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Full-length article

Distribution of cysteinyl leukotriene receptor 2 in human traumatic brain injury and brain tumors[1]

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Key words

cysteinyl leukotriene receptor 2; brain injuries; brain neoplasms; vascular smooth muscle cell

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Abstract

Aim: To determine the distribution of cysteinyl leukotriene receptor 2 ($CysLT_2$), one of the cysteinyl leukotriene receptors, in human brains with traumatic injury and tumors. **Methods:** Brain specimens were obtained from patients who underwent brain surgery. $CysLT_2$ in brain tissues was examined using immunohistochemical analysis. **Results:** $CysLT_2$ was expressed in the smooth muscle cells (not in the endothelial cells) of arteries and veins. $CysLT_2$ was also expressed in the granulocytes in both vessels and in the brain parenchyma. In addition, $CysLT_2$ was detected in neuron- and glial-appearing cells in either the late stages of traumatic injury or in the area surrounding the tumors. Microvessels regenerated 8 d after trauma and $CysLT_2$ expression was recorded in their endothelial cells. **Conclusion:** $CysLT_2$ is distributed in vascular smooth muscle cells and granulocytes, and brain trauma and tumor can induce its expression in vascular endothelial cells and in a number of other cells.

Introduction

Cysteinyl leukotrienes (CysLTs), including LTC_4, LTD_4 and LTE_4, are potent inflammatory mediators. In peripheral inflammatory diseases such as asthma and rhinitis, CysLTs can induce smooth muscle constriction, microvascular leakage, eosinophilic recruitment and other responses[1–3]. In the central nervous system (CNS), the level of CysLTs increases after brain injuries such as cerebral ischemia, brain trauma and tumors[4–6]. The increase in CysLTs after traumatic brain injury peaks at 4 h and again at 7 d, and is related to edema and cellular inflammatory responses in the rat brain[5]. In addition, the increase in CysLTs in metastatic tumors and gliomas is considered to be a factor promoting peritumoural edema[4,7].

The cloned CysLT receptors include $CysLT_1$ and $CysLT_2$, both of which are classic G protein-coupled receptors with seven transmembrane domains[8,9]. Human $CysLT_2$ is highly expressed in the spleen, placenta, heart, and peripheral blood leukocytes, and weakly expressed in the brain, prostate, skeletal muscle, kidney and ovary[8–10]. Using a ribonuclease protection assay, the highest expression of murine $CysLT_2$ was detected in the spleen, adrenal gland and thymus, and weaker expression was recorded in the kidney, brain and peripheral blood leukocytes[11]. In human and murine brains $CysLT_2$ is expressed much more than $CysLT_1$ using Northern blot and RT-PCR[11,12]. However, the distribution of $CysLT_2$ in the brains of animals including humans is still unknown.

Recently, we examined $CysLT_1$ expression in human brain specimens from patients with traumatic brain injury and brain tumors[13]. We found that $CysLT_1$ is mainly distributed in the vascular endothelium, which is consistent with the inhibiting effects of $CysLT_1$ antagonists on plasma extravasation and brain edema in the brains of focal cerebral ischemic rats[14]. In the present study, we examined the distribution of $CysLT_2$ in human brains after traumatic injury and in brains with tumors.

Materials and methods

Human brain specimens This study was approved by the ethics committee of the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China. Brain specimens were obtained from 24 patients who underwent brain surgery because of traumatic brain injury, brain tumors, or benign meningioma (Table 1). The diagnosis of each patient with brain tumor was based on criteria pertaining to the clinical MRI picture, appropriate laboratory data and biopsy findings. Astrocytomas and gangliogliomas were classified as low (grades I–II) or high (grades III–IV) grade according to the Daumas-Duport criteria.

Immunohistochemistry Brain tissues were fixed in 4% formaldehyde for 24–48 h, and then embedded in paraffin. A 6 μm thick paraffin section was stained with hematoxylin and eosin (HE). Another section was incubated with a primary polyclonal antibody against $CysLT_2$ for 1 d at 4 °C (5 mg/L, rabbit IgG, Cayman, USA). This section was sequentially treated with anti-rabbit IgG biotinylated secondary antibody and avidin biotin complex (Zymed, USA). Finally, the section was visualized with 0.01% diaminobenzidine tetrahydrochloride (DAB) and 0.005% H_2O_2 in 50 mmol/L Tris-HCl, pH 7.6. Control sections were treated with normal goat serum instead of the primary antibody to test the specificity of the immunohistochemical reaction. Nuclei of cells were counter stained using hematoxylin.

Results

The control sections treated with normal goat serum showed no positive immunostaining (data not shown). $CysLT_2$ was highly expressed in the smooth muscle cells of both arteries and veins, but not in the endothelial cells (Figure 1A,1B). Brain tissues surrounding benign meningioma appeared relatively normal using MRI and HE staining (data not shown). In such relatively normal brain tissues, $CysLT_2$ was not detected in microvascular endothelial cells or in other cells (Figure 1C, Table 1).

$CysLT_2$ expression in human brains after traumatic injury Within 3 d after traumatic brain injury, mild expression of $CysLT_2$ was frequently detected in the neuron- and glial-appearing cells in a number of human brain specimens (Figure 2A, Table 1), but rarely in the microvascular endothelial cells (Figure 2B, Table 1). However, 8 d after trauma, microvascular regeneration was observed and $CysLT_2$ was highly expressed in the regenerated microvascular endothelial cells and glial-appearing cells (Figure 2C, Table 1), but no $CysLT_2$ positive cell was found in the necrotic regions (Figure 2D). In addition, in one patient, MPO-positive granulocytes were found within vessels and in the brain parenchyma (data not shown), and $CysLT_2$ was highly expressed in the granulocytes both within vessels (Figure 2E) and in the brain parenchyma (Figure 2F).

$CysLT_2$ expression in human brain tumors In brain tumors, no $CysLT_2$ was detected in the center of glioma, ganglioglioma and metastatic carcinomas (Figure 3A,3B, Table 1), but strong $CysLT_2$ immunostaining was found in neuron- and glial-apprearing cells surrounding tumors (Figure 3C, Table 1).

Discussion

In this study, the first finding is the specific distribution pattern of $CysLT_2$ in brain vessels. $CysLT_2$ was highly ex-

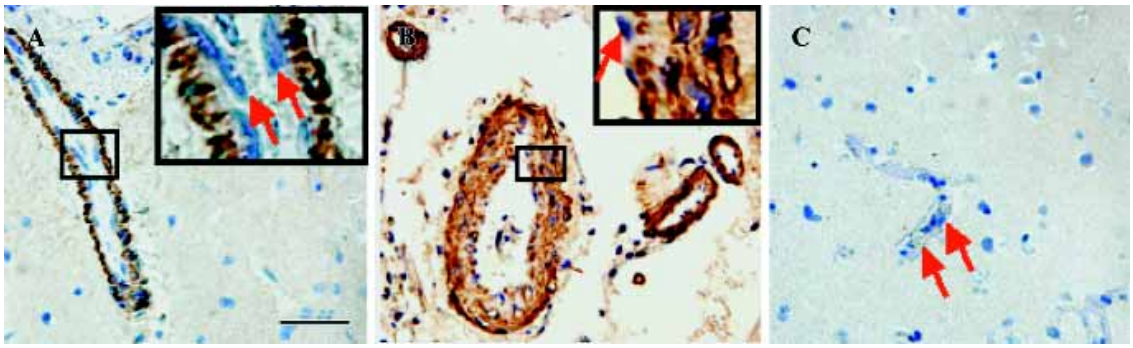

Figure 1. $CysLT_2$ expression in the vessels of the human brain. Brain samples were obtained from two patients with astrocytoma (Patient numbers 21 and 22 from Table 1 in A and B, respectively) and one patient with benign meningioma (Patient number 1 from Table 1 in C). $CysLT_2$ expression was detected using immunohistochemistry. $CysLT_2$ immunoreactivity (brown) was found in the smooth muscle cells of veins (A) and arteries (B), but not in endothelial cells (red arrows), microvascular endothelial cells (C, red arrows) or other cells within the brain tissues. The inserts are amplifications from the black boxes in A and B. Scale bar is 50 μm.

Table 1. Patient profiles and the immunohistochemical results for $CysLT_2$. NA, not applicable; -, absent; ±, weak or few; +, mild; ++, marked; ?, not detected; L, left; R, right; T, temporal lobe; F, frontal lobe; P, parietal lobe. Samples were from the tissues surrounding (*) and within (**) the injured regions or the tumors; NAC, neuron-appearing cells; GAC, glial-appearing cells; VEC, microvascular endothelial cells.

	Patient number	Age/sex	Duration from onset	Side/ lobes	Grade	Adjacent to lesions*			Center of lesions**		
						NAC	GAC	VEC	NAC	GAC	VEC
Control samples											
Beside benign meningioma	1	51/F	NA	L/T	NA	-	-	-	NA	NA	NA
Traumatic brain injury	2	55/M	6 h	R/T	NA	NA	NA	NA	-	-	-
	3	15/F	9h	L/T	NA	NA	NA	NA	-	-	-
	3	51/M	9 h	L/T	NA	NA	NA	NA	±	±	-
	4	26/F	9h	R/F	NA	NA	NA	NA	-	-	-
	5	30/M	14 h	R/P	NA	NA	NA	NA	±	±	-
	6	52/M	15 h	R/T	NA	NA	NA	NA	-	-	±
	7	49/M	20 h	R/L/F	NA	NA	NA	NA	-	-	-
	8	52/M	24 h	R/L/F	NA	NA	NA	NA	-	-	±
	9	21/M	24 h	R/L/F	NA	NA	NA	NA	±	±	-
	10	41/M	24 h	R/F	NA	NA	NA	NA	-	-	-
	11	51/M	24 h	R/F	NA	NA	NA	NA	-	-	-
	12	57/F	30 h	L/F	NA	NA	NA	NA	±	±	-
	13	71/M	2.5 d	R/T	NA	NA	NA	NA	±	±	-
	14	21/F	3 d	L/T	NA	NA	NA	NA	±	±	-
	15	39/M	8 d	R/T	NA	NA	NA	NA	?	++	++
Astrocytoma	16	42/F	NA	L/P	II	++	++	?	?	-	-
	17	38/F	NA	R/T	III	++	++	?	?	-	-
	18	82/M	NA	L/T	III	++	++	?	?	-	-
	19	64/M	NA	L/P	II	++	++	?	?	-	-
	20	29/F	NA	L/F	II	++	++	?	?	-	-
	21	38/M	5 y	L/T	II	++	++	?	?	-	-
	22	71/M	2 y	L/T	II	++	++	?	?	-	-
Ganglioglioma	23	52/M	NA	L/T	II	++	++	?	?	-	-
Metastatic carcinoma	24	55/M	NA	L/T	NA	++	++	?	-	-	-

pressed in the smooth muscle cells of arteries and veins, but rarely in vascular and microvascular endothelial cells in relatively normal brain tissues or in tissues within 3 d of brain trauma. This pattern is different from that of $CysLT_1$, which is primarily expressed in endothelial cells as found in our previous study[13]. However, strong expression of $CysLT_2$ was found in the regenerated microvascular endothelial cells 8 d after trauma, suggesting an inducible $CysLT_2$ expression. This result is similar to that of the human heart, in which $CysLT_2$ mRNA has been detected in myocytes, fibroblasts and vascular smooth muscle cells, but not in endothelial cells[15]. Moreover, $CysLT_2$ has been reported to be expressed primarily in human umbilical vein endothelial cells (HUVECs)[16], and may play a role in inflammation during atherogenesis or leukocyte infiltration into tissues[17]. A recent study using $CysLT_2$-deficient mice confirmed that $CysLT_2$ mediates an increase in vascular permeability in IgE-dependent passive cutaneous anaphylaxis[18]. Our present results showed that unlike peripheral tissues (HUVECs), $CysLT_2$ was only expressed in the endothelial cells of injured brain tissues after a longer duration. This result implies that $CysLT_2$ might be involved in inflammatory responses in the CNS.

The second finding of this study is the inducible $CysLT_2$ expression in neuron- and glial-appearing cells after brain trauma and in brain tumors. We have recently reported inducible $CysLT_1$ expression in neuron- and glial-appearing

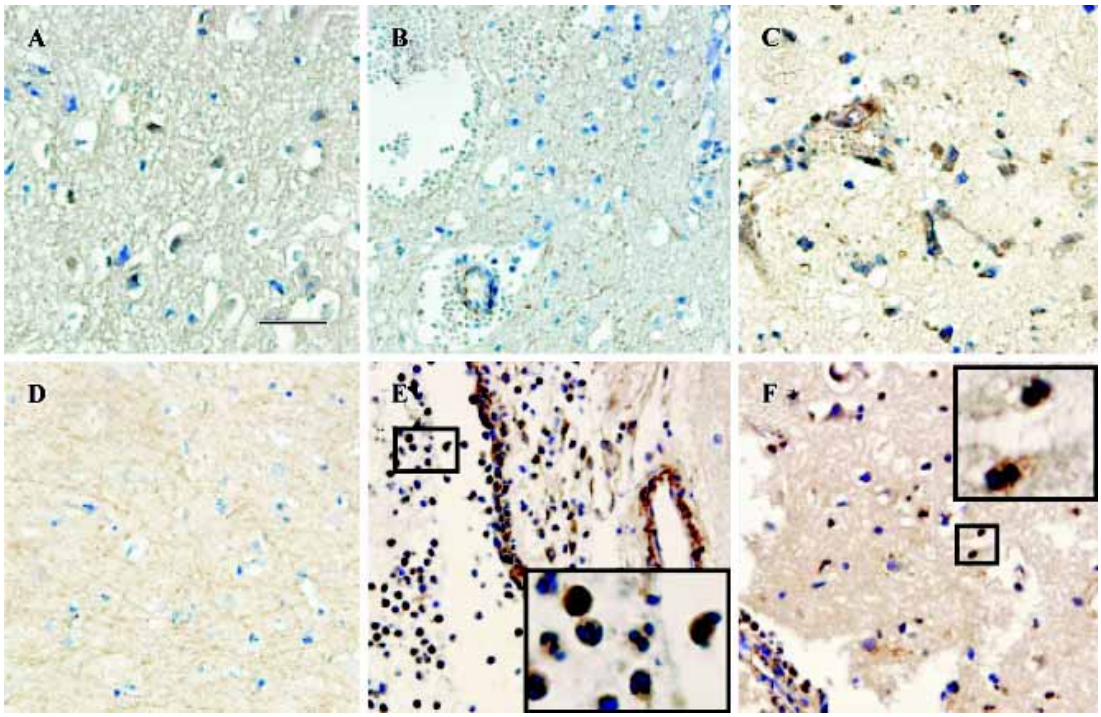

Figure 2. CysLT2 expression in human brains after traumatic brain injury. Brain samples were from patients 14 h (A; patient number 5), 15 h (B, E, F; patient number 6), or 8 d (C, D; patient number 15) after traumatic brain injury. At 14 or 15 h after injury, mild expression of CysLT2 were found in neuron- and glial-appearing cells (A), and in microvascular endothelial cells (B). At 8 d after injury, microvessels had regenerated, and CysLT2 was highly expressed in the microvascular endothelial cells, glial-appearing cells and granulocytes (C), but not in the necrotic region (D). CysLT2 was also highly expressed in granulocytes both within vessels (E) and within brain tissues (F). The inserts are amplifications from the black boxes in E and F. Scale bar is 50 μm.

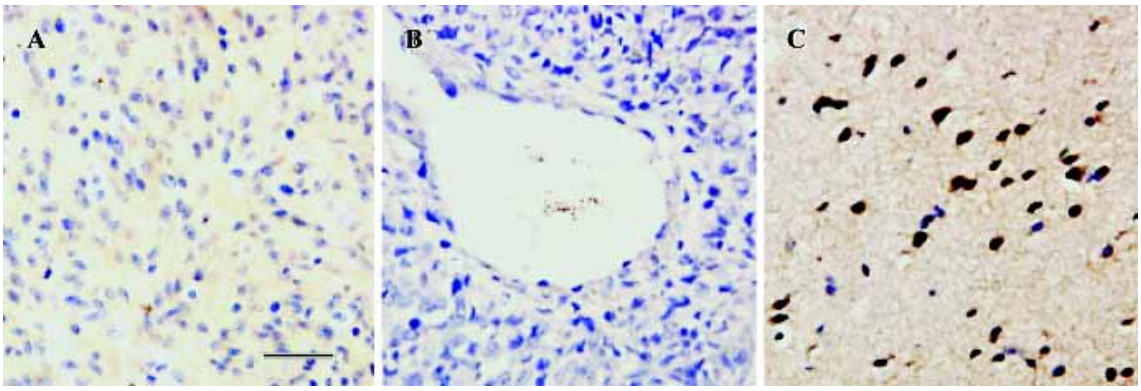

Figure 3. $CysLT_2$ expression in human brains with tumors. Samples were obtained from patients with astrocytoma (A, C; patient number 19) or metastatic carcinoma (B; patient number 24). $CysLT_2$ was not found within astrocytomas (A) or metastatic carcinomas (B), but was highly expressed in neuron- and glial-appearing cells surrounding the astrocytoma (C). Scale bar is 50 μm.

cells after traumatic brain injury and brain tumors[13]. Unlike $CysLT_1$, the inducible $CysLT_2$ expression in these cells was much reduced within 3 d after trauma, but was strong 8 d after trauma and surrounding the tumors. The pathophysiological implications of $CysLT_2$ in the brain are unknown, but $CysLT_2$ may be involved in intracerebral cell proliferation because CysLTs can promote astrocyte and intestinal epithelial cell proliferation[19,20]. Recently it has been reported that bleomycin-induced pulmonary fibrosis is increased in $CysLT_1$-deficient mice, but decreased in $CysLT_2$-deficient-

mice [18,21]. Therefore, the inducible $CysLT_2$ expression 8 d after trauma might be responsible for cell regeneration and/or proliferation.

In addition, $CysLT_2$ was highly expressed in the granulocytes in both vessels and brain tissues. This result is consistent with previous reports that show that $CysLT_2$ is expressed in peripheral blood leukocytes[10] and is responsible for chemotaxis[22]. However, according to the results of guinea pig brain perfusion with human neurtrophils, Di Gennaro *et al*[23] hypothesized that $CysLT_1$ in leukocytes and $CysLT_2$ in endothelial cells might be involved in the adherence and intrusion of leukocytes in brain inflammatory reactions. However, our results examining the distributions of $CysLT_1$ and $CysLT_2$ in human brains do not support their hypothesis.

In summary, we found that $CysLT_2$ was expressed in smooth muscle cells and granulocytes, suggesting that $CysLT_2$ might play a role in cerebral circulation and the inflammatory response that occurs in human brains after injury. The induced $CysLT_2$ expression in microvascular endothelial cells, and neuron- or glial-appearing cells after traumatic injury and in brain tumors requires further investigation.

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