Aberrant Co-localization of Synaptic Proteins Promoted by Alzheimer’s Disease Amyloid-β Peptides: Protective Effect of Human Serum Albumin

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Abstract. Amyloid-β (Aβ), Aβ40, Aβ42, and, recently, Aβ25-35 have been directly implicated in the pathogenesis of Alzheimer’s disease. We have studied the effects of Aβ on neuronal death, reactive oxygen species (ROS) production, and synaptic assembling in neurons in primary culture. Aβ25-35, Aβ40, and Aβ42 significantly decreased neuronal viability, although Aβ25-35 showed a higher effect. Aβ25-35 showed a more penetrating ability to reach mitochondria while Aβ40 did not enter the neuronal cytosol and Aβ42 was scarcely internalized. We did not observe a direct correlation between ROS production and cell death because both Aβ40 and Aβ42 decreased neuronal viability but Aβ40 did not change ROS production. Rather, ROS production seems to correlate with the penetrating ability of each Aβ. No significant differences were found between Aβ40 and Aβ42 regarding the extent of the deleterious effects of both peptides on neuronal viability or synaptophysin expression. However, Aβ40 elicited a clear delocalization of PSD-95 and synaptotagmin from prospective synapsis to the neuronal soma, suggesting the occurrence of a crucial effect of Aβ40 on synaptic disassembling. The formation of Aβ40- or Aβ42-serum albumin complexes avoided the effects of these peptides on neuronal viability, synaptophysin expression, and PSD-95/synaptotagmin disarrangement suggesting that sequestration of Aβ by albumin prevents deleterious effects of these peptides. We can conclude that Aβ borne by albumin can be safely transported through body fluids, a fact that may be compulsory for Aβ disposal by peripheral tissues.

Keywords: Alzheimer’s disease, Aβ disposal, amyloid-beta, PSD-95, serum albumin, synaptophysin, synaptotagmin

INTRODUCTION

Alzheimer’s disease (AD) is characterized by the formation of plaques composed of amyloid-β peptides (Aβ) [1]. The more frequent Aβ found in these plaques are Aβ40 and Aβ42 which are formed from AβPP (amyloid-β protein precursor) by β- and γ-secretases [2]. Recently it has been found that these peptides can be detached from the plaques by a
spontaneous mechanism that is not dependent on enzymatic catalysis [3]. In addition, the shorter Aβ peptide that remains toxic, Aβ25-35, has also been found in the brain of AD patients, presumably coming from Aβ40 cleavage [4]. Thus, normal aging would promote racemization of serine26 of Aβ40, which may result in the formation of truncated forms of Aβ40 including Aβ25-35 [5]. This suggests that Aβ25-35 together with Aβ40 and Aβ42 can play a role in the pathogenesis of AD. In this context, Aβ25-35 can reach mitochondria where it may cause the disarrangement of respiratory machinery [6]. This can be accomplished by the occlusion of TIM23/TOM40 mitochondrial protein permease system as caused by AβPP overproduction [7] or by the interaction of Aβ with the mitochondria permeability transition pore which may lead to the increase mitochondrial permeability and subsequent apoptosis [8]. Finally, an abnormal interaction of Aβ with Drp1 in neurons from AD patients can result in the inhibition of mitochondrial fission [9], a fact that can change mitochondrial number together with their distribution around synapsis (for a review see [10]).

One of the early events in neurodegeneration associated with AD is synaptic disarrangement, a phenomenon that presumably precedes Aβ plaque formation [11, 12]. Indeed, Aβ preferentially accumulates in synapsis [13, 14], which can result in the decrease in neuronal interconnections [15–17]. In agreement with this, it has been described that Aβ caused morphological and functional changes in synapsis [18, 19] and inhibited neuronal plasticity [20, 21].

Monomeric or oligomeric Aβ can be transported from brain interstitial compartment to the cerebrospinal fluid (CSF) [22] and from this to peripheral blood circulation [23, 24]. Most of the Aβ in CSF and blood is associated with serum albumin [25, 26], which may prevent Aβ polymerization [27]. Moreover, it has been proposed that serum albumin plays an important role in Aβ clearance and elimination [28]. Aβ concentrations in the blood correlated with those observed in the brain, leading to the proposal of the “peripheral sink hypothesis” for disposal of the peptides [28]. According to this hypothesis, the “peripheral sink” would drive Aβ efflux from the brain. Indeed, serum albumin can play an important role in this process because Aβ is transported as Aβ-albumin complex by body fluids. In addition, Aβ disposal by the kidney may be mediated by serum albumin because megalin, i.e., a serum albumin receptor in the apical membrane of proximal tubular cells [29], has shown a protective effect promoting Aβ disposal [30–33]. Thus, Aβ would be transported by serum albumin from the brain to the kidney where its disposal is facilitated by the albumin-megalin endocytosis mechanism. It should be mentioned that neprilysin, a putative Aβ-protease, is abundant in the kidney brush border membrane [34], suggesting the occurrence of a convenient allocation of Aβ disposal machinery. In agreement with the role played by serum albumin in Aβ clearance from the brain, treatment of AD patients by repeat plasma replacements with Aβ-free human serum albumin decreased brain Aβ accumulations and ameliorated cognitive test results [35].

In a previous work, we showed that the presence of serum albumin promoted neuronal survival in the presence of Aβ25-35, which can be explained because serum albumin prevented amyloid entry into neurons [36]. In this work, we have studied the protective effect of human serum albumin on the changes caused by Aβ25-35, Aβ40, and Aβ42 in neuronal viability, reactive oxygen species (ROS) production, and synaptic protein expression and localization in neurons in primary culture.

MATERIALS AND METHODS

Neuronal cultures

Albino Wistar rats were obtained from the animal house of the University of Salamanca (Spain) and were used according to local and EU Ethical Committee guidelines. Cell cultures were carried out as previously described [37]. Briefly, fetuses at 17.5 days of gestation were delivered by rapid hysterection after cervical dislocation of the mother. Animals were decapitated and their brains immediately excised. After removing the meninges and blood vessels, the forebrains were placed in Earle’s balanced solution (EBS) containing 20 μg/ml DNase and 0.3% (w/v) BSA. The tissue was minced, washed, centrifuged at 500 × g for 4 min and incubated in 0.025% trypsin (type III) and 60 μg/ml DNase I for 15 min at 37°C. Trypsinization was terminated by the addition of DMEM containing 10% FCS. The tissue was then dissociated by gentle passing it eight times through a siliconized Pasteur pipette, and the supernatant cell suspension was recovered. This operation was repeated and the resulting cell suspension was centrifuged at 500 × g for 5 min. The cells were then resuspended in DMEM containing 10% FCS and plated on Petri dishes coated with 10 μg/ml of poly-
L-lysine at a density of 1.0 \times 10^5 \text{cells/cm}^2. Neurons were maintained at 37°C and 5% CO2. One day after plating, cytosine arabinoside was added to avoid glial cell proliferation.

**Cellular treatments**

Aβ peptides (30 μM) were purchased from Bachem (Bubendorf, Switzerland) and were prepared in sterile deionized water. This concentration was chosen because dose-response experiments showed that 30 μM caused maximal effects with the three Aβ (see Supplementary Figure 1). Human Serum Albumin was obtained from Grifols (Barcelona, Spain). In order to prepare the HSA-Aβ complexes, Aβ peptides were gently dissolved in an HSA solution.

**Neuronal viability assay**

3 days in vitro neurons (3 DIV) were maintained in a serum-free medium (Hanks medium, pH = 7.4) with the different treatments for 20 h. Then, neuronal viability was determined by the MTT reduction assay [38]. Briefly, MTT (Thermo Fisher, Waltham, USA) was diluted in Hanks medium (0.5 mg/ml) and added to the cells. After 75 min of incubation (37°C, 5% CO2, in the darkness), the medium with MTT was replaced by dimethyl sulfoxide and cells were gently shaken for 10 min in the darkness. Finally, the absorbance was measured at 570 nm. Data are presented as percentages of cell viability as compared to non-treated cells.

**Reactive oxygen species production**

Production of ROS was measured using the fluorogenic 2',7'-dichlorodihydrofluorescein-diacetate probe (H2DCFDA, Thermo Fisher) [39]. 3 days in vitro neurons (3 DIV) were incubated in Hanks medium containing 10 μM H2DCFDA for 20 h treatments. Fluorescence at 535 nm was measured at the beginning and at the end of the experiment. The fluorescence difference was normalized using cell viability data and was expressed as the percentage of ROS production as compared to non-treated cells.

**Immunocytochemistry**

Immunocytochemistry was essentially carried out as described by Tabernero et al. [40]. After the treatments, cells were fixed in 4% paraformaldehyde for 20 min. In those experiments where mitochondria were localized, neurons were incubated with 100 nM MitotrackerRed (Thermo Fisher) for 20 min before fixation. Once fixed, cells were washed with PBS and permeabilized with 0.25% Triton X-100 for 1 h or 100% methanol for 10 min at –20°C. Then, neurons were incubated overnight at 4°C with primary antibodies (1:200) against Aβ25-35 (LSBio, Seattle, USA), Aβ40 (Novus Biologicals, Littleton, USA), Aβ42 (LSBio), Glut3 (Novus Biologicals), APP (Sigma-Aldrich, Madrid, Spain), PSD-95 (Thermo Scientific), or synaptotagmin (Synaptic Systems, Goettingen, Germany). Then they are incubated for 2 h at room temperature with (1:1000) secondary antibodies anti-rabbit or anti-mouse Alexa Fluor 488, 594, or 647 (Thermo Fisher). Finally, nuclei were stained with TOPRO3 (Thermo Fisher). Images were taken using a Leica DM-IRE 2 TCS-SP2 confocal microscope with LCS Lite Software (Leica Microsystems, Wetzlar, Germany). Fluorescence and co-localization were analyzed using Image J software (NIH, Bethesda, MD, USA).

**Western blot analysis**

Cell proteins were extracted using a lysis buffer containing 5 mM Tris-HCl (pH 6.8), 2% SDS, 2 mM EDTA, 2 mM EGTA, 1 mM PMSF, and cocktail protease inhibitors (Calbiochem, Darmstadt, USA). Lysates were centrifuged at 14,000 \times g for 15 min at 4°C. 20 μg of protein extract was analyzed in 10% precast commercial gels (NuPAGE Novex 10% Bis-Tris Midi Gel 1.0 mm). The buffer used for protein electrophoresis was NuPAGE MOPS SDS Running Buffer 20X. NuPAGE Sample Reducing Agent 10X and NuPAGE LDS Sample Buffer 4X were used to prepare the samples. Electrophoresis was run at room temperature using a constant voltage. After electrophoresis, gels were washed in transfer buffer (10% methanol, 0.1% NuPAGE Antioxidant diluted in NuPAGE Transfer Buffer 2X) for 10 min. Then, the proteins were transferred to a nitrocellulose membrane (iBlot Gel Transfer Stacks Nitrocellulose) for 10 min, applying a constant voltage. All products used for electrophoresis and subsequent electrotransfer were purchased from Invitrogen (Thermo Fisher). After blocking for non-specific binding, the membranes were incubated overnight at 4°C with mouse monoclonal antibody against synaptophysin 1:2500 (Santa Cruz Biotechnology, Dallas, USA), Mouse monoclonal antibody against α-tubulin (Sigma-Aldrich) was used to nor-
malize and quantify protein expression. After several washes, membranes were incubated with secondary antibody against mouse immunoglobulin conjugated with peroxidase. Finally, membranes were incubated for 1 min with peroxidase substrates, which afforded a chemiluminescence reaction. The signal collected on the autoradiographic film was proportional to the amount of protein in the membrane. The bands were quantified using an image analysis program.

**Statistical analysis**

All results are presented as the mean ± SEM of at least three independent experiments \( (n \geq 3) \). Data were analyzed for statistical significance with one-way ANOVA, followed by an appropriate *post-hoc* test. Dunnett test was used to compare all the values with the control and Tukey test to compare all the values among themselves. Values were considered significant when \( p < 0.05 \).

**RESULTS**

**Effects of Aβ peptides on cell viability in neurons in primary culture**

The three Aβ assayed promoted cellular death in neurons in primary culture although Aβ25-35 showed a more conspicuous deleterious effect (Fig. 1). The presence of serum albumin *per se* was able to decrease neuronal death caused by Aβ25-35 but not by Aβ40 or Aβ42 (Fig. 1). However, if the Aβ-serum albumin complexes were previously formed the effect of Aβ40 or Aβ42 on cell viability were fully avoided although Aβ25-35-albumin complex still showed a low deleterious effect (Fig. 1).

**Effects of Aβ peptides on ROS production in neurons in primary culture**

The presence of Aβ25-35 sharply increased ROS production in neurons in primary culture (Fig. 2). The effect of Aβ42 on ROS production was lower as compared to Aβ25-35. ROS production showed by the presence of Aβ40 was negligible (Fig. 2). In all the cases including controls the presence of serum albumin *per se* strongly prevented ROS production confirming the role of serum albumin as an inhibitor of ROS production [41]. Unexpectedly, Aβ40- or Aβ42-albumin complexes were unable to prevent ROS production suggesting that the changes in albumin conformation caused by binding to these amyloids are enough to avoid the role of albumin as an inhibitor of ROS production [41].
Fig. 2. Effect of amyloid-beta peptides on ROS production in the absence or the presence of HSA. Neurons in primary culture (3 DIV) were incubated for 20 h in Hanks medium with three different Aβ peptides: Aβ25-35, Aβ40, or Aβ42 (30 μM) in the absence or the presence of human serum albumin (HSA; 30 μM) or as HSA-Aβ complexes (30 μM). Results were normalized using cell viability data and are expressed as percentages compared to non-treated cells. Results are means ± SEM (n ≥ 6). One-way ANOVA and Tukey Test were applied in order to compare different groups. Distinct characters are used to indicate statistically different groups (p < 0.05).

Cellular localization of Aβ peptides in neurons in primary culture

Aβ25-35 was clearly internalized by neurons (Fig. 3) and reached mitochondria as showed by its co-localization with MitotrackerRed (yellow), a specific marker of mitochondria (Figs. 3 and 4). Under these circumstances, Aβ40 mostly remained outside the cell and formed discrete aggregations subtly bound to the neuronal membrane (Fig. 5). Instead, Aβ42 was found inside the cell (Fig. 3) mostly clustered in lumpy aggregations firmly pasted to the neuronal membrane (Fig. 5). These results are in agreement with Nagele et al. [42] who found an accumulation of exogenous Aβ42 but not Aβ40 in neuroblastoma cells.

Fig. 3. Cellular localization of amyloid-beta peptides in neurons in primary culture. Neurons in primary culture (3 DIV) were incubated for 5 h in Hanks medium with three different Aβ peptides: Aβ25-35, Aβ40, or Aβ42 (30 μM). After incubation, neurons were stained with MitotrackerRed, a mitochondrial marker (in red) and then were fixed. Immunocytochemistry against each Aβ was performed (in green) and nuclei were stained with TOPRO (in blue). Images were taken using confocal microscopy. Scale bar: 30 μm.
The presence of albumin per se did not change Aβ40 or Aβ42 cellular localization (see Supplementary Figure 3) but both Aβ-albumin complexes (C 40 and C 42; Fig. 5) did not enter neurons nor stick to their membranes but apparently they were mostly washed during the preparation of immunocytochemistry (Fig. 5).

Effects of Aβ peptides on synaptic generation and assembling

It has been suggested that an early event in AD is synaptic disruption, which resulted in the decrease in neuronal connections [15–17]. In agreement with this, our results show that the three Aβ assayed decreased synaptophysin expression (Fig. 6). The effect of Aβ25-35 cannot be prevented by albumin per se but it is mostly not observed in the presence of Aβ25-35-albumin complex (Fig. 6). Albumin per se decreased the effect of Aβ40 or Aβ42 on synaptophysin expression while Aβ-albumin complexes avoided their effects (Fig. 6).

In addition, we decide to investigate the possible effect of Aβ on the localization of synaptic proteins. Since our aim was to study the effects of Aβ on synaptic assembling, we challenged neurons with Aβ before this process was completed. Indeed, preliminary experiments showed that the effect of Aβ was observed as early as after 4 days in culture. Consequently, these experiments were carried out in 4 DIV neurons. In order to know if Aβ peptides elicited some short-term effects on synaptic assembling, we studied the short-term (2 h) effects of the three amyloids on pre-synaptic (synaptotagmin) and post-synaptic (PSD-95) proteins expression (Fig. 7). Synaptotagmin fluorescence staining decreased in the presence of Aβ25-35 but not significantly in the presence of Aβ40 or Aβ42 (Fig. 7). PSD-95 slightly decreased in the presence of Aβ25-35 or Aβ40 but not in the presence of Aβ42 (Fig. 7). Co-localization between both markers measured by yellow fluorescence decreased in the presence of Aβ25-35 but not in the presence of Aβ40 or Aβ42 (Fig. 7). More intriguing were the changes in the dis-
Fig. 6. Effect of amyloid-beta peptides on synaptophysin expression in the absence or the presence of HSA. Neurons in primary (3 DIV) culture were incubated for 20 h in DMEM medium with three different Aβ peptides: Aβ25-35, Aβ40, or Aβ42 (30 μM) in the absence or the presence of HSA (30 μM) or as HSA-Aβ complexes (30 μM). Then proteins were obtained and analyzed by western blot. Results are expressed as percentages compared to non-treated cells and are means ± SEM (n ≥ 7). One-way ANOVA and Dunnett Test were applied in order to compare different treatments vs. control. Distinct characters are used to indicate statistically different groups as compared to the control (p < 0.05). See Supplementary Figure 2 for raw western blot.

DISCUSSION

There is increasing evidence that Aβ and others AβPP metabolites may perform as antimicrobial agents or play a physiological role regulating protein expression as transcription factors [45–48]. However, there is a consensus that Aβ accumulation is the main event in the development of neurodegeneration observed in AD. In this context, the three Aβ assayed significantly decreased neuronal viability but Aβ40 elicited the higher effect (Fig. 1). This may be due to a more penetrating ability performed by Aβ25-35, since under our experimental circumstances, Aβ25-35 was internalized by the neuron and it reached mitochondria (Figs. 3 and 4). This result may be relevant in the context that it has been proposed that short Aβ peptides may be released from AD plaques enhancing neurodegeneration [3]. In agreement with this, Aβ25-35 fragments have been found in the brain of AD patients [4], probably synthesized by a senile degradative process that includes racemization of serine26 in Aβ40 [5]. If so, it is reasonable to suggest that short Aβ-fractions containing Aβ25-35 amino acid sequence may be released by AD plaques contributing to neurodegeneration observed in AD. Thus, these peptides would be internalized by neurons reaching mitochondria where they interfered with the respiratory machinery [6]. It should be mentioned that mitochondrial dysfunction is an early event in the development of AD [49], a fact that has been explained by the deleterious effects
Fig. 7. Quantification of PSD-95, synaptotagmin fluorescence, and co-localization. Fluorescence (Integrated density) was measured in confocal photographs as those depicted in Fig. 8 using NIH Image J Software. Co-localization fluorescence is referred to concurrence points of green (PSD-95) and red (synaptotagmin) fluorescence. Results are expressed as percentages as compared to non-treated cells and are means ± SEM (n ≥ 24). One-way ANOVA and Dunnett Test were applied in order to compare different treatments vs. control. Distinct characters are used to indicate statistically different groups as compared to the control (p < 0.05).

Fig. 8. Effect of amyloid-beta peptides on PSD-95 and synaptotagmin localization. Neurons in primary culture (4 DIV) were incubated for 2 h in Hanks medium in the absence or the presence of 30 μM Aβ25-35, Aβ40, Aβ42, HSA, or HSA-Aβ complexes (C 25–35, C 40, C 42). After incubation, cells were fixed and immunocytochemistry against PSD-95 (in green) and against synaptotagmin (in red) was carried out. Images were taken using confocal microscopy. Orthogonal projections along the z-axis of the images are shown at the bottom and right. Scale bar: 20 μM.

Fig. 8. Effect of amyloid-beta peptides on PSD-95 and synaptotagmin localization. Neurons in primary culture (4 DIV) were incubated for 2 h in Hanks medium in the absence or the presence of 30 μM Aβ25-35, Aβ40, Aβ42, HSA, or HSA-Aβ complexes (C 25–35, C 40, C 42). After incubation, cells were fixed and immunocytochemistry against PSD-95 (in green) and against synaptotagmin (in red) was carried out. Images were taken using confocal microscopy. Orthogonal projections along the z-axis of the images are shown at the bottom and right. Scale bar: 20 μM.

of Aβ on mitochondria. Indeed, Aβ interaction with mitochondrial transition pore [8] together with the inhibition of mitochondrial fission [9] would result in mitochondrial respiratory impairment and the subsequent increase in neuronal death (Fig. 1). It should be mentioned that our results are not concurrent with the idea that there is a direct correlation between ROS production (Fig. 2) and cell viability (Fig. 1), because both Aβ40 and Aβ42 increased cell death (Fig. 1) while Aβ40 did not change ROS production (Fig. 2). Conversely, we observed that those amy-
loids that are able to cross the neuronal membrane (Aβ25-35 and Aβ42) increased ROS production but those remained outside (Aβ40) did not change ROS generation (Figs. 2 and 3). This is in agreement with Boldyrev et al. [50] who did not find a correlation between cell death and ROS production in dissociated granular cell neurons exposed to Aβ. Moreover, Aβ variants unable to enter neurons do not impair synaptic transmission [51].

In our hands, no significant differences were found between Aβ40 and Aβ42 regarding the effect of both peptides on neuronal viability. This is in agreement with the idea that the enhanced deleterious effect of Aβ42 as compared to Aβ40 that has been reported [52] must be due to the lower clearance rate of the former through the blood-brain barrier [53]. In this context, soluble forms of Aβ are released into the CSF [22] and transported to the blood [23, 24] by a process in which serum albumin may play a crucial role. In fact, albumin not only showed a high affinity for Aβ but also prevented Aβ polymerization [27]. In agreement with this, our results showed that Aβ-albumin complexes avoided the effects of Aβ on cell death (Fig. 1), a fact according with the idea that albumin not only helps to transport Aβ through body fluids but also protects brain structures against the deleterious effects of Aβ. In agreement with this, repeated replacements of plasma by Aβ-free serum albumin (Albutein®) ameliorated cognitive decline in AD patients [35], which is consistent with the idea that albumin plays an important role in Aβ clearance and disposal. It should be mentioned that other serum proteins such as immunoglobulins (IVIG) have also shown to perform a protective role on preventing oxidative damage associated to AD [54, 55].

It has been proposed that neurodegeneration occurring in AD started by the decrease in dendritic spines and subsequent synaptic damage resulting in the impairment of neurotransmission [2]. In this context, Aβ preferentially accumulated in synapsis [13, 14] and the decrease in synaptic function correlates with cognitive decline in AD [15–17]. In agreement with this, our results show that both Aβ40 and Aβ42 decreased synaptophysin expression (Fig. 6), suggesting that neurotransmitter storage and/or release may be compromised in these circumstances [18, 19]. Moreover, a short exposition of neurons to Aβ40 or Aβ42 did not change PSD-95 and synaptotagmin expression (Fig. 7), but Aβ40 elicited a clear delocalization of PSD-95 and synaptotagmin from cell membrane to the soma (Fig. 8). This suggests the occurrence of a short-term effect of Aβ40 on synaptic disassembly. It should be mentioned that PSD-95 and synaptotagmin co-localization in adjacent membranes between neurons is a clear indication of prospective synapsis (Fig. 8 None, yellow points) [43, 44] suggesting that under our experimental conditions, Aβ40 is disrupting synaptic structure. According to Savioz’s model, as far as dendrites are disarranged with the progress of the disease, PSD-95 decreases in dendritic spines while its presence in the soma becomes apparent [56]. In this context, our results clearly indicate that in the presence of Aβ40, PSD-95, and synaptotagmin abnormally co-localized in the cell soma before the disarrangement of neurites (Fig. 8). This suggests that abnormal co-localization of PSD-95-synaptotagmin aggregates caused by Aβ40 precedes dendrite disarrangement. If so, the presence of Aβ40 may cause the disruption of synaptic connections by leading PSD-95 and synaptotagmin to an aberrant co-localization in cell soma instead of between adjacent neurites. In our experiments, this phenomenon takes place as early as 2 h after the exposition of the neurons to Aβ40, consistent with the idea that delocalization of PSD-95 and synaptotagmin can be the first step in the process causing neurodegeneration in AD.

Aβ40- or Aβ42-serum albumin complexes avoided the effects of these peptides on neuronal viability (Fig. 1), synaptophysin expression (Fig. 6), and PSD-95/synaptotagmin disarrangement (Fig. 8) suggesting that sequestration of Aβ by albumin prevents deleterious effects of these peptides in neurons. This may be a consequence of the conformation adopted by Aβ when it is bound to albumin that avoids toxic effects. In addition, the protective effect of albumin may be also due to the fact that Aβ-serum albumin complexes inhibit Aβ polymerization and/or their internalization into neurons. This may prevent Aβ to adopt a toxic conformation and/or to reach the undesirable target. In agreement with this, our results show that Aβ-serum albumin complexes are mostly not detected by immunohistochemistry (Fig. 5), a fact consistent with the idea that Aβ were washed out as Aβ-serum albumin complex before staining. Consequently, the adverse effects of Aβ were not observed when the amyloids were bound to albumin suggesting that Aβ-serum albumin complex may be formed just when amyloids are synthesized, as the first step of a process designed not only for brain Aβ-clearance but also for transporting Aβ through body fluids safely. It should be mentioned that AD patients showed low serum albumin concentrations as compared to those healthy counterparts [57], a fact that may contribute...
to the accumulation of Aβ in the brain and to the neurodegeneration associated with this disease.

Taken together, our results show that unlike Aβ25-35 and Aβ42, Aβ40 does not enter the neuronal cytoplasm nor increases ROS production but causes delocalization of synaptic proteins, a phenomenon that may account for synaptic disarrangement and subsequent loss of inter-neuronal connections observed in AD.

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SUPPLEMENTARY MATERIAL

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