Remote morphological changes in the white matter after ischaemic stroke

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Acute phase of stroke is the focus of most experimental and clinical studies on cerebral ischaemia. The scarcity of data on remote changes led us to examine the morphological pictures of brains after ischaemic insults. We paid special attention to the white matter capillaries. We microscopically evaluated 10 brains of patients who died after one month to fourteen years after the ischaemic stroke. Morphological examinations involved the application of routine histological stains and immunohistochemical reactions with antibodies against human albumin, GFAP, macrophage antigen CD 68 and lectins (Ulex europaeus, Wheat Germ agglutinin and Bandeirea simplicifolia). The results showed a swelling of the endothelial cells and their invagination into the vessel lumen. Postapoplectic cavities and white matter spongiosis decreasing with increase in distance from the cavity were observed. Immunohistochemical study showed that there was no segmental immunoreactivity to lectins on the capillary wall. Immune reaction to albumin revealed protein extravasation to the rarefied brain parenchyma.

Our results indicate that progressing damage of the white matter after ischaemia may be caused not only by degeneration of axons of neurones destroyed by stroke, but also by pathological changes in small blood vessels, especially in capillaries. Hence, vascular leukoencephalopathy is probably caused by arteriolar damage as well as by microangiopathy.

key words: cerebral ischaemia, leukoencephalopathy, microangiopathy, lectins

INTRODUCTION

Acute phase of stroke is the focus of most experimental and clinical papers about cerebral ischaemia. Morphological changes in acute ischaemic injury both in humans and in animals are well known. Morphological events in ischaemic lesion and various pathogenic factors that cause brain tissue damage were microscopically examined during the first few hours or days after the ischaemic insult in experimental studies, in human – during a few first weeks.

Scarcity of morphological data especially concerns changes in the late period after stroke. Clinical observations have shown that neurological status of 14% of survivors deteriorates between the 3rd and the 20th month after stroke [16]. Increasing motor dysfunction [8] and cognitive disturbances have also been described [15]. In patients with stroke, significant progression of white matter lesion and small deep (lacunar) infarcts were found in CT [17].

It is known that progressing neurological deficit after stroke may be associated with delayed neuronal death. But this phenomenon is observed in a relatively short period after ischaemia [4]. In literature, data describing structural changes, particularly in the white matter, during progressing ischaemic injury are on rare
disorders such as subcortical hypertonic encephalopathy of Binswanger’s type and CADASIL (Cerebral Autosomal Dominant Arteriopathy, Subcortical Infarcts, Leukoencephalopathy). There are no data on common stroke. Clinical observations and scarcity of data in literature led us to examine morphological pictures of brains several months/years after ischaemic stroke. We paid special attention to the white matter capillaries. We used a variety of lectins to assess the microvessels. Lectins are proteins synthesised from plants that bind to specific carbohydrate residues (glycoconjugates) on the surface of a variety of cells, including the brain microvascular endothelium. Glycoconjugates are biochemical components of various receptors. They also form the cell glycoprotein coat and play an important role in the transport of ions across the plasma membrane of the endothelial cells. These glycoconjugates can be detected and localised with lectins [9–11, 13].

The presence of glycoprotein coat on the luminal surface of the endothelium is of great importance to the normal functioning of the blood-brain barrier (BBB) [3]. In ischaemia changes in the distribution of endothelial surface glycoconjugates associated with altered permeability of the brain microvessels have been described [12, 13]. In ME observation with lectin-gold complex technique, the decoration of luminal surface of endothelium was evidently less intense in the leaking microvessels than in uninjured one [14].

RESULTS

In all the examined cases, except postapoplectic cavities, enlargement of the perivascular space and white matter spongiosis was found (Fig. 1). Intensity of the spongiosis decreased with the increase in distance from the cavities. Sometimes, single, CD68(+) macrophages were seen in the area surrounding the necrotic foci. They were located mainly around small blood vessels and could be found even in brains of patients who died many years after the stroke.

In areas adjacent to cavities, moderate hypertrophy and proliferation of astrocytes was observed. Reaction of astrocytes on the distant white matter was mild or weak. Many small focal lesions with distinctive spongiosis were devoid of GFAP immunoreactive astrocytes.

<table>
<thead>
<tr>
<th>nr</th>
<th>Sex/age</th>
<th>Area of the infarction</th>
<th>Risk factors</th>
<th>Disability after stroke</th>
<th>Duration from stroke onset to death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/76</td>
<td>Left MCA</td>
<td>AH, DM, IHD,</td>
<td>Right hemiparesis</td>
<td>1 month</td>
</tr>
<tr>
<td>2</td>
<td>F/73</td>
<td>Left MCA</td>
<td>Chronic circulatory insufficiency</td>
<td>Right hemiparesis, aphasia</td>
<td>4 months</td>
</tr>
<tr>
<td>3</td>
<td>F/91</td>
<td>Left MCA</td>
<td>AH, IHD</td>
<td>Right hemiparesis</td>
<td>5 months</td>
</tr>
<tr>
<td>4</td>
<td>F/86</td>
<td>Left MCA</td>
<td>AH</td>
<td>Exaggerated right tendon reflexes</td>
<td>1 year</td>
</tr>
<tr>
<td>5</td>
<td>M/71</td>
<td>Left PCA</td>
<td>AF, DM</td>
<td>Exaggerated right tendon reflexes</td>
<td>2 years</td>
</tr>
<tr>
<td>6</td>
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<td>Left MCA</td>
<td>AF</td>
<td>Right hemiparesis</td>
<td>3 years</td>
</tr>
<tr>
<td>7</td>
<td>F/88</td>
<td>Left MCA</td>
<td>AF, IHD</td>
<td>Right hemiparesis</td>
<td>4 years</td>
</tr>
<tr>
<td>8</td>
<td>F/82</td>
<td>Right MCA</td>
<td>AF, AH</td>
<td>Symptomless</td>
<td>5 years</td>
</tr>
<tr>
<td>9</td>
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<td>Left MCA</td>
<td>AH</td>
<td>Right hemiparesis</td>
<td>10 years</td>
</tr>
<tr>
<td>10</td>
<td>F/75</td>
<td>Right MCA</td>
<td>AF, AH</td>
<td>Left hemiparesis</td>
<td>14 years</td>
</tr>
</tbody>
</table>

MCA — middle cerebral artery, PCA — posterior cerebral artery, AF — atrial fibrillation, AH — arterial hypertension, DM — diabetes mellitus, IHD — ischaemic heart disease
Gliarial scar bordering the ischaemic foci was not noticed in any of the examined brains.

Immunohistochemical study of the blood microvessels in the white matter surrounding the postapoplectic cavity and the distal from the cavity revealed that there was no segmental immunoreactivity to the examined lectins (Fig. 2–4). Swollen endothelium and invagination of the endothelial nuclei into the vessel lumen were observed in many capillaries (Fig. 5). Immune reaction to albumin sometimes revealed mild protein extravasation to the rarefied brain parenchyma (Fig. 6).

In both hypertensive and normotensive patients, the described changes were observed independent of the duration after the stroke onset.

**DISCUSSION**

Evident scattered changes in the surrounding white matter were observed in all the examined cases, apart from the focal ischaemic lesions. These changes involved rarefaction of the white matter, sometimes, with pronounced diffuse or focal spongiosis, état cribré, many lacunas as well as astrocytic reactivity of various intensity and a presence of single macrophages. White matter rarefaction and spongiosis near the postnecrotic cavities were more pronounced than those in distant areas. This finding indicates that the process of necrosis is gradual and slowly progresses similarly to a process of "maturity" of acute ischaemic lesion [5].

In our material, segmental reduction of lectin bindings were observed in many capillaries located in rarefied white matter. But the immune reaction to albumin revealed that only a relatively small part of the capillaries had increased BBB permeability. This discrepancy suggests that partial loss of lectins is not a main cause of BBB damage. The damage is probably associated with a deep structural or functional injury of the endothelial cells.

Morphological changes observed in the examined brains were similar to changes found in Binswanger’s
subcortical hypertonic encephalopathy, in which hyalination and sclerosis of arterioles connected with the white matter are described. It is important that in our material this type of encephalopathic changes were noticed also in cases without arterial hypertension. Since it is believed that Binswanger’s encephalopathy is associated with pathology of the arterioles, pathologically changed capillaries might also be responsible for diffuse white matter damage after ischaemic stroke.

Except disturbances in BBB permeability, there are at least three additional factors that can influence progress of the white matter injury. The first one is axonal degeneration of the nerve fibres triggered by necrosis of the cortical neurons and it is responsible for tissue rarefaction. The second one is associated with astroglial reactivity. Glial scar bordering focal necrotic lesions and preventing the surrounding tissue from negative influences of agents diffused into or secreted by damaged cells was not found in any of
the examined cases. Lack of the glial scar was probably associated with decrease in age of the astroglial reactivity [2, 7]. This may make the white matter more vulnerable to detrimental effects of various cytokines, chemokines and growth factors and may also facilitate its damage. The third factor playing a role in progressing white matter damage seems to be insufficient blood supply. In humans, vascular architecture of the white matter is different from other CNS areas; vessels are spiral and coiled [6]. We observed irregularities in the capillary lumen and swollen endothelium, which make the flow of blood in microvessel worse. The anatomical properties of white matter vessels overlapping with structural changes on the capillary wall can promote parenchymal damage over a period of time. Similar findings were demonstrated in experimental studies in which microvascular obstruction producing increased perfusion deficits in the ischaemic penumbra led to extension of the infarction [1].

Figure 5. Swollen endothelial cell invaginating into the capillary lumen and forming “functional” microembolus composed of erythrocytes. Visible segmental differences in the lectin immunoreactivity on the vessel wall. Anti-BSA × 1000.

Figure 6. Albumin leakage from the permeable capillary vessel in the white matter distal from the necrotic cavity. Anti-albumin × 250.
CONCLUSIONS
Progressing damage of the white matter after ischaemic stroke may be caused not only by a degeneration of axons of neurons destroyed during stroke, but also by pathological changes in small blood vessels, especially in capillaries. Hence, vascular leukoencephalopathy is probably caused by arteriolar damage as well as by microangiopathy.

REFERENCES