

Stress protein expression in the Alzheimer-diseased choroid plexus

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Abstract. Abnormal patterns of stress protein expression are found in the cerebral cortex and hippocampus of Alzheimer (AD) subjects. In this study, expression of various stress proteins in the Alzheimer-diseased choroid plexus (CP) was assessed immunohistochemically. We observed decreased HO-1 immunoreactivity in the AD CP, commensurate with our earlier report of suppressed HO-1 protein levels in AD cerebrospinal fluid (Schipper et al., *Neurology* 54:1297–1304, 2000). Heat shock protein (HSP) 90 was up-regulated in the AD CP relative to controls. There was a trend towards increased expression of HSP60, a mitochondrial stress protein; this is compatible with mitochondrial pathology recently documented in AD CP. Up-regulation of HSP90, a steroid receptor chaperone, in the AD CP may indicate abnormal hormone receptor expression in this secretory tissue. Glucose-regulated protein (GRP) 78 and 94 immunostaining was diminished in AD CP, implicating possible derangements in glucose or calcium homeostasis. Oxidative stress, per se, is probably not responsible for our observations because: i) there were no noticeable differences in the expression of HSP 70, ubiquitin, and alpha-B crystallin in the AD CP; and ii) augmentation, rather than the noted suppression, of HO-1 immunoreactivity would have been expected.

Keywords: Alzheimer's disease, choroid plexus, alpha-B crystallin, heat shock protein, oxidative stress, heme oxygenase-1, ubiquitin

1. Introduction

Choroid plexus (CP) epithelium maintains the brain extracellular milieu by secreting a broad spectrum of biologically active molecules including growth factors, neuropeptides, inflammatory mediators, and metabolites [13,14,40]. By forming the cerebrospinal fluid (CSF), the CP regulates fluid dynamics in the developing and injured brain [9]. Growth factor expression

is modified in the CP and CSF system of Alzheimer-diseased (AD) brains [42]. Many studies have found altered concentrations of regulatory molecules in the CSF of AD patients [2,16,17,32], including elevated levels of the tau protein and reduced levels of β -amyloid protein [2]. AD investigations have revealed enhanced concentration of tau protein in choroid plexus concurrent with that in cortex, prompting the interpretation of a pathogenetic relationship [19,20]. Given the significant role of the CP-CSF nexus in CNS homeostasis, it is important to gain further understanding on how the plexus helps the brain adjust to various stressors [33, 40].

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In response to cellular insults (including sublethal exposure to heat, reactive oxygen species, metal ions, amino acid analogues, denatured proteins and sulfhydryl agents), neural and other cells elaborate a number of highly-conserved stress or heat-shock proteins. The superfamily of stress proteins includes high molecular weight heat shock proteins such as HSP90 and HSP72, certain low molecular weight peptides (e.g. HSP27, ubiquitin and heme oxygenase-1) and a group of glucose-regulated proteins (e.g. GRP94). The latter appear to respond to a more restricted range of stimuli such as glucose deprivation and calcium ionophores but not to generalized intracellular oxidative stress. HSPs are thought to protect cells undergoing stress by preventing damage to the translational apparatus, maintaining lipid membrane integrity, accelerating degradation of misfolded or denatured proteins and preventing deleterious protein aggregation by binding to exposed hydrophobic surfaces [24]. Thus, HSPs provide a wide spectrum of protection against neurodegeneration.

Overexpression of stress proteins, including HSP70, HSP27, alpha B-crystallin, ubiquitin and HO-1, has been demonstrated in neuronal and glial cells, and within hallmark neuropathological inclusions (senile plaques, neurofibrillary tangles) in AD-affected regions [26,29,39,46]. Up-regulation of these stress proteins may be partly due to augmented levels of oxidative stress in Alzheimer-diseased neural tissues [35]. Schipper et al. (2000) reported decreased HO-1 protein in sporadic-AD CSF, suggesting that choroid plexus regulates heat shock proteins in cerebral extracellular fluid [36]. Moreover, HO-1 interacts with tau, a major protein upregulated in the choroid plexus of AD patients [19,20]. Because HO-1/tau interactions may be detrimental or beneficial [43,44], depending on specific conditions, it is important to have information for HO-1 and tau expression patterns in non-neuronal cells, like choroid epithelium, as well as neurons.

Although stress protein expression in the brain parenchyma of AD patients has been amply documented [12,23,27,30,37,39,41,46], the role of altered proteins in the choroid plexus-CSF of the aging/degenerating CNS needs more attention due to potential impact on neuronal function [33]. In this study, we determined whether and to what extent the expression of various cellular stress proteins is perturbed in the choroid plexus of Alzheimer patients relative to normal, elderly control subjects.

2. Materials and methods

2.1. Human tissues

Formalin-fixed, paraffin-embedded samples of choroid plexus were obtained from normal adult human brains ($n = 5$) and those of pathologically confirmed AD ($n = 10$), with postmortem intervals ranging from 1 to 24 h. The diagnosis of AD was made with widely accepted criteria [15,45]. The pathological severity of disease was rated on a scale of I-VI in accordance with the staging criteria of Braak and Braak [6]. A clinical synopsis of each case is given in Table 1.

2.2. Immunohistochemistry

Before immunostaining, tissue sections were deparaffinized with xylene and rehydrated through graded alcohol and water. Sections were then incubated with 30% H₂O₂ for 10 min to block endogenous peroxidase, microwaved for 15 min in citrate buffer for antigen retrieval, and blocked with 5% non-immune serum for 1 h. After pretreatment, the sections were incubated overnight in monoclonal and polyclonal antibodies directed against HO-1 (1/100 in block buffer; SPA-895), HSP60 (1/100; SPA-806), HSP90 (1/500; SPA-830), GRP78 (1/250; SPA-826), GRP94 (1/500; SPA-850), HSP70 (1/100; SPA-810), ubiquitin (1/100; SPA-201) and alpha-B crystallin (1/50; SPA-222) (StressGen Biotechnologies, Victoria, British Columbia).

Following rinsing with FTA hemagglutination buffer, a biotinylated secondary reagent and streptavidin-horseradish peroxidase conjugate were each applied for 20 min and rinsed in FTA buffer after each step. DAB chromagen was applied for 5 min, after which sections were rinsed with distilled-deionized water. Slides were assessed blindly for staining distribution and intensity using a scale of 0–5, where 0 = complete absence of staining, 1 = 0–20% of choroid plexus epithelial cells staining, 2 = 20–40% of cells staining, 3 = 40–60% of cells staining, 4 = 60–80% of cells staining, and 5 = a robust, homogeneous staining pattern of 80–100% of cells. For a given antibody, the mean staining intensity in AD was compared to control by Student's *t* test.

A staining intensity comparison was done for post-mortem delay intervals. Intervals for our specimens ranged from 1–24 hr. Therefore, we divided the samples into 4 groups: 1–6 hr, 7–12 hr, 13–18 hr, and 19–24 hr; and then did a 1-way factorial ANOVA. This was followed by the Tukey HSD test for post-ANOVA pair-wise comparisons.

Table 1
Clinical information on Alzheimer patients and controls

Group	Braak staging	Age (y)	Sex	Cause of death
AD	VI	63	F	aspiration pneumonia
	V	77	M	cardiac failure
	VI	88	F	myocardial infarction
	VI	92	F	cardiac failure
	VI	70	M	gastric ulcer
	VI	91	F	cachexia
	VI	69	F	cardiac failure
	VI	75	M	bronchopneumonia
	IV	65	M	aspiration pneumonia
	VI	77	F	bronchopneumonia
Control		91	F	myocardial infarction and pericardial effusion
		86	M	myocardial infarction
		93	F	cardiac failure
		51	F	rectal adenocarcinoma
		53	M	corpus callosum glioma

3. Results

For all antibodies analyzed, the staining intensity did not vary with postmortem delay interval ($p > 0.05$). Immunohistochemistry revealed variable expression of the stress proteins surveyed within the choroid plexus epithelial cells and stroma of AD patients (Figs 1 and 2). Thus, in AD the expression of some HSPs increased, whereas that of others decreased:

HO-1. Control specimens were marked by focal HO-1 staining of epithelial cells and diffuse staining of stromal tissue (mean intensity = 3.8). AD choroid plexus tissue exhibited less immunoreactivity (Fig. 1), with only faint staining of the epithelium and stroma (mean intensity = 2.8).

HSP60. Low-level HSP60 immunoreactivity was observed in the epithelium and stroma of controls (mean intensity = 2.4). AD tissue exhibited slightly stronger nuclear and cytoplasmic staining of epithelial cells (mean intensity = 3.2).

HSP90. Very faint HSP90 staining of stromal tissue was seen in control plexuses (mean intensity = 1.4). In AD tissue, staining was markedly increased, characterized by strong cytoplasmic staining of the epithelium with lighter staining of the stroma (mean intensity = 3.1). HSP90 immunoreactivity was also present in vascular endothelial cells (Fig. 1).

GRP78. In controls, GRP78 immunoreactivity was confined predominantly to epithelial cytoplasm (mean intensity = 4.2), with faint staining of the stroma. In AD tissue, there was only sparse focal staining of the epithelium and faint staining of the stroma (mean intensity = 2.4).

GRP94. Immunoreactivity was strong in control CP epithelial cytoplasm (Fig. 2) and stroma (mean intensity

= 3). GRP 94 only faintly stained the epithelium of AD tissue (mean intensity = 1.4).

HSP70. There were no noticeable differences in HSP70 immunoreactivity between controls (mean intensity = 0.4) and AD tissues (mean intensity = 0.7). In both groups, faint HSP70 immunostaining was limited to the CP stroma (Fig. 2).

Ubiquitin and alpha-B crystallin. Immunoreactivity for these small heat shock proteins was undetectable in the CP epithelium of both control and AD plexuses (mean intensity = 0 for both). Very faint staining of the stroma was present in all cases.

4. Discussion

In this investigation, immunohistochemistry revealed markedly altered stress protein expression in the AD choroid plexus relative to that observed in non-demented elderly controls. These observations are commensurate with deranged patterns of HSP expression previously documented in neurons and glia within AD-affected brain regions. Increased oxidative stress, possibly provoked by toxic β -amyloid deposits or pro-inflammatory cytokines, has been traditionally invoked as a major stimulus for altering expression of HSPs in AD-affected regions. These HSPs, in turn, may confer cytoprotection by limiting the aggregation of hydrophobic proteins, tagging potentially deleterious peptides for rapid, proteosomal degradation and preventing injury to the translational machinery within affected neurons and glia [24]. The present results, however, argue that augmented oxidative stress *per se* is unlikely to be solely responsible for abnormal stress protein expression observed in the AD choroid plexus. Although

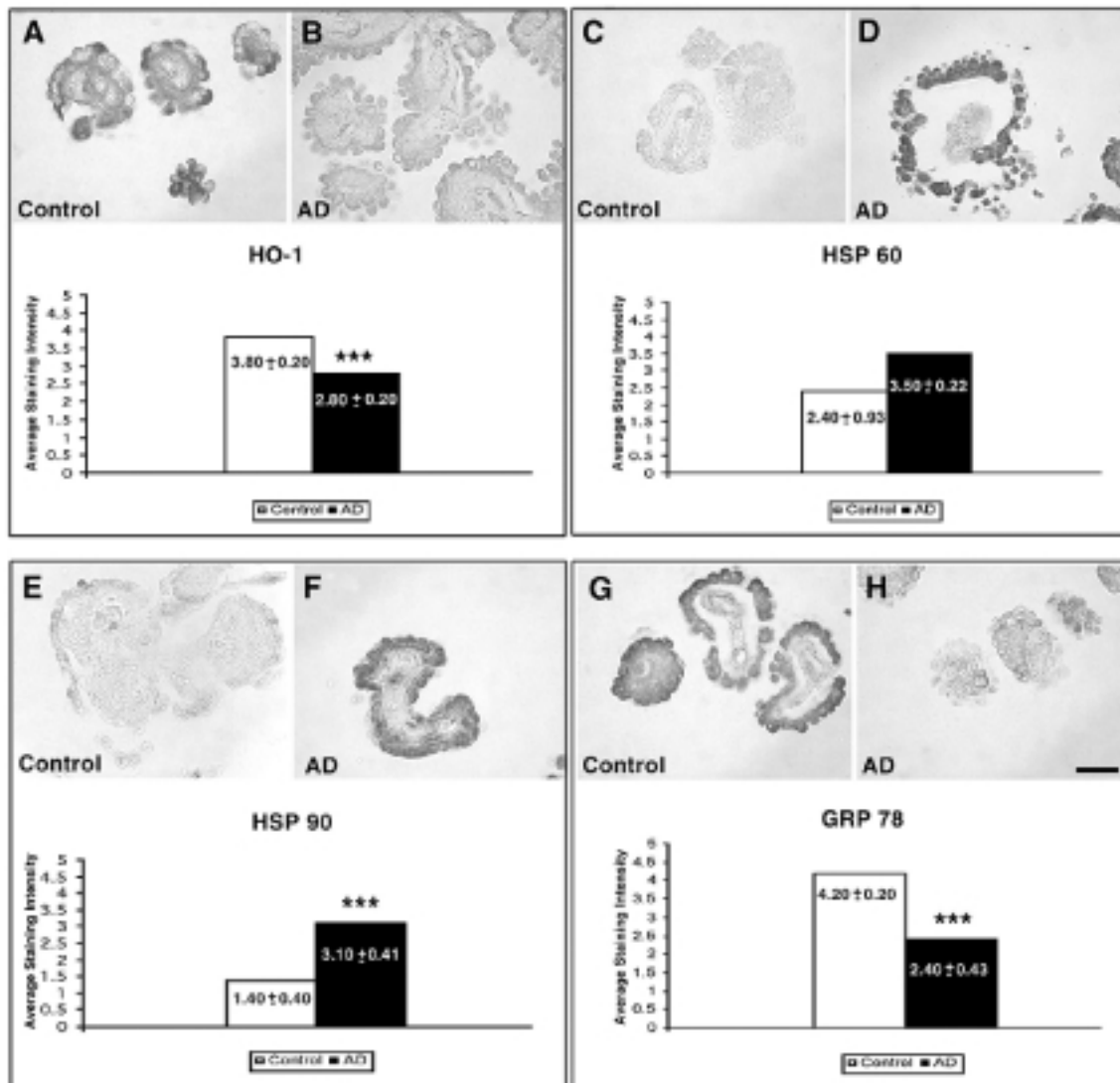


Fig. 1. Stress protein staining in AD and control tissue: Immunostaining images from control and AD choroid plexuses showing the average staining intensities for HO-1, HSP 60, HSP 90, and GRP 78. Staining intensity was graded according to criteria described in Methods. Means \pm SEM are given in each bar for 10 AD tissues and 5 controls. *** $p < 0.05$, by Student's *t* test. Scale bar, 20 μ m.

levels of immunoreactive HSPs 60 and 90, respectively were marginally and clearly elevated in the AD choroid plexus, there were no discernible differences between AD and control specimens in the expression of HSP70, ubiquitin, and alpha-B crystallin, stress proteins sensitive to up-regulation by oxidative stress. Moreover, augmentation, rather than the observed suppression, of HO-1 immunostaining would be expected in the AD choroid plexus in the face of oxidative challenge [24].

The observed suppression of HO-1 staining in the AD choroid plexus is particularly intriguing in light of previous reports of distorted HO-1 expression in both

central and peripheral AD tissues. HO-1 immunoreactivity is markedly increased in neurons and astrocytes of AD hippocampus and temporal cortex relative to age-matched non-demented controls [37]; conversely, CSF and plasma HO-1 protein levels (measured by ELISA) are significantly decreased in patients with sporadic AD [36]. We also determined that mononuclear cell HO-1 mRNA in AD patients (assayed by Northern blotting and quantitative RT-PCR) is chemically unstable and prone to accelerated ex vivo denaturation relative to NEC and to subjects with other neurological and medical conditions [36,38]. Oxidative DNA and RNA

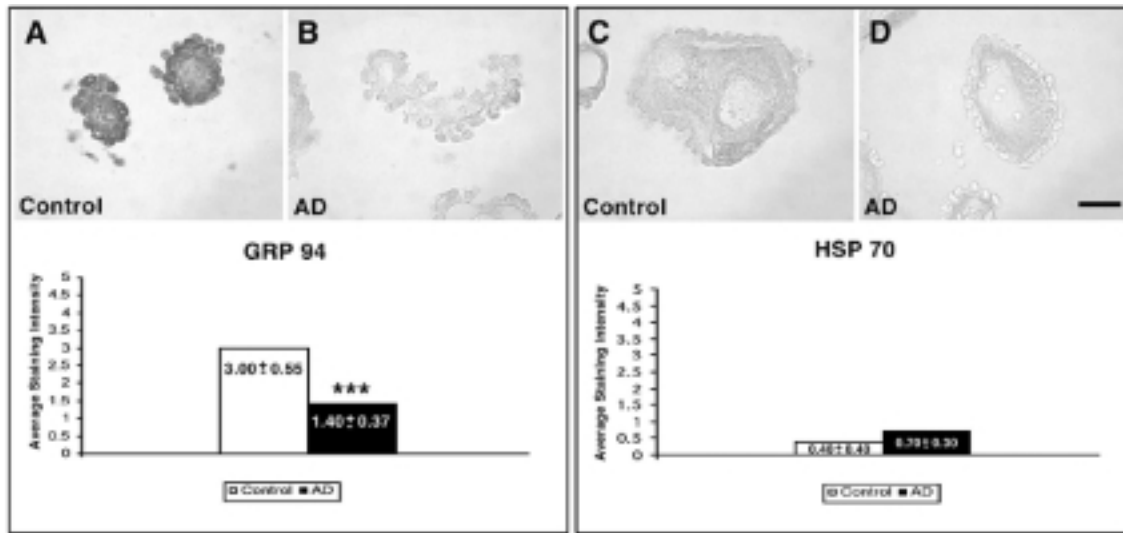


Fig. 2. Stress protein staining in AD and control tissue: Immunostaining images from control and AD choroid plexuses showing average staining intensities for GRP 94 and HSP 70. Means \pm SEM are given in each bar for 10 AD tissues or 5 controls. *** $p < 0.05$, by Student's *t* test. Scale bar, 20 μ m.

damage has been documented in various central and peripheral AD tissues [18,25,34] and may be responsible for the HO-1 mRNA instability observed in AD cells. Experiments in progress are determining if impaired translation of oxidatively modified HO-1 mRNA is responsible for diminished expression of HO-1 protein in AD choroid plexus and CSF. Moreover, alterations in HO-1 (present study) and tau expression [20] in AD choroid plexus need to be elucidated in regard to possible functional interaction, as in neurons [43].

HSP60 is a mitochondrial stress protein which facilitates proper folding and assembly of polypeptides as the latter are imported from the cytosol into mitochondria [21,22]. There is a growing literature implicating mitochondrial structural damage and enzyme defects (bioenergetic failure) in the pathogenesis of Alzheimer's disease [5,8,11,35]. Our present findings revealed slightly increased HSP60 immunoreactivity in epithelial cells of the AD choroid plexus relative to control specimens. Such results are consistent with a prior report of mitochondrial enzyme insufficiency in AD choroid plexus [8]. These observations raise the possibility that bioenergetic failure and cellular ATP depletion in AD choroid plexus epithelial cells are responsible for the aberrant CSF dynamics and constituents reported in this disease [25].

Up-regulated HSP90 expression found in AD CP may further contribute to pathological CSF secretion. HSP90 forms complexes with several intracellular protein kinases and induces the activity of heme-regulated

e1F-2 alpha kinase. The latter, in turn, is implicated in the generalized down-regulation of gene transcription characteristic of heat-shocked cells [1,31]. HSP90 induction may act by this mechanism to alter protein biosynthesis and secretion in the AD CP. The well-documented function of HSP90 as a steroid receptor chaperone protein could be relevant [28]. Theoretically, HSP90 overexpression in AD-stressed CP engenders pathological interactions between sex steroids or glucocorticoids and the CSF secretory apparatus.

The mechanism(s) responsible for down-regulation of GRP78 and GRP94 observed in the AD CP awaits clarification. Based on considerations above, the general impairment of protein biosynthesis or oxidative nucleic acid damage may curtail the expression of glucose-regulated proteins in the AD CP. Alternatively, more specific derangements in glucose metabolism [10], calcium homeostasis [3,7] or signal transduction pathways [4,28] characteristic of AD-affected neural tissues might explain the abnormal pattern of GRP expression in AD CP.

The present immunocytochemical investigation reveals patterns of stress protein expression that are markedly altered in the CP of AD subjects relative to age-matched, non-AD controls. It is important to expand upon this immunostaining information with immunoblot analyses to corroborate that HSPs are altered in the CP-CSF system stressed by AD. Transgenic mouse models of AD and cultured CP epithelial cells should provide insight on factors that govern expression

of heat shock and glucose-regulated proteins in the AD plexus and how these choroidally-synthesized proteins play a role in regulating CSF-brain parameters.

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