Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Increased expression of aquaporin-4 in human traumatic brain injury and brain tumors*

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Received July 10, 2004; revision accepted Aug. 3, 2004

Abstract: Objective: To characterize the expression of aquaporin-4 (AQP4), one of the aquaporins (AQPs), in human brain specimens from patients with traumatic brain injury or brain tumors. Methods: Nineteen human brain specimens were obtained from the patients with traumatic brain injury, brain tumors, benign meningioma or early stage hemorrhagic stroke. MRI or CT imaging was used to assess brain edema. Hematoxylin and eosin staining were used to evaluate cell damage. Immunohistochemistry was used to detect the AQP4 expression. Results: AQP4 expression was increased from 15 h to at least 8 d after injury. AQP4 immunoreactivity was strong around astrocytomas, ganglioglioma and metastatic adenocarcinoma. However, AQP4 immunoreactivity was only found in the centers of astrocytomas and ganglioglioma, but not in metastatic adenocarcinoma derived from lung. Conclusion: AQP4 expression increases in human brains after traumatic brain injury, within brain-derived tumors, and around brain tumors.

Key words: Aquaporin-4 (AQP4), Traumatic brain injury, Astrocytoma, Ganglioglioma, Metastatic adenocarcinoma, Brain edema

doi:10.1631/jzus.2005.B0033 **Document code:** A **CLC number:** R978.2; Q71

INTRODUCTION

Brain edema is associated with common brain injuries such as head trauma, brain tumor, stroke, brain abscess and circulation failure. Water transport across microvasculature is a main cause of brain edema, a phenomenon that was once believed to be the result of simple diffusion. A recently described family of water channel proteins (aquaporins, AQPs) facilitates the passage of water and other small solutes across membranes and through cells, including the vascular endothelium, so study of aquaporins may shed light on the treatment of brain edema (Denker *et al*., 1988).

AQP4 belongs to the aquaporin family, which includes 11 subtypes (AQP0 to AQP10). Among these, AQP4 is unique because of its exceptionally high intrinsic water permeability and predominant location in brain (Yang and Verkman, 1997). AQP4 is expressed in astrocyte foot processes adjacent to vascular endothelial cells, and in the basolateral membrane of the ependymal cells but not in neurons (Nielsen *et al.*, 1997; Rash *et al.*, 1998; Saadoun *et al.*, 2002). AQP4 is proposed to play an important role in water homeostasis in the brain and in formation of brain edema (Nielsen *et al.*, 1997). Recently, AQP4 expression has been investigated to clarify its role in the development of brain injury. It has been reported

* Project supported by the National Natural Science Foundation of China (No. 30271498) and the National Basic Research Program (973) of China (No. G1999054000)

that AQP4 is increased at the injury site in rat traumatic brain injury (Sun *et al.*, 2003), and is up-regulated following non-traumatic brain injury such as stroke or water intoxication in rodents (Taniguchi *et al.*, 2000). In AQP4 gene knockout mice, there is less brain swelling 24 h after brain ischemia (Manley *et al.*, 2000). In a human brain autopsy study, AQP4 immunoreactivity was found increased after cerebral infarction (Aoki *et al.*, 2003). However, the expression characteristics of AQP4 in human traumatic brain injury are unknown. Furthermore, in human brains, AQP4 expression increases in edematous human astrocytomas, and there is significant correlation between up-regulation of AQP4 expression and breakdown of the blood-brain barrier (Aoki *et al.*, 2003; Saadoun *et al.*, 2002). Most malignant brain tumors, including gliomas and metastatic adenocarcinoma, are associated with considerable brain edema. Whether gliomas and metastatic adenocarcinoma activate astrocytes and increase AQP4 expression remains unknown.

The aim of this study was to clarify the properties of AQP4 expression in human traumatic brain injury and brain tumors (astrocytoma, ganglioglioma and metastatic adenocarcinoma) using biopsy specimens from brain surgery. This may help determine the mechanisms responsible for brain edema associated with brain injury and brain tumors.

MATERIALS AND METHODS

Patients

This study was approved by the ethics committee of the Second Affiliated Hospital, School of Medicine, Zhejiang University. Brain specimens were obtained from 17 patients (Nos. 3~19 in Table 1) who underwent brain surgery because of traumatic brain injury or brain tumors. Two relatively normal specimens were from patients with benign meningioma (No. 1 in Table 1) and hemorrhagic stroke in an early stage (No. 2 in Table 1). MRI or CT images were taken to evaluate brain edema. A neuropathologist without knowledge of the results described here examined the patients. The diagnosis of each brain tumor was established according to the usual criteria pertaining to the clinical MRI picture, appropriate laboratory data and biopsy findings. Astrocytomas and ganglioglioma were classified as low (I–II) or high grades (III–IV) according to the Daumas-Duport criteria (Table 1).

Materials

Primary antibody against AQP4 was purchased from Santa Crutz, USA. Anti-goat IgG biotinylated secondary antibodies, avidin biotin complex and diaminobenzidine tetrahydrochloride were purchased from Zymed, USA. Other reagents were commercial products with analytic purity.

Table 1 Patients information and summary of AQP4 expression in the brain

Histology	No.	Age and sex	Duration from onset	Side	Grade	Adjacent to lesions	Center of lesions
Control							
–Benign meningioma	1	51 F	NA	L	NA	+	ND
–Hemorrhagic stroke	2	63 F	6 h	L	NA	+	ND
Traumatic brain injury	3	55 M	6 h	R	NA	ND	+
	4	51 M	9 h	L	NA	ND	++
	5	30 M	14 h	R	NA	ND	+
	6	52 M	15 h	R	NA	ND	++
	7	52 M	1 d	R/L	NA	ND	++
	8	21 M	1 d	R/L	NA	ND	+++
	9	71 M	2.5 d	R	NA	ND	+++
	10	21 F	3 d	L	NA	ND	++
	11	39 M	8 d	R	NA	ND	+++
Astrocytoma	12	42 F	NA	L	II	+++	+++
	13	38 F	NA	R	III	+++	+++
	14	82 M	NA	L	III–IV	+++	++
	15	64 M	NA	L	II–III	+++	++
	16	29 F	NA	L	II	+++	++
Ganglioglioma	17	52 M	NA	L	II	+++	+++
Metastatic adenocarcinoma	18	55 M	NA	L	NA	+++	–
	19	51 F	NA	R	NA	+++	–

NA: not applicable; ND: not detected; –: no expression, +: mild, ++: moderate, +++: strong

Histopathological examination and immunohistoc-hemistry

Brain tissues were fixed in 4% formaldehyde for 24~48 h and then embedded in paraffin. Six μm sections were cut and stained with hematoxylin and eosin (HE). Adjacent sections were incubated with a primary antibody against AQP4 (1:300) for 1 d at 4 °C. Sections were sequentially incubated with anti-goat IgG biotynylated secondary antibodies and avidin biotin complex. Sections were finally visualized with 0.01% diaminobenzidine tetrahydrochloride (DAB) and 0.005% H_2O_2 in 50 mmol/L Tris-HCl, pH 7.6. To test the specificity of the immunohistochemical reaction, control sections were treated with normal goat serum instead of the primary antibody. Nuclei were counterstained by hematoxylin.

RESULTS

AQP4 expression in non-edematous brain

No edema was found around the benign meningioma by using MRI (Fig.1a), and HE staining showed normal structure (Fig.1b). The brain specimen from the patient with hemorrhagic stroke in an early stage was similar (data not shown). AQP4 immunoreactivity (brown deposit) was detected over cell processes surrounding microvessels in these brain samples (Fig.2a and Nos. 1 and 2 in Table 1).

AQP4 expression in traumatic brain injury

Brain edema was found in the area surrounding traumatic brain injury by using CT (Fig.1c). HE

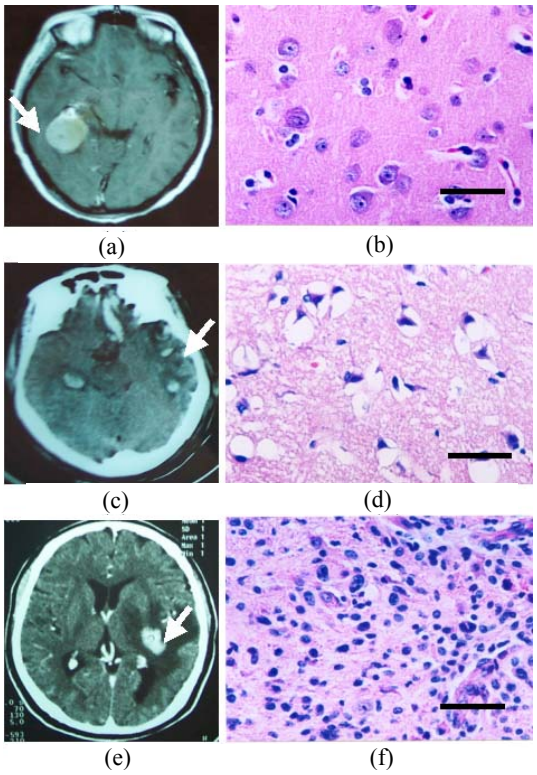

Fig.1 MRI or CT images and photomicrographs of representative samples. For a patient with benign meningioma (No. 1 in Table 1), MRI shows no brain edema around the tumor (a) and HE staining is normal adjacent to the tumor (b). For a patient with traumatic brain injury (No. 6 in Table 1), CT shows lesions that include edema (c) and HE staining demonstrates tissue injury (d). For a patient with astrocytoma (No. 13 in Table 1), MRI image (e) and HE staining (f) show the pathological changes of the tumor. The white arrows in (a), (c) and (e) indicate the analyzed using HE staining. Bars=50 μm

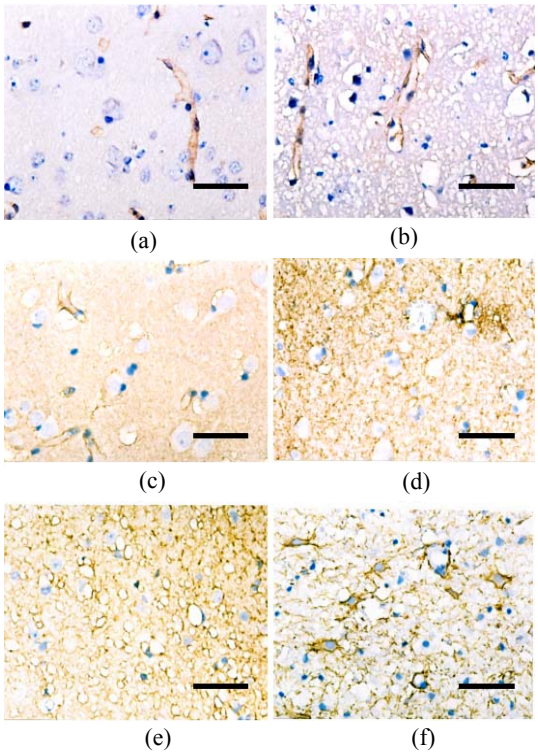

Fig.2 Photomicrographs of AQP4 immunostaining in normal and traumatic brain tissues obtained from the cerebral cortex of a patient with benign meningioma (No. 1 in (a)) and five patients with traumatic brain injury (No. 3 in (b), No. 6 in (c), No. 7 in (d), No. 9 in (e) and No. 11 in (f)). AQP4 is expressed around the microvessels in cerebral tissue (a) suggested to be normal by MRI and HE staining (Fig.1). Six hours after brain injury, AQP4 expression does not change (b); 15 h after brain injury, expression increases (c); 24 h after injury, it peaks (d). Up to 3 (e) and 8 d (f) after injury, AQP4 is still expressed at higher levels. Bars=50 μm

staining suggested that neurons and glial were shrunken 15 h after traumatic brain injury (Fig.1d). AQP4 expression showed no apparent change at the injury site 6~14 h after brain injury (Fig.2b and Table 1), but it was increased from 15 h and even more 8 d after brain injury (Figs.2c–2f and Table 1).

AQP4 expression in brain tumors

Brain edema was present around the tumors according to MRI (Fig.1e), and HE staining showed high density of abnormal cells within astrocytomas (Fig.1f). AQP4 expression in the brain tumors varied according to the kind of tumor. In astrocytomas and gangliogliomas, AQP4 immunoreactivity was increased both in the center of the tumors and adjacent areas (Figs.3a–3d and Table 1). However, in metastatic adenocarcinoma, AQP4 immunoreactivity increased robustly around the tumor, but was not detected in the center of the tumor (Figs.3e–3g and Table 1).

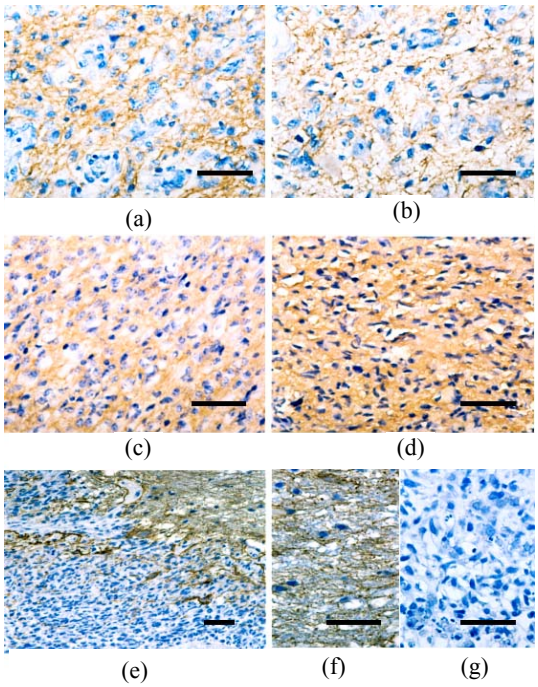

Fig.3 Photomicrographs of AQP4 immunostaining in brains from patients with various brain tumors. The tissues were obtained from the cerebral cortex of three brain tumor patients (No. 13 in (a) and (b), No. 17 in (c) and (d), No. 18 in (e), (f) and (g)). AQP4 is highly expressed in central (a) and adjacent (b) regions of astrocytoma, ganglioglioma (c, d). In metastatic adenocarcinoma, AQP4 is highly expressed in the area adjacent to the tumor (e, f) but not in the center of the tumor (e, g). Bars=50 μm

DISCUSSION

The main finding of this study was that AQP4 expression increased after traumatic brain injury, in peritumoral brain tissue and in the centers of tumors derived from brain tissue. In normal brain tissue, AQP4 was found around microvessels. This is consistent with its location in rat brain, in which AQP4 was detected on astrocyte foot processes adjacent to endothelial cells, and the basolateral membrane of ependymal cells by immunohistochemistry and immunoelectromicroscopy (Nielsen *et al.*, 1997; Rash *et al.*, 1998). Our finding demonstrate the characteristics of AQP4 expression in human brains, where AQP4 is expressed exclusively in astrocytes around vessels (Aoki *et al.*, 2003; Taniguchi *et al.*, 2000).

We found that AQP4 expression gradually increased in human brains after traumatic brain injury, with some increase apparent 15 h after brain injury and an even greater increase 8 d after brain injury. This was consistent with that in a rat model in which AQP4 mRNA expression did not change at 1 or 4 h; but significantly increased at the site of injury and decreased in the area adjacent to the injury 24 h after traumatic brain injury (Sun *et al.*, 2003). As some injury properties of brain trauma and brain ischemia are similar (Leker and Shohami, 2002), it is interesting to compare the two. In a rat brain ischemia model, AQP4 mRNA expression increased in the peri-infarcted cortex during the observation period (1~7 d, maximal on 3rd day) after middle cerebral artery occlusion, with the change being related to the generation and resolution of brain edema (Taniguchi *et al.*, 2000). Thus, it is possible that the increased expression of AQP4 at the injured site in traumatic brain injury might be involved in brain edema formation. Because only a small number samples were used and quantitative analysis was not performed in this study, the time dependence of AQP4 expression after traumatic brain injury remains unclear. In future studies it would be valuable to determine whether time dependent changes in AQP4 expression varies according to the location and severity of injury.

We found that AQP4 expression in brain tumors increased but that pattern of increased expression was different depending on the kind of tumor. Expression of AQP4 in astrocytoma and ganglioglioma increased obviously at their centers and adjacent areas of the

tumors. But in metastatic adenocarcinoma, increased AQP4 expression was seen only in areas adjacent to the tumor, and not in the center. This extends results in which AQP4 expression was simply described to be increased in metastatic adenocarcinoma (Saadoun *et al.*, 2002). Because AQP4 is expressed in astrocytes, the absence of AQP4 in the center of metastatic adenocarcinoma derived from lungs may be due to the lack of astrocytes. Since most brain tumors exhibit high vascular permeability and peritumoral edema (Verkman, 2002), and AQP4 is believed to participate in the transport of water in brain tumors (Saadoun *et al.*, 2002), the increased AQP4 expression within or around brain tumors might result in edema of malignant brain tumors.

In summary, our study showed that AQP4 expression increases in human traumatic brain injury, and around and within tumors derived from brain. Since AQP4 may be responsible for the brain edema in traumatic brain injury and peritumoral edema, AQP4 inhibition might be a new therapeutic strategy for the treatment of brain edema.

ACKNOWLEDGEMENTS

We thank Prof. Chen Pei-Hui, Department of Pathology, Second Affiliated Hospital, School of Medicine, Zhejiang University, for his help in the analysis of brain sections. We also thank Dr. Steven A. Thomas at the Department of Pharmacology, University of Pennsylvania for critically reading and commenting on this manuscript.

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