INTRODUCTION

One of the main pathological features of Alzheimer’s disease (AD) is the abnormal production of the amyloid beta peptide (Aβ) which accumulates in the brain as senile plaques leading to neuronal death. Among the current therapeutic strategies, the most interesting are those based on binding and sequestration of Abeta avoiding its fibrillation and accumulation. It is noteworthy that Abeta and plasma proteins are in a state of dynamic equilibrium through the blood brain barrier (BBB), and that peripheral sequestration of Abeta may shift this equilibrium towards the blood, eventually drawing out the excess from the brain (“sink effect”) (Fig. 1).

Nanoparticles (NPs) are an attractive mean for these tasks, being suitable vehicles for imaging probes and therapeutic agents and for the possibility to functionalize their surface with target-specific ligands and eventually to cross the BBB (Modi et al, 2009). The possibility that NPs could sequester Abeta at central level, reducing the amount of fibril formation, is an attractive therapeutic strategy for AD. Several kinds of NPs are now under investigation, among them polyethylene glycol (PA)-incorporated (20 mol%) sphingomyelin/cholesterol liposomes exhibit affinity for beta amyloid (Aβ) 1-42 peptide.

PATIENTS AND METHODS

After informed consent approved by the local ethical committee, patients with AD and controls were recruited at Department of Neurology, S.Gerardo Hospital, University of Milano-Bicocca (Tab. 1).

Plasmas were obtained after centrifugation of blood at 3660g x 20 min. Samples were stored at -80°C until assay.

All human plasma samples, after dilution (1:50) in dissociation buffer (PBS +1.5% BSA +0.2 mol/L glycine-acetate, pH 3.5), were incubated for 20min at room temperature (RT) and then centrifuged at 16000g for 25min at RT in Centinfic centrifugal filter device 50 Kd.

Human lyophilized Abeta 1-42 was reconstituted in 200ul of HFIP to obtain a final concentration of 1 μg/ml. To favour lyophilization tubes have been rotated.

For oligomers preparation lyophilized Abeta has been resuspended in DMSO (1ul DMSO for 10ug Abeta). Following a sonication in water bath for 10min, cold PBS was added. Solution has been incubated for 24h at 4°C and then put on ice to stop the oligomerization. Sample will be centrifuged at 14000g for 10 min at 4°C. Supernatant contains oligomers.

For monomers preparation lyophilized Abeta has been resuspended in DMSO (1ul DMSO for 10ug Abeta). Following a sonication in water bath for 10min, cold water was added. Non-denaturing western blot was performed to verify the Abeta aggregation status (monomers, oligomers and fibrilis) (Fig.2).

Lipid-based nanoparticles were added in a concentration range of 2.5-100 nM at different times (0-4 h), while Abeta 1-42 (Phoenix) was added in a concentration range of 2.5-0.25 μM.

Measurements of Abeta 1-42 plasma levels were performed by commercial ELISA immunoassay kits.

RESULTS

To better analyze the capacity of nanoparticles to sequester Abeta we performed dose and time course experiments both for NPs and Abeta and we chose: Abeta 2.5μM, nps 25μM and two hours of incubation; for our purpose the best Abeta species was the monomeric one. Since fibrils amount of Abeta are probably the less important in AD formation (as shown in several previous works, e.g. Finder et al, 2007) and they are not a good target in a peripheral model such as plasma.

ABETA CONTENT IN PLASMA

To show the real Abeta content in plasma we used the dissociation procedure as described in a previous work (Storace et al, 2010); in the procedure can be considered effective to dissociate Abeta from plasma proteins (i.e. probably antibodies) and Fig. 3 shows a significant increase (p<0.05) in Abeta 1-42 plasma levels after diluting plasma in dissociation buffer pH 3.5; as negative control we treated plasma with buffer at pH 7 and a reduction of Abeta 1-42 concentration can be seen; nevertheless the value is not in the same range of the non dissociated one: probably the filter used during the procedure has a role in this separation, holding back a part of the plasma proteins and amplifying the Abeta concentration in the collected solution.

NANOPARTICLES-ABETA BINDING

We tried to search for a decrease in Abeta 1-42 concentration in non dissociated plasma, adding the lipid-base nanoparticles, but no difference was shown (Fig.4), so we decided to administer an exogenous amount of Abeta 1-42 and there was a relevant difference in that way; probably these lipid-based nanoparticles are not effective enough to sequester small concentration of Abeta. After the addition of exogenous Abeta 1-42 liposomes showed a significant capacity (p=0.0073) to sequester Abeta considering the whole group of AD and controls (Fig. 5).

Finally, we compared results obtained from AD and controls separately: as shown in Fig.6 liposomes are able to reduce Abeta amount even if there is not a statistical difference; probably it is due to the low number of cases, further studies on a larger population could confirm our results.

CONCLUSIONS

• The procedure used to dissociate Abeta from plasma proteins is effective in evaluating the real Abeta content in plasma.

• Adding plasma with exogenous Abeta 1-42, lipid-based nanoparticles showed to have enough affinity to bind and sequester Abeta, reducing its concentration in a significant way; to confirm our results the capacity of liposome to sequester Abeta 1-42 and to eventually evidence a difference between patients and controls, further studies on a larger population will be necessary.