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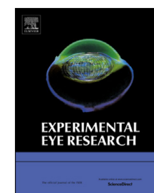

Absence of amyloid-beta in lenses of Alzheimer patients: A confocal Raman microspectroscopic study

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A B S T R A C T

We have compared the protein profiles in plaques and tangles in the hippocampus of post-mortem Alzheimer brains and in opaque and clear regions in the deep cortex of eye lenses of the same donors. From the 7 Alzheimer donors studied, 1 had pronounced bilateral cortical lens opacities, 1 moderate and 5 only minor or no cortical opacities. We focused on beta-sheet levels, a hallmarking property of amyloid-beta, the major protein of plaques and tau protein, the major protein of tangles in Alzheimer brains. Confocal Raman microspectroscopy and imaging was used in combination with hierarchical cluster analysis. Plaques and tangles show high levels of beta-sheets with a beta-sheet to protein ratio of 1.67. This ratio is 1.12 in unaffected brain tissue surrounding the plaques and tangles. In the lenses this ratio is 1.17 independently of the presence or absence of opacities. This major difference in beta-sheet conformation between hippocampus and lens is supported by Congo red and immunostaining of amyloid-beta and tau which were positive for plaques and tangles in the hippocampus but fully negative for the lens irrespective of the presence or absence of opacities. In line with a previous study (Michael et al., 2013) we conclude that cortical lens opacities are not typical for Alzheimer patients and are not hallmarked by accumulation of amyloid-beta, and can thus not be considered as predictors or indicators of Alzheimer disease as claimed by Goldstein et al. (2003).

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1. Introduction

In previous papers Goldstein et al. (2003) and Moncaster et al. (2010) described accumulation of amyloid-β (Aβ) in supra-nuclear (cortical) cataracts of patients with Alzheimer disease (AD) and Down syndrome. They conclude from these studies that "The process (i.e. the accumulation of Aβ) that's going on in the brain is also going on in the eye", cited by Gardner (2005). Since then non-invasive *in vivo* ophthalmological methods (quasi-elastic light scatter and fluorescence ligand screening) have been developed by Goldstein and coworkers to identify the presence of Aβ in cortical cataract and advocate its presence as an (early) predictor of AD (Goldstein, 2008; Grohol, 2009).

Because of the paramount clinical importance of this conclusion, we decided to study a large population of cortical cataracts in Alzheimer and non-Alzheimer donors to verify the presence of Aβ in human cataract lenses. Based on histochemical (Congo red and Thioflavin-S staining) and immunohistochemical (Aβ antibody clone 6F/3D) evidence we came to a conflicting conclusion: full absence of Aβ in cortical cataracts in AD and non-AD donors (Michael et al., 2013). In contrast with the Goldstein et al. (2003) and Moncaster et al. (2010) studies we included in our study frontal cortex sections from brain donors with Alzheimer disease as positive controls. The brain slices showed strong positive Congo red staining with a red-to-apple green shift (birefringence) under polarized light, Thioflavin-S fluorescence and positive immunostaining. We discussed this discrepancy at length and have readdressed the question of Aβ in the lens in the present study.

The neuropathology of Alzheimer disease is hallmarked by the presence of amyloid-β plaques and neurofibrillary tangles in the cerebral cortex and hippocampus. As recently reviewed by

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Table 1
Lens opacity findings, donor information and AD classification.

Donor number	Lens circumference affected by cortical opacity[a]	Extension of cortical opacity towards the optical axis[b]	Nuclear cataract[c]	Age	Sex	AD classification neurofibrillary tangles[d]	AD classification neuritic plaques[d]
311a	XXXXX	XXXXX	1	80	f	V	C
311b	XXXXX	XXXXX	1				
321a	XX	XX	2	63	f	V	C
321b	XX	XX	2				
316b	XX	XX	4	91	f	VI	C
316a	X	X	4				
305a	X	X	6	91	m	VI	C
305b	0	0	6				
326a	0	0	4	81	m	VI	C
326b	Not available						
308a	0	0	1	79	f	IV	B
308b	0	0	1				
330a	X	X	2	78	f	V	B
330b	Not available						

Cases collected between May and December 2011.
Hippocampus tissue received from donors 311, 316 and 321.
m: male; f: female.
[a] Degree of lens circumference affected by cortical opacity: 0% = 0; 1–20% = x; 21–40% = xx; 41–60% = xxx; 61–80% = xxxx; 81–100% = xxxxx (100% means entire lens circumference affected.).
[b] Extension of the cortical opacity towards the optical axis is expressed as smallest diameter of a theoretical pupil where the cortical opacity would be visible inside the pupillary area: no cortical opacity = 0; 8.1–10 mm = x; 6.1–8.0 mm = xx; 4.1–6.0 mm = xxx; 2.1–4.0 mm = xxxx; 0–2 mm = xxxxx.
[c] Nuclear cataracts graded in a combined scale for opacity and coloration scales from 0 to 10.
[d] AD classification from brain autopsy, based on neurofibrillary tangles (stages I–VI) according to Braak et al. (2006), and based on neuritic plaques (scores A–C) according to Mirra et al. (1991).

Friedman (2011) the pathological accumulation of Aβ in the brain is due to the enzymatic cleavage by α, β and γ secretases of Aβ peptides from the amyloid precursor protein (APP). Most common are the $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides against which most of the immunohistochemical antibodies are raised. These monomeric peptides are normally broken down by the ubiquitin-proteasome pathway or by phagosomes and lysosomes (Forman et al., 2004). In old age, however, this breakdown is inhibited and the monomeric peptides tend, for unknown reasons, to aggregate to oligomers and eventually to polymers which form amyloid fibrils. These fibrils are molecularly characterized by their regularly aligned β-pleated sheet configuration and form the main components of the plaques in AD. Tau is a neuronal microtubule-associated protein whose expression is strongly up-regulated during neuritogenesis (von Bergen et al., 2006). Upon aging the originally unfolded random coil tau protein is altered by several processes and forms fibrils with a regularly aligned β-pleated sheet configuration (von Bergen et al., 2005). The tau fibrils, localized in the neuronal cytoplasm, are the main components of the tangles which, as the Aβ plaques, obstruct the normal function of the neurons involved.

Although most proteins, including lens crystallins, have β-sheets as part of their molecular conformation, the Aβ peptides in plaques and tau in tangles in AD brains are hallmarked by a high occurrence of these β-sheets (Koudinov et al., 1999) which are present as fibrils. In the present study we have analyzed the molecular conformation of the proteins in plaques and tangles in the hippocampus of neuropathologically verified Alzheimer donors and in the lenses of the same donors using Raman microspectroscopy and imaging. Raman microspectroscopy is a sensitive quantitative analytical method to detect specific molecular conformational bonds in proteins. The protein amide bands in the spectral fingerprint regions around the vibrational bands 1250 cm^{-1} (Amide III) and 1670 cm^{-1} (Amide I) reflect the presence and the amount of α-helical and β-pleated sheet conformations in proteins and thus are appropriate to analyze possible differences between Aβ in AD plaques and the crystallins in the eye lens. In previous studies in human lenses we have shown that the method indeed enables detection of local conformational changes in proteins and lipids and in water content and of local accumulations of these macromolecules. The use of a confocal scanning Raman set up further enables the imaging of these changes and to correlate them with local differences in the (ultra)structure of the lenses but also in individual cells and other tissues (Duindam et al., 1998; Siebinga et al., 1991, 1992; Uzunbajakava et al., 2003a; Uzunbajakava et al., 2003b; van Manen et al., 2008).

Apart from a monolayer of epithelial cells at the anterior pole and developing fibers in a small equatorial zone the lens is a homogenous tissue mainly consisting of mature fibers filled with a high density (30–40%) of specific proteins (mainly α, β and γ crystallins) surrounded by membranes. In the vast majority of mature lens fibers organelles are absent. This means that Raman imaging over extended areas in several regions of the eye lens tissue enables to draw conclusions on the local differences in protein content and molecular conformation. In contrast, brain tissue is a rather in-homogenous tissue with neuronal, astrocytic, oligodendrocytic and microglial cell bodies, vessels, and neuropil consisting of dendrites, axons and cellular processes of glial cells.

The aim of this study was to analyze the differences between hippocampus and lens regarding the hallmarking β-sheet configuration of Aβ. In order to observe potential local differences in brain tissue, we adopted Raman imaging. In the case of plaques and tangles, which are mostly smaller than the imaged area, specific Raman information related to the chemical composition will reflect structural information based on Raman contrast. The extensive Raman data sets typically consisting of 4096 spectra from areas of 900 μm^2 were analyzed with hierarchical cluster analysis (HCA). This method combined with Raman imaging data matrices visualizes regions in the tissues with high Raman spectral similarities. For a correct comparison between lens and brain tissue we have used HCA applied to data sets of both tissues.

2. Material and methods

Brain and eye tissue from seven donors was provided by the Neurological Tissue Bank of the Biobank-Hospital Clinic-IDIBAPS (NTB-IDIBAPS), Barcelona in collaboration with the Banco de Ojos para Tratamientos de la Ceguera, Barcelona. Written informed consent for removal of the brain and the eyes for diagnostic and

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