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Immunodistribution of amyloid beta protein (Aβ) and advanced glycation end-product receptors (RAGE) in choroid plexus and ependyma of resuscitated patients

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Abstract

RAGE (receptor for advanced glycation end-products) participates in the influx transport of glycated Aβ (amyloid beta) from the blood to the brain. Because little is known of the RAGE operating in brain barriers such as those in the choroid plexus and ependyma, the aim of the present study was to examine the immunodistributions of RAGE and Aβ peptides in the choroid plexus where the blood-cerebrospinal fluid barrier (B-CSF) is located, and in ependyma of the brain ventricles associated with functions of the cerebrospinal fluid-brain barrier (CSF-B). The study was performed on patients over 65 years successfully resuscitated after cardiac arrest with survival a few weeks. The control group consisted of age-matched individuals who were not resuscitated and died immediately after cardiac arrest.

In resuscitated patients, but not in controls, RAGE receptors were localized in choroid plexus (CP) epithelial cells and in ependymal cells bordering the brain ventricles. These cells form the B-CSF and CSF-B barriers. The presence of Aβ was detected within the CP blood vessels and in the basement membrane of the CP epithelium. In numerous cytoplasmic vacuoles of CP epithelial and ependymal cells Aβ protein was found and our observations suggest that the contents of those vacuoles were undergoing progressive digestion.

The results demonstrated that CP epithelium and ependymal cells, equipped with RAGE receptors, not only play an important role in the creation of amyloid deposits in the brain but are also places where Aβ may be utilized. The RAGE transportation system should be a main target in the therapy of brain amyloidosis, a well-known risk factor of Alzheimer disease.

Key words: *RAGE (receptor for advanced glycation end-products), Aβ (amyloid beta protein), choroid plexus, ependyma, cardiac arrest.*

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Introduction

Glycation is a non-enzymatic process which causes post-translational modifications of various proteins by reducing sugars to form advanced glycation end-products (AGEs), such as amyloid beta protein (Aβ), tau, transthyretin and other proteins associated with several neurodegenerative diseases [11,6]. Although brain compartments are effectively isolated from the plasma proteins by the blood-brain barrier (BBB), localized on the endothelium of the brain capillaries, there are specialized receptors at this barrier that may shuttle all these proteins in efflux and influx directions [1,2]. Efflux transport of AGEs through the BBB is mediated by the endothelial LRP-1 (lipoprotein receptor-related protein-1), whereas influx transport involves RAGE (receptor for advanced glycation end-products). The proper function for both types of receptors at the BBB is critical for the regulation of protein homeostasis in the brain [14].

In aging, dysfunction of the BBB and increased plasma levels of AGEs may lead to accumulation in the brain of neurotoxic proteins such as Aβ [8]. Because little is known of the RAGE operating in brain barriers such as those in the choroid plexus and ependyma, the aim of the present study was to examine the immunodistributions of RAGE and Aβ peptides in the choroid plexus where the blood-cerebrospinal fluid barrier (B-CSF) is located, and in ependyma of the brain ventricles associated with functions of the cerebrospinal fluid-brain barrier (CSF-B).

Material and methods

The study was performed on thirty patients over 65 years old who were affected by total ischaemia caused by cardiac arrest, successfully resuscitated and survived a few weeks before they died and following autopsy their brains were used for the study. The control group consisted of thirty age-matched individuals who were not resuscitated and died immediately after cardiac arrest. The morphological changes in the brains of all patients were described previously [10]. Brain samples containing the choroid plexus and ependyma were drawn from the file or dissected from the brains during new autopsies. The brains were fixed and embedded in paraffin or Epon. RAGE and Aβ proteins were detected in the tissue using immuno-histochemical methods and specific antibodies generated against different domains of RAGE and Aβ proteins. Distribution of the antigens was examined by light or electron microscope.

Light microscopy

Brain samples containing the choroid plexus and ependyma were fixed in 4% neutral formalin, dehydrated in absolute ethanol, cleared in xylene and then embedded in paraffin. The paraffin blocks were cut into 5-μm sections that were stained with Harris haematoxylin, counterstained with eosin and then evaluated by light microscope. To detect distribution of RAGE or Aβ proteins, sections were incubated in different solutions of polyclonal or monoclonal antibodies generated against RAGE or Aβ protein of human origin. The antibodies were purchased from Santa Cruz Biotechnology, Inc and Sigma-Aldrich (USA). For incubation, all antibodies were diluted 1 : 50. Immunoreactions were visualized using appropriate biotinylated secondary antibodies and an alkaline phosphatase-avidin-biotin conjugate (Santa Cruz, USA) as previously described [7]. For negative controls, primary antibodies were replaced with an appropriate normal immunoglobulin isotope fraction at matched protein concentration. These were included for the examination of each specimen and consistently produced negative results.

Electron microscopy

Samples of choroid plexus or ependyma were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). Specimens were post-fixed with 1% osmium tetroxide, dehydrated through ascending series of ethanol, and embedded in Epon. The serial semi-thin sections were stained with 1% toluidine blue and examined with a light microscope.

The thin sections were incubated in the solution containing Aβ antibody as described above but antigen in the tissue was visualized by gold particles (Chemicon Internat. Inc.) or were double-contrasted with uranyl acetate followed by lead citrate, and viewed and photographed with a transmission electron microscope (Hitachi Ltd, Tokyo, Japan).

Results

The selective transport of Aβ to the cerebrospinal fluid requires special receptors such as RAGE. In the choroid plexus (CP) and ependyma of resuscitated patients, but not in controls, we found RAGE recep-

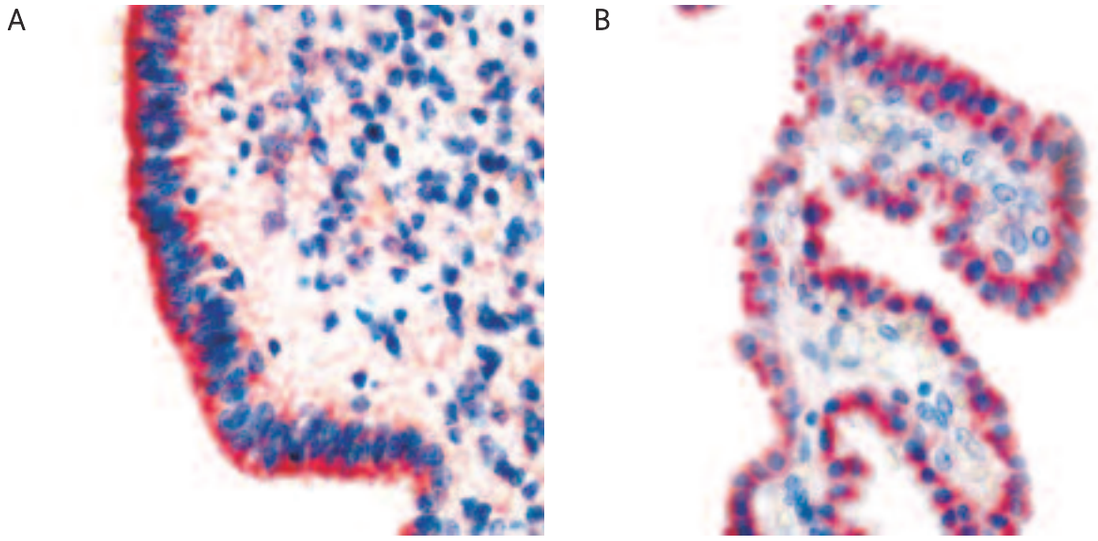

Fig. 1. Immunodistribution of RAGE (red colour) in resuscitated patients in: **A)** choroid plexus epithelial cells, **B)** ependyma cells bordering the brain ventricle. Orig. magn. 200×.

tors precisely in cells that form the barriers. Thus, RAGE were localized in epithelial cells of CP (Fig. 1A) and in ependyma cells bordering the brain ventricles (Fig. 1B). No RAGE receptors were detected on the endothelium of CP blood vessels. Using electron microscopy we demonstrated the presence of Aβ within numerous CP blood vessels (Fig. 2A) and in the basement membrane of the CP epithelium (Fig. 2B). We also found Aβ protein in numerous cytoplasmic vacuoles of epithelial and ependyma cells (Fig. 2C). Our observations suggest that the contents of these vacuoles were undergoing progressive digestion. The peripheral part of the vacuole content appeared undigested and formed characteristic rings or scrolls (Fig. 3), all being immunopositive with Aβ antibodies.

Discussion

The choroid plexus (CP) is a small and complex organ which plays pivotal roles in an extraordinary range of processes that establish, survey and maintain the biochemical and cellular status of the central nervous system (CNS) under both normal and pathological conditions. Although distinct choroidal pathology has been observed in different brain disorders including stroke and Alzheimer's disease (AD), the causal relationship between choroidal changes and some medical conditions is poorly known. Choroid plexus closely cooperates with ependyma in the transportation of numerous compounds from the circulating blood into the brain, and the results of our present study document that both of these structures can participate in the transport of Aβ protein as well. The CP epithelium and ependyma cells, like the endothelium of brain capillaries, are equipped with the RAGE receptor. RAGE is the key receptor for the influx transport of glycated proteins including Aβ [1]. In contrast to the BBB, the CP capillaries have larger diameters than regular brain capillaries, and at an ultrastructural level fenestrations can be seen in their endothelium [12]. Thus, numerous compounds can easily cross the wall of these vessels and penetrate the core matrix of the CP. However, the tightly sealed monolayer of CP epithelium and ependyma cells makes them the essential component of the blood-cerebrospinal fluid (B-CSF) and the cerebrospinal fluid-brain (CSF-B) barriers that prevent the passive exchange of solutes between the blood and the brain. Within the CP, the B-CSF barrier function is shifted from the vasculature to the epithelium; therefore, we demonstrated the presence of Aβ within numerous CP blood vessels, in the basement membrane of epithelium and in cytoplasm vacuoles of these cells. Our observations suggest that the contents of vacuoles undergo successive degradation (digestion), leaving at the periphery of some vacuoles the undigested part of

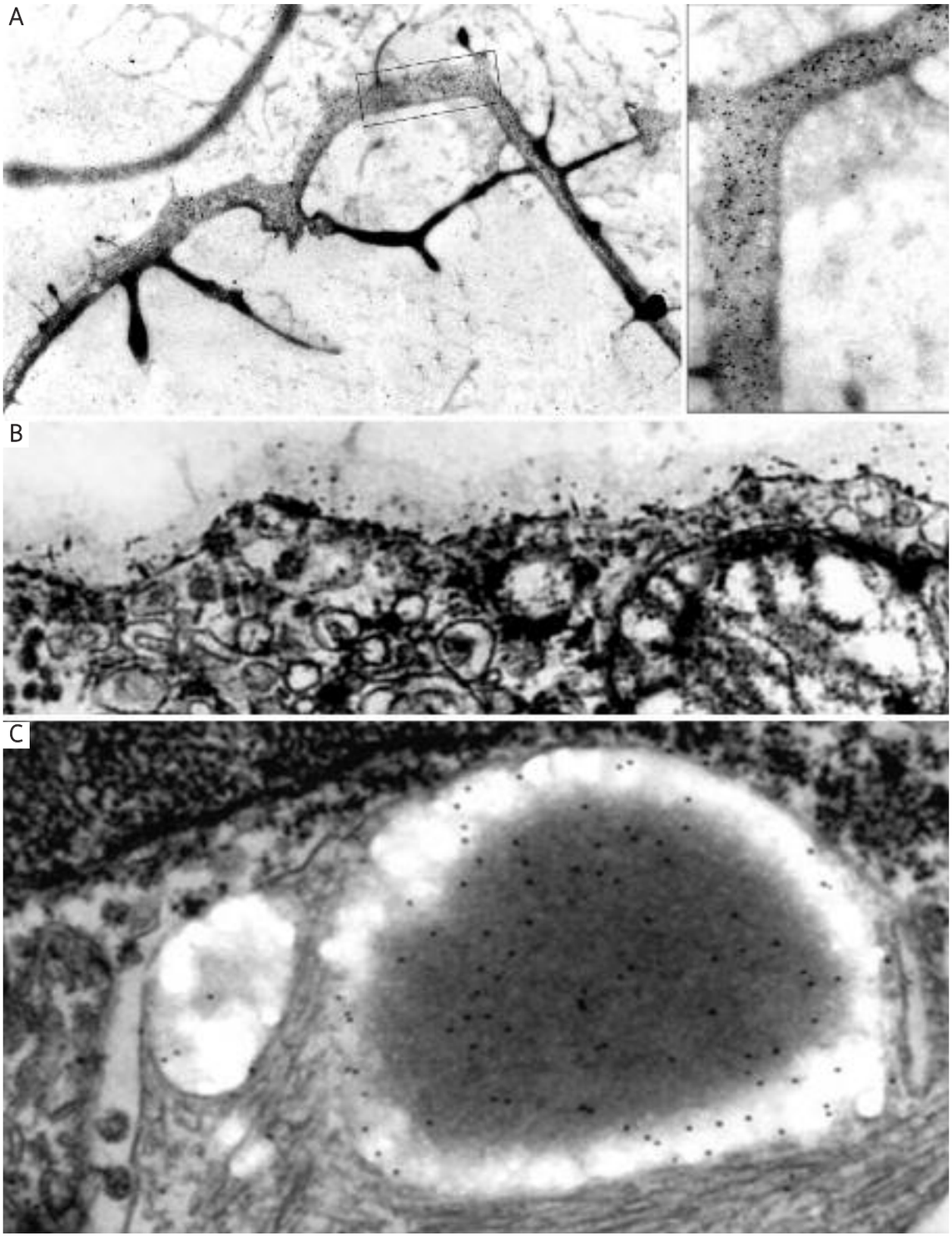

Fig. 2. Immunodistribution of Aβ visualized by 18 nm colloidal gold particles in: **A)** choroid plexus blood vessels, **B)** basement membrane of choroid plexus epithelium, **C)** cytoplasmic vacuoles of choroid plexus epithelium. Orig. magn. 5000×.

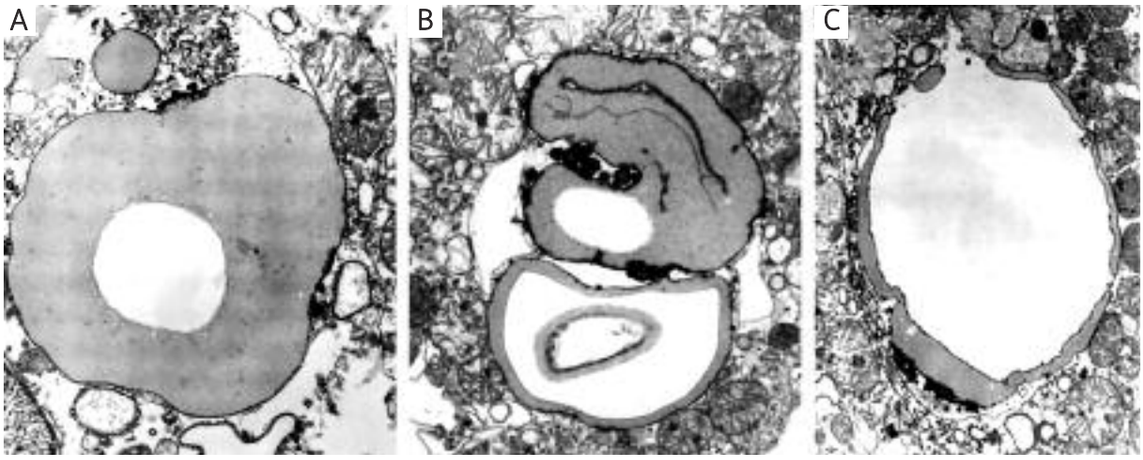

Fig. 3. Different stages of digestion of vacuole content: **A)** initial stage starting in the centre of the vacuole, **B)** formation of ring- and scroll-like structures during digestion within two vacuoles, **C)** undigested material localized at the periphery of the vacuole. Orig. magn. 5000×.

this material, which has a ring- and scroll-like shape. Such structures in epithelium of human CP were previously described by Biondi in 1933. Authors of the subsequent studies thought that these intracellular inclusions, called Biondi bodies (Bb), resemble the cerebral neurofibrillary tangles and extracellular plaques of Alzheimer's disease. Although it was revealed that Bb contain amyloid-beta protein epitopes, [3] the arrangement of their fibres was found to be different than in plaques and tangles [4,5,13]. In the present studies, we observed that the formation of such intracellular inclusions and their characteristic shapes are an effect of unsuccessful digestion of the CP vacuoles' contents. This digestion as well as the homeostatic and secretory functions of the CP is linked to energy-dependent mechanisms and there is compelling evidence that the ageing tissue is unable to maintain appropriate energy output. On the other hand, ageing and ischaemia are known risk factors but not the causes of amyloid-beta accumulation in the tissue. Thus, we found Biondi bodies only in patients affected by both of these factors but not in the age-matched controls. This means that severe ischaemia was a more potent factor for the induction of RAGE and influx of amyloid-beta protein to the CP than just regular aging of the control patients who suddenly died. It was recently suggested that RAGE, which mediates transfer of Aβ through the endothelial cells [1], can be upregulated during ageing [14], but it was also observed that in aging, the rate of Aβ transport by RAGE across the brain barriers was determined as five- to six-fold lower than the rate for the transport of large neutral amino acids [9].

Independently of the age of patients, severe ischaemia initiates expression of RAGE and increases degradation of amyloid precursor protein (APP) in the brain and in all peripheral organs. Then, amyloid-beta protein is produced in the brain and in the periphery by a number of different cell types and is transported to the brain across the brain barriers via receptor-mediated transcytosis. Altered management of Aβ at the brain barrier level is one of the crucial links between ischaemia, ageing and AD [2].

Our results confirm the observations that severe ischaemia may stimulate the expression of RAGE in cells forming barriers and suggest that CP epithelial and ependyma cells not only play an important role in the creation of amyloid deposits in the brain, but are also places where Aβ may be utilized.

In conclusion, our results strongly support the observations that the RAGE transportation system should be a main target in the therapy of brain amyloidosis, which is a known risk factor for Alzheimer's disease.

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