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Glycosaminoglycans (GAGs) in Human Cerebral Tumors

Part 1. Biochemical Findings*

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Summary. GAG electrophoretic pattern and concentration have been studied in 38 human cerebral tumors and in specimens of normal nervous tissue. Gray matter had higher total GAG concentration and higher CA to HA ratio than white matter. In both, DS was present in very small amount whereas HS represented 15% of GAGs. All benign gliomas displayed the same electrophoretic pattern and contained more GAGs than normal nervous tissue. Dermatan sulfate was detected in malignant gliomas, as well as in other oncotypes, due to their mesodermic component. Ependymomas were particularly rich in HS and meningiomas were poor in HA.

Key words: Biochemistry – Cerebral tumors – Electrophoresis – Glycosaminoglycans

Introduction

Glycosaminoglycans (GAGs) are involved in many cell processes such as tissue hydration, cellular adhesion, contact inhibition, antigenicity, other surface related phenomena, etc. (Margolis and Margolis 1979). In tumors they seem to play a very important role (Chiarugi et al. 1978); however, up to now GAGs have been biochemically studied only in animal brain (Margolis and Margolis 1979), in normal human brain (Federico and Di Benedetta 1979; Singh and Bachhawat 1968) and in mucopolysaccharidoses (Dawson 1979); only partial biochemical data are available on cerebral tumors (Anghileri et al. 1977), whereas histochemistry has yielded many interesting results (Engelhardt 1980; Böck and Jellinger 1981).

The results of quantitative and electrophoretic analysis of GAGs in 38 cerebral tumors are presented in this report.

Material and Methods

We have studied 38 human brain tumors and 7 samples of histologically normal white and gray matter, taken from peritumoral areas. Each specimen was subdivided in two parts: the first was processed for histology and histochemistry, the second (wet weight 300–4,500 mg) was frozen for biochemical studies after elimination of necrotic and hemorrhagic areas.

The brain tumors were: 2 fibrillary astrocytomas, 2 protoplasmic astrocytomas, 1 anaplastic astrocytoma, 3 oligodendrogliomas, 3 cerebellar spongioblastomas, 2 glioblastomas, 1 gliosarcoma, 1 neurinoma, 16 meningiomas, 1 choroid plexus carcinoma, 3 metastases and 1 leukemic brain infiltration.

Extraction of GAGs

The specimens were homogenized, digested by papain and deproteinized with trichloroacetic acid. GAGs were precipitated by adding 4 vol of cold ethanol, dialyzed, lyophilized and dissolved in distilled water (Cappelletti et al. 1980).

Quantitation of GAGs

GAGs were quantified by the carbazole method using glucuronelactone as standard (Bitter and Muir 1962). The concentration was expressed as microgram uronic acid per g of wet tissue.

Electrophoresis

Electrophoresis was performed according to the method of Cappelletti et al. (1979a, b) using 1 M Ba acetate buffer, pH 5. Sheets were stained with Alcian Blue 8GX 0.15% for 2 min and destained in 0.05 M Na acetate pH 3.7 for 45 min.

Glycosaminoglycan reference standards [1], extracted and purified by M. B. Mathews and K. J. A. Cifonelli (Dept. of Pediatrics,

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1 The abbreviations used are: GAGs glycosaminoglycans; CC chondroitin-6-sulfate; CA chondroitin-4-sulfate; HA hyaluronic acid; HS heparan sulfate; DS dermatan sulfate; HP heparin; KS keratan sulfate

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Table 1. *GAG* Electrophoresis reproducibility and sensitivity

	CC	CA	HA	HS	DS	HP
Lowest detectable quantity (ng)	8	12	3	20	4	8
Densitometric sensitivity (ng)	50	50	40	300	50	100
Densitometric reproducibility, % ± SD[a]	13.2 ± 1.8	10.9 ± 1.6	11.3 ± 1.9	42.4 ± 4.5	16.5 ± 2.4	5.7 ± 1.4

[a] Results of 12 electrophoreses of a GAG standard mixture performed on 12 different days

Table 2. Electrophoretic pattern of GAGs in cerebral human tumors

	No. of cases	CC	CA	HA	HS_{II}	DS	HS_{I}
White matter	4	0	24 (16–30)	60 (56–63)	16 (13–21)	< 1	0
Gray matter	3	0	41 (34–51)	44 (35–55)	15 (10–21)	< 1	0
Astrocytomas	4	0	23 (5–62)	70 (26–90)	7 (5–10)	< 1	0
Oligodendrogliomas	2	0	40 (38–42)	45 (43–47)	15 (14–16)	< 1	0
Spongioblastomas	3	0	45 (40–48)	37 (34–39)	16 (11–18)	< 1–4	0
Anaplastic astrocytoma	1	0	47	39	14	< 1	0
Glioblastomas	2		31 (29–33)	39 (31–47)	20 (16–24)	9 (7–11)	< 1–2
Gliosarcoma	1	0	37	38	11	14	< 1
Ependymomas	3		32 (23–38)	21 (15–27)	44 (39–50)	< 1–7	< 1
Neurinoma	1	0	13	54	23	10	0
Meningiomas	16	0	35 (19–64)	7 (3–18)	28 (10–42)	24 (6–42)	5 (0–13)
Choroid plexus carcinoma	1		48	21	24	7	0
Metastases	3	0	37 (35–39)	20 (15–23)	26 (24–29)	13 (9–18)	4 (0– 8)
Myeloid leukemia	1	5	78	2	8	7	0

Values are expressed in percentage

University of Chicago) were: CC, from the cranial cartilage of the river sturgeon; CA, from the notochord of the river sturgeon; HA from human umbilical cord; HS, from bovine lung; DS, from hog mucosal tissue; HP from bovine lung; KS1 from bovine corneal tissue; KS2 from human costal cartilage (generous gift of Prof. V. Chiarugi, Institute of General Pathology, University of Florence).

Identification of Electrophoretic Bands

The bands were identified by their comigration with standard GAGs and by chemical and enzymatic procedures. HP and HS were identified by nitrosation on the electrophoretic sheet (Cappelletti et al. 1980). CC, CA, HA and DS were identified by chondroitinases ABC, and AC (Sigma, St Louis, MO, USA), and fungal hyaluronidase (Calbiochem, San Diego, CA, USA), as described elsewhere (Bertolotto et al. 1982).

The presence of KS was only electrophoretically investigated (Cappelletti et al. 1979b).

Densitometric Analysis

Electrophoretic sheets stained with Alcian Blue 8GX were analyzed with a CliniScan densitometer (Helena Laboratories Corp., Beaumont, TX, USA). The values of GAGs are expressed as percentages. The analytical results for a standard GAGs mixture are yielded in Table 1.

Results

Table 2 shows the electrophoretic patterns of GAGs in brain tumors and Table 3 their quantitation. The concentration of GAGs and the CA to HA ratio was higher in the gray than in the white matter. The electrophoretic patterns of astrocytomas, oligodendrogliomas and spongioblastomas were similar, except for 2 protoplasmic astrocytomas, in which 90% of GAGs

Table 3. Quantitative determination of GAGs in cerebral tumors

	No. of cases	μg uronic ac./ g wet tissue[a]
White matter	4	123 (105–134)
Gray matter	2	148 (145–152)
Astrocytomas	3	376 (148–527)
Oligodendrogliomas	2	193 (125–261)
Spongioblastomas	3	321 (203–464)
Anaplastic astrocytoma	1	683
Glioblastomas	2	613 (483–743)
Gliosarcoma	1	622
Ependymomas	3	312 (278–342)
Neurinoma	1	644
Meningiomas	16	379 (138–756)
Choroid plexus carcinoma	1	385
Metastases	3	625 (364–982)
Myeloid leukemia	1	3156

[a] The values are the mean of three determinations

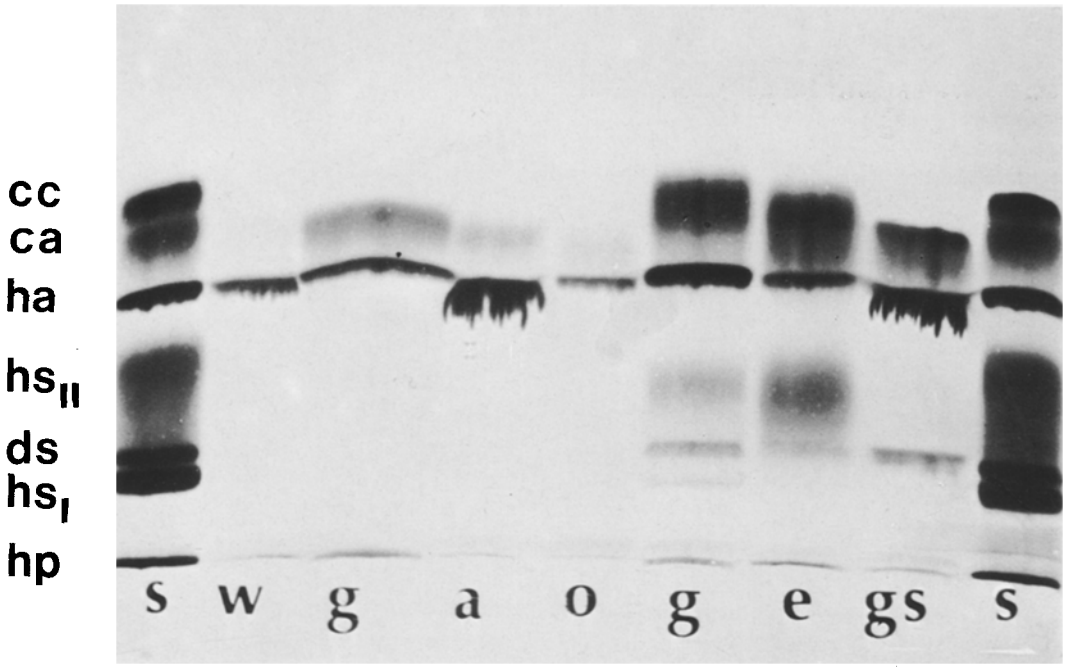

Fig. 1. Electrophoretic pattern of normal nervous tissue and several gliomas. *s* standard GAGs; *w* normal white matter; *g* normal gray matter; *a* astrocytoma; *o* oligodendroglioma; *g* glioblastoma; *e* ependymoma; *gs* gliosarcoma

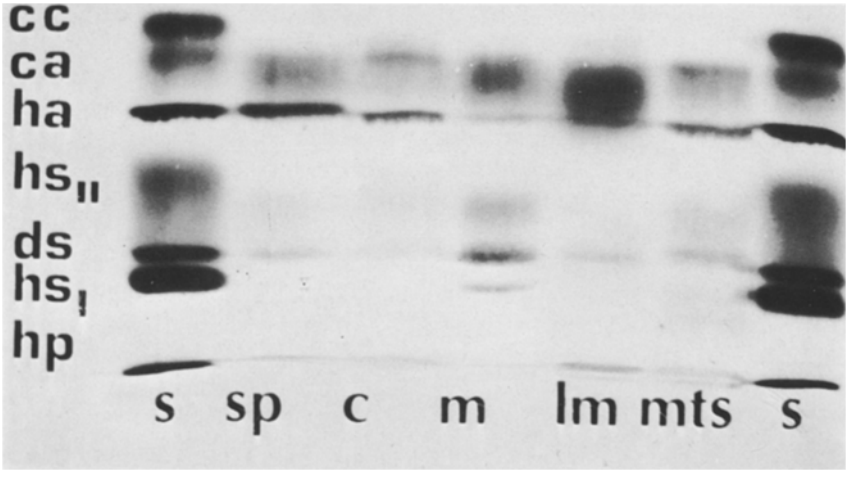

Fig. 2. Electrophoretic pattern of several tumors. *s* standard GAGs; *sp* spongioblastoma; *c* choroid plexus carcinoma; *m* meningioma; *lm* myeloid leukemia; *mts* metastasis

was represented by HA. A clear band of DS was present only in 1 spongioblastoma. The GAG concentration was lower and had a wider range in benign gliomas than in glioblastomas; in the latter, besides a large single band of CC and CA, a DS band was evident. In gliosarcomas the DS band was particularly prominent. Ependymomas demonstrated a high concentration of HS_{II} and a single large band of CC and CA; in one case, DS represented 7% of GAGs. In neurinomas HA constituted about 50% and DS 10% of the total. Meningiomas had a low percentage of HA and a high percentage of DS and HS; the 3 metastases, all of unknown origin, showed similar patterns. The case of leukemic infiltration displayed a clear CC band (5%) and large CA band (78%).

HP was occasionally present only in traces (< 1%). A band comigrating with standard KS was never observed.

Nitrosation and chondroitinase digestion demonstrated the presence in some cases of Alcian-positive, non-glycosaminoglycidic bands, migrating either near HS_I, or between HS_{II} and HA or between DS and

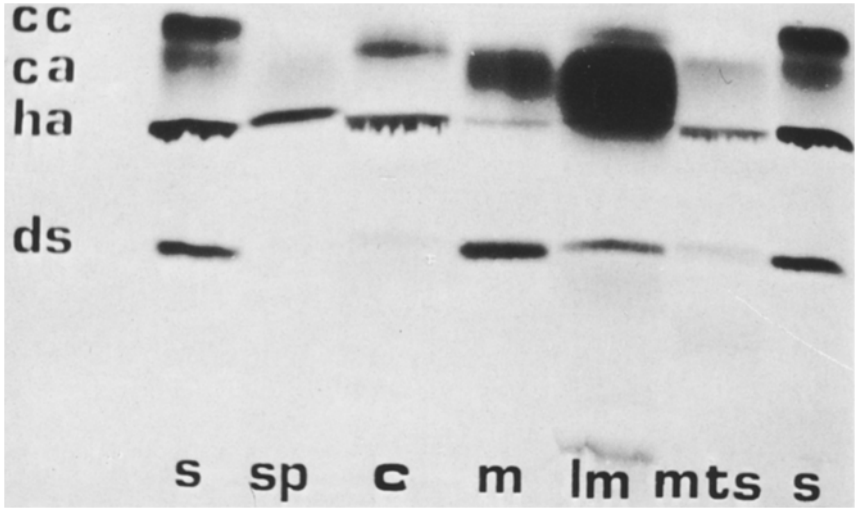

Fig. 3. Effect of nitrosation applied directly on the sheet. Samples and standard GAG mixture as in Fig. 2. Disappearance of HS_{II}, HS_{I} and HP. The pale band still present in samples lm and mts disappears after treatment with neuraminidase

HS_{II}. They probably are RNA or oligosaccharides (Bertolotto et al. 1982). Electrophoretic patterns of some tumors are shown in Figs. 1, 2 and 3.

Discussion

The electrophoretic method used made it possible to separate clearly all the GAG standards and was found to be highly sensitive and reproducible (Bertolotto et al. 1982). Its high sensitivity allowed us to analyse small specimens, containing low concentrations of GAGs. Moreover, nitrosation directly applied on electrophoretic sheets simplified the identification of the GAG bands.

Our quantitative data indicate a higher GAG concentration in the gray matter than in the white matter, in agreement with the observations in bovine (Margolis 1967) and in human brain (Federico et al. 1977). Discordant findings (Federico et al. 1980, 1981) may be explained by the different water contents in the various tissue samples, since GAG concentration was referred to wet weight.

The CA to HA ratio was higher in the gray than in the white matter. The sheep brain shows the same ratio, even though the total amount of GAGs is similar in gray and white matter (Singh and Bachhawat 1965).

In normal nervous tissue we found a large CA band, whereas no CC band was present. Since our standard CA contains 6% of 6S disaccharide, the GAGs comigrating in electrophoresis with standard CA could contain about 6% of 6S disaccharide. Therefore, our observation is consistent with the values (about 10%) obtained by enzyme digestion or infra-red analysis (Clausen and Hansen 1963; Margolis 1967; Singh and Bachhawat 1968; Singh et al. 1969; Saigo and Egami 1970; Margolis and Margolis 1972).

Concerning DS concentration in normal CNS there is no general agreement. Some authors claim that DS is present only in traces (Saigo and Egami 1970; Margolis and Margolis 1972; Dietrich et al. 1976; Toledo and Dietrich 1977), while others maintain that it may represent up to 8% of the total GAGs (Singh and Bachhawat 1968; Goldberg and Cunningham 1970; Margolis and Margolis 1979). Our data indicate a very low DS concentration both in white and gray matter.

HS represented about 15% of GAGs in normal CNS, in agreement with previous reports (Singh and Bachhawat 1968; Margolis and Atherton 1972).

Concentration and electrophoretic pattern of GAGs in cerebral tumors are influenced by several factors: GAG distribution in nervous tissue; intrinsic characters of tumor cells; presence of intercellular matrix; quantity and quality of blood vessels. Moreover the localization of the tumor, the occurrence of necrosis and degeneration and the amount of normal tissue included in the neoplasm are of paramount importance.

All but two glial tumors contained a higher GAG concentration than normal nervous tissue; analogous increase was already observed in brain tumors (Anghileri et al. 1977) and in hepatic tumors (Kojima et al. 1975). The high GAG concentration in the two glioblastomas may be related to their rich vascularization. The wider range of values observed in benign glial tumors, where vascularization is scanty, could be explained by the histochemical findings (Part 2) alone. Striking differences in the electrophoretic patterns were found between benign and malignant glial tumors; in the latter a clear band of DS was present and chondroitin sulfates contained a higher percentage of 6S disaccharide. The same high percentage of 6S disaccharide was observed in ependymomas, leukemic in-

filtration, choroid plexus carcinoma and in some meningiomas. The meaning of these findings is not clear. They might indicate a relationship between cell proliferation and CC concentration (Chiarugi et al. 1978); however, a high CC concentration has been found also in normal vessels (Toledo and Dietrich 1977).

Some tumors showed special patterns: ependymomas. for instance, contained a high percentage of HS_{II}, meningiomas low percentages of HA and high percentages of DS and so on. The results may depend on the tissue of origin, since different tissues are generally characterized by different patterns (Toledo and Dietrich 1977).

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