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Immunohistochemical expression of glutathione S-transferase placental type (GST-π), a detoxifying enzyme, in normal arachnoid villi and meningiomas

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Summary. Human normal dura mater containing arachnoid villi were examined for their expression of glutathione S-transferase placental type (GST-π), a detoxifying enzyme, using an immunohistochemical method. All of the arachnoid villi and arachnoid cells in five normal cases were found to have expression of GST-π, although no positive reaction for the enzyme was present in other tissues of the dura mater. The results show a possible role for arachnoid tissues in protecting human brain from hazardous xenobiotics in the cerebrospinal fluid. Twenty-six meningiomas were also examined for expression of the enzyme. Tissues of meningotheliomatous meningiomas were always positive for expression of the enzyme. Transitional meningiomas also showed the expression in their meningotheliomatous components. No staining reaction of GST-π was recognized in fibroblastic meningiomas except for two cases with a tendency to meningotheliomatous differentiation. The findings suggest a functional similarity between the arachnoid tissues and meningotheliomatous components of meningiomas.

Key words: Arachnoid villi – Meningioma – Glutathione S-transferase placental type – Cerebrospinal fluid

Introduction

Arachnoid villi are known to function as part of the circulatory mechanism of the cerebrospinal fluid. They protrude into the walls of the dural vein and sinuses and secrete cerebrospinal fluid into vessels. However, it is not known if they have a detoxifying function. Glutathione S-transferases (GSTs) are a family of detoxifying enzymes that protect organs or tissues from the toxic effect of xenobiotics, catalyzing the conjugation of a wide variety of hydrophobic electrophilic substances with glutathione. A variety of isozymes of GST of rats has been studied extensively (Kitahara and Sato 1981; Mannervik and Jensson 1982; Hayes 1983; Kitahara et al. 1983). The placental form of GST (GST-P) has been used as a marker for preneoplastic or neoplastic lesions in chemical hepatocarcinogenesis in rats, since normal hepatocytes of rats lack the enzyme activity (Satoh 1985; Tatematsu et al. 1985, 1988; Sato 1988). The human placental form of GST (GST-π), which is closely related to GST-P immunologically, has also been recognized as a useful marker for preneoplastic or neoplastic lesions of colon (Kodate et al. 1986), uterine cervix (Shiratori et al. 1987) and lung (Di Ilio et al. 1988). However, the enzyme is also present normally in some organs, including the kidney, pancreas and lung (Tateoka et al. 1987).

This study has been conducted to examine the expression of the detoxifying enzyme, GST-π in normal arachnoid villi. Tissues from meningiomas were also investigated.

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Materials and methods

Five normal dura mater and 26 cases of human meningiomas were used. Normal dura mater was obtained from autopsy cases without meningeal abnormalities. All specimens were taken from the midportion of the superior sagittal sinus. The tissues were fixed in 10% formalin and routinely embedded in paraffin. Tissues from meningiomas were obtained by surgical resection (Table 1). The meningiomas were composed of four types; meningotheliomatous (7 cases), fibroblastic (6 cases), transitional (10 cases) and angiomatous types (3 cases). Twenty-two of the tumors were fixed in 10% formalin and routinely embedded in paraffin. Four meningiomas were fixed in cold acetone for 40 min and also embedded in paraffin. Three-micron-thick sections were cut and used for haematoxylin and eosin staining and for immunohistochemical studies.

For immunohistochemistry, anti-human GST-π antibody (rabbit immunoglobulin) was purchased from Bioprep Co. (Dublin, Ireland). GST-π used to obtain the antiserum was purified from human placenta by affinity column. The antibody cross-reacts with mouse and rat GST placental form, but does not cross-react with α- or μ-classes of enzyme (data from the manufacturer). For the

Table 1. Meningiomas used in the immunohistochemical analysis

Patient no.	Sex	Age	Histologic type	Location
1	m	55	M	r-sphenoid ridge
2	f	33	M	l-parietal convexity
3	f	67	M	l-frontal convexity
4	f	50	M	l-frontal base
5	f	40	M	l-parietal convexity
6	f	62	M	l-sphenoid ridge
7	f	61	M	l-parietal convexity
8	m	49	T	r-parietal convexity
9	m	55	T	l-frontal convexity
10	f	53	T	l-sphenoid ridge
11	f	72	T	r-parietal convexity
12	f	57	T	l-falx
13	f	62	T	l-falx
14	f	32	T	l-parietal convexity
15	f	45	T	suprasellar
16	f	57	T	l-frontal convexity
17	f	70	T	r-frontal convexity
18	m	57	F	l-parasagittal
19	m	48	F	r-olfactory groove
20	f	69	F	l-cerebellar tent
21	f	64	F	l-cerebellar tent
22	f	36	F	r-parietal convexity
23	f	62	F	r-cerebellopontine angle
24	m	70	A	r-frontal convexity
25	f	54	A	l-sphenoid ridge
26	f	40	A	r-frontal convexity

m: male; f: female; M: meningotheliomatous; T: transitional; F: fibroblastic; A: angiomatous

Table 2. Staining reaction of GST-π in the normal dura mater and meningiomas

Tissue types	No. of cases	No. of cases with positive or negative staining responses		
		++	+	−
Normal dura mater	5	5		
Meningiomas:				
M	7	7		
T	10	9	1	
F	6		2	4
A	3	3		

M: meningotheliomatous; T: transitional; F: fibroblastic; A: angiomatous; ++ = positive; + = weakly positive; − = negative

immunohistochemical analysis, the regular peroxidase-antiperoxidase (PAP) technique was adopted (Sternberger et al. 1970). To diminish nonspecific staining, sections were treated with methanol containing 0.3% hydrogen peroxide for 30 min. After washing in phosphate-buffered saline solution (PBS), each section was treated at room temperature sequentially with normal swine serum for 20 min, rabbit anti-GST-π antibody (1:100) for 40 min, swine anti-rabbit antibody (Dako, Santa Barbara, Calif) for 30 min, and PAP (Dako) for 30 min. The sections were carefully rinsed in PBS after each step. The peroxidase binding sites were detected by the staining with a solution of 3-amino-9-ethylcarbazole. Counterstaining was performed using Mayer's haematoxylin. Human full-term placenta was used as a positive control for immunohistochemistry. Negative control staining was obtained after absorption of immune serum with purified GST-π (Bioprep Co., Dublin, Ireland) and after replacement of the primary antibody with non-immunized rabbit serum.

Results

Positive immunoreactivity for GST-π was graded semiquantitatively as follows: negative (−), weakly positive (+), and positive (++). In the normal dura mater, arachnoid villi protruded into the walls of the superior sagittal sinuses in berrylike shapes, commonly showing positive staining response for GST-π (Fig. 1a, b). The staining reaction of these arachnoid villi was uniform. In general, the cytoplasms of the arachnoid cells were diffusely stained, although cell nuclei lacked enzyme expression. Other structures in the dura mater had perfectly negative staining response.

The results of the immunohistochemical survey of the meningiomas are summarized in Table 2. Seven meningotheliomatous meningiomas showed positive staining reaction for GST-π. The enzyme expression was recognized diffusely in the cytoplasm (Fig. 2a). In fibroblastic meningiomas, four had negative reaction (Fig. 2b); however, two meningiomas of this type showed a partially positive response. In these cases only the tumor cells with relatively abundant cytoplasm suggesting meningotheliomatous differentiation exhibited a weakly positive staining response in their cytoplasm. All of the transitional meningiomas had the meningotheliomatous components positively stained (Fig. 2c, d). The fibroblastic components were negative. The staining reaction of meningotheliomatous components of the transitional meningiomas was similar to that of normal arachnoid villi with enzyme expression in the cytoplasm. Three angiomatous meningiomas showed positive staining reaction for GST-π in their meningotheliomatous components.

In this study, immunohistochemical analysis was done with tissues fixed in formalin as well as cold acetone. However, the difference in fixation did not give rise to an obvious change in the staining response.

Discussion

Russell and Rubinstein (1989) have described some of the biological functions of arachnoid villi or arachnoid cells. In particular, arachnoid villi protrude into the walls of the dural veins and sinuses and secrete the cerebrospinal fluid. Arachnoid cells also have a phagocytic role against foreign particles.

A possible detoxifying function for arachnoid tissues has not been investigated. One of the detoxifying enzymes GSTs, which catalyze the conjugation of various hydrophobic electrophilic substances with glutathione (Jakoby 1978; Chasseaud 1979; Mannervik 1985), was shown to be present in arachnoid villi under normal conditions. The results suggest that the arachnoid tissues may have a role in detoxifying the cerebrospinal fluid when contaminated by some toxic agents.

This study also demonstrated the expression of GST-

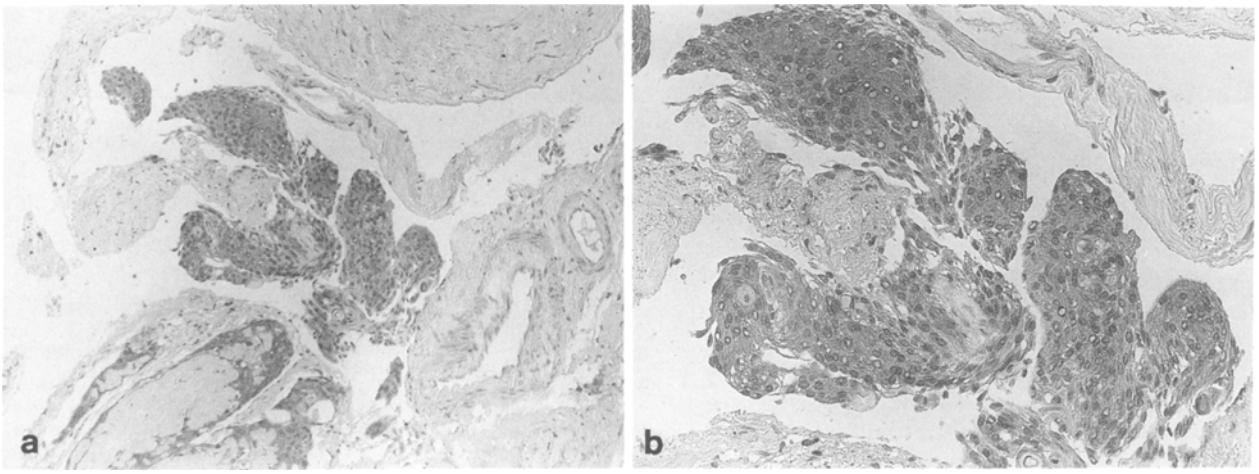

Fig. 1 a, b. Normal arachnoid villi protruding into the walls of the superior sagittal sinus show positive immunoreaction for glutathione S-transferase placental type (GST-π). Other structures of the dura mater had a perfectly negative response; anti-GST-π and counterstaining with Mayer's haematoxylin. (**a** $\times 75$; **b** $\times 150$)

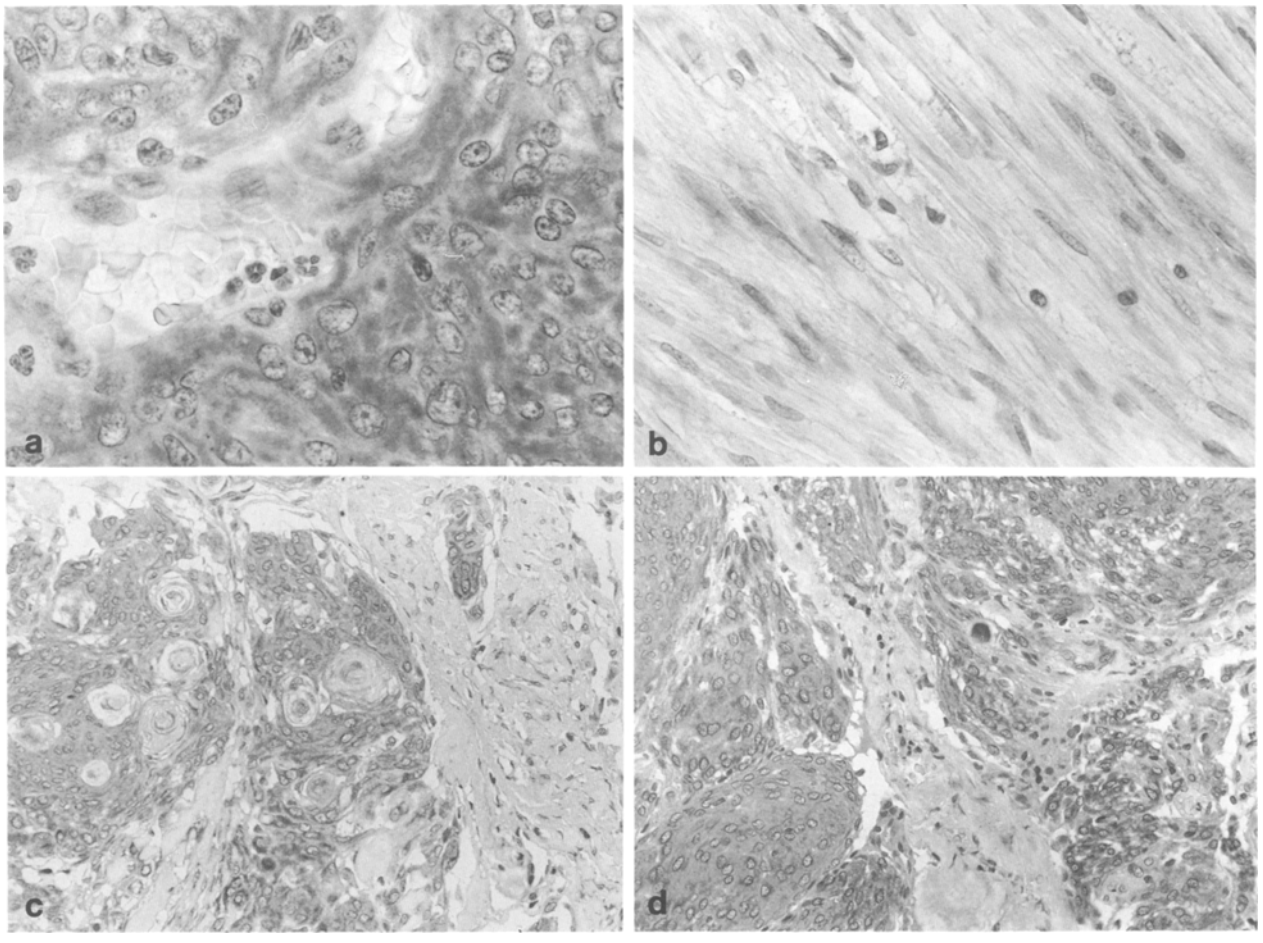

Fig. 2. a Meningotheliomatous meningioma (case 2) shows positive staining reaction for GST-π diffusely in the cytoplasm. The enzyme is not present in endothelium and erythrocytes; anti-GST-π and counterstaining with Mayer's haematoxylin ($\times 300$). **b** Fibroblastic meningioma (case 21) shows negative immunoreaction for GST-π; anti-GST-π and counterstaining with Mayer's haematoxylin ($\times 300$). **c** Transitional meningioma (case 9) shows positive GST-π response in its meningotheliomatous component and negative response in its fibroblastic region; anti-GST-π and counterstaining with Mayer's haematoxylin ($\times 150$). **d** Another transitional meningioma (case 10) also shows positive immunoreaction in its meningotheliomatous component; anti-GST-π and counterstaining with Mayer's haematoxylin ($\times 150$)

π in some meningiomas. Meningiomas are derived from arachnoid villi (Russell and Rubinstein 1989) and are classified into several types. Meningotheliomatous, fibroblastic and transitional types are frequent, and the transitional type is comprised of both meningotheliomatous and fibroblastic components. In the present immunohistochemical analysis, the meningotheliomatous components of the meningotheliomatous meningiomas and the transitional meningiomas, resembling the structure of normal arachnoid villi, were positive for GST-π, whereas fibroblastic components of the fibroblastic meningiomas and transitional meningiomas were negative except for two cases. These cases, which had relatively enriched cytoplasm indicating a tendency to meningotheliomatous differentiation, showed partially positive expression of the enzyme. The expression of GST-π in the normal arachnoid villi and meningotheliomatous components of meningioma thus confirms a relationship between the two elements.

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