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Interaction between β-amyloid protein and heparan sulfate proteoglycans from the cerebral capillary basement membrane in Alzheimer's disease

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Abstract

Proteoglycans are important in the pathogenesis of senile dementia of Alzheimer type (SDAT) by participating in amyloidogenesis. Knowledge about specific proteoglycan subtypes in SDAT may be of therapeutic advantage. In this study, we examined proteoglycan constituents of SDAT brains with reference to hyaluronic acid, heparan sulfate (HS), dermatan sulfate and chondroitin sulfate subtypes. Total proteoglycans showed a 1.6-fold increase in the hippocampus and 4.3-fold increase in the gyrus frontalis superior compared to non-demented elderly subjects. The HS subtype showed a 9.3-fold increase in hippocampus and a 6.6-fold increase in gyrus frontalis superior. Immunohistochemical studies of senile plaques revealed the expression of heparan sulfate proteoglycan (HSPG) in a portion of the core of typical plaques. β-amyloid expression was positive in senile plaques and the degenerated neuronal processes and capillary basement membrane, but was negative in endothelial cells. Microglial cells adjacent to senile plaques were positive for HLA-DR expression, and astroglial cells positive for glial fibrillary acidic protein were scattered around the microglial cells. Immunoelectron microscopic examination showed an electron-dense reaction for HSPG in the thickened basement membrane adjacent to the endothelial cells of capillary vessels, but not inside the endothelial cells. These findings suggest that a markedly increased HSPG in SDAT brains is most likely caused by HSPG from the blood capillary basement membrane and that the degenerated processes around senile plaques may arise from microglial or astroglial cells.

Keywords: Heparan sulfate proteoglycan; β-amyloid protein; Alzheimer's disease; Immunohistochemistry; Immunoelectron microscopy

1. Introduction

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder that leads to profound memory loss, cognitive decline, and preferential loss of cholinergic and cholinoceptive neurons in the cerebral cortex and hippocampus. Pathological features include an abundance of focal extracellular deposits of amyloid (amyloid plaques) and neurofibrillary tangles. A principal component of amyloid plaques is β-amyloid a peptide fragment composed of the 40 or 42 amino acid residues derived from the selective proteolytic cleavage of the amyloid precursor protein (APP) by the sequential actions of β-secretases and γ-secretases.[1,2] Despite extensive research documenting amyloid deposition within the brain, a clear causal relationship between the increased deposition of amyloid β 42 (Aβ42) and AD is yet to be defined. Aβ42, the longer isoform of amyloid that is preferentially concentrated in senile plaque amyloid deposits in AD, is resistant to degradation and accumulates as

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insoluble aggregates in late endosomes or lysosomes. The breakdown of the lysosomal membrane may be a key pathogenic event, leading to the release of heparan sulfate and lysosomal hydrolases into the cytosol.[3]

APP is stimulated by an interaction with specific proteoglycans. APP binds to at least two major proteoglycans, heparan sulfate proteoglycan (HSPG) and chondroitin sulfate proteoglycans. The interaction of specific APP-binding proteoglycans with amyloid plaques may disturb the normal function of APP-binding proteoglycans and contribute to the neuritic degeneration commonly seen around the amyloid plaque cores.[4]

The HSPG perlecan accumulates primarily with microglia/macrophages within the amyloid infusion site following infusion of β-amyloid protein into the rodent hippocampus *in vitro*.[5] At least three distinct classes of proteoglycans are localized to AD lesions. These include neuritic plaques, cerebrovascular amyloid deposits and neurofibrillary tangles.

Although HSPGs are still the only class of proteoglycans immunolocalized to amyloid fibrils within the neuritic plaques of AD, information on specific fractions of proteoglycans such as keratan sulfate (KS), dermatan sulfate (DS), and chondroitin sulfate (ChS) in the AD brain is limited. These particular proteoglycans may have distinct and important roles in neuritic plaque pathogenesis.[6] We examine the concentrations of these classes of proteoglycans and assess the localization of HSPG and β-amyloid protein by immunohistochemical methods and the involvement of astroglial and microglial cells in the formation of senile plaques and surrounding degenerated neuronal processes.

2. Materials and methods

2.1. Tissues

Formalin-fixed post-mortem samples of cerebral tissues from the right hippocampal gyrus and right superior frontal gyrus were used. The senile dementia of Alzheimer type (SDAT) brain samples from the Tokyo Metropolitan Geriatrics General Research Center and Hatsuishi Hospital were obtained from 25 patients (8 males, 17 females) between 60 and 97 years of age (mean 81.7 years) with a clinical and histopathological diagnosis of AD. The SDAT brain mass averaged 1094 g (range 750–1380 g). Brain samples of non-demented elderly (NDE) patients were obtained from autopsies conducted at the Department of Pathology, Nippon Medical School. There were 10 patients (7 males, 3 females) between 75 and 101 years of age (average: 87.7 years). The average brain mass was 1248 g (range 1010–1350 g).

2.2. Proteoglycan analysis

Quantitation of proteoglycans in formalin-fixed cerebral tissues was performed[7] with minor modification. We delipidated post-mortem samples from the right hippocampal gyrus and right superior frontal gyrus with several changes of acetone followed by extraction with chloroform/methanol (3:1 volume). We carried out exhaustive proteolytic digestion of these preparations with papain (p3125, Sigma, St Louis, MO, USA) in 0.01 M cysteine ethylene diamine tetra-acetic acid (EDTA) buffer and then with pronase (Kaken Seiyaku, Tokyo, Japan) in 0.01 M cysteine EDTA buffer to liberate proteoglycans. The digests were then treated with trichloroacetic acid to obtain a more concentrated proteoglycan solution. For quantification of proteoglycans, the mixture was fractionated by two-dimensional electrophoresis on a cellulose acetate membrane followed by staining with Alcian blue. The resulting proteoglycan spots were cut from the membrane, dissolved in 1 mL of dimethyl sulfoxide and each fraction of proteoglycan including hyaluronic acid (HA), HS, DS and ChS was quantified at 677 nm. Each proteoglycan was analyzed five times using different aliquots.

2.3. Immunohistochemistry

Paraffin sections for immunohistochemical studies were prepared from the cerebral tissues adjacent to those collected for quantitative measurement. Monoclonal antibodies to HSPG (1:50 dilution; Chemicon, Temecula, CA, USA) and was developed with diaminobenzidine. The specimens were then fixed in 1% osmium tetroxide, washed in phosphate buffer (pH 7.6), dehydrated in a series of ethanol solutions and embedded in Epok 812 resin. Ultrathin sections (60 nm) were cut and examined unstained under a transmission electron microscope (JEOL; Nippon Denshi, Osaka, Japan) at an accelerating voltage of 80 kV.

3. Results

3.1. Proteoglycan analysis

Quantitation of proteoglycans was performed for HA, HS, DS and ChS (Fig. 1). Total proteoglycan levels in the hippocampus were 2.67 μg for an SDAT brain, in contrast to 1.70 μg for a NDE brain (Fig. 1A). Proteoglycan levels in the gyrus frontalis superior were 2.68 μg for an SDAT brain in contrast to 0.63 μg for a NDE brain (Fig. 1B). Total proteoglycan levels were increased 1.6-fold in the hippocampus and 4.3-fold in the gyrus frontalis superior compared to NDE subjects. The amounts of HA, HS, DS, and ChS in the hippocampus of SDAT brains were 1.52 μg, 0.37 μg, 0.55 μg, and 0.23 μg, in contrast to 0.87 μg, 0.04 μg, 0.43 μg, and 0.37 μg in NDE brains, respectively. The amounts of HA, HS, DS, and ChS in the gyrus frontalis of SDAT brains were 1.27 μg, 0.59 μg, 0.57 μg, and 0.25 μg, in contrast to 0.37 μg, 0.09 μg, 0.43 μg, and 0.10 μg in NDE brains, respectively.

In SDAT patients, the mean concentration of HA was increased 1.7-fold in the hippocampus and 3.4-fold in the gyrus frontalis superior as compared to NDE brains. Furthermore, the mean amount of DS was increased 1.3-fold