular environment (Lambert et al. 1998). Both the forms, or their intermediates, produce different damages to cell membrane and different organelles inducing alteration of physiological biochemical pathways and leading to oxidative stress, inflammation and, at the end, to cell death via apoptosis (Chauhan and Chauhan 2006; Weiner and Frenkel 2006). Put together, biological and biophysical knowledge aim to develop strategies that utilize Aβ as a target for therapeutic agents in an attempt to reduce its production, inhibit aggregation, and neurotoxicity.

The origin of beta amyloid

The major constituent of the amyloid deposits, in AD disease, is an Aβ, the beta amyloid (Aβ), derived by the proteolysis from a large membrane spanning precursor protein, called APP (Glenner and Wong 1984; Masters et al. 1985).

In the APP molecule, the future 4 kDa polypeptide is located in the N-terminal region and in particular within the transmembrane domain. The Aβ is derived by the action of two protease referred as β- and γ-secretase that cleave APP in different steps (Nunam and Small 2000; Findeis 2007). APP is first cleaved by β-secretase allowing its large ectodomain to be released into the extracellular fluid and leaving a membrane-bound C-terminal fragment. This 99 amino acid long fragment is successively cleaved by γ-secretase producing Aβ to be released in the extracellular space. The C-terminal 12 amino acid residues of the Aβ peptide are hydrophobic and confer to the peptide the ability to self-aggregate and polymerise into amyloid fibrils. The term β of Aβ indicates, indeed, its propensity to form partial β-plated sheet structures once it aggregates into amyloid fibrils. Depending on the exact point of cleavage by γ-secretase, three principal forms of Aβ, comprising 39, 40, and 42 amino acid residues, respectively, are produced. The relative amount of β-amyloid 42 is particularly important because more prone to oligomerize and form amyloid fibrils respect than the shorter peptides. (Burdick et al. 1992; Jarrett et al. 1993). Production of β-amyloid is a normal process but in a small number of individuals, the overproduction of Aβ or an increased ratio of the 42 amino acid form appear sufficient to cause early onset AD (Citron et al. 1992; Cai et al. 1993; Suzuki et al. 1994; Kumar-Singh et al. 2006). Inherited mutation within the presenilin 1 (PS1) and presenilin 2 (PS2) genes increases the $A\beta_{42}/A\beta_{40}$ ratio through life and causes very early and aggressive forms of AD (Kumar-Singh et al. 2006; Bentahir et al. 2006). Both PS1 and PS2 are integral membrane proteins containing multiple transmembrane domains. They are involved both in γ-secretase and NOTCH receptor protein activity. γ-secretase is, indeed, a multiprotein complex consisting of PS1 or PS2, nicastrin PS1, APH1, and PEN2 (Haass and Steiner 2002). Moreover, recent studies have demonstrated that APP mutation enhances the preference of γ-secretase for the cleavage in position 42 in comparison with the position 40 and that the amount of γ-secretase governs the ratio of $A\beta_{42}/A\beta_{40}$ (Yin et al. 2007a, b). Further, several stress conditions upregulate APP expression and amyloidgenic processing of APP to generate Aβ (Atwood et al. 2003). Recent evidence indicates that microRNA (miRNA) pathways, implicated in gene transcriptional control, participate in the regulation of APP gene expression, suggesting that variations in miRNA presence, maybe due to stress, could contribute to changes in APP expression in the brain during AD disease (Hébert et al. 2008). Moreover, using a sensitive qRT-PCR platform, it has been demonstrated that deregulated brain miRNAs play a role in activation of pathway related to APP processing (Cogswell et al. 2008).

Aβ fibril formation

Amyloid beta peptide is present in the brain and cerebrospinal fluid (CSF) of healthy humans throughout life (Walsh et al. 2000) and the mere presence of Aβ does not mean neuropathy. In contrast, when neuronal injury appears, the aggregation of physiologically secreted soluble Aβ into oligomers and large Aβ fibrils is currently considered to be a crucial event in AD onset (Geula et al. 1998). Of course, the main question, not yet answered, remains why the Aβ peptide, which normally circulates in soluble form in the CSF and in plasma, becomes prone to aggregate, forming highly toxic oligomers and protofibrils and mature fibrils accumulating in plaques. Aβ peptide can interact in vivo with many different molecules which could play a significant role in the onset and progress of AD. However, as starting point, great effort has been spent to understand the mechanism of fibrillogenesis in vitro in simple conditions. The effect of sequence on the ability to form fibrils has been studied and has brought to identify a hydrophobic core in the group of residues 17–21 and a major role in the aggregation played by the C-terminus (Tycko 2003). This explains the seminal role shown by Aβ42 and other shorter peptides with full-length C-terminus, as outlined by examination of deposits in senile plaques. The structural analysis of the final ex vivo products from the deposited plaques helps to elucidate the Aβ aggregation mechanism. In fact, both electron microscopy and X-ray diffraction experiments on ex vivo Aβ amyloid fibrils, together with solid state NMR measured on in vitro samples have led to a structural

model for the core structure of an Aβ fibril (Tycko 2003). The search of a feasible model is simplified by the fact that amyloid fibrils are primarily β-sheet structures and also by the condition that the β-sheets form a cross-β motif, as clearly indicated by X-ray diffraction data on several amyloid systems (Sunde et al. 1997; Tycko 2003). Aβ molecules self-associate to form a protofilament made from a sequence of peptides stacked and helically wrapped around a principal axis. The Aβ molecules are in hairpin conformation with two beta-strands forming separate parallel β-sheets. The cross-β unit is therefore a double-layered β-sheet structure characterized by the typical intra- and inter-molecular distances detected by X-ray diffraction (4.7 and 10 Å). Data are consistent with a model for Aβ peptide fibrils, where two protofilaments pack face-to-face the C-terminus strands of the cross-β units. Dimensions of this filament can be of about 5 nm, in agreement with the thinnest fibrils seen by electron microscopy. Mature fibrils can be composed of more filaments and be 1 μm long. Actually, this model can be valid for all the fibrils, from a variety of amyloidoses, which yield a diffraction pattern remarkably similar to Aβ fibrils (Kirschner et al. 1986). Different amyloid fibrils may be composed of protofilaments built from a different number of β-sheets and the filaments of a different number of protofilaments. Furthermore, a super-helical structure can be obtained by wrapping around more fibrillar filaments. In vitro studies have been and are still essential to obtain kinetic models of amyloid fibril formation. The understanding of all key steps in the process (preliminary conformational changes, oligomerization, nucleation, elongation and branching) can help to choose appropriate therapeutic strategies. No single experimental method can be considered exhaustive and reveal all aspects of the whole process. Furthermore, the structural properties of the Aβ peptide in physiological conditions are still uncertain, due to low solubility of the molecule.

The low solubility of the Aβ peptide has a low solubility and easily forms fibrils when preformed seeds are added to the soluble peptide. For this reason it has been necessary to establish a protocol for Aβ preparation which assured experimental repeatability and also the possibility to compare and put together different kinetic results (Fezoui et al. 2000; Teplow 2006). Due to the evident presence of β-sheet structure in the ordered aggregates, a model involving a conformational change of the Aβ peptide, from an α-helix or random coil to a β-sheet structure has been proposed (Serpell 2000). Conformational changes are favoured by particular conditions of the system (pH, temperature, cosolvents, and salt concentration) or by the presence of instability in the sequence or by high concentration. As matter of fact, the fibrillogenesis tendency shows a dependence on pH, with a peak at around five, the peptide isoelectric point, and decreasing at increasing pH. It is generally accepted that fibril formation is a multistep process, whose onset is dependent on an initial nucleation step, which is the rate limiting step for the growth process, similar to any crystallization process (Lomakin et al. 1996). In the early stages of the aggregation, formation of intermediate species, dimers, and oligomers up to decamers has been detected by PAGE electrophoresis, SEC, fluorescence depolarization ratio, and sedimentation experiments (Sorenghan et al. 1994; Huang et al. 2000; Garzon-Rodigrez et al. 1997). Different Aβ peptide isoforms, for example, $A\beta_{40}$ or $A\beta_{42}$, show different oligomeric paths, although the instability of such species makes uncertain the determination of their population (Bitan et al. 2003). In fact, direct identification and characterization of intermediates in their native state are difficult. The reasons for this difficulty are the short lifetimes of these species, their low concentrations, and the lack of methods to resolve them from large mass Aβ fibrils. To overcome these experimental constraints, a chemical stabilization (cross-linking) during oligomer formation and Aβ fibrillogenesis has also been induced by freezing the oligomers. Afterwards, the solutions have been examined by PAGE electrophoresis and the oligomers identified (Bitan et al. 2001). Protofibrils and fibrils have been detected during amyloid aggregation, separated by SEC experiments, and characterized by electron microscopies (Harper et al. 1997). Aβ has been found to have surfactant ability in a surface tension study, and it has been suggested that above the critical Aβ concentration of 0.1 mM, at acid pH, the nucleation event is the result of Aβ micelle formation (Lomakin et al. 1997). At pH 3.1, a non-cooperative elongation process has been observed as monitored by static and dynamic light scattering, suggesting that under this experimental condition any preventive nucleation is not a rate limiting step of the process. Fibril formation mechanism has been successfully described in this particular case as a linear colloidal aggregation driven by diffusion and assembly of different size growing aggregates (Carrotta et al. 2005). Recently, conformational studies using the 11–28 fragment of Aβ, containing the core region responsible for Aβ aggregation, have demonstrated that the formation of α-helical structures is preceded by creation of 3(10)-helix/3(10)-turn structures (Juszczyk et al. 2009).

Aβ soluble oligomers

While oldest observations and investigations demonstrated that β amyloid aggregation was the principal cause to impart toxicity, more recently, a number of studies suggest that non-fibrillar, soluble forms of Aβ are also toxic to cultured neuron (Hoshi et al. 2003). The effect of three distinct assembly forms of synthetic Aβ high molecular weight oligomers, Aβ-derived diffusible ligands, the so-called ADDLs, and fibrillar Aβ was examined and it was found

that all three preparations were toxic to primary human cortical neurons, but the extent and mechanism of toxicity differed (Demuro et al. 2005; Deshpande et al. 2006). Studies using synthetic Aβ peptide, Aβ-containing cell culture medium, APP transgenic mouse, and human brain demonstrate that Aβ toxicity is a complex and multifaceted phenomenon that may be induced by multiple assembly forms of Aβ and which can result in a variety of effects leading to neuronal loss. Moreover, small oligomers have also been found in the intracellular environment as well as in the CFS of AD patients. These evidences, together with the low correlation between brain cell damage and plaques formation, support the hypothesis that "soluble oligomers" rather than mature amyloid fibrils are the more dangerous agents in AD (Kienlen-Campard et al. 2002; Gong et al. 2003; Verdile 2004).

Moreover, to begin to understand which are more toxic, oligomers or aggregates, a simple model system has been used, such as sea urchin embryo. Sea urchin embryos were separately incubated with a recombinant $A\beta_{42}$ peptide dissolved under different conditions at pH 7 or pH 3, to obtain small oligomers or large aggregates, respectively. By the percentage of the survived or morphologically altered embryos it was possible to demonstrate that small oligomers are more toxic than large aggregates (Carrotta et al. 2006). However, the question is still open, but the most recent finding obtained both in vitro and in transgenic mice studies supports the idea that soluble Aβ oligomers are the toxic intermediates rather than fibrillar insoluble Aβ aggregates, at least at the onset of the pathology.

Aβ and different neurotoxic paths

Another intriguing problem is to understand whether oligomers and aggregates activate the same o different biochemical pathways leading to cell death. Aβ peptide induces neurotoxic effects via extracellular and intracellular ways. The toxicity of oligomers or large fibril aggregates appears, indeed, in the ability to impair fundamental cellular processes. Understanding the mechanism leading to aggregate formation is not sufficient to explain how and why these aggregates are toxic for the neuron. If the toxicity is due to the plaques external to the neurons, aggregates could not interfere directly with the cell metabolism. The interaction of plaques with cellular membranes could cause damage by mechanical blockage of cellular exchanges with the outer environment, or free radical oxidative stress increase or inflammation but no damage to internal organelles. However, some evidences indicate that extracellular Aβ contributes to the intracellular pool of Aβ with an internalization mechanism, utilizing an endocytic pathway involving caveolae/lipid rafts (Saavedra et al. 2007). Thus, some external Aβ, probably under mono/oligomeric forms, enters inside the cell and begins its dangerous work. Further, intracellular accumulation of Aβ is the result of more processes such as Aβ decreased degradation, increased intracellular generation of Aβ together with the increased uptake of Aβ from an external source (D'Andrea et al. 2002).

Studies based on level of Ca^{++} and cell death mechanism have suggested that the toxicity of misfolded and aggregate states of different proteins, such as Aβ peptide, proceeds through the modification of the same specific biochemical proprieties and such modification depends on the type of aggregates rather than on the specific amino acid sequences (Bucciantini et al. 2004). Further, the cascade of events triggered by different aggregated polypeptides leading to cell death, starts, in many cases, with the alteration of the same cellular parameters such as imbalance of ion homeostasis and/or impairment of well determined signalling pathways (Bucciantini et al. 2004).

Aβ and apoptosis cell death

As described above Aβ in oligomers, aggregates, plaques and intermediate forms resides extracellularly or intracellularly. In both these cases, Aβ is able to induce neurodegeneration. Thus, the involved cell death pathway must have the ability to be induced both from the extracellular or the intracellular environment. Apoptosis is a well-known cell death mechanism that answers to this request and neurodegeneration has been often associated with apoptosis. This mechanism controls the normal homeostasis of cell population in normal development and inflammation through programmed cell death. Failed control of cell numbers through apoptosis is common in cancer; on the contrary, excessive apoptosis is considered to play a role in different neurological disorders, including AD, stroke, and Parkinson's disease. Proteolitic enzymes of the caspase family play a central role in initiating and sustaining the event resulting in apoptotic cell death. As mentioned earlier, apoptosis covers two pathways starting from the external (extrinsic) or internal (intrinsic) environment of the cell. In some forms of apoptosis, the extrinsic apoptotic pathway is initiated by activation of caspase 8 after death receptor ligation placed on cell membrane; in others, activation of the intrinsic apoptotic pathway is initiated by signalling molecules recruited by mitochondria that produce the cytochrome C release from mitochondrial matrix to cytoplasm and activation of the caspase 9 (Kerr 2002). Both these pathways are able to activate the executrix caspase-3 involved in the death process. Apoptosis is considered a mechanism involved in neurodegenerative diseases, because it is selective at the individual cell level. In AD, neuronal loss is prominent in the cerebral cortex and the limbic lobe, while different neuronal population is vulnerable in other neurodegenerative disease. Some studies have demonstrated the

involvement of Aβ in activation of apoptotic mechanism and recent evidence has indicated that soluble protofibrils and oligomers induce cell death utilizing this mechanism (Dickson 2004). Thus, the toxicity is independent of the complexity of the aggregate, and suggests that different forms might activate different intracellular or extracellular pathways that induce degeneration. Some experimental studies suggest that Aβ can activate caspases through the extrinsic pathway, by binding of extracellular Aβ to receptors, while other studies suggest that the intrinsic pathway that involves endoplasmic reticulum (ER) stress or mitochondrial stress may be more relevant suggesting an intracellular role in neurodegeneration (Glabe 2001; Oddo et al. 2003). Recently, utilizing a recombinant $A\beta_{42}$ peptide, under oligomeric and fibrillar aggregate forms, and neuroblastoma LAN5 cell line, it has been demonstrated that fibrillar aggregates are able to activate extrinsic apoptotic pathway by inducing caspase 8 and 3, whereas oligomers are able to activate mainly intrinsic apoptotic pathway by inducing caspase 9 and 3. Results demonstrate that different Aβ aggregation forms activate different apoptotic pathways from different environments: the extracellular and the intracellular (Picone et al. 2009). Due to its structure Aβ is able to bind a variety of biomolecules, including lipids, proteins, and proteoglycans. It has been supposed that Aβ peptides secreted into extracellular fluid never leave the membrane lipid bilayer and after fibrillation modify its electric property and alter ion channels. This could allow lethal concentration of calcium to enter into the cell and induce apoptosis (Marchesi 2005). Moreover, a subset of membrane proteins such as SEC-R (serpin-enzyme complex), integrins, RAGE (receptor for advanced glycosylation end-product), the α7nAChR (α7nicotinic acetylcholine receptor), the insulin receptor, and the same APP are able to bind monomeric and/or fibrillar forms of Aβ. Perturbation of their functions could induce degenerative pathways (Verdier et al. 2004).

Accumulation of Aβ within the lumen of the ER, where APP is synthesized and processed, may activate apoptotic mechanism through unfolded protein response inducing ER stress. Some mechanisms appear to contribute to apoptosis in response to ER stress such as activation of ER-specific caspase-12 (Nakagawa et al. 2000; Ishige et al. 2007). Another possibility is that intracellular Aβ may bind to alcohol dehydrogenase inside the mitochondria and activate apoptosis through stress and activation of caspase 9. However, the different soluble protofibrils, oligomer forms, or plaques could activate different apoptotic pathway depending by the amount of the different forms outside or inside the cells.

Aβ and oxidative stress

Apoptosis is the last activated pathway addressing cell death, but other biochemical pathways are activated earlier. Increasing evidence indicates that both fibrillar Aβ and soluble oligomers stimulate reactive oxygen species (ROS) formation establishing a link between Aβ and neurodegeneration (Praticò 2008).

Oxidative stress is a major feature in the pathophysiology of AD and it occurs when generation of free radicals during normal metabolic processes in the cell overpowers the anti-oxidative defense system. Oxidative stress is produced by free radicals, i.e., ROS that are generated by oxygen- and nitrogen-based molecules that have unpaired electrons. Due to the presence of unpaired electrons, free radicals are very unstable and highly reactive. If not removed or neutralized, they react with lipids, proteins, and nucleic acids and damage cellular functions. Generally, oxidative damage to the cellular components results in alteration of the membrane properties such as fluidity, ion transport, enzyme activities, and protein cross-linking. Excessive oxidative damage eventually leads to apoptosis, inflammation, and neurodegeneration.

Aβ-mediated increase in oxidative stress could be due to either increase in ROS production, or decrease in the enzyme activities involved in the antioxidant defence system, or dysfunctional mitochondria. A direct role of Aβ-mediated oxidative stress has been observed in APP/PS-1-transgenic mice in which a significant increase in protein oxidation and lipid peroxidation was seen (Mohmmad et al. 2004). In addition, inflammatory mediators are attracted by amyloid deposits that can further speed up the generation of an oxidative micro-environment (Behl 2005).

A relevant role is played by mitochondrion where cell energy is produced. ROS are minor cytotoxic products of normal mitochondrial metabolism. Imbalance between mitochondrial ROS production and the intracellular level of antioxidant defences leads to oxidative stress (Hockenbery et al. 1993). Moreover, dysfunction in mitochondria can lead to cell death. Defective adenosine triphosphate (ATP) production and increased oxygen radicals may induce mitochondria-dependent cell death in AD, because damaged mitochondria are unable to supply energy to the cell (Zhu et al. 2004). ROS can also cause oxidative damage to nuclear and mitochondrial DNAs. It includes base modification, deoxyribose oxidation, strand breakage, and DNA cross-linking (Wiseman and Halliwell 1996), resulting in the generation of more ROS through increased leakage of electrons, and further cell damage.

Several reports suggest that Aβ increases oxidative stress by increasing lipid peroxidation. Because lipids are membrane components, the interaction of Aβ oligomers and protofibrils with membrane causes the impairment of cellular processes originating oxidative stress and increasing free calcium ion concentration that eventually leads to apoptotic cell death (Kirkitadze and Kowalska 2005).

Extensive evidence suggests that not only Aβ can increase oxidative stress but oxidative stress can also stimulate Aβ generation and its aggregation (Kim et al. 2003; Chauhan and Chauhan 2006).

Aβ and metals

Metals have been detected around amyloid plaques in AD brain, and it has been seen that aggregation of Aβ is mediated by interaction with metals, in particular zinc (Zn) copper (Cu), and iron (Fe) (Bush 2003). The interest in studying the role of metals in the development of AD strongly increased after discovering that Cu^{++} and Zn^{++} chelators can be used to solubilize Aβ aggregates (Opazo et al. 2003). As referred earlier, both Cu^{++} and Zn^{++} ions are known to promote aggregation Zn^{++} always being more effective than Cu^{++} (Comai et al. 2003); moreover, Cu^{++} effectively inhibits the Aβ aggregation by competing with Zn^{++} for the histidine residues present in the Aβ sequence (Suzuki et al. 2001). Two distinct ways in which the metal ion can bind the peptide have been suggested, generically termed as inter- and intra-molecular modes, respectively (Curtain et al. 2001). In the inter-molecular mode, Aβ-peptides are supposed to be cross-linked with ''Aβ-metal-Aβ'' bridges, while in the intra-molecular mode the atoms participating in the metal coordination all belong to the same peptide. Different structural models for each of the two binding modes have been proposed in which different numbers of histidine residues are involved in the metal coordination. With a combination of complementary experimental techniques, the atomic structure around the metal binding site of Aβ peptides complexed with either Cu^{++} or Zn^{++} has been determined, and it has been observed that Cu^{++} bind to three histidines, whereas Zn^{++} prefers to be complexed to four histidines. Lacking a fourth histidine along the Aβ-peptide sequence, this geometrical arrangement hints at a Zn^{++} promoted inter-peptide aggregation mode (Stellato et al. 2006; Minicozzi et al. 2008). These results are in agreement with the hypothesis that assigns different physiological roles to the two metals, with zinc favoring peptide aggregation, and as a consequence, plaque formation.

Altered metal homeostasis may be an important factor leading to AD pathogenesis. Some evidences suggest that metals concentrated in amyloid deposits may also contribute to the oxidative insults observed in AD-affected brains. Aβ is also able to catalyse the reduction of Cu^{++} and Fe^{+++}, which, in the absence of sufficient antioxidant mechanisms, could lead to the production of toxic ROS (Huang et al. 1977). Oxidative stress in turn may contribute to Aβ accumulation by generating modified Aβ species that have a high tendency to aggregate and are resistant to clearance. Further, neuroprotective effect of soluble Aβ oligomers when associated with Cu^{++} and Fe^{++} has been proposed. They may be a transient specie preceding the formation of amyloid aggregates that prove to be less toxic than soluble oligomers (Maynard et al. 2005).

Aβ and inflammation

Extracellular amyloid deposit in senile plaques also trigger neuroinflammation that can contribute to neuronal loss through production of ROS and cytokines such as TNF-α and IL-1β (Akiyama et al. 2000; Weiner and Frenkel 2006).

Amyloid-beta deposition activates a potentially pathological innate immune response in AD. Inflammation is a response to eliminate the initial cause of cell injury as the necrotic cells and tissues resulting from the original insult. If tissue health is not restored in short time inflammation becomes chronic and continues to damage surrounding tissues. Brain inflammation is a pathological hallmark of AD. Oligomerization of beta-amyloid and deposition in the brain lead to microglial-cell, and astrocyte activation (Akiyama et al. 2000). Microglia, astrocytes, and neurons are, indeed, responsible for the reaction activating inflammatory mediators such as cytokines and interleukines (McGeer and McGerr 2001). In turn, inflammatory mediators and stress conditions enhance APP production and its amyloidogenic processing to generate Aβ (Atwood et al. 2003). On the other hand, Aβ induces expression of proinflammatory cytokines in glia cells in a various cycle (Lindberg et al. 2005; Vasto et al. 2007, 2008). Pratically, inflammatory response is at the same time the cause and effect of Aβ-induced toxicity and regulates soluble and insoluble amounts of Aβ.

Development of therapeutic strategies which target Aβ

Alzheimer's disease prevalence is ~1% between 65 and 69 years and it is higher than 50% in individuals above 95 years. As the human population continues to age and the number of patients affected by AD increases, requirements for therapies preventing the disease progression become more and more urgent. The progressive accumulation of Aβ aggregates is widely believed to be fundamental to the initial development and progression of AD, but Aβ toxicity is a complex and multifaceted phenomenon that may be induced by multiple assembly forms of Aβ and that triggers a cascade of biochemical events such as neurotoxicity, oxidative damage, and inflammation. Thus, the strategies to utilize, for an efficient therapy, should target both the oligomerization process and the different activated biochemical pathways inducing toxicity and degeneration. At the moment, many therapeutic efforts have been concentrated in reducing or modulating Aβ production, including secretase inhibition, increase of Aβ clearance with amyloid

vaccines, or block of Aβ aggregation with different sources such as antibodies, breaker peptides, or small organic and natural molecules that selectively bind and inhibit Aβ aggregation, and fibril formation (Walshe and Selkoe 2007).

Preventing the formation of cytotoxic oligomers should be an important goal for treating AD. While information is lacking regarding the range of Aβ assemblies present in human brain, therapeutic intervention should target the earliest stages of oligomerization to remove all potential Aβ aggregation forms rather than a single Aβ assembly. The decrease of the production of soluble Aβ monomer is particularly attractive, because it may be possible to titrate Aβ down to concentrations that will not permit oligomerization. However, different approaches have been investigated which target Aβ at different stages of the amyloid formation from its production from APP to its deposition.

The first level at which it could be important to act is to inhibit the accumulation of Aβ obtained by APP proteolysis. The development of potent highly selective inhibitors of β- and γ-secretase that can readily enter the brain and lower Aβ production is being actively pursued. The inhibitor, N *N*-[*N*-3,5-difluorophenacetyl)-l-alanyl]-*S*-phenylglycine *t*-butyl ester, for example, has been shown to reduce brain Aβ level when administred orally to APPV717F transgenic mice (Dovey et al. 2001).

Another experimental strategy, whose objective is to disrupt fibrillogenesis, is the so-called chelation therapy. This strategy leads to develop molecules that inhibit Aβ toxicity by preventing oligomerization. A Cu/Zn chelator such as clioquinol is able to distrupt Aβ fibrillar formation by chelating copper and zinc ions (Cherny et al. 2001). Experimentally clioquinol was administered orally to 21-month-old APP transgenic mice and a decrease in brain Aβ deposition and an increase in soluble Aβ were observed compared with control mice (Cherny et al. 2001). Although the mice utilized showed no obvious adverse side-effects, administration of vitamin B12 supplements has been successively demonstrated to be necessary (Tateishi 2000). Moreover, interaction between Aβ and metals ions generate ROS production, hallmark of oxidative stress (Liu et al. 1999; Chauhan and Chauhan 2006), and it has been demonstrated that Zn availability may regulate mRNA cytokine expression, influencing inflammatory response (Vasto et al. 2007). Thus chelation therapy may have a multiple effect on different Aβ caused cellular damages.

Inhibition of fibrillogenesis has been also studied using β-sheet breaker peptides (BSB), and, by employing a computational methods (docking), it has been found that a single molecule of BSB does not influence the interaction among the amyloid peptides while two BSB molecules are sufficient to prevent the aggregation process (Hetényi et al. 2002; Chini et al. 2008).

Although not yet well developed, a research area about employment of small chemical or natural molecules which bind and stabilize Aβ monomer preventing oligomerization and allowing the natural removal of monomer by the brain has been undertaken. Many inhibitors of in vitro Aβ aggregation have been identified, although molecules capable of disrupting pre-formed oligomers have not yet come to clinical trials (Walsh et al. 2005). Recent studies on animals using a small molecule inhibitor of in vitro fibrillogenesis, scyllo-cyclohexanehexol (AZD-103), are promising. Furthermore, natural molecules have been utilized to destabilize preformed Aβ fibrils. Ferulic acid (FA), a phenolic compound and a major constituent of fruit, inhibits Aβ fibril formation and also destabilizes preformed Aβ fibrils; this makes it a potential key molecule to employ in AD therapy (Ono et al. 2005). Moreover, FA has antioxidant effect as demonstrated by experiments in which free FA or entrapped in solid lipid nanoparticles (SNL), utilized as drug delivery system, reduces ROS production in neuroblastoma cell line (Bondì et al. 2009). Using a natural polycyclic pigment, hypericin (Hyp), it has been demonstrated by circular dichroism and fluorescence that Hyp can associate with precursors of the mature fibrils and perturb the aggregation process through intermolecular interactions with the Aβ peptides (Sgarbossa et al. 2008).

The vaccine approach is an emerging area as a result of the finding that Aβ immunization could clear amyloid plaques. The attraction of immunotherapy of AD lies in the possibility to vaccinate large segment of the aging population to treat or prevent the devastating effect of this neurological disorder. It has also been shown that antibodies against Aβ can effectively bind and neutralize neurotoxic Aβ oligomers, reverting memory deficit and improving cognitive functions in transgenic mice (Dodart et al. 2002; Klyubin et al., 2005). Some anti-Aβ antibodies are able to recognize multiple different toxic Aβ assemblies. The immune response could prevent cytotoxicity, facilitating the removal of soluble and deposited Aβ by promoting microglial clearance and/or by redistributing Aβ from the brain to the systemic circulation (Schenk et al. 1999). This strategy has been encouraged by studies showing that the induction of antibodies against residues 4–10 of $A\beta_{42}$ in TgCRND8 mice, overexpressing the APP Swedish and V717F mutation, inhibits both Aβ fibrillogenesis and cytotoxicity, without the inflammatory response observed after immunisation with full-length $A\beta_{42}$ (McLaurin et al. 2002). Vaccines have been also experimented on patients with clinically diagnosed mild to moderate AD with the results that these patients showed significantly slower rates of decline in cognitive function and daily living, over the 1-year period of assessment (Hock et al. 2003).

Treatment with antioxidants is a promising approach for slowing progression of AD to the extent that oxidative

damage may be responsible for the cognitive and functional decline observed in AD. Although a number of epidemiological studies have not found a clear link between antioxidant intake and reduced incidence of dementia and cognitive decline in elderly populations, some antioxidants such as Vitamins E and C (Ayasolla et al. 2004), gossypin (Yoon et al. 2004) melatonin (Zatta et al. 2003) curcumin (Shishodia et al. 2005) and Ginkgo biloba (Yao et al. 2001) are reported to have protective effect against Aβ neurotoxicity. It is suggested that a combination of antioxidants might be of greater potential benefit for AD, especially if these agents work in different cellular compartments or have complementary activity.

Inflammation has been shown to contribute to neurodegeneration in AD. The use of anti-inflammatory compounds in mouse models and human clinical trials has provided some promising results. Non-steroidal anti-inflammatory drugs (NSAID) including the ibuprofen, naproxene, ketoprofen, the curry spice curcumin, sulimdac, and indomethacin have been shown to be selective Aβ agents in a variety of model systems and their role in reducing inflammation, oxydative damage, and plaque formation has been demonstrated (Weggen et al. 2001; Lim et al. 2001).

The spice curcumin is an interesting molecule that at the same time inhibits fibrillogenesis and has potent anti-inflammatory and antioxidant activities. Curcumin is a major active component of the food flavor turmeric. It is extracted from the powdered dry rhizome of *Curcuma longa Linn* (Zingiberacee), a perennial herb, widely cultivated in tropical regions of Asia. It has been used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions and other diseases. Curcumin is a phenolic compound with a polar groups and it has been suggested that because of its chemical structure can cross the blood-brain barrier. At low doses, curcumin, indeed, is able to disaggregate Aβ as well as prevent fibril and oligomer formation (Yang et al. 2005). In vivo studies showed that curcumin injected peripherally into aged Tg2576 mice crossed the blood-brain barrier and bound plaques. When fed to aged Tg2576 mice with advanced amyloid accumulation, curcumin reduced amyloid levels and plaques.

Curcumin has NSAID activities independent of cyclooxygenase inhibition and reduces amyloid accumulation in vivo (Lim et al. 2001) but fails to reduce Aβ42 production in vitro (Sagi et al. 2003). Further, it has been seen that curcumin has chelation propriety for both iron and copper, but not zinc, and its treatment has been proposed as one mecha-

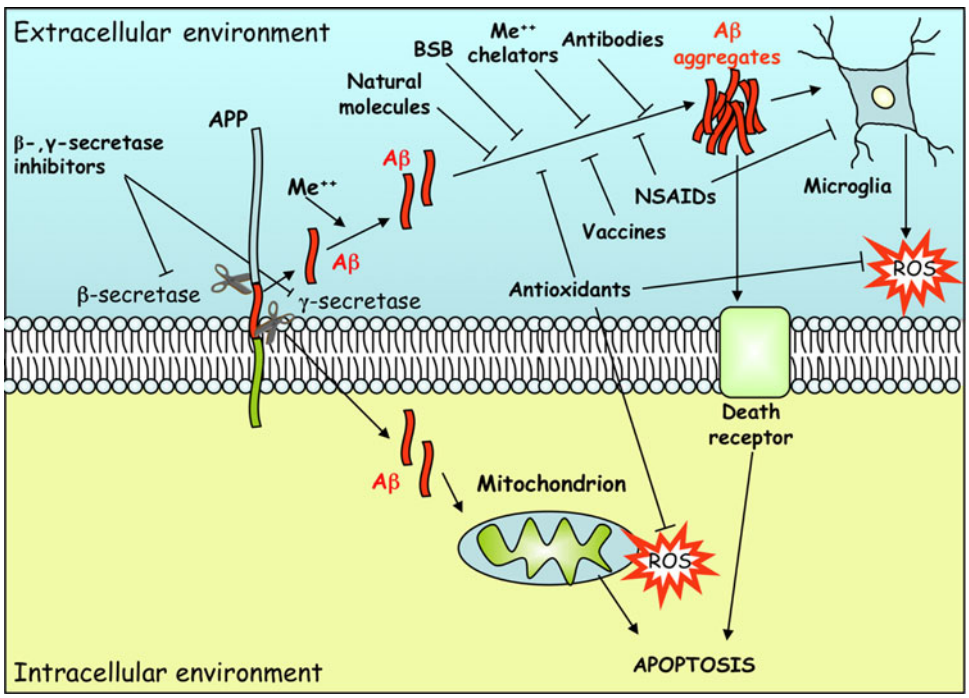

Fig. 1 Scheme indicating the production and aggregation of Aβ and its effect on cellular mechanisms and the potential action of some therapeutic agents. Aβ is produced by the proteolitic cleavage of β- and γ-secretases. In the extracellular environment, the Aβ peptide is able to aggregate and form fibrillar aggregates (Aβ aggregates) and to produce inflammation by microglia induction, oxidative stress by ROS production, and apoptosis. Metal ions (Me^{++}) can promote aggregation. Aβ is also present in a non-fibrillar soluble portion into the intracellular environment and can induce mitochondrial damage, oxidative stress, and apoptosis. The strategies for potential therapies target Aβ production, fibrillogenesis, and specific biochemical pathways. Further β- and γ-secretase inhibitors, metal chelators, natural molecules, antioxidants, β-sheet breaker peptides (BSB), non-steroidal anti-inflammatory drugs (NSAID), vaccines, and antibodies are also used

nism potentially contributing to amyloid reduction in animal models (Baum and Nu 2004). Several evidences, besides, indicate that oral curcumin treatment reduces, in central nervous system, the concentration of nitric-oxide synthase, inflammatory cytokines, and inhibits lipid peroxidation better than vitamin E (Chan et al. 1998; Zhao et al. 1989). Moreover, curcumin in central nervous system inhibits AP-1-mediated transcription in vivo (Luo et al. 1999) and activates genes such as heme oxigenase-1 (HO-1), fundamental for defending neurons by oxidative damage (Scapagnini et al. 2006). Thus, curcumin results to be a potent antioxidant and anti-inflammatory molecule. However, it is not possible to exclude that other phenolic antioxidants found in plant extracts, including gingko biloba, FA, and resveratrol from red wine might be similarly protective and potentially contribute to AD risk reduction as curcumin.

Conclusions

It is largely accepted that Aβ amyloid plaques are not the only causes of AD set in. Aβ can exist in different aggregation forms of different sizes and all these forms produce toxicity. The origin of cell death pathway can be different and depend on the localization and, probably, by the association with other molecules such as metal or specific proteins. Pharmacological strategies should be able to affect more points of origin of the dangerous stimulus including fibrillogenesis and the different biochemical pathways. A model of Aβ different activated pathways and potential therapeutic targets is proposed in Fig. 1. Employing molecules, such as curcumin or other natural antioxidants, inhibiting fibrillogenesis and protecting against different cellular damages, could be a promising therapy to defeat AD and probably other neurodegenerative disease.

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