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ORIGINAL ARTICLE

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Ultrastructure of capillary endothelium in pilocytic astrocytomas

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Abstract The aim of this study was to assess the capillary ultrastructure of pilocytic astrocytomas with gadolinium contrast enhancement on magnetic resonance imaging (MRI). Cysts were identified in all cases with pilocytic astrocytoma. Histological investigation focusing on the vascular structure was performed by light microscopy and electron microscopy in four pilocytic astrocytomas. In the pilocytic astrocytomas, light microscopic examination demonstrated vascular abnormalities (fibrosis, hyalinization, and vascular proliferation), and electron microscopic examination revealed fenestration and vesicles in the capillary endothelium. Fenestration of the vessels and vascular abnormalities with degeneration are suggested to develop both contrast enhancement on neuroimaging and cystic formation in pilocytic astrocytomas.

Key words Pilocytic astrocytoma · Capillary · Ultrastructure

Introduction

The contrast-enhanced magnetic resonance imaging (MRI) technique has become established for the detection and characterization of brain tumors. Contrast agents such as gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) cause MRI contrast enhancement due to extravascular leakage. Therefore, enhancement with contrast materials is considered to be caused by a defect in the function of the vascular barrier to the materials in the tumors. However, few studies have specifically focused on the histological aspect of contrast enhancement on MRI in central nervous system (CNS) tumors. At the morphological level, it is widely recognized that the anatomical basis of the blood-brain barrier rests on the unique structure of the normal cerebral capillary endothelial cells, which have a lack of fenestration, intercellular gaps with continuous belts of tight junctions, and scanty endocytotic vesicles in capillary endothelial cells.[1–3] Infiltrative low-grade astrocytomas (fibrillary astrocytomas) are often demonstrated as nonenhanced lesions, which are considered to have infiltrated and proliferated in the brain as the blood-brain barrier remains intact. On the other hand, most anaplastic astrocytomas and glioblastomas are contrast enhanced because of disruption of the blood-brain barrier or neovasculalization without the barrier to contrast materials. However, pilocytic astrocytomas are demonstrated as enhancing masses in spite of their benign nature.[4,5] Histopathologically, perivascular lymphocytes and vascular degeneration are frequently seen in pilocytic astrocytomas.[6,7] However, few studies have specifically focused on the vascular ultrastructure of pilocytic astrocytomas. Therefore, we investigated the vascular characteristics of pilocytic astrocytomas using electron microscopy.

Materials and methods

Four tumor specimens obtained from four patients (one male and three females, aged 8–18 years) with pilocytic astrocytomas were investigated. In each patient, axial T_1-weighted spin echo images were obtained (slice thickness, 5 mm) using a 1.5 T MR system (Signa Horizon, General Electric Medical System, Milwaukee, WI, USA) before the contrast injection. Post-contrast-enhanced T_1-weighted images were obtained approximately two minutes after the intravenous administration of Gd-DTPA (Magnevist, Nihon Schering, Osaka, Japan), followed by a 20-ml saline flush. All tumors were surgically proved and applied in our histopathological study. All tumor specimens had been routinely fixed in formalin and embedded in paraffin. For

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hematoxylin and eosin (H&E) staining, paraffin sections were mounted on poly-L-lysine adhesive-coated slides. The sections were deparaffinized in a graded series of alcohol and xylene. HE staining was performed to examine vascular abnormalities (fibrosis, hyalinization, vascular cell proliferation, and vascular tufts) and extravascular lymphocytic infiltration in the tumors. For electron microscopic study, small pieces of tumor tissue were fixed immediately in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in Epon-Araldite. Semithin sections (1 μm thick) were stained with toluidine blue to select an appropriate specimen and field. Thereafter, ultrathin sections, contrasted with uranyl acetate and lead citrate, were employed in the ultrastructural investigation of the tumors.

Results

A summary of the clinical and histopathological findings in this study is given in Table 1. These tumors were homogeneously enhanced and well demarcated (Fig. 1A and B). Cyst formation was seen in all pilocytic astrocytomas. In light microscopic study, vascular abnormalities, including fibrosis (identified as abundant fibrous tissue in the vascular wall), hyalinization, vascular cell proliferation, vascular tufts, and extravascular lymphocytic infiltration, were found in all four pilocytic astrocytomas (Table 1).

Electron microscopic study revealed that fenestration and some vesicles were found in the cytoplasm of the endothelial cells in the pilocytic astrocytomas (Fig. 2). There were few tight junctions in the interendothelial junction. There were no definitive neuronal tissues, such as neurons, myelin, synaptic formation, and neurosecretory granules, in any cases.

Discussion

Histologically, the endothelial cells of capillaries with the blood-brain barrier in the brain differ from those in other

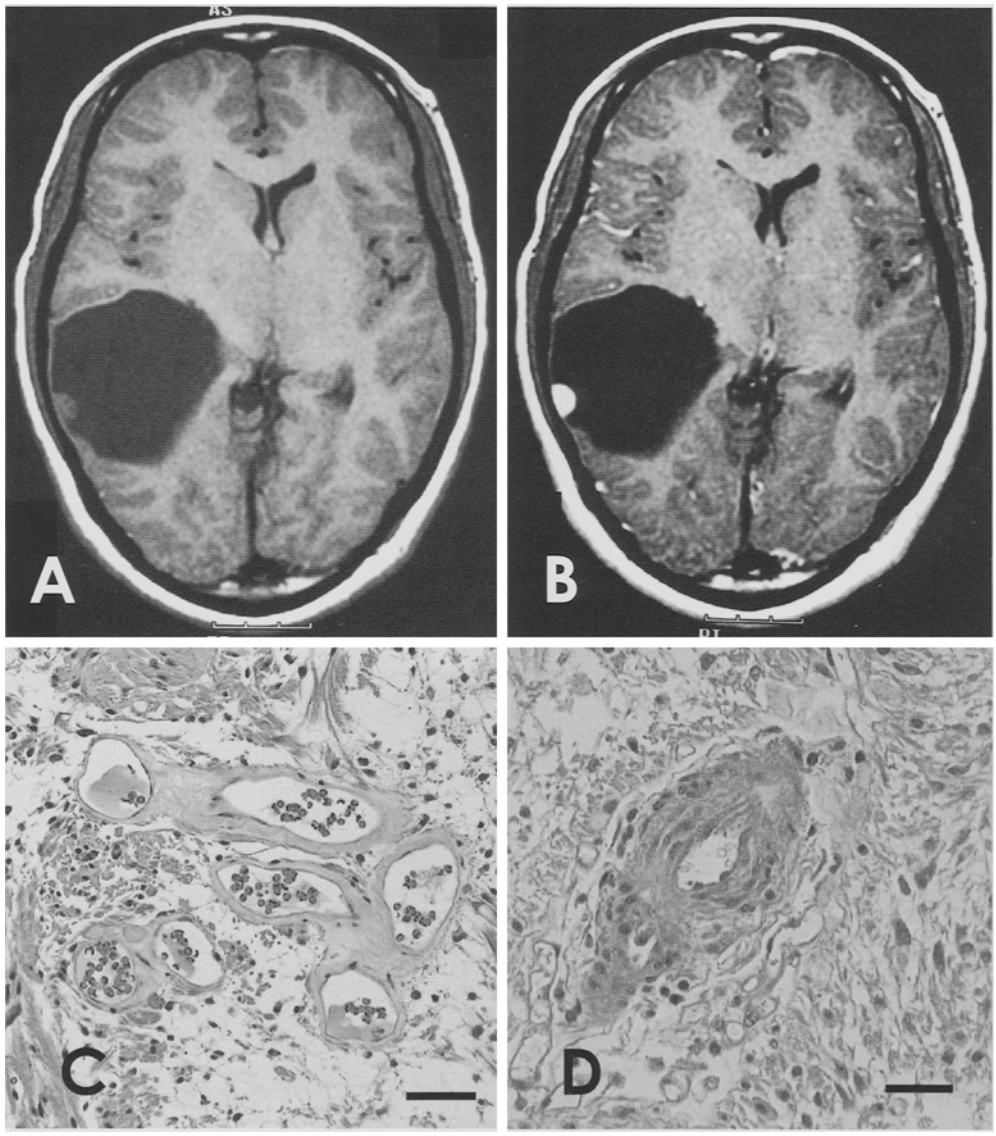

Fig. 1. Magnetic resonance images (MRI) and photomicrographs of pilocytic astrocytomas (case 3). **A**, **B** Pre- and post-contrast T_1-weighted MRI (**A** and **B**, respectively) showing an intensely enhanced mural nodule in the right temporal lobe. **C** Photomicrograph showing vascular proliferation with hyalinization and tumor cells with Rosenthal fiber. *Bar*, 70 μm. **D** Photomicrograph showing vascular cell proliferation with fibrosis. *Bar*, 35 μm

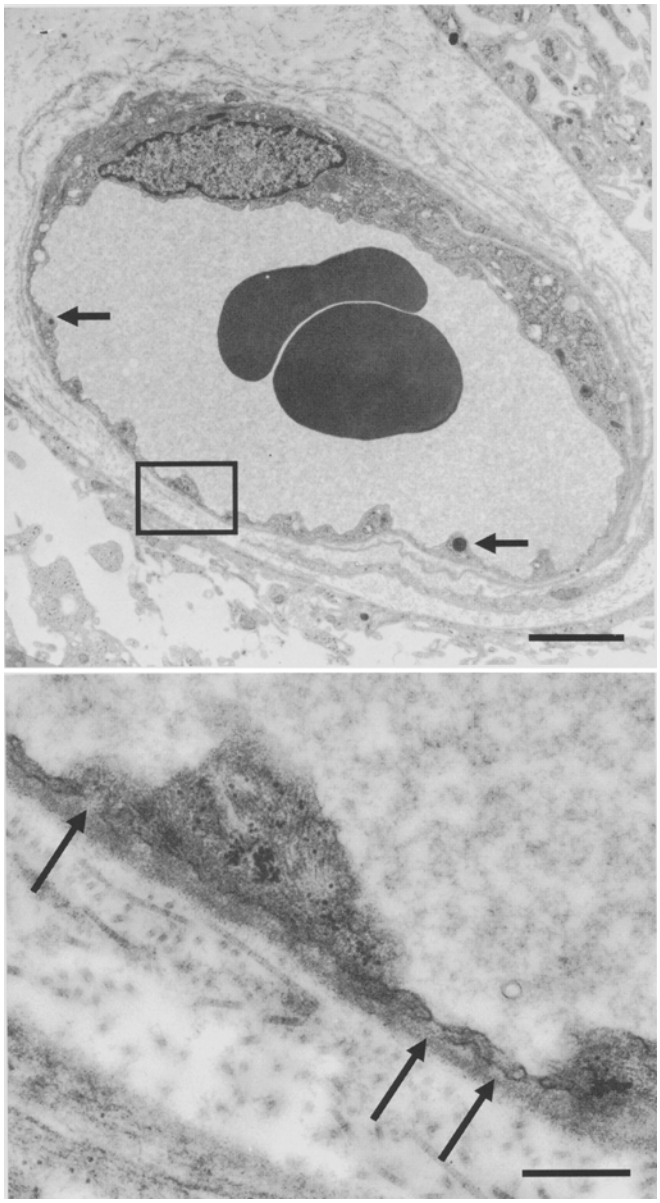

Fig. 2. Ultrastructure of a capillary in the enhanced pilocytic astrocytoma (case 3). **Upper**: Electron micrograph showing a capillary with fenestration (*box*) and vesicles (*arrows*) in the endothelium. *Bar*, 3 μm. **Lower**: Electron micrograph showing high-magnification view of endothelial fenestration (*arrows*) enlarged from section of capillary wall indicated in the box. *Bar*, 0.45 μm

organs in two important ways. These differences account for the ability of the blood-brain barrier to exclude certain molecules. First, peripheral endothelial cells are either fenestrated or have junctions of low resistance between the cells. In contrast, brain endothelial cells are joined by tight junctions of high electrical resistance. These highly resistant junctions present an effective barrier, even to ions.[2] The use of electron microscopy and electron-dense tracers such as horseradish peroxidase has demonstrated that the blood-brain barrier of vertebrates is located in the specialized endothelial cells of capillaries in the brain.[3] Tight junctions of the vascular endothelium play a major role in the function of the blood-brain barrier. Second, in peripheral endothelial cells, molecules move across the cells by two means: nonspecific process endocytosis and receptor-mediated endocytosis. Brain endothelial cells lack both of these mechanisms.[2]

In MRI study, T_1 enhancement after administration of Gd-DTPA is common in pilocytic astrocytomas.[4,5] In our study, fenestration and vesicles were present in the capillary endothelial cytoplasm of the enhanced pilocytic astrocytomas. These findings suggest that contrast agents are transported from the vascular lumen to the extravascular space via fenestration or by endocytosis. In addition, our electron microscopic observation revealed that there were few tight junctions in the interendothelial junction of pilocytic astrocytomas, which suggests that the tight junction plays an important role in preventing extravascular leakage of contrast agents. Long[8,9] demonstrated that endothelial cell tight junctions were often absent and junctions between cells were widely patent, allowing the passage of tracer materials in malignant brain tumors such as glioblastomas or metastatic carcinomas, meningiomas, and schwannomas.

The blood-brain barrier is disrupted by the degeneration of vessels and inflammation, such as that caused by infection and autoimmune reactions.[1,10] Histopathologically, vascular proliferation, lymphocyte infiltration, and degenerative changes are often observed in pilocytic astrocytomas.[6,7] Fulham et al.[4] reported that glucose metabolism of pilocytic astrocytomas was higher than that of low-grade astrocytomas and was similar to that of anaplastic astrocytomas. Our previous report demonstrated two cases of optic gliomas with spontaneous regression associated with apoptosis.[11] Chemical mediators such as glutamine,

Table 1. Summary of clinicopathological findings of pilocytic astrocytomas

Characteristic	Case			
	1	2	3	4
Age (yr)/sex	14/F	8/F	18/F	15/M
Location	Chiasm	Cerebellum	Temporal lobe	Cerebellum
Light microscopic findings				
Fibrosis	+	+	+	+
Hyalinization	+	+	+	+
Vascular cell proliferation	+	+	+	+
Vascular tufts	+	+	+	+
Lymphocyte infiltration	+	+	+	+
Ultrastructural findings of capillary endothelium				
Fenestration	+	+	+	+
Vesicles	−	−	+	+

serotonin, free radicals, and lysosomal enzymes are released after cell death.[12] These findings suggested that the inflammatory reaction and increase in capillary permeability might be associated with expression of the glucose metabolism and transporter in pilocytic astrocytomas.

Cysts are a common feature of pilocytic astrocytoma.[6,7] In our study, fenestration in the capillary endothelium is suggested to result in cystic formation, as all cases with pilocytic astrocytoma had peritumoral cysts and fenestrated capillaries. Inflammatory reaction in association with apoptosis and/or degenerative changes as longstanding lesions may also increase the vascular permeability and result in cystic lesions. Therefore, enhancement of astrocytomas on neuroimaging may not always only represent malignant findings such as necrosis and vascular proliferation, but also degenerative or inflammatory changes.

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