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Glycosaminoglycan changes in human gliomas. A biochemical study

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Summary

Glycosaminoglycans (GAGs) were isolated, separated by electrophoresis and quantified in 36 neurosurgical specimens of human gliomas and in 8 samples of normal white and gray matter. Gliomas of various degrees of malignancy exhibited different GAG patterns. Total GAG concentration was three times higher in low grade gliomas than in normal white matter. The mean percentage of single GAG classes was usually similar in both tissues, although in certain tumor samples a higher percentage of hyaluronate was found. GAG patterns in anaplastic astrocytomas, however, more closely resembled normal white and gray matter, both quantitatively and qualitatively. Glioblastomas, on the other hand, showed high GAG concentrations, in particular of heparan sulfate and dermatan sulfate. This finding could be secondary to the abundant vessels and mesodermal material associated with this oncotype. The hyaluronate/sulfated GAGs ratio was lower in oligodendrogliomas than in low grade astrocytomas. This biochemical feature may be correlated with the alcianophilia found in the honey-comb degeneration of oligodendrogliomas. The significance of these findings as they relate to tumor histology and biology have been discussed.

Introduction

Histochemical differences have been shown between glycosaminoglycans (GAGs) in normal central nervous system (CNS) and in human and experimental gliomas (1–9). Considering the many functions attributed to GAGs in the CNS (10), such differences could have important functional significance. These compounds are the only extracellular matrix components synthetized by neurons and glial cells (10). Apart from a chondroitin sulfate-proteoglycan observed in mature neurons and glial cells (11), they seem to be localized on cell membranes and in the extracellular space (12–13). Consequently GAGs could be involved in intercellular contacts and in cell-extracellular matrix interactions; moreover they could influence water and electrolyte distribution in the extracellular space (13).

Beside a previous report from our group on a few cases (14), there are few data available on the GAG distribution in human gliomas. This study was performed in order to verify the histochemical findings, using biochemical techniques, and to quantify the single GAG classes, since every class of GAGs possesses characteristic biological properties (15).

Materials and methods

Papain (E.C. 3.4.4.10) was purchased from Hoechst Italia, Milan and Titan III Zip Zone Cellulose Acetate Plate from Helena Laboratories, Beaumont, Texas. yM5 Diaflo ultrafiltration mem-

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branes were obtained from Amicon Co., Massachusetts. Alcian Blue 8GX; Chrondroitinase ABC (E.C. 4.2.2.4) from *Proteus vulgaris*; Chondroitinase AC (E.C. 4.2.2.5) from *Arthrobacter aurescens*; Chondroitin-6-sulfate (CC) from shark cartilage; Chondroitin-4-sulfate (CA) from whale cartilage and Hyaluronic acid (HA) from bovine vitreous humor were purchased from Sigma Chemical Co., St. Louis, Missouri and fungal Hyaluronidase (E.C. 3.2.1.25) from Hoechst Italia, Milan.

The following GAGs, extracted and purified by Professors M.B. Mathews and K.J.A. Cifonelli (Dept. of Pediatrics, University of Chicago) were a generous gift of Prof. V. Chiarugi (Institute of General Pathology, University of Florence): Dermatan sulfate (DS), from hog mucosal tissue; Heparan sulfate (HS) from bovine lung; Heparin (HP) from bovine lung; Keratan sulfate from bovine corneal tissue (KS1) and from human costal cartilage (KS2).

Human gliomas

36 surgical specimens of human gliomas were subdivided into two parts; the first was utilized for histological diagnosis and histochemical study of GAGs, the second was frozen at −20 °C within 15 min after neurosurgical resection and later utilized for biochemical analysis.

The brain tumors were: 12 low grade astrocytomas, 3 cerebellar spongioblastomas, 3 oligodendrogliomas, 2 recurrent low grade astrocytomas, 5 anaplastic astrocytomas, 8 glioblastomas and 3 gliosarcomas.

As normal control we utilized postmortem samples of cortex and white matter of frontal, parietal, temporal and occipital lobes taken from a 57-year-old man without neurological disease.

Isolation of glycosaminoglycans

Two groups of tumor samples were studied. The first group of 13 samples were homogenized, digested by papain and deproteinized by trichloroacetic acid. GAGs were then precipitated with ethanol, dialysed and electrophoresed. In the second group (23 cases of gliomas and 8 samples of autoptic normal white and gray matter), samples were defatted by chloroform and methanol (16), dried with acetone and weighed. The acetone-dried powder was dissolved in 0.2 M Tris HCl, pH 7.5, digested 3 times by papain with 0.005 M cystein and 0.005 M EDTA at 60 °C for 3 days. A few drops of octanoic acid were added to prevent bacterial growth. After protein precipitation by trichloroacetic acid, the supernatant was neutralized, dialysed in an Amicon Cell with yM5 membrane against distilled water, and finally electrophoresed.

Electrophoresis and quantitation of GAGs

GAGs were electrophoresed according to the method of Cappelletti and Del Rosso (17) allowing GAG analysis in samples containing proteins. Sheets were stained with Alcian Blue 8GX.

GAG quantitation was performed according to a new densitometric method (18). A densitometer (CliniScan™, Helena Laboratories, Beaumont, Texas) was used to compare samples with 150 ng of a HA standard run of the same sheet, and provided both total GAG concentrations and percentage concentration of every single band. The error due to different affinity of each class of GAGs for Alcian Blue was evaluated with standard GAGs and corrected for. As unknown aliquots of GAGs of the first group were utilized in a previous study (14), only the second group of samples was used for the evaluation of the total GAG concentration (expressed as μg/g dried defatted tissue). Both groups were included in the evaluation of the percentage contribution of individual classes.

Identification of GAG electrophoretic bands

GAG electrophoretic bands were identified by: 1) comigration with standard GAGs; 2) susceptibility to nitrous acid treatment (19); 3) digestion with Chondroitinase ABC, AC and fungal Hyaluronidase (20).

Histochemical study

A part of all the surgical samples was fixed in Carnoy and embedded in paraffin. GAGs were studied by using Alcian Blue 8GX (Sigma) 0.1% in acetate buffer pH 5.7, with increasing molarity of $MgCl_2$, according to the Critical Electrolyte Concentration (CEC) method of Scott and Dorling (21). This method is considered specific for sulfated GAGs (21).

Results

The overall concentration of GAGs, particularly of the CS class (Chondroitin-4- and -6-sulfated), in post-mortem human brain was higher in gray than in white matter with no differences between lobes (Fig. 1 and Table 1, 2). Hyaluronic acid (HA) concentration was similar in all samples of white and gray matter (Table 1).

Glial tumors differed both quantitatively and qualitatively from the normal white matter in terms of their GAG content.

Low grade astrocytomas showed a concentration of GAGs three times higher than the normal white matter (Student's 't' test $p<0.001$) (Table 1). This was due to a higher amount HA ($p<0.001$), CS ($p<0.01$) and heparan sulfate (HS) ($p<0.05$) (Table 1). The mean percentage distribution of single GAG classes was similar to that of the normal white matter (Table 2), apart from two samples of protoplasmatic astrocytomas with 90% HA and a fibrillary astrocytomas with 58% chondroitin sulfate A (CA). Cerebellar spongioblastomas presented a mean CS/HA ratio higher than cerebral astrocytomas (Table 2).

The GAG pattern of oligodendrogliomas was markedly different from that of astrocytomas because of a higher percentage of sulfated GAGs, particularly of HS and DS (Table 2).

We considered as a particular group two cases of recurrent low grade astocytomas in which histology showed abundant infiltrated white matter. These two cases presented a peculiar pattern: the GAG concentration was 6 times higher than in the normal white matter and HA represented 65% of the total (Table 1, 2).

Our 5 case of anaplastic astrocytomas showed the following histological feature: high number of

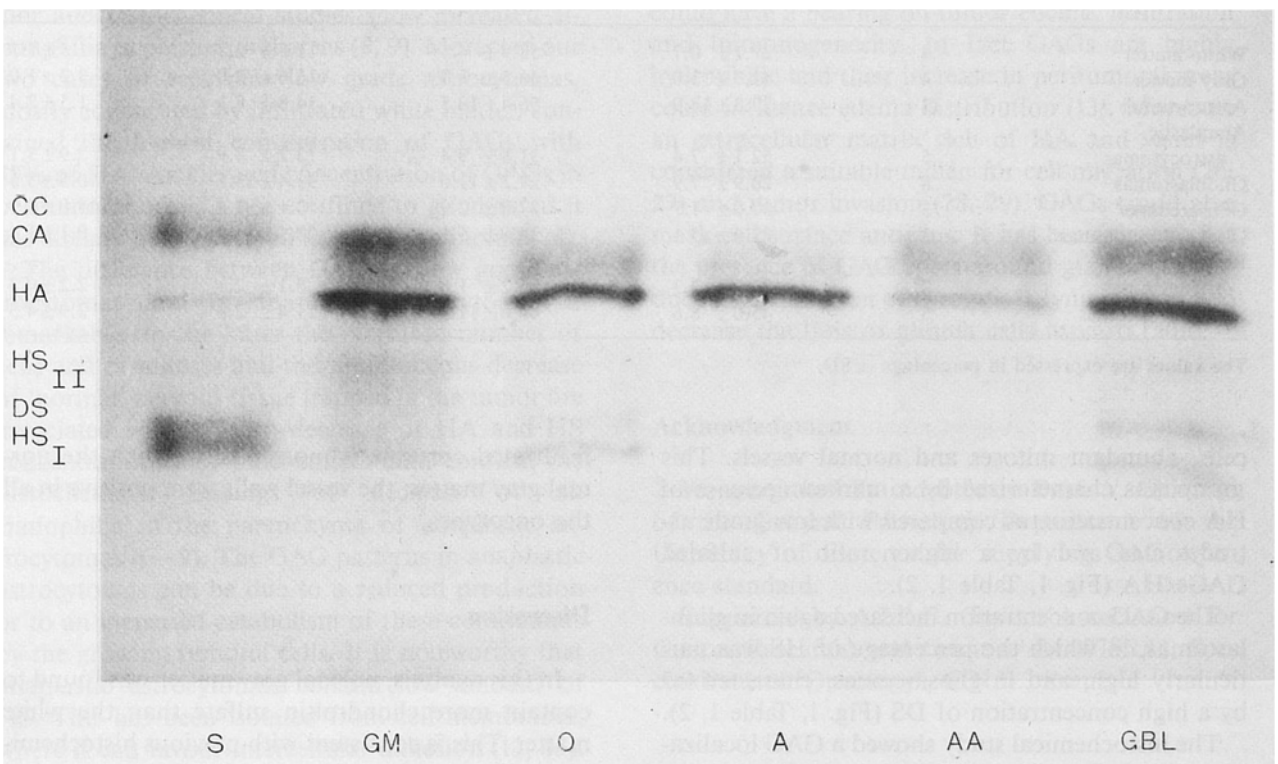

Fig. 1. Electrophoresis of glycosaminoglycans isolated from gliomas. S: mixture of standard glycosaminoglycans containing chondroitin-6-sulfate and -4-sulfate (CA), hyaluronic acid (HA), heparan sulfate (HS_{II} and HS_{I}) and dermatan sulfate (DS). GM: post-mortem normal gray matter; O: oligodendroglioma; A: low grade astrogytoma; AA: anaplastic astrocytoma; GBL: glioblastoma.

Table 1. Glycosaminoglycan concentration in human gliomas.

	Cases	μg/g·dried defatted tissue (mean ± SD)				
		Chondroitin sulfate	Hyaluronic acid	Heparan sulfate	Dermatan sulfate	Total
White matter	4	374± 27	743± 74	241± 31	21± 20	1378± 88
Gray matter	4	892± 36[a]	748± 92[c]	340± 54[c]	65± 22[c]	2046± 158[a]
Astrocytomas	6	1284±423[b]	2164±355[a]	738±397[d]	43± 32[e]	4230± 796[a]
Anaplastic astrocytomas	4	972±234[b]	546±258[e]	202± 20[e]	75± 80[e]	1721± 390[e]
Glioblastomas	7	879±301[b]	956±620[e]	1158±409[a]	342±163[b]	3335± 899[b]
Gliosarcomas	3	1841±662[b]	1842±560[b]	459± 69[e]	764±321[b]	4909±1584[b]
Oligodendrogliomas	1	1888	1636	1855	433	5813
Recurrences of astrocytomas	2	1821± 39	5186±957	644±268	167± 6	7820±1290

Statistical differences between the white matter and the other samples were evaluated by Student's 't' test: a<0.001; b<0.01; c<0.02; d<0.05; e:NS.

Table 2. Glycosaminoglycan percentage in human gliomas.

	Cases	Chondroitin sulfate	Hyaluronic acid	Heparan sulfate	Dermatan sulfate
White matter	4	27.1± 0.7	53.9± 4.1	17.5± 2.7	1.5±1.3
Gray matter	4	43.7± 3.0	36.5± 1.7	16.6± 2.0	3.2±1.0
Astrocytomas	12	27.3±14.9	56.4±19.5	14.8± 8.1	1.5±2.4
Anaplastic astrocytomas	5	52.1± 7.4	31.8± 9.4	13.2± 3.9	2.9±3.1
Glioblastomas	8	26.9± 7.9	29.2±11.6	33.4±13.8	10.5±5.5
Gliosarcomas	3	37.0± 1.7	37.8± 2.9	10.1± 2.4	15.0±3.9
Oligodendrogliomas	3	28.4± 5.1	34.0± 5.1	28.5± 4.9	9.1±1.4
Recurrences of astrocytomas	2	23.5± 3.3	66.2± 1.6	8.1± 2.1	2.2±0.3
Spongioblastomas	3	45.0± 8.6	40.6±11.7	11.4± 2.8	2.9±4.3

The values are expressed in percentage ±SD.

cells, abundant mitoses and normal vessels. This group was characterized by a marked decrease of HA concentration as compared with low grade astrocytomas and by a higher ratio of sulfated GAGs/HA (Fig. 1, Table 1, 2).

The GAG concentration increased again in glioblastomas, in which the percentage of HS was particularly high, and in gliosarcomas, characterized by a high concentration of DS (Fig. 1, Table 1, 2).

The histochemical study showed a GAG localization identical to that already described *in extenso* by our group (8). Briefly, the Alcian Blue CEC method revealed a progressive reduction of the alcianophilia in the tumoral parenchyma that paralleled the increasing degree of malignancy. The infiltrated cortex was more positive than the normal gray matter; the vessel walls were positive in all the oncotypes.

Discussion

In this analysis, normal gray matter was found to contain more chondroitin sulfate than the white matter. This is consistent with previous histochemical (8), biochemical (22), and immunocytochemical (11) studies.

GAG concentrations and patterns in gliomas are probably influenced by the presence of degeneration, quantity and quality of vessels, and by the

synthesis or catabolism of GAGs by tumoral and peritumoral cells. Further biochemical and immunocytochemical studies are necessary to define the importance of each factor. However the comparison of previously reported histochemical studies (1–9) and our biochemical and histochemical data may suggest which factors are involved in the different glioma oncotypes.

In low grade astrocytomas we found GAG concentrations higher than in the white matter. Histochemically this oncotype presents a strong alcianophilia (6–7), particularly in microcysts (8). The high GAG content can be due to new synthesis by tumoral cells. In fact in vitro glial cells are able to synthetize GAGs and most of the glioma lines produce more HA and less sulfated GAGs than normal glial lines (23). The high GAG amount in low grade astrocytomas can also be due to synthesis by peritumoral tissue progressively infiltrated by the growing neoplasia. In fact in this oncotype a large amount of 'normal' tissue is trapped in the tumor and histochemical studies show increased alcianphilia in peritumoral areas (8, 9). Moreover our two cases of recurrent low grade astrocytomas, mostly constituted by infiltrated white matter, contained the highest concentration of GAGs with 65% of HA. An elevated concentration of GAGs in peritumoral areas is not confined to gliomas, as it has already been observed in colon carcinoma (24).

The difference between GAGs in low grade astrocytomas and in anaplastic astrocytomas is remarkable. In the latter the increased number of cells and of mitoses and the simultaneous decrease of 'normal' nervous tissue trapped in the tumor are associated with a sharp decrease of HA and HS concentration. Our biochemical data confirm the histochemical findings of decrease of alcianophilia in the parenchyma of anaplastic astrocytomas (6–9). The GAG patterns in anaplastic astrocytomas can be due to a reduced production or to an increased catabolism of these compounds by the growing tumoral cells. It is noteworthy that anaplastic astrocytomas contain low amount of HS. This has been isolated from cell membranes, where it can favour intercellular adhesion (12, 15).

In glioblastomas we found a GAG concentration higher than in anaplastic astrocytomas. This increase was mainly due to HS and DS. Histochemically the tumoral parenchyma is alcian-negative, whereas the vessels and the mesodermal component are strongly positive (2, 6–9). Moreover the Alcian CEC method revealed the presence of sulfated GAGs in these structures (6–9) and the biochemical analysis of cerebral vessels demonstrated a high content of HS and DS (25). All these data suggest that the high concentration of sulfated GAGs in glioblastomas is mainly associated with the abundant mesodermal component of this oncotype and, as a consequence, the tumoral parenchyma contains low amount of sulfated GAGs. The high percentage of sulfated GAGs in gliosarcomas is consistent with this hypothesis.

Our biochemical data revealed a high percentage of CS, HS and DS in oligodendrogliomas. This pattern can be correlated with the honey-comb degeneration, typical of oligodendroglioma, as this degeneration is histochemically very rich in sulfated GAGs (1–9).

In conclusion, this study suggests that primary brain tumors with various degrees of anaplasia present different GAG patterns. These differences could have a bearing on tumor edema, infiltration and immunogenecity. In fact GAGs are highly hydrophilic and their increase in peritumoral areas could influence edema distribution (13). Moreover an extracellular matrix rich of HA and water is considered a suitable milieu for cell migration (26, 27) and tumor invasion (28, 29). GAGs could also mask cell surface antigens. It has been shown that the presence of GAG coats around glioma cells reduce the generation of cytolytic T lymphocytes and decrease the lysis of glioma cells in vitro (30).

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