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Ischemic blood-brain barrier and amyloid in white matter as etiological factors in leukoaraiosis

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Abstract
Background Pathology of white matter, which is observed in ischemic brain, indicates that similar processes contribute to Alzheimer's disease development. These injuries have been seen in the subcortical and periventricular regions. Periventricular white matter changes in ischemic and Alzheimer's disease brain, referred to as leukoaraiosis, are responsible for changes in memory, cognition and behavior. It is not clear whether the blood-brain barrier in ischemic periventricular white matter is altered in aged animals.
Methods We studied blood-brain barrier changes with amyloid precursor protein staining around blood-brain barrier vessels. Rats were made ischemic by *cardiac arrest*. Blood-brain barrier insufficiency, accumulation of amyloid precursor protein and platelets around blood-brain barrier vessels were investigated in ischemic periventricular white matter up to 1-year survival.
Findings Ischemic periventricualr white matter demonstrated enduring blood-brain barrier changes. Toxic fragments of amyloid precursor protein deposits were associated with the blood-brain barrier vessels. Moreover our investigation revealed platelet aggregates in- and outside blood-brain barrier vessels. Toxic parts of amyloid precursor protein and platelet aggregates correlated very well with blood-brain barrier permeability.
Conclusions Progressive injury of the ischemic periventricular white matter may be caused not only by a degeneration of neurons destroyed during ischemia but also by damage in blood-brain barrier. Chronic ischemic blood-brain barrier insufficiency with accumulation of toxic components of amyloid precursor protein in the periventricular white matter perivascular space, may gradually over a lifetime, progress to leukoaraiosis and finally to severe dementia.

Keywords Leukoaraiosis · Blood-brain barrier · Axonal leakage · Amyloid precursor protein

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Introduction

Alzheimer's disease is progressive disorder with unknown etiological mechanisms. Recent clinical and experimental studies propose connection of ischemic processes with Alzheimer's dementia [6, 8]. Slowly developing ischemic- and Alzheimer's-type dementia probably has connection with white matter changes, too. Several reports have been suggested that axonopathy and axonal transport deficits may also play a causative role during progression of Alzheimer's disease [2, 4, 11]. Here the overlap of ischemia with Alzheimer's disease will be a central issue, as will the white matter changes frequently seen in dementia. Periventricular white matter injury occurs in both neuropathological processes and is termed leukoaraiosis. In this paper we will try understand the role of ischemic blood-brain barrier permeability for platelets and toxic fragments of amyloid precursor protein in leukoaraiosis process.

Materials and methods

In female Wistar rats (n=24, 3 months old, 150–180 g body weight) blood-brain barrier, amyloid precursor protein and

platelets pathology were investigated following 10 min of ischemic brain injury due to *cardiac arrest* [7]. Experiments were performed with rats aged 2 and 7 days and 6 and 12 month. Sham-operated control animals (n=16) were sacrificed at the same time points. Animals were perfusion fixed for light and electron microscopic analysis.

Horseradish peroxidase served as an indicator of blood-brain barrier permeability. It was injected *i.v.* and was allowed to circulate for 30 min. Left hemisphere was cut at 50–80 μm slices in the coronal plane with a vibratome and incubated in a solution of 3,3′-diaminobenzidine tetrahydrochloride and examined by light microscopy [7]. Sections from the right hemisphere were selected for electron microscopy studies [7]. Antibodies recognizing different parts of amyloid precursor protein were used to stain paraffin sections [7].

Results

Until one year after ischemic brain injury periventricular white matter regions contained single and scattered areas of horseradish peroxidase extravasations (Fig. 1a) (Table 1). Horseradish peroxidase extravasations involved arterioles, capillaries, venules and veins. Extravasated horseradish peroxidase appeared to be restricted to branches and bifurcations of leaking blood-brain barrier vessels. In summary ischemic periventricular white matter presented random blood-brain barrier permeability (Fig. 1a).

After short-term survival following experimental *cardiac arrest* N- and C-terminal of amyloid precursor protein and β-amyloid peptide immunoreactivity were found around the blood-brain barrier vasculature (Table 1). Following long-term survival, staining only for the neurotoxic β-amyloid peptide and C-terminal of amyloid precursor protein was found (Fig. 1c) (Table 1). Multiple and abundant β-amyloid peptide and C-terminal of amyloid precursor protein accumulations embraced or adjoined the blood-brain barrier vessels (Fig. 1c). The staining size was different and irregular in shape. Blood-brain barrier vessel lumens and their inner and outer side of walls were also stained. The halo of β-amyloid peptide and C-terminal of amyloid precursor protein immunoreactivity in the perivascular space of blood-brain barrier vessels suggests that both proteins can easy cross walls of blood-brain barrier vasculature. In general, perivascular deposits of different parts of amyloid precursor protein took the same forms as extravasated horseradish peroxidase.

Investigation of leaky sites of blood-brain barrier vessel demonstrated single or aggregating platelets sticking and adhering to the blood-brain barrier vessel walls at all time points [7, 8]. Intravascular aggregates of platelets predominated in blood-brain barrier branches and bifurcations,

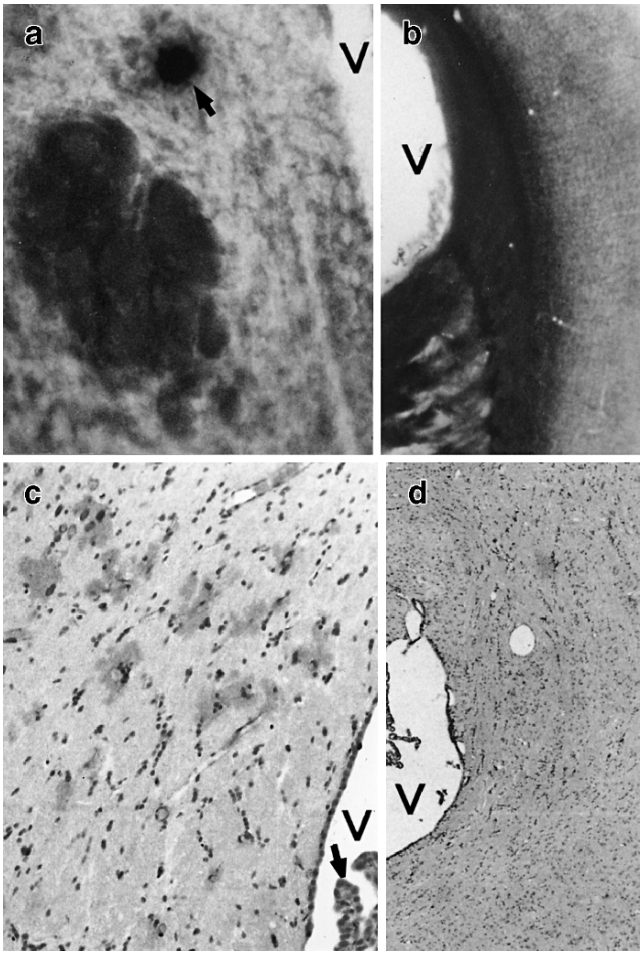

Fig. 1 **a**. Vibratome section reacted for horseradish peroxidase, extravasated horseradish peroxidase (arrow) is noted in periventricular space. 10-min brain ischemia, 1 year survival. (× 60). **b**. No staining for horseradish peroxidase in vibratome section is seen in periventricular space. Sham-operated rat, 1 year survival. (× 40). **c**. Numerous perivascular, extracellular and intracellular C-terminal of amyloid precursor protein deposits in periventricular space (brown color). C-terminal of amyloid precursor protein-positive staining in plexus chorioideus (arrow). 10-min brain ischemia, 1 year survival. (× 100). **d**. C-terminal of amyloid precursor protein staining is not identified in perivascular, extracellular and intracellular space in periventricular area. Sham-operated rat, 1 year survival. (× 60). Ventricles of brains (v) are shown

which correlated very well with blood-brain barrier permeability. Many vessels were plugged by platelets completely and completely blocked blood flow. Moreover single and aggregating platelets were noted on the abluminal side of blood-brain barrier vessels [7, 8]. Platelets pathology was single, scattered, and random. These kind of alterations occurred in arterioles, capillaries, venules and veins independent of survival time. Toxic components of amyloid precursor protein and platelet aggregates correlated well with blood-brain barrier permeability.

Control animals showed no horseradish peroxidase leakage (Fig. 1b) (Table 1) and no luminal or abluminal

Table 1 Immunoreactivity for N-terminal of amyloid precursor protein (NAPP), β-amyloid peptide (βA) and C-terminal of amyloid precursor protein (CAPP) and horseradish peroxidase (HRP) in the periventricular white matter perivascular space following 10-min brain ischemia

Group	NAPP	βA	CAPP	HRP
Controls	–	–	±	–
Short-term survival				
2 days	++	++	++	+
7 days	++	++	++	+
Long-term survival				
6 months	–	+++	+++	+
12 months	–	+/++	+/++	+

The staining intensity was categorized into five grades as follows: - no staining; ± staining for cytoplasm of single cells; + a single and diffuse areas; ++ a few and diffuse areas; +++ many strong and diffuse areas

platelet aggregation around blood-brain barrier vessels [7, 8]. In control brains staining for different fragments of amyloid precursor protein was not observed (Fig. 1d) except for weak immunoreactivity for the C-terminal of amyloid precursor protein in the cytoplasm of single cells (Table 1).

Discussion

We identified a novel lesion in periventricular white matter myelinated fibers caused by blood-brain barrier permeability for toxic components of amyloid precursor protein in ischemic brain. Therefore it is possible that the focal abnormal levels of β-amyloid peptide and C-terminal of amyloid precursor protein may locally destroy axon membranes [7, 10, 13] and induce local axonal leakage e.g. for amyloid precursor protein (Fig. 2). Moreover, if an axon is destroyed at certain sites [7], amyloid precursor protein may leak out and, *via* proteolysis of amyloid precursor protein, β-amyloid peptide may be produced in a *vicious cycle* [1, 3] (Fig. 2). Both circulatory and intraaxonal components, including amyloid precursor protein, may leak out of ischemic blood-brain barrier and ischemic axons and produce leukoaraiosis, in same way as in Alzheimer's brain (Fig. 2). This process may be involved in the formation of amyloid plaques that may also play a causative role in the cognitive deficits in Alzheimer's disease [6, 8] (Fig. 2).

In the present study we found that swollen axons are accompanied by ischemic blood-brain barrier changes and accumulation of the C-terminal of amyloid precursor protein and β-amyloid peptide [7]. The occurrence of astrocytic and axonal swelling in perivascular space one year following brain ischemia [7] represents vasogenic edema. On the other hand the available evidence indicates that impaired axonal transport is a key process inducing axon swelling in an experimental model of Alzheimer's disease [11]. Axonal swelling phenotypes that resemble the dystrophic neurites related to senile plaques have been described not only in Alzheimer's disease mouse models but also in the brains of aged and Alzheimer's disease patients. It is possible that the swollen axons [7] with local membrane damages [10, 13] may in turn affect axonal transport and make axonal leakage worse. Amyloid precursor protein is a functional membrane protein in axons that is produced in the cell body and transported to the distal part of axons [5]. If amyloid precursor protein leakage occurs at the site of axonal leakage, then β-amyloid

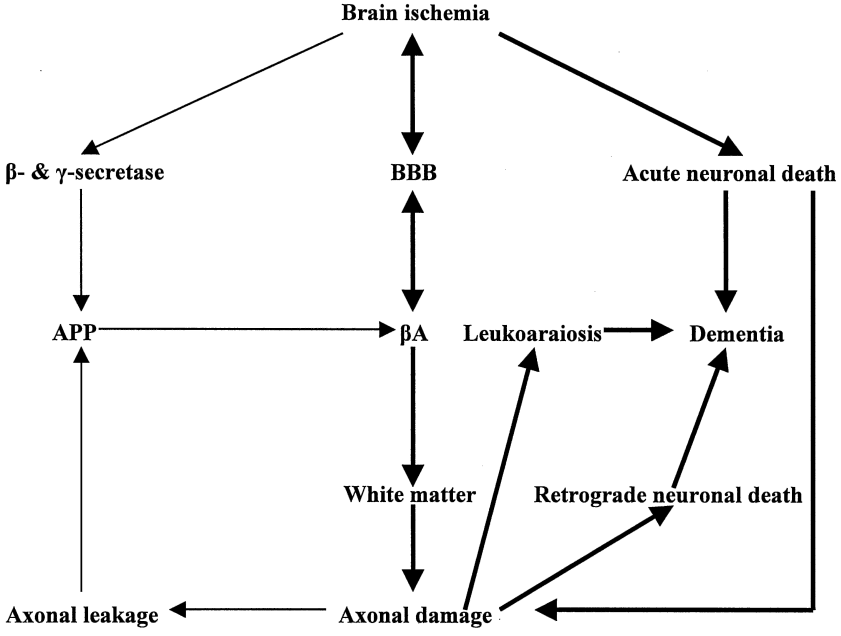

Fig. 2 Schematic diagram of the ischemic pathological processes in leukoaraiosis development. BBB-Blood-brain barrier; APP-amyloid precursor protein; βA-β-amyloid peptide

peptide production and deposition may occur in the extraaxonal space by proteolysis of amyloid precursor protein [1, 3] (Fig. 2). Accumulation of β-amyloid peptide and other products of amyloid precursor protein cleavage, at the sites of axonal injury after traumatic experimental brain lesion, suggest that this may also be the case in Alzheimer's disease [1, 3, 11]. The uncontrolled leakage of amyloid precursor protein and other proteins may decrease the availability of these components in the distal part of axons, resulting in dystrophic neurites and diminished neurotransmitters at the axon terminals. Axonal leakage may be an additional factor in the development of leukoaraiosis and retrograde secondary neuronal death in ischemic injury and dementia (Fig. 2).

If ischemic blood-brain barrier with different fragments of amyloid precursor protein and axonal leakage appear together around the neurites as we observed in our experimental model, this may explain the formation of amyloid plaques with dystrophic neurites typical of Alzheimer's disease. It is understandable that axonal leakage is due to direct and indirect ischemic membrane damage of axons. Any factor that affects membrane stability and results in severe blood-brain barrier [7, 12] and axonal leakage, may precipitate Alzheimer's disease-like neuropathological change. Our findings indicate that blood-brain barrier and axonal leakage may be a key pathological change that would help to explain mechanisms of leukoaraiosis. These changes may possibly lead to the development of amyloid plaques and neurofibrillary tangles and may contribute to Alzheimer's dementia (Fig. 2). Finally we can observe age-related loss of myelinated fibers and neurons [9]. The development of substances stabilizing axonal transport might represent a novel therapeutic target to prevent cognitive decline in neurodegenerative disorders such as ischemia and Alzheimer's disease.

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Conflict of interest statement We declare that we have no conflict of interest.

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