apoptosis in stroke which may initiate a new aspect of the role of antithrombotic drug in the treatment of acute ischemic stroke.

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TDP-43 inclusion bodies formed in bacteria are structurally amorphous, non-amyloid and inherently toxic to neuroblastoma cells

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Accumulation of ubiquitin-positive, tau- and a-synuclein-negative intracellular inclusions of TAR DNA binding protein (TDP-43) in the central nervous system represents the major hallmark correlated to amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U). Such inclusions have variably been described as amorphous aggregates or more structured deposits having an amyloid structure. Therefore, it is not yet clear the structure adopted by TDP-43 in such deposits. Following the increasing observations that bacterial inclusion bodies generally consist of amyloid aggregates, we have overexpressed TDP-43 in *E. coli* cells, purified the resulting TDP-43 containing inclusion bodies (TDP-43 IBs) and subjected them to a number of biophysical analyses to assess their structure and morphology, along with control IBs. We show that the TDP-43 aggregates contained in the bacterial IBs do not bind thioflavin T and Congo red, possess a largely disordered secondary structure, as determined with circular dichroism and infrared spectroscopy, and are highly susceptible to proteinase K digestion, thus possessing none of the distinctive hallmarks for amyloid. In addition, atomic force microscopy imaging revealed that TDP-43 IBs have an irregular structure and a rough surface. However, the TDP-43 IBs were able to severely impair the viability of cultured neuroblastoma cells, when added to their extracellular medium and, even more markedly, when transfected into their cytosol. These data reveal an inherently high propensity of TDP-43 to form amorphous aggregates, which possess, however, an inherently high ability to cause cell dysfunction. This indicates that a gain of toxic function caused by TDP-43 deposits is effective in TDP-43 pathologies, in addition to possible loss of function mechanisms originating from the cellular mistrafficking

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Aβ42 traffic through plasma membrane: role of P-glycoprotein

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Alzheimer's disease (AD) is the most common neurodegenerative pathology that affects more than 25 millions of older people around the world. Although the cause of AD is unknown, the amyloid cascade hypothesis postulates that the deposition of the amyloid-β peptide (Aβ42) in the brain is a central event in Alzheimer's disease pathology. This hypothesis proposes that the reason for the Aβ42 deposition is an imbalance between the production at neuronal level and its elimination trough the blood-brain-barrier (BBB). In this context, it has been proposed that P-glycoprotein (P-gp), expressed in endothelial cells of the BBB, plays a role in the elimination of Aβ42. However, the role of the P-gp remains controversial.

The aim of this study is to establish whether Aβ42 is transported by P-glycoprotein or not. The first and very important point is the method for Aβ42 solubilization. The nature of used solvent as well as the conditions of solubilization plays an important role in the behavior of the peptide in solution, which directly affects his way of spontaneous aggregation.

After the analysis of an important number of bibliographic sources, we established a protocol for the solubilization of synthetic amyloid peptide with a preliminary use of organic solvents hexafluoroisopropanol (HFIP) and DMSO. The kinetics of the aggregation of Aβ42 was followed by SDS PAGE and microspectrofluorescence using Thioflavin T.

The toxicity of Aβ42 was studied. For this purpose, K562 cells overexpressing or not P-gp (K562/ADR and K562 respectively) were incubated during 3 days with the peptide solution aggregated during 24 hours. Our results show that the toxicity is similar between the two cells lines. Furthermore, we have study the transport of the pirarubicin by the P-gp in the presence or absence of Aβ42. The transport of this P-gp substrate was not modified, suggesting that P-glycoprotein is not involved in Aβ42 transport.

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Study of the process of transthyretin aggregation in presence and absence of polyphenols and other molecules

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Transthyretin (TTR) is a plasma protein secreted by hepatocytes into the blood and cerebrospinal fluid, where it transports thyroid hormones, thyroxine (T4) and triiodothyronine (T3) and cotrasport of vitamin A with Retinol Binding Protein (RBP). The TTR is an amyloidogenic protein implicated in diseases such as senile systemic amyloidosis (SSA) and familial amyloid polyneuropathy (FAP), both characterized by extracellular deposition of insoluble amyloid fibrils in heart, peripheral nerves and other organs. In particular, fibrils in FAP patients are composed of variants Leu55 → Pro55 (L55P) is the most amyloidogenic and it forms amyloid fibrils *in vitro*. It is suspected that the single-point mutations accelerate amyloidogenesis by destabilizing the monomeric partially unfolded amyloidogenic intermediate state rather than by altering the tetrameric native state. TTR fibrils have been considered direct responsible of tissue impairment in FAP and SSA, but the unstable fibril precursors are increasingly considered the main responsible of cell suffering and tissue impairment in amyloid diseases. In particular, the early unstable oligomeric intermediates are highly toxic due to their ability to interact, disassemble and permeabilize cell membranes. Moreover, increasing information on polymorphism of pre-fibrillar and fibrillar assemblies has led to propose that apparently similar fibrils can display different stability and efficiency in generating toxic species. These data suggest the opportunity to search natural or synthetic molecules interfering with amyloid aggregation by stabilizing the TTR native state by hindering the appearance of toxic species, or by favoring the growth of less toxic assemblies. We have recently described a natural compound (oleuropein) which is protective in Tg animal models of Abeta deposition and cultured cells by stim-