Unraveling the possible cellular mechanism behind leptomeningeal amyloidosis using as model a highly unstable transthyretin tetramer

Estefania Azevedo[1], Fernando Palhano[1], Juliana Freire[1], Leonardo Palmieri[1], Luís Maurício Lima[2], Flávia Lima[3], Vivaldo Moura Neto[3] and Débora Foguel[1]

[1]Inst. Bioq. Médica, [2]Faculdade de Farmácia, [3]Depto. de Anatomia, ICB,UFRJ, Rio de Janeiro, Brazil.

Transthyretin (TTR) is a 127-residue homotetrameric β-sheet-rich protein that transports thyroxine in the blood and cerebrospinal fluid (CSF). Among all TTR variants, A25T is the most unstable tetramer. Its great instability induces TTR degradation in the endoplasmic reticulum of the hepatocytes, while thyroxine (a natural ligand of TTR) leads to A25T secretion in the CSF by the choroid plexus. In the present work we aimed to study A25T dissociation, aggregation and its role in leptomeningeal amyloidosis (LA). Our data showed that A25T was less stable to high pressure treatment than the wt and L55P (most aggressive variant). Besides, this variant was also the most amyloidogenic, aggregating in conditions where wt and L55P remained mostly soluble. Using HPLC and native PAGE we monitored acrylodan-labeled TTR aggregation in the plasma. The aggregates formed, when analyzed by Atomic Force Microscopy, Thioflavin T and Congo Red binding displayed amyloid structure. We solved the crystal structures of the wt and A25T and the comparative analysis shed light into the mechanism behind A25T highly amyloidogenicity: an expanded tetramer which is stabilized by a lower number of H-bonds and hydrophobic interactions. Interestingly, in the presence of thyroxine and lumiracoxib (a COX-2 inhibitor), two ligands of TTR, the structure of A25T was similar to that displayed by the wt protein. Since LA symptoms suggest a local inflammation, we questioned ourselves whether A25T aggregates can induce an inflammatory response in the CNS. Microglia primary cultures incubated for 48 h with increasing concentrations of aggregates composed of A25T, underwent activation and presented a dose dependent nitric oxide secretion. Exposure of microglia to A25T aggregates stimulated phagocytosis, which could be blocked by cytochalasin D. These results suggest that amyloid fibrils may have a role in LA pathogenesis, exacerbating tissue damage.

Keywords: amyloid, crystallography, transthyretin, microglial, inflammation
Support: CNPq and FAPERJ.