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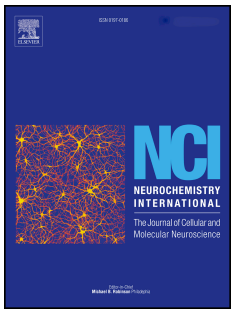

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MULTIFUNCTIONAL LIPOSOMES INTERACT WITH ABETA IN HUMAN BIOLOGICAL FLUIDS: THERAPEUTIC IMPLICATIONS FOR ALZHEIMER'S DISEASE

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Abstract

The accumulation of extracellular amyloid beta (Abeta42) both in brain and in cerebral vessels characterizes Alzheimer's disease (AD) pathogenesis. Recently, the possibility to functionalize nanoparticles (NPs) surface with Abeta42 binding molecules, making them suitable tools for reducing Abeta42 burden has been shown effective in models of AD.

Aim of this work consisted in proving that NPs might be effective in sequestering Abeta42 in biological fluids, such as CSF and plasma. This demonstration is extremely important considering that these Abeta42 pools are in continuum with the brain parenchyma with drainage of Abeta from interstitial brain tissue to blood vessel and plasma.

In this work, liposomes (LIP) were functionalized as previously shown in order to promote high-affinity Abeta binding, *i.e.*, either with, phosphatidic acid (PA), or a modified Apolipoprotein E-derived peptide (mApo), or with a curcumine derivative (TREG); Abeta42 levels were determined by ELISA in CSF and plasma samples.

mApo-PA-LIP (25 and 250 µM) mildly albeit significantly sequestered Abeta42 proteins in CSF samples obtained from healthy subjects ($p<0.01$). Analogously a significant binding (~20%) of Abeta42 ($p<0.001$) was demonstrated following exposure to all functionalized liposomes in plasma samples obtained from selected AD or Down's syndrome patients expressing high levels of Abeta42. The same results were obtained by quantifying Abeta42 content after removal of liposome-bound Abeta by using gel filtration chromatography or ultracentrifugation on a discontinuous sucrose density gradient.

In conclusion, we demonstrate that functionalized liposomes significantly sequester Abeta42 in human biological fluids. These data may be critical for future in vivo administration tests using NPs for promoting sink effect.

1. Introduction

Alzheimer's disease (AD) represents the most frequent cause of dementia in the elderly population. Key event in AD pathogenesis is the deposition and accumulation both in brain and in microvessels of beta amyloid (Abeta) at the extracellular level. This deposition eventually leads to synaptic failure and consequent neurodegeneration. Accumulation of the most fibrillogenic isoform, Abeta 1-42, may start in normal subjects at about 40 years of age and raises with advancing age, possibly due to a disequilibrium between the production and catabolism of this peptide. Abeta oligomers, more than fibrils, seem to be the direct cause of synaptic dysfunction (Walsh & Selkoe 2007). In fact, only a weak correlation between severity of dementia and Abeta fibrils density was shown in AD patients. On the other hand, recent studies show a strong correlation between soluble Abeta oligomer levels and the extent of synaptic loss and the severity of cognitive decline (Sakono & Zako 2010). Currently available therapies do not seem to be able to modify this pathogenic mechanism but are largely considered as symptomatic drugs. The possibility to reduce the amount of cerebral Abeta burden represents a potential therapeutic strategy under investigation. Brain and peripheral soluble Abeta are in equilibrium across the blood brain barrier (BBB); several clearance systems are responsible for the removal of Abeta from the brain. Receptors as LDLR, α2M and RAGE are involved in Abeta bidirectional passage through the BBB. Moreover, also perivascular drainage pathways represent clearance mechanism (Tarasoff-Conway *et al.* 2015). It has been proposed that a reduction in peripheral Abeta may result in decreased soluble levels in the brain, thereby reducing the formation of plaques (Matsuoka *et al.* 2003).

In recent years, many efforts to halt or reverse disease progression by immunomodulation strategies have been employed. Both active and passive immunization against Abeta has been tried first on animal model than in clinical trials on AD patients with alternating results (Wisniewski & Goñi 2015). De Mattos and colleagues (DeMattos *et al.* 2001) suggested that only a small number of anti-Abeta antibodies administered via passive immunization in a mouse model of AD entered the central nervous system (CNS) and the degradation and clearance of central Abeta is plausibly

reached by “peripheral sink” effect. Despite immunization of animal models gave promising results, clinical trial on human subject have not yet shown a real clinical benefit on disease progression, revealing immune toxic effects in some cases (Wisniewski & Goñi 2015). In particular, during immunotherapies, the risk to develop transient but detrimental effects as vasogenic edema and microhemorrhages, known as amyloid related imaging abnormalities (ARIA), has been demonstrated. Moreover, this risk is associated to the presence of ApoE4 genotype (Salloway *et al.* 2009).

More recent strategies rely on the use of nanoparticles (NPs) as innovative vehicles able to localize and directly interact with Abeta. The possibility to functionalize NPs surface with molecules able to bind Abeta allows, in fact, in prospective, to have available tools of enormous potential for attempting Abeta burden reduction via the sink effect.

Studies on animal models of AD demonstrated that liposomes functionalized for Abeta binding and BBB crossing were able to reduce both total brain insoluble and oligomeric Abeta42, resulting in amelioration of impaired cognition (Balducci *et al.* 2014). Moreover, liposomes functionalized only for Abeta binding induced a reduction in brain and plasma Abeta via peripheral sink effect in APP/PS1 transgenic mice (Ordóñez-Gutiérrez *et al.* 2015). Lastly, bifunctionalized liposomes induced an efflux of Abeta oligomers from the “brain” side to the blood compartment in an in vitro model of BBB (Mancini *et al.* 2016).

In this work we tested the capacity of liposomes functionalized with different molecules to sequester Abeta42 in human biological fluids, *i.e.*, both plasma and cerebrospinal fluid (CSF). This proof of concept, in fact, is critical for thinking to future therapeutic applications of NPs in AD patients.

2. Materials and methods

2.1 Recruited subjects

Following approval by the ethical committee of the S. Gerardo Hospital (Monza, Italy), 20 AD patients were recruited (mean age ± SD: 75.5 ± 5.5, sex: 10M/10F, MMSE mean ± SD 18 ± 5.1). AD specialists diagnosed probable AD according to the NINCDS-ADRDA criteria (McKhann *et al.* 1984). Brain imaging and an extensive neuropsychological test battery excluded alternative diagnoses. Blood samples (5 ml) were collected in K_2EDTA tubes (4.08 mM final concentration), after overnight (ON) fasting. Plasma was obtained by centrifugation (3700 *g*, 20 min) and stored at -80°C until assay. Five Down's syndrome (DS) patients were included in the study as well: they were recruited from the "Eugenio Medea" Institute,"Associazione La Nostra Famiglia" Bosisio Parini (Lecco, Italy) and characterized by trisomy 21 chromosomal analyses.

Cerebrospinal fluid (CSF) was obtained from 10 control subjects, using a 21-gauge needle and collected in 10-mL polypropylene tubes. Part of the CSF was used for routine analysis (including leukocyte and erythrocyte count, glucose and total protein concentration). The remaining CSF was aliquoted into new polypropylene tubes, and stored at -20°C for 24 hours and then at -80°C until analysis.

2.2 Preparation and characterization of liposomes and Abeta42

Liposomes (Lipo) composed of sphingomyelin from bovine brain (Sm, Sigma-Aldrich), cholesterol (Chol, Sigma-Aldrich), (1:1 molar ratio) and functionalized with dimyristoylphosphatidic acid (PA, Sigma-Aldrich) or bi-functionalized with PA and the peptide CWGLRKLRKRLLR-NH_2 (MW 1698.18 g/mol, mApo) derived from the receptor-binding domain (a.a. residues 141–150) of human Apolipoprotein E, were prepared and characterized as previously described (Gobbi *et al.* 2010, Bana *et al.* 2014).

Briefly, lipids were resuspended in chloroform/methanol (2:1, v:v) and dried under gentle stream of nitrogen followed by a vacuum pump for 3 h to remove traces of organic solvent. The resulting lipidic film was resuspended with 10 mM phosphate-buffered saline (PBS), pH 7.4, and extruded 10 times at 40 °C through a 100-nm pore size polycarbonate filter (Millipore Corp., Bedford, MA)

under 20 bar nitrogen pressure with an extruder (Lipex Biomembranes, Vancouver, Canada). Lipid recovery after extrusion was assessed by phosphorous assay using the method of Stewart (Stewart 1980). mApo was added to maleimide containing liposomes in PBS to give a final peptide-to-maleimide molar ratio of 1.2:1. The mixture was incubated overnight at 25°C. Peptide-bound NL was separated from the unbound peptide using a PD-10 column (GE Healthcare, Uppsala, Sweden). The yield of coupling and the amount of coupled peptide was assessed by tryptophan fluorescence intensity measurements, as reported (Re *et al.* 2011).

TREG liposomes were prepared and characterized as previously described (Sancini *et al.* 2013). They were composed of a matrix of Sm/Chol (1:1 molar ratio) mixed with 10 molar% of a PEGylated lipid containing an azido terminus (3-deoxy-1, 2-dipalmitoyl-3-(4`-methyl(O-(2-azidoethyl)- heptaethylenglycol-2-yl)-ethylcarbamoylmethoxy ethylcarbamoyl-1H- 1`,2`,3`-triazol-1`-yl)-sn-glycerol) (Mourtas *et al.* 2011),for the coupling with TREG, a curcumin-derivative with a terminal alkyne group (N-propargyl 2-(3', 5'-di(4-hydroxy-3-metoxystyryl)-1H-pyrazol-1`-yl)-acetamide) synthesized as previously described (Airoldi *et al.* 2011). For the coupling of TREG to liposomes, CuSO4 (8 mM), sodium ascorbate (145 mM) and TREG (100 mM in DMSO) were added to liposomes prepared as described above, and the reaction was stirred for 6 h, pH 6.5, at 25°C. The resulting mixture was purified by gel filtration through a Sepharose 4B-CL column (Sigma-Aldrich, Milano, Italy).

All liposomes were characterized in terms of size and polydispersity using a ZetaPlus particle sizer (Brookhaven Instruments Corporation, Holtsville, NY, U.S.A.) at 25 °C and 0.25 mM total lipid concentration. Standard deviations were calculated from at least three measurements.

Liposomes functionalized with TREG were also prepared and characterized as previously described (Sancini et al. 2013).

Lyophilized human Abeta42 peptide (Phoenix Pharmaceuticals) was solubilized in cold Tris–Buffer (pH 9) to obtain a 1 mg/ml solution of non-fibrillary Abeta42, as previously shown (Zoia *et al.* 2011).

Biocompatibility of different liposomes used in the present work has already been tested on different cell lines (Sancini et al. 2013, Orlando *et al.* 2013, Bana et al. 2014).

Moreover, biocompatibility of PA-LIP, TREG-LIP and mApo-PA-LIP liposomes was also preliminarily tested on fibroblasts cultures in a wide concentration range (10, 100, 250, 500 µM) for 2 and 24 hours. At any concentration used liposomes were not able to affect cell viability (data not shown). Then, liposomes were added to CSF or plasma in a concentration range of 25-250 µM for 10 minutes, vehicle treated plasma represents basal condition and the vehicle is saline solution, while 0.5 µM Abeta42 (Phoenix) was added to plasma in ultracentrifugation on a discontinuous sucrose density gradient.

2.3 Ex vivo Abeta42 sequestration

CSF and plasma Abeta42 levels were measured with sandwich enzyme-linked immunosorbent (ELISA) assay according to the manufacturer's instructions (Millipore).

CSF samples from each subject were incubated with or without 25 µM, 250 µM mApoE-PA-LIP or 250 µM control LIP at 37°C for 15 minutes before the ELISA assay. Abeta42 content in treated samples was expressed as percentage of the corresponding vehicle-treated samples (controls).

Five selected AD patients that showed Abeta content above 500 pg/ml and 5 DS patients were treated with PA-LIP (25 and 100 µM), TREG-LIP (25 and 100 µM) and mApo-PA-LIP (100 and 250 µM) at 37°C for 15 minutes prior to ELISA.

2.3.1 Binding of liposomes to Abeta42 investigated by Molecular Exclusion Chromatography

Samples treated with mApo-PA-LIP 250 µM were also submitted to Molecular Exclusion Chromatography (18 cm x1 cm column, packed with Sepharose CL-4B resin, Sigma Aldrich, eluted with saline solution) prior to ELISA in order to distinguish fractions containing liposomes bound to Abeta42 and fractions with residual Abeta42. Each fraction eluted from the column was tested for

both liposome content and protein content by using Dynamic Light Scattering and Bradford Assay. (**Figure 1**), For Bradford Assay, 5 µL of each fraction was tested for protein content by adding 200 µL of Bradford reagent (BioRad, Munich, Germany) diluted 1: 5 in water, and the absorbance at 595 nm was measured by Victor3 1420 multilabel counter (Perkin Elmer). Liposomes containing fractions and protein containing fractions were concentrated to 200 µL each, and the amount of Abeta42 in the two samples (Abeta42 bound to liposomes or free Abeta42) was quantified by ELISA.

2.3.2 Binding of liposomes to Abeta42 investigated by ultracentrifugation on a discontinuous sucrose density gradient.

Plasma added with 0.5 µM Abeta42 was incubated with mApo-PA liposomes (250 µM) at 37°C for 15 minutes. After incubation, liposomes-bound Abeta42 was separated from free peptide by flotation in a discontinuous sucrose density gradient (60% sucrose on the bottom, 50% sucrose in the middle, sucrose-free buffer on the top) performed as described in Gobbi et al. (Gobbi et al. 2010). The samples were centrifuged in a Beckman MLS 50 rotor at 140,000 g for 2 h in polycarbonate tubes; 10 fractions of 450 µL each were collected from the top of the gradient and assayed for Abeta42 content by ELISA assay. As control, plasma added with 0.5 µM Abeta42 without liposomes was subjected to the same procedure. The experiment was performed on plasma from 4 subjects and repeated three times. The amount of Abeta42 in the fractions samples was quantified by ELISA.

2.4 Statistical Analyses

Data are reported as mean ± standard error (SEM). Statistical analysis was performed with GraphPad Prism, version 4.00 program. Either, unpaired sample, or repeated measures analysis of variance (ANOVA) tests were used as appropriate and followed by the opportune post hoc tests.

3. Results

Liposomes functionalized with PA or TREG, or bi-functionalized with PA and mApo were prepared and characterized by using different procedures already optimized by the authors (Bana et al. 2014, Sancini et al. 2013). Final preparations were monodispersed, with size of 120 ± 4 nm, 179 ± 5 nm, 123 ± 3 nm, respectively for PA liposomes, TREG liposomes and PA-mApo liposomes.

Preliminary experiments on plasma samples from AD patients were not able to demonstrate the capacity of PA or TREG liposomes to sequester Abeta42 because the plasma levels of this peptide were close to the lower determination capacity of our ELISA curve (mean value ± SD: 34 ± 19 pg/ml; data not shown). For this reason, we tested liposomes in plasma from specifically selected AD patients (n=5) and DS patients (Conti *et al.* 2010) (n=5) that naturally expressed high Abeta42 levels (concentration >500 pg/ml; Abeta42 basal concentrations 1221 ± 646.9 pg/ml mean ± SD, 597.5- 2,556 pg/ml, range). A significant sequestration of Abeta42 ($p<0.001$) in plasma treated with PA or TREG (25 µM or 100 µM/15 min at 37°C) or mApo-PA (100 µM or 250 µM/15 min at 37°C) functionalized liposomes was demonstrated (see **Figure 2**) (PA 25µM: -17 ± 3.6%, PA 100 µM: -15 ± 2.3%; TREG 25 µM: -19 ± 6.6%, TREG 100 µM: -20 ± 5.4%; mApo-PA 100 µM: -16 ± 2.6%, mApo-PA 250 µM: -12 ± 7.8%). These results were obtained without separating liposome-bound Abeta from free Abeta, hypothesizing that the first, hindered by liposomes, would not been recognized by ELISA antibodies. To verify this hypothesis, we performed ELISA experiments also after removing liposome-bound Abeta, to evaluate the real content of residual Abeta42. To reach this aim we treated plasma, after liposomes incubation, with Molecular Exclusion Chromatography (MEC) in order to obtain two fractions, one containing liposomes bound to Abeta (liposome fractions) and one constituted by plasma containing residual Abeta (plasma fractions). After this procedure, we assessed Abeta42 levels and we demonstrated that mApo-PA-LIP were able to significantly reduce the peptide level (-20 ± 8.7%; $p<0.05$; see **Figure 3**).

A confirmation of such binding comes from an experiment in which we investigated the binding of mApo-PA 250 µM liposomes to Abeta42, by using ultracentrifugation on a discontinuous sucrose

density gradient. Plasma added with exogenous Abeta42 was incubated or not with mApo-PA liposomes. After incubation, the mixtures were submitted to ultracentrifugation on discontinuous sucrose density gradient, and 10 fractions were collected from the top of the gradient and assayed for Abeta42 content by ELISA assay. As already shown (Gobbi et al. 2010), when loaded alone on the gradient, liposomes are recovered within the upper, low density, fractions; Abeta42, when loaded alone, remained in the bottom fractions. Our results showed that after incubation with mApo-PA liposomes, the amount of Abeta42 recovered in the bottom fractions decreased of about 20% (range 8-30%) (fractions from 7 to 10 $p<0.01$ Abeta 42 in plasma+mApo-PA liposomes 250 mM with respect to Abeta 42 in plasma; see **Figure 4**). Finally, we also evaluated the capacity of functionalized liposomes to sequester Abeta42 in CSF obtained from control subjects (n=10; Abeta42 basal concentrations 724.0 ± 505.6, 129,0-1,480 pg/ml, mean ± SD, range; **Figure 5**), showing a significant binding of Abeta42 content in mApo-PA-LIP (25 µM: -16.7 ± 6.7%; 250 µM: -10.6 ± 2.6%) treated samples respect to control samples ($p<0.01$) and samples treated with unfunctionalized Lipo ($p<0.05$).

4. Discussion

The Abeta peptide plays a key role in AD and represents one major target of therapies that are currently under investigation. Abeta oligomers toxicity has been demonstrated in several studies (Cerasoli *et al.* 2015). An innovative and promising therapeutic strategy under investigation is based on NPs directly targeting Abeta. Our study tested the capacity of liposomes functionalized with different molecules to sequester Abeta42 in human biological fluids, both plasma and CSF, including samples obtained from AD patients. To this purpose, we have previously synthetized and characterized liposomes functionalized with PA or TREG able to bind Abeta42, and bi-functionalized with PA and mApo-PA able both to bind with high affinity Abeta42 and to cross BBB (Gobbi et al. 2010, Bana et al. 2014). Moreover, mApo-PA liposomes have already been shown to bind Abeta42 in an in vitro BBB cellular model (Mancini et al. 2016).

Here, we demonstrated that Abeta-binding liposomes are able to sequester Abeta42 also in human biological fluids, both plasma and CSF. One major problem consisted in the fact that Abeta42 largely binds to plasma protein, (Bohrmann *et al.* 1999) so that the measurement of plasma Abeta42 is plausibly underestimated, resulting at the lower level of the ELISA curve sensitivity with consequent difficulties for correct quantification. To overcome this problem we selected those plasma samples from AD and DS patients that naturally displayed Abeta42 content above 500 pg/ml. In plasma, the amount of the bound peptide was independent from the dose and kind of liposomes used. To explain this issue we have to consider that, during systemic administration, liposomes are covered by plasma protein leading to the formation of the so called "protein corona" that could lead to opsonization and activation of phagocyte system, which in turn lead to the removal of liposome from the bloodstream (Palchetti *et al.* 2016). To achieve stealth properties and to avoid the protein corona formation, our liposomes underwent to PEGylation. NPs used in this study have both stealth properties and the capacity to bind Abeta, but the high plasma protein content might potentially interfere, at least in part, with the PEG covering and undo eventual Abeta-binding properties related to concentration of NPs used. Moreover, in blood only a portion of Abeta is free, and thus available to be linked and sequestered by our liposomes. In future experiments we can think to change the density of Abeta ligands on liposomes surface, to see if in this way liposomes can bind more Abeta42, due to multivalency. We do not think as surprising that liposomes functionalized with PA, TREG, or mApoE-PA have the same ability to bind Abeta. In fact, in previous experiments, we showed a similar binding affinity of the three kind of liposomes towards Abeta42 fibrils (K_D=0.8 µM, 0.6 µM and 0.6 µM, respectively, as assessed by Surface Plasmon Resonance) (Bana et al. 2014).

Further experiments on CSF samples clearly confirmed the capacity of functionalized liposomes to sequester Abeta42 from these fluids.

Our results seems to suggest that the ELISA antibody recognizes a portion of Abeta peptide that is involved in the binding of the peptide to the lipidic surface. Thus, when Abeta is bound to the

liposomes, this portion of the peptide is hindered. Considering that Abeta interacts extensively with lipidic membranes and apolipoproteins in vivo (Morgado & Garvey 2015, Namba *et al.* 1991, D'Errico *et al.* 2008), our results is of particular interest to avoid underestimation of the amount of Abeta in ex vivo experiments. Finally, it has been demonstrated that cholesterol acts as a promoter for Abeta-membrane interactions, which would facilitate Abeta aggregation and membrane insertion (Yu & Zheng 2012). Since cholesterol is a constituent of our liposomes this further strengthen the concept that plasma lipid concentration might contribute to underestimate the quantified amount of Abeta. Further experiments are currently ongoing to confirm this suggestion.

The literature concerning the ability of NP to sequester Abeta in human biological fluids is scanty, and only few works report the use of magnetic NP for detection of AD biomarkers (Wang *et al.* 2015, de la Escosura-Muñiz *et al.* 2015) and not for potential therapeutic strategy. Moreover, it has to be taken into account that commonly used animal models of AD carry mutations in AD-related genes that better reproduce the familial forms of the disease, characterized by higher Abeta levels than the sporadic and more diffuse forms of AD (Gidyk *et al.* 2015).

Our data report the capacity of functionalized liposomes to sequester Abeta42 in human biological fluids, including samples obtained from sporadic AD, suggesting the possibility that they may exploit the “sink effect” to reduce Abeta toxicity as a potentially useful therapeutic strategy. Consistently, a reduction in brain and plasma Abeta via peripheral sink effect was already demonstrated with PA functionalized liposomes in APP/PS1 transgenic mice (Ordóñez-Gutiérrez et al. 2015). In this scenario, we conclude reporting the first demonstration of the ability of functionalized nanoliposomes to bind Abeta42 in human biological fluids, including those from sporadic AD patients. This proof of concept paves the way for further and more comprehensive studies on NP therapeutics in AD.

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Figure legends

Figure 1

The elution of liposomes from molecular exclusion chromatography was checked by Dinamyc Light Scattering (solid line), while the elution of plasma protein (included Abeta42) was followed by Bradford Assay (broken line).

Figure 2

Abeta42 plasma levels were significantly reduced in samples treated with PA, TREG or mApo-PA functionalized liposomes. n=5 AD and 5 DS samples with Abeta42 basal levels >500pg/ml. $p<0.0001$ at Repeated Measures ANOVA, followed by Bonferroni's Multiple Comparison Test (**$p<0.001$ vs. CTRL); plasma Abeta42 levels are expressed as percentage of the cognate vehicle-treated plasma samples considered as control (CTRL).

Figure 3

Plasma samples from 5 AD and 5 DS subjects, treated with mApo-PA functionalized liposomes (250 µM), underwent to Molecular Exclusion Chromatography. “Plasma fractions” bar represents fractions of plasma containing residual Abeta, “liposome fractions” represents fractions containing Abeta-bound liposomes. Abeta42 plasma levels, expressed as percentage of the cognate control plasma samples, were reduced in samples treated with mApo-PA functionalized liposomes. $P=0.0004$ at Repeated Measures ANOVA, followed by Bonferroni's Multiple Comparison Test (*$p<0.05$ vs. CTRL).

Figure 4

Plasma samples from 4 control subjects added with 0,5 µM Abeta 1-42 and with mApo-PA functionalized liposomes (250 µM), ultracentrifugation on a discontinuous sucrose density gradient . Incubation mixtures of Abeta42 with liposomes were purified by ultracentrifugation on a

discontinuous sucrose density gradient and 10 fractions were collected from the top of the gradient. Distribution of Abeta42 in the gradient fractions (1-10) of plasma loaded alone on the gradient (black line) or after incubation with mApo-PA liposomes (grey line) were reported $P<0.0001$ at Two-way RM ANOVA, followed by Bonferroni post-tests (*$p<0.01$ and **$p<0.001$ Abeta 42 in plasma+ mApo-PA liposomes 250mM vs. Abeta 42 in plasma)

Figure 5

CSF Abeta42 percentage levels were significantly reduced in CTRL samples (n=10) treated with mApo-PA functionalized liposomes (25 and 250 µM) respect to control CSF considered as basal. $p<0.0001$ at Repeated Measures ANOVA, followed by Bonferroni's Multiple Comparison Test (*$p<0.01$ mApo-PA-LIP 25 and 250 µM vs. CTRL, \$$p<0.05$ mApo-PA-LIP 25 µM vs. unfunctionalized LIP).

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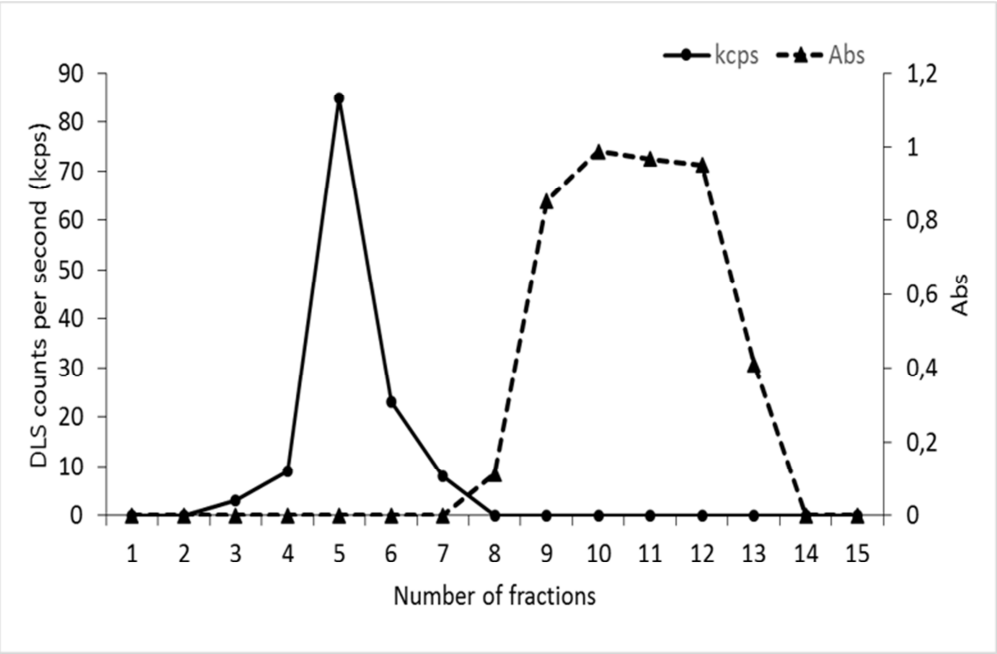

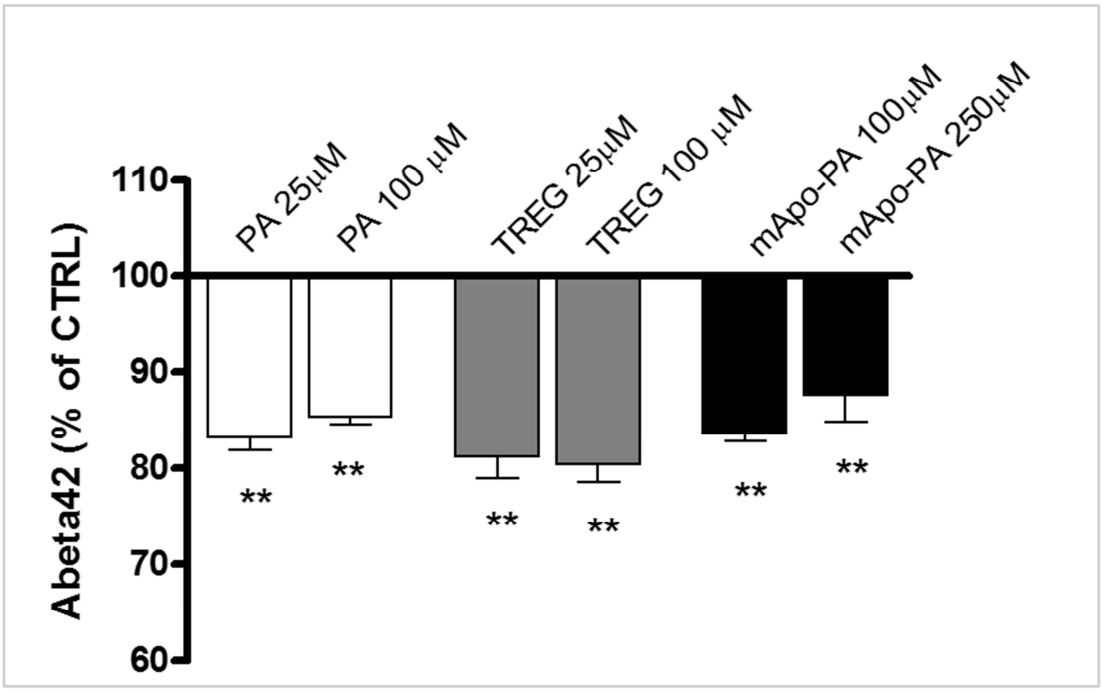

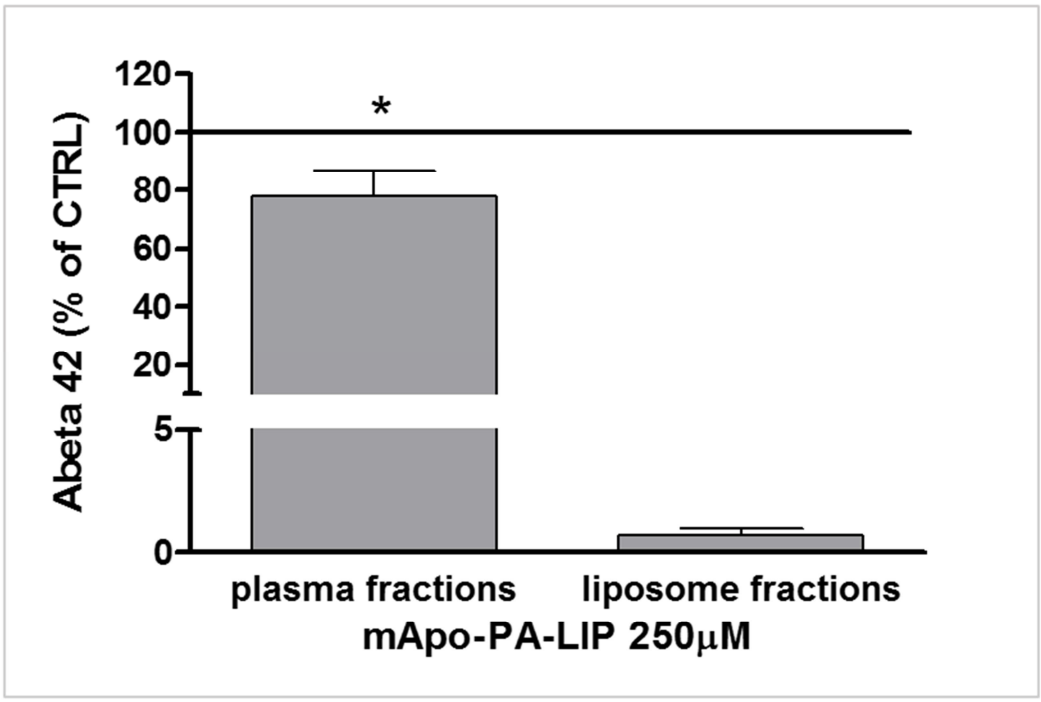

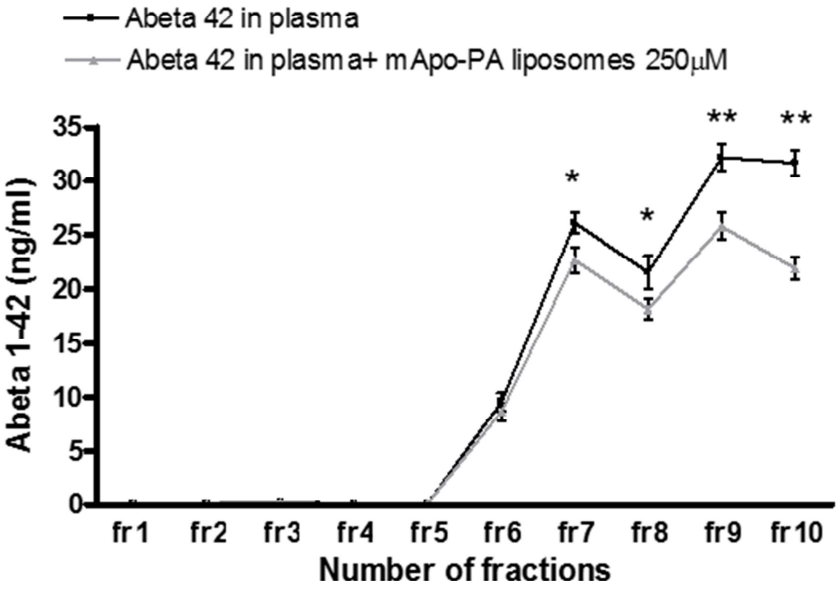

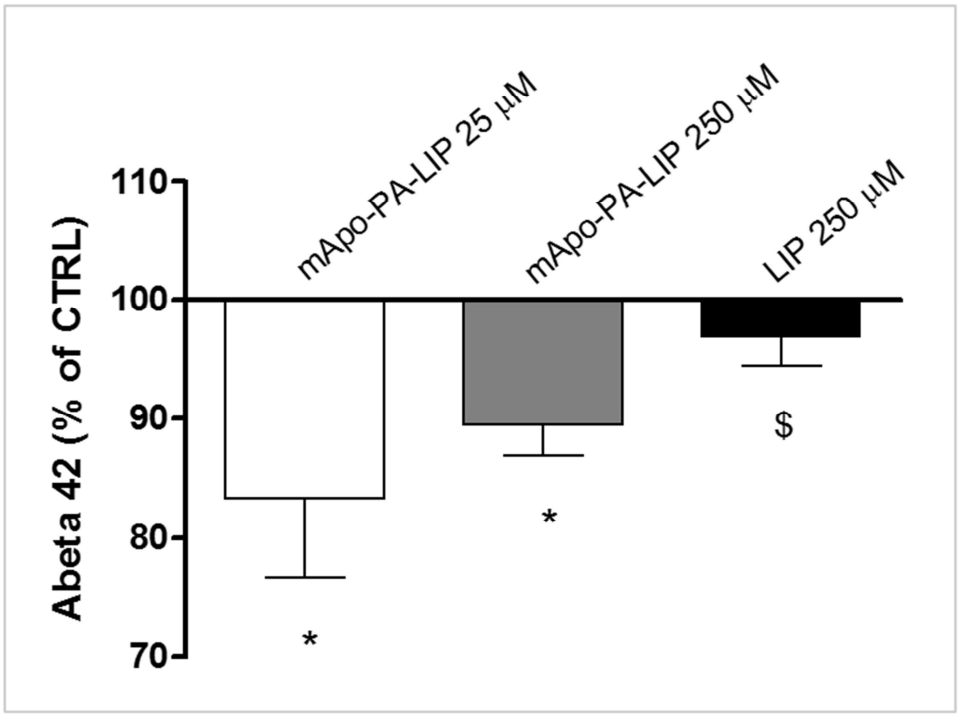

- The use of high affinity Abeta binding liposomes is proposed in human biological fluids
- Functionalized liposomes significantly interact with Abeta in plasma and CSF from AD patients
- Liposomes may exploit the Abeta “sink effect” as a potential therapeutic strategy