Radioimmunoassay of Astroprotein (an Astrocyte-Specific Cerebroprotein) in Cerebrospinal Fluid From Patients with Glioma. A Preliminary Study

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Summary

Astroprotein, an astrocyte-specific cerebroprotein, in cerebrospinal fluid (CSF) and cyst fluid of tumors from 20 patients with brain tumors as well as other diseases has been measured by radioimmunoassay, and the following results were obtained:

- 1) Remarkably great amounts of astroprotein were detected in both CSF and cyst fluid of tumor from patients with glioblastoma.
- 2) Astroprotein in CSF from patients with brain tumors originated from other than astrocyte was found increased when the tumor was huge and was accompanied with severe fibrous gliosis.
- 3) Astroprotein in CSF from patients with other diseases than brain tumors was found generally minimal.

Key Words; Astroprotein (an astrocyte-specific cerebroprotein), Brain tumors, Immunological diagnosis, Cerebrospinal fluid, Radioimmunoassay

Introduction

Bogoch (1966) reported remarkable increase of an acidic cerebroprotein fraction, group 10B, in the brains of Tay-Sachs' disease as well as in glioma.²⁾ With the immunofluorescence study of human brain tumors, Mori, Mogami, Benda and Sweet (1968) and Benda, Mori and Sweet (1970) revealed subsequently that an unknown particular cerebroprotein of this group 10B is localized specifically in glial cells.^{5,1)} Eventually Mori (1970) proved this particular cerebroprotein to be astrocyte-specific and designated it as "astroprotein".^{6,7)} The purpose of the present study is to detect astroprotein in CSF by radioimmunoassay, attempting to clarify the diagnostic value of astroprotein for brain tumors.

Materials and Methods

1) Preparation of astroprotein

Specimens of human glioblastoma and gemistocytic astrocytoma were obtained at surgery or necropsy, and stored at -60°C until use. Glioma tissues were minced and homogenized with 0.005M phosphate buffer, pH 7.2 and then sonicated at 20 kc/second, 150 watt for 5 minutes. All procedures were carried out at 4°C .

The supernatant was collected by centrifugation at $75,000 \times g$ for 30 minutes. Group 10B cerebroprotein were fractionated with DEAE-Sephadex A25 column chromatography, according to the elution scheme of Bogoch.²⁾ Group 10B cerebroproteins were further fractionated by gel filtrations of Sephadex G75, Sephadex G200 and finally, astroprotein was obtained by preparative acrylamide gel electrophoresis. In order to prevent aggregation of astroprotein in the course of procedures, 0.1% sodium lauroyl sarconate was used in some cases.

2) Preparation of anti-astroprotein

Antisera to astroprotein were produced in rabbits by four successive injections of antigen over the period of 28 days. Freund complete adjuvant was only applied for the first immunization. The rabbit antisera were absorbed with normal human plasma and the anti-astroprotein activities were checked by double gel diffusion.

3) Iodination of astroprotein

15 μg of astroprotein was iodinated with ^{125}I by chloramine T method and the labelled astroprotein with specific activity of 44 $\mu\text{Ci}/\mu\text{g}$ was further fractionated with Sephadex G200 column chromatography. Radioimmunoassay was performed by the double antibody technique.

Table 1. Procedures of radioimmunoassay.

astroprotein	100 μ l
test sample	100 μ l
anti-astroprotein	100 μ l
buffer solution	100 μ l
(4°C, 2 days)	
NRS $\times$ 100	100 μ l
anti-R- γ -G	100 μ l
(4°C, overnight)	
Centrifuge	
Count	

Briefly, 100 μ ml of each hot astroprotein, test sample, anti-astroprotein and buffer solution were mixed and incubated for 48 hours at 4°C. Furthermore, 100 μ ml of each goat antiserum to rabbit γ -G and normal rabbit serum (diluted 100 times) were added and incubated overnight, followed by centrifugation and counting (Table 1.)

4) Test samples (Table 2)

CSFs were obtained from 18 patients with or without brain tumor, and cyst fluid were aspirated from two patients with glioblastoma during surgery. Test samples collected were preserved at -60°C until radioimmunoassay.

Results

A. Iodination of astroprotein

Sephadex G200 column chromatography revealed two or three groups of radioiodinated astroprotein with different molecular weight. However, they were identical to anti-astroprotein antiserum, suggesting that the difference of their molecular weight was due to the aggregation of astroprotein. As a matter of fact, by adding sodium lauroyl sarconate to astroprotein solution, the heterogeneity within the iodinated astroprotein was minimized remarkably (Fig. 1). As a rule, astroprotein with small molecular weight was used as tracer in radioimmunoassay. Fig. 2 shows the standard curve.

B. Astroprotein in CSF and cyst fluid

Table 2 represents astroprotein in CSF and cyst fluid measured by radioimmunoassay. A great amount of astroprotein (over 5,000 ng/ml) in CSF was detected in five cases. Two of them

Fig. 1. Sephadex G200 column chromatography of both 125 I-astroprotein (----) and 125 I-astroprotein with 0.1% sodium lauroyl sarconate (—).

Fig. 2. Standard curve for radioimmunoassay of astroprotein.

were obtained from patients with glioblastoma, while the other three were from patients with ependymoma and metastatic brain tumor from the lung. All these tumors were huge and were accompanied with severe extensive fibrous gliosis. On the contrary, minimal amount of astroprotein (below 10 ng/ml) was generally detected in CSF of the patients with other than brain tumors (meningitis, epilepsy and appendicitis). Two cyst fluid samples of glioblastoma also contained a great amount of astroprotein.

Discussions

Astroprotein is the first particular cerebro-

Table 2. The amount of astroprotein in CSF and cyst fluid measured by radioimmunoassay.

Case	Clinical diagnosis	CSF (ng/ml)
1 T. O.	Glioblastoma	5000 or more
2 Y. O.	Glioblastoma	5000 "
3 S. M.	Glioblastoma	5000* "
4 K. O.	Glioblastoma	5000* "
5 K. S.	Metastatic tumor	5000 "
6 T. I.	Ependymoma	5000 "
7 Y. T.	Chordoma	5000 "
8 A. K.	Craniopharyngioma	230
9 S. H.	Glioblastoma	160
10 T. M.	Pineal tumor	160
11 N. H.	Post fossa meningioma	160
12 Y. K.	Pituitary adenoma	140
13 C. M.	Metastatic tumor	35
14 M. Y.	Medulloblastoma	30
15 K. K.	Olfactory meningioma	25
16 N. I.	Pituitary adenoma	10 or less
17 H. K.	Epilepsy	10 "
18 Y. I.	Ependymoma	10 "
19 S. K.	Meningitis	10 "
20 H. G.	Appendicitis	10 "

*Cyst fluid in glioma.

protein known to be specific to glial cells, as immunofluorescence study on normal brains of human beings as well as of rats and mice has demonstrated clearly that astroprotein is localized in the cytoplasm of fibrous astrocytes.⁵⁻⁷⁾ In brain tumors, astroprotein has been detected at fibrous, gemistocytic and pilocytic astrocytoma cells as well as at reactive astrocytes neighboring to tumor tissues.⁵⁻⁷⁾ Years later than Mori's first paper of astroprotein,⁵⁾ Eng *et al.*⁴⁾ also successfully isolated a protein fraction from brain tissues with severe gliosis and named it as "Glial Fibrillary Acidic Protein" (GFAP), as it was proved to be specific to fibrous (fibrillary) astrocytes by immunofluorescence.³⁾ However, from our studies in gel immunodiffusion, immunofluorescence and chemical analysis, GFAP seems to be identical to astroprotein.⁸⁾

Although it has been evident that protein content of CSF will be increased in cases of brain tumors, no one has ever demonstrated the leakage of such proteins originated from brain tumor into CSF. In the present study, a great amount of astroprotein was detected in CSF from five patients. Two of them were glioblastoma multiforme, while the other three had neither glioblastoma multiforme nor astrocy-

toma, but their intracranial tumors were huge and accompanied with severe and extensive fibrous gliosis, indicating that astroprotein in CSF might increase not only in cases of astrocytoma and glioblastoma multiforme but also in some cases with extensive gliosis. Since astroprotein is not a tumor-specific antigen, results of the present study are reasonably accepted. However, from an other view-point, it is strongly suggested that measurement of astroprotein in CSF might be applicable not only as a screening test for glioma but also as an indicator for the degree and extent as well as prognosis of brain damage.

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References

- 1) Benda, P., Mori, T., and Sweet, W. H.: Demonstration of an astrocyte-specific cerebroprotein by an immunofluorescence study of human brain tumors. *J. Neurosurg.* 33: 281-286, 1970.
- 2) Bogoch, S. and Belval, P.: Brain proteins in the sphingolipidoses: Tay-Sachs' disease protein. In "Inborn Disorders of Sphingolipid Metabolism". Aronson, S. and Volk, B. W. (Eds.), Pergamon Press, New York, 1966, pp. 273-278.
- 3) Bignami, A., Eng, L. F., Dahl, D., and Uyeda, C. T.: Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. *Brain Research.* 43: 429-435, 1972.
- 4) Eng, L. F., Vanderhaeghen, J. J., Bignami, A., and Gerstle, B.: An acidic protein isolated from fibrous astrocyte. *Brain Research.* 28: 351-354, 1971.
- 5) Mori, T., Mogami, H., Benda, P., and Sweet, W. H.: An astrocyte-specific cerebroprotein in normal brain and in human glioma. *Neurologica medico-chirurgica.* 10: 103-104, 1968.
- 6) Mori, T.: Studies on astrocyte specific antigen (astroprotein). *Neurochemistry (Tokyo).* 9 (suppl.) 75-78, 1970.
- 7) Mori, T. and Morimoto, K.: Astroprotein, an astrocyte-specific cerebroprotein, in normal brain and glioma. Fourth International Meeting of the International Society for Neurochemistry., Tokyo, 1973 (Abstracts pp. 174).
- 8) Mori, T. and Morimoto, K.: Studies on the identity of astroprotein (Mori) and Glial Fibrillary Acidic Protein (Eng). *Igaku-no-ayumi.* 92: 16-17, 1975.