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Morphological alterations of the choroid plexus in late-onset Alzheimer's disease

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Abstract Anomalies of the cerebrospinal fluid flow rate and composition that have been reported in patients suffering from Alzheimer's disease (AD) could be related to alterations of the choroid plexuses (CD). Here we report a photonic and electron morphometric study in which we compared the height of CP epithelial cells and the thickness of their basement membrane on post-mortem samples from AD patients, age-matched controls and two new-borns. Ageing appeared associated with epithelial atrophy and basement membrane thickening, but these features were significantly accentuated in AD. These data suggest that a dramatic alteration of the secretion and filtration could be involved in the multiparametric pathogenesis of late-onset AD.

Key words Alzheimer's disease · Basement membrane · Choroid plexus · Epithelial cells

Introduction

Alzheimer's disease (AD) is characterized by the formation, in brain tissue, of numerous senile plaques composed of extracellular fibrils of precipitated β-4 amyloid protein. In early onset AD, mutations on genes encoding amyloid precursor protein, presenilin 1 or presenilin 2 induce an overproduction of amyloid protein. The pathogenesis of late-onset AD is, by contrast, poorly understood. The main risk factor considered is aging, but the possible age-related changes leading to AD are not known. Recent data have focused on oxidative damage, inflammatory processes and ApoE4 neurotoxicity.

Amyloid fibril formation can be inhibited in vitro by cerebrospinal fluid (CSF) [25], the composition of which is very similar to that of brain interstitial fluid [7]. CSF is largely dependent on the filtration and secretion role of choroid plexuses (CP), which are highly vascularized structures, located in the lateral ventricles of the brain, composed of convoluted villi with capillaries, connective tissue and a monolayer of ciliated epithelial cells [5]. CP have multiple functions of synthesis, secretion (of CSF), active transport and selective reabsorption of deleterious substances. CP constitute a selective blood-brain barrier that could also participate in the immuno-surveillance of the brain. CSF-transthyretin, the main CSF thyroxin carrier protein, is almost exclusively of CP origin, where it is secreted by the epithelial cells lining the ventricle's lumen [1]. CSF-transthyretin constitutes 50% of the proteins secreted by the CP. CP also transport folate, vitamin B6, vitamin B12, vitamin C, and probably vitamin E [6, 24].

The levels of transthyretin, vitamin B12, vitamin E and folate have been reported to be lowered in the CSF of AD patients [9, 11, 17, 18, 22]. Morphological studies further demonstrated CP fibrosis in deceased AD patients [10]. This suggests that CP alterations, leading to an abnormal production of CSF, could be involved in the pathogenesis of late-onset AD.

Here we examined possible morphological alterations of this CSF-producing secretory system. We were able to evidence a significant thickening of the CP basement membrane (BM) and a significant flattening of epithelial cells in AD. These observations suggest that impaired functions of the CP could be involved in the pathogenesis of late-onset AD.

Patients and methods

Brain tissue and CP were obtained post-mortem from 21 patients (Table 1): 2 3-months-old children with sudden infant death syndrome, a 46-year-old man who died from traumatic hemiplegia

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Table 1 Epithelium height and BM thickness in choroid plexuses of new-borns, middle-age control, elderly controls and AD expressed as mean ± SD (*n* is given in parentheses) (*BM* basement membrane, *CV* cerebrovascular, *MID* multi-infarct dementia, *AD* Alzheimer's disease)

Diagnosis	Sex	Age (years)	Epithelial cells height (μm)	BM thickness (nm)
New-born I	M	0.25	15.2 ± 3.6 (62)	094.6 ± 024.6 (20)
New-born II	M	0.25	14.7 ± 2.8 (65)	116.6 ± 030.9 (20)
Middle-age control	M	46	14.3 ± 2.1 (43)	219.9 ± 063.6 (20)
Elderly controls				
Prost. Cancer	M	82	13.7 ± 2.9 (61)	310.8 ± 140.5 (20)
Colonic cancer	F	86	13.7 ± 1.9 (30)	345.9 ± 118.5 (20)
Lung cancer	F	85	14.3 ± 1.3 (40)	315.7 ± 073.6 (20)
CV Stroke	F	88	13.2 ± 2.7 (78)	249.4 ± 141.2 (20)
MID	F	96	13.3 ± 1.9 (30)	304.8 ± 071.9 (20)
MID	M	86	12.6 ± 2.3 (36)	157.7 ± 042.5 (20)
MID	F	95	14.4 ± 2.8 (49)	274.6 ± 112.1 (20)
MID	F	95	13.9 ± 2.7 (52)	225.6 ± 065.6 (20)
AD patients				
AD	M	82	07.4 ± 1.7 (34)[a]	365.9 ± 342.1 (20)[b]
AD	F	82	10.6 ± 2.6 (38)[a]	210.4 ± 104.2 (20)[a]
AD	F	87	10.1 ± 2.0 (37)[a]	430.6 ± 267.3 (20)[b]
AD	F	73	12.9 ± 1.8 (37)	266.3 ± 107.8 (20)
AD	M	83	09.4 ± 1.9 (30)[a]	418.4 ± 200.7 (20)[b]
AD	M	89	10.5 ± 1.9 (52)[a]	325.4 ± 197.5 (20)
AD	F	77	09.2 ± 1.6 (40)[a]	440.7 ± 237.8 (20)[b]
AD	F	84	10.6 ± 1.9 (30)[a]	387.9 ± 176.9 (20)[b]
AD	F	93	11.2 ± 2.6 (35)[a]	298.6 ± 166.5 (20)
AD	F	92	12.7 ± 2.5 (45)[a]	341.7 ± 220.8 (20)[b]

[a] Individual values < mean values of the control group; $P < 0.05$

[b] Individual values > mean values of the control group; $P < 0.05$

and 18 elderly patients (13 females and 5 males) aged between 73 and 96 years. Ten of them (7 females and 3 males; 84.2 ± 5.9 years old) suffered from definite AD diagnosed according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's and Related Disorders Association work group [14]. Among the other 8 patients, 4 had a multi-infarct dementia, 1 died from colonic cancer, 1 from lung cancer, 1 from prostatic cancer and 1 from cerebrovascular stroke. They were considered as age-matched controls (6 females, 2 males; 88.3 ± 4.8 years).

All samples were obtained within 24 h post-mortem, after informed consent was obtained from the families of the patients. In all elderly patients, requisite sections of hippocampus, middle temporal gyrus, middle frontal gyrus and inferior parietal lobule cortex as well as both ventricular choroid plexuses were removed. The cortex sections were snap-frozen in liquid nitrogen and maintained at –80 °C until tested. Part of the CP also was stored snap-frozen, and the remainder was fixed in 2% glutaraldehyde for 2 h, rinsed in buffer, dehydrated, and embedded in Epon 812. Only CP were studied in the infants and in the middle-age control.

In all brain samples, the presence and number of senile plaques and neurofibrillary tangles were investigated using the classic indirect immunofluorescence method on frozen-cut 4-μm-thick sections with non-conjugated mouse antisera to human β-4 amyloid (Dako-Glostrup, Denmark) and tau-1 (Boehringer Mannheim Biochemica, Mannheim, Germany), with FITC-conjugated anti-mouse Ig (Dako) as second-step reagent. These sections were obtained at –30 °C, collected on clean glass slides, air dried and rapidly fixed for 45 s in a microwave oven. Incubations were carried out for 60 min at room temperature in a moist chamber, and after three series of washes in phosphate-buffered saline (PBS), the sections were mounted in PBS-glycerol and examined under UV light with an Olympus BH2 (Olympus, Tokyo, Japan) microscope equipped with a Ploem system of epi-illumination. Semithin (1.5 μm) and ultrathin (80–90 nm) sections of Epon-embedded CP were cut with a glass knife on a Reichert OMU 2 (Reichert, Wien, Austria) ultramicrotome. Semithin sections were stained with Azur II blue and examined using an Olympus BH2 light microscope. Ultrathin sections were stained with 4% uranyl acetate and lead citrate Reynolds' stain. These sections were examined and the BM measured with a Philips CM 12 transmission electron microscope (Philips, Eindhoven, Netherlands) on 20 randomly selected fields for each specimen. The height of epithelial cells was measured by photonic microscopy on at least 30 randomly selected fields, on semithin sections for each CP sample using the Biocom system (Biocom, Les Ulis, France) for quantitative imaging. BM thickness and epithelial cell height were then expressed as mean values representative of the characteristics of each group, including the infant controls. We compared the mean values of the AD group and of each AD patient to the mean values of the elderly control group using Student's *t*-tests performed with the Prism software (Grafpad, San Diego, Calif.). Statistical significance was considered for *P* values lower than 0.05. Results for all patients are expressed as means ± SD and summarized in Table 1.

Results

AD patients and control subjects were selected both on the basis of clinical signs, and the presence of β-amyloid and tau immunoreactivity of frontal, parietal, temporal neocortical areas ad hippocampus. All of the ten AD patients had many senile plaques with similar frequency in the three neocortical regions, as well as numerous threads and neurofibrillary tangles (NFT). A different immunoreactivity was observed in the eight controls. Seven had sparse senile plaques and less than 1 NFT/mm^2. The other control presented three large cerebral cortical infarcts on autopsy and microscopic immunoanalysis of his brain tissue showed, exclusively in the temporal area, moderate amyloid deposits and exceptional NFT (< 1/mm^2). The diagnosis of "definite AD" according to the criteria for AD

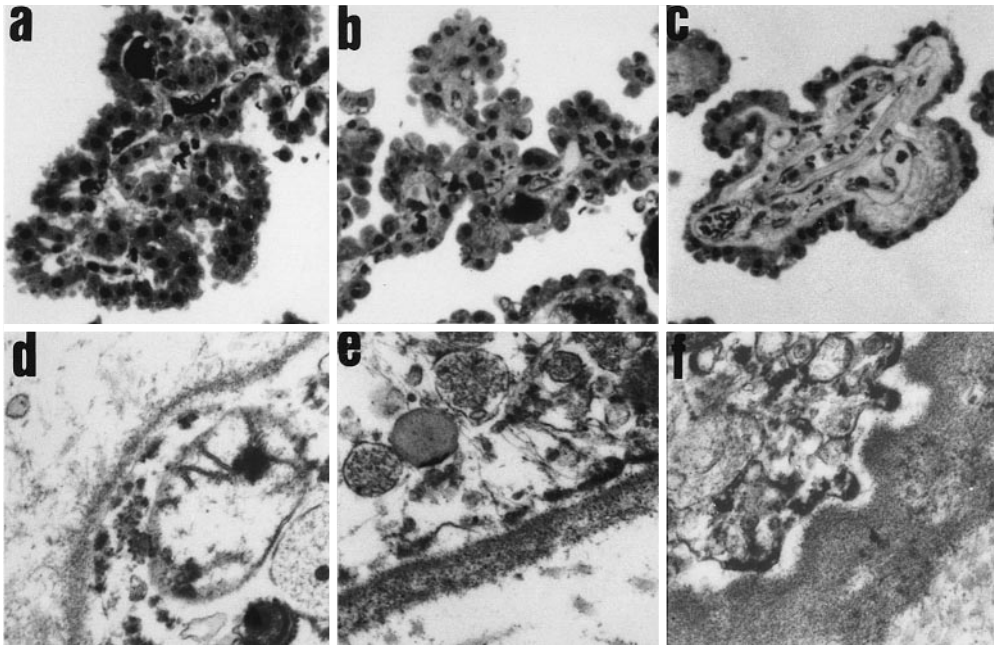

Fig. 1 Semi-thin (**a–c**) and ultrathin (**d–f**) sections of human CP. The *upper column* shows CP epithelial cells in an infant (**a**), a mentally healthy elderly individual (**b**) and a patient (**c**) with AD. The *lower column* shows transmission electron microscopy revealing the thin CP epithelial BM of an infant (**d**), thickened epithelial BM of a mentally healthy elderly individual (**e**) and the irregular and extremely thick epithelial BM of an AD patient (**f**) (*CP* choroid plexus, *AD* Alzheimer's disease, *BM* basement membrane). **a–c** Azur II blue, × 150; **d–f** × 10,000

of the Consortium to Establish a Registry for AD is based upon evidence of senile plaques in the three neocortical areas without specification for them being diffuse or neuritic plaques [16]. The concomitant presence of numerous senile plaques and NFT confirmed that all ten patients and none of the controls suffered from "definite AD".

In the CP of the two infants, epithelial cells were cubic and clearly protruded into the lumen of the ventricle (Fig. 1 a). Their mean height was, respectively, 14.6 ± 2.8 and 15.2 ± 3.6 μm. In the middle-age control subject, the height of CP epithelial cells was similar at 14.3 ± 2.1 μm. In elderly controls (Fig. 1 b), the CP epithelial cells appeared flattened with a mean height of 13.7 ± 2.6 μm and often contained lipofucsin deposits. Epithelial cells were even flatter in AD patients (Fig. 1 c) at 10.5 ± 2.5 μm, significantly different from elderly controls ($P < 0.001$).

In the two infant CP samples, the epithelial BM (Fig. 1d) was regular and with a mean thickness of 116.7 ± 30.3 and 94.2 ± 24.6 nm thick, respectively. In the middle-age control the epithelial BM of CP was thicker at 219.9 ± 63.6 nm. A maximum thickness of 352.7 ± 227.7 nm was seen in the BM of CP in AD patients (Fig. 1 f), which was significantly different ($P < 0.001$) from the mean values obtained for the elderly controls (Fig. 1 e) of 274 ± 117.7 nm. Epithelial BM had typical features in the four conditions studied. They were linear and uniform in the infants (Fig. 1 d), slightly irregular and thicker in the middle age control, irregular, undulated and often inhomogeneous, with electron-luscent areas and coarser feltwork in elderly individuals (Fig. 1 e). The latter features were accentuated in samples from AD patients (Fig. 1 f), where epithelial BM were extremely irregular, and often associated with very thick fibrosis of the underlying connective tissue.

Discussion

This study reports on severe morphological alterations of the CP in elderly patients with AD, which differed from those of age-matched controls.

Our data in control subjects are consistent with previous reports such as that from Dohrmann [5], who reported a height of about 15 μm for CP epithelial cells of newborn. Ageing is associated with a flattening of several types of epithelial cells, such as renal tubules [15] or salivary glands acini [4], as well as with altered functions of these cells [8], consistent with the flattened CP epithelial cells we observed in mentally healthy elderly patients. BM thickening is also a frequent phenomenon associated with ageing. Again, our results are within the range of values reported by others for seminiferous tubules [26] and kidney BM [3, 12].

Both epithelial cells and BM are important structures of the CP, involved in the proper production and filtration of CSF. Their modifications, with age, are likely to be related with the lower CSF secreton rate of elderly individuals, which has been reported to be only 0.19 ml/min while it is of 0.41 ml/min in young healthy subjects [13]. Under physiological ageing conditions, the lowered secretion appears be sufficient to maintain proper brain functions, but brain homeostasis could become impaired if these alterations were more severe. Indeed, this seems to be the case in AD patients, who have been reported to display such CSF hydraulic disorders, substantiated by isotopic cisternography, as reverse flow with ventricular reflux or delayed clearance [2]. We suggest that there could be a direct relationship between CP BM and epithelial cells alterations and AD. Indeed, CP epithelial cells are responsible for the secretion of transthyretin [1], a molecule reported to be the major β-amyloid sequestering pro-

tein [19]. CSF transthyretin levels are decreased in AD patients [18, 22], which might result from a decreased activity of severely modified epithelial cells. Lowered levels of transthyretin in the CSF could favor β-4 amyloid precipitation, a typical feature of AD. The rates of CP production of vitamin C and E, both important chain-breaker anti-oxidants, could be also lowered and participate to the oxidative stress described in the brain of AD patients [23].

The mechanisms inducing an increased thickening of epithelial BM of CP and epithelial cells flattening in AD are unknown. However, we have previously reported [20, 21] the presence of potentially harmful autoantibodies in the serum of AD patients and linear deposits of IgG and complement along the CP epithelial BM of late-onset AD patients. The occurrence of autoimmune reactions at this sensitive site of CSF production could be involved, in AD, in an exacerbation of age-related CP modifications.

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