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Glycosaminoglycans of Cardiac Tumors[1] (40077)

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Cardiac tumors are rare neoplasms that originate mostly in connective tissue (1). The nature of the connective tissue, especially the glycosaminoglycans (GAG), in heart tumors has not been well established, but a comparison of normal heart GAG to that of cardiac tumors should help reveal the histogenesis of these neoplasms. In this study, several tumors were compared to normal human heart tissue for GAG composition.

Methods and Materials. Tumors were collected during surgery or from necropsy and fixed in formalin or frozen until studied. They were obtained from Charity Hospital and Touro Infirmary in New Orleans, LA; Mayo Clinic in Rochester, MN; and Lafayette Medical Laboratory in Lafayette, LA. Three of the six tumors were myxomas, one was a malignant mesenchymoma, one was a rhabdomyoma, and one was a primary angiosarcoma that we obtained in three parts: (a) tumor of the left ventricle, (b) residual tumor and left ventricular wall, (c) tumor of the right atrial cavity. Control tissues were collected shortly after necropsy from portions of the free wall of the left and right ventricles and the ventricular septum from Charity Hospital patients who did not show clinical evidence of heart disease.

Isolation of GAG. The control cardiac and tumor tissues were minced, defatted, dehydrated with acetone, and air-dried. GAG were extracted with 2% sodium hydroxide for 48 hr from dry-defatted tissue by methods described earlier (2). The extracts were neutralized with 7.35 *N* phosphoric acid and digested with pronase for 72 hr while being dialyzed against 0.1 *M* phosphate buffer pH 7.8. The digests were then treated with trichloroacetic acid, filtered through celite, and dialyzed against distilled water. The samples were concentrated, and the total content of GAG isolated from the tissue was estimated by uronic acid analysis (3).

Fractionation of GAG by column chromatography. The isolated GAG were fractionated on Dowex-1X2 (AG 200–400 mesh) Cl^- column, 1.0 × 50 cm, by eluting with a stepwise-increasing concentration of NaCl (0.5–4.0 *M*) (4). The effluent fractions were dialyzed, concentrated, and analyzed for uronic acid content. Briefly, the resin technique fractionates GAG, due to their polydispersity and varying degrees of sulfation, essentially into groups of compounds that require further chemical analyses for quantitation of individual GAG. Similar fractionations have been done on GAG preparations from a number of sources, including skin, aorta, heart, kidney, and liver (5–9). After studies of four tumors and control tissue were completed, another myxoma and the rhabdomyoma became available. Fractionations of GAG from these tissues were conducted by an automated column chromatographic procedure (10). The results of individual GAG obtained by both fractionation procedures are comparable.

Analysis of fractions. The GAG were then quantitated by additional analyses by an approach which has been described in detail earlier (11). Fractions obtained from resin columns were analyzed for uronic acids and hexosamines by gas–liquid chromatographic techniques (GLC) (12, 13). For hexosamine analysis the GAG were hydrolyzed with 4 *N* HCl for 16 hr in sealed tubes at 100–105°. We prepared trimethylsilyl (TMS) derivatives by a method previously described using hexamethyldisilazane and dimethylformamide (12). Samples to be analyzed for uronic acid were hydrolyzed with concentrated formic acid at 100–105° for 20 hr in sealed tubes under nitrogen. We prepared the TMS derivatives of uronic acid by the method of Sweeley *et al.* (14) and estimated chondroitin 4-

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sulfate (Ch 4-SO_4) and chondroitin 6-sulfate (Ch 6-SO_4) according to Mathews and Inouye (15). We further identified GAG fractions by electrophoresis on cellulose acetate in pyridine–formic acid buffer pH 3.0. Mixtures of chondroitin sulfates and dermatan sulfate obtained in the automated chromatographic procedure were analyzed for Ch 4-SO, Ch 6-SO_4 and dermatan sulfate (DS) by an enzymatic method described by Saito *et al.* (16).

Results. The amounts of total GAG extracted from control cardiac and tumor tissues are expressed in Table I as mg uronic acid per gm of dry-defatted tissue. Assuming uronic acid accounts for approximately 30% of the GAG, we multiply these values by three to estimate the average content of GAG. The control tissues, from right and left ventricles, contained similar concentrations of GAG. In contrast, the myxoma and malignant mesenchymoma tissue contained seven to ten times more GAG than the control tissues; the rhabdomyoma contained four times more; and the angiosarcoma tissue ranged from approximately one and a half to twice the amount isolated from control cardiac tissue.

An example of the multiple analyses of the different GAG fractions isolated from myxoma #1 after fractionation on Dowex Column is shown in Table II. Most of the GAG were eluted at 0.5, 1.25, and 1.5 *M* NaCl. The fraction eluted at 0.5 *M* NaCl contained glucuronic acid and glucosamine

TABLE I. TOTAL GAG CONTENT OF VARIOUS CARDIAC TUMORS.

Type of tumor	Patients' Sex and Age	Total GAG (mg uronic acid/g dry-defatted tissue)
Myxoma (#1)[a]	F 29 years	4.39
Myxoma (#2)	F 33 years	3.42
Myxoma (#3)	F 53 years	4.43
Malignant Mesenchymoma	—[c] 17 years	3.31
Rhabdomyoma	M 10 days	2.14
Angiosarcoma[b]	— —[c]	
(a) Angiosarcoma of left ventricle		0.77
(b) Residual tumor of (a) and normal left ventricle wall		0.79
(c) Angiosarcoma in right atrial cavity		1.08
Control Hearts (N = 5)	M 21–56 years	
Left ventricle		0.46 ± 0.09[d]
Right ventricle		0.51 ± 0.06[d]

[a] Myxomas were from different individuals.
[b] All angiosarcomas were from the same individual.
[c] — information not available.
[d] SE. GAG = glycosaminoglycans; N = Number of tissues

TABLE II. AN EXAMPLE OF ANALYSIS OF FRACTIONS FROM DOWEX 1 × 2 Cl^- COLUMN OF GAG FROM MYXOMA (#1).[a]

Fractions from column *M* NaCl	mg per g dry-defatted tissue	Gas-liquid chromatography: % of total hexosamine GlcN	GalN	% of total uronic acid GlcUA	IdUA	Electrophoretic identification	% of GAG fractions: HA	HS	DS	Ch 4-SO_4[b]	Ch 6-SO_4[b]
0.50	0.96	100	tr	100	tr	HA	100	—	—	—	—
0.75	0.19[c]										
1.00	0.58[c]	8.0	92.0	82.8	17.2	HS, CS/DS[d]	—	8.0	17.2[e]	25.8	49
1.25	1.32[c]										
1.50	1.20[c]										
2.00	0.13[c]	tr	100	74.0	26.0	CS/DS[d]	—	—	26[e]	13	61
4.00	0.01[c]										

[a] Thirteen milligrams uronic acid acid were applied to Dowex 1 × 2 Cl^- column and 11 mg recovered.
[b] Calculated by method of Mathews and Inouye (15).
[c] Combined for analysis.
[d] CS and DS cannot be resolved in the buffer used.
[e] Estimated from the iduronate content. Since DS also contained small amounts of glucuronate this value might be slightly lower than the actual content. Ch 4-SO_4 = chondroitin 4-sulfate; Ch 6-SO_4 = chondroitin 6-sulfate; CS = chondroitin sulfates; DS = dermatan sulfate; GalN = galactosamine; GAG = glycosaminoglycans; GlcN = glucosamine; GlcUA = glucuronic acid; HA = hyaluronic acid; HS = heparan sulfate; IdUA = iduronic acid

only and was considered as hyaluronic acid (HA). The combined fractions that eluted at 0.75, 1.0, and 1.25 *M* NaCl contained both glucosamine and galactosamine, and glucuronic and iduronic acids. The fractions eluted at 1.5, 2.0 and 4.0 *M* contained galactosamine as the only hexosamine but contained both glucuronic and iduronic acids. Based on these analyses as well as enzymatic analyses, individual GAG fractions were calculated.

The GAG compositions of human cardiac tumor tissues and control myocardium are reported in Table III. HA and heparan sulfate (HS) were the major GAG found in the cardiac tissue, although small amounts of other GAG were also present. The observations are similar to earlier ones of cardiac tissue (7), and these GAG are more like those preparations from medial and outer wall sections of the aorta than like those from intimal preparations of aorta. Of the myxomas studied, two had high Ch 6-SO_4 (about 40–50%) and low HA (about 20%), as seen in myxomas #1 and #2; and the other had high HA (about 50%), low Ch 6-SO_4 (about 20%), and low DS (about 5%), shown by myxoma #3. In all three tumors HS was present in smaller amounts than in control tissue, and about 15% of total GAG was Ch 4-SO_4. The GAG of malignant mesenchymoma tumor was similar to that of myxoma #3 with high HA and low Ch 6-SO_4.

In contrast to these observations of myxomas and malignant mesenchymoma, the angiosarcoma of the left ventricle and the right atrial cavity in the same heart showed the major GAG to be HA, HS, and Ch 4-SO_4 with lesser amounts of Ch 6-SO_4 and DS. While the compositions of GAG in angiosarcoma tissues from the left ventricle and right atrial cavity were very similar, the compositions of GAG from the residual tumor with normal left ventricular wall was somewhat similar to that of control heart tissues with larger amounts of HA and smaller amounts of the chondroitin sulfates. The rhabdomyoma, however, with low HA and DS and very high Ch 6-SO_4, appeared different from other tumors.

Discussion. Although the clinical and anatomic features of cardiac tumors have been described in many studies (17–19), much less is known about the biochemical changes that occur in different types of tumors or even cardiac tumors of similar histologic classification. Earlier studies have shown that HA and HS, as the major GAG found in normal myocardium, increase in hearts involved in amyloidosis (7). Certainly, variations in GAG composition occur in different cardiac structures (7, 20).

Myxoma is the most common primary intracavitary tumor; however, the exact nature of atrial myxomas is disputed. Some investigators consider them atrial thrombi in various stages of organization while others suggest they are true neoplasms (18). The myxoma is composed mostly of plump endothelial-like

TABLE III. COMPOSITION OF GLYCOSAMINOGLYCANS IN HUMAN CARDIAC TUMORS.

Type of tumor	Percent of total GAG				
	HA	HS	Ch 4-SO_4	DS	Ch 6-SO_4
Myxoma (#1)	21.8	3.8	17.1	16.1	41.2
Myxoma (#2)	19.8	—[a]	12.7	18.5	48.9
Myxoma (#3)	49.2	7.0	15.5	5.1	23.2
Malignant Mesenchymoma	50.2	12.1	10.0	12.7	15.0
Rhabdomyoma	16.7	27.5	—[a]	4.2	51.6
Angiosarcomas:					
(a) Angiosarcoma of left ventricle	21.9	27.8	25.2	11.4	13.7
(b) Residual tumor of (a) and normal left ventricle wall	55.3	27.8	—	6.0	10.9[b]
(c) Angiosarcoma in right atrial cavity	24.0	28.1	24.7	10.4	12.8
Control Hearts[c]					
(N = 5)	66.0 ± 15.1	24.3 ± 8.1	—	2.8 ± 1.5[d]	6.4 ± 2.1[d, b]

[a] Not detected.

[b] Includes small amount of Ch 4-SO_4.

[c] No difference in GAG distribution was observed between left and right ventricles.

[d] SE, standard error. Ch 4-SO_4 = chondroitin 4-sulfate; Ch 6-SO_4 = chondroitin 6-sulfate; DS = dermatan sulfate; GAG = glycosaminoglycans; HA = hyaluronic acid; HS = heparan sulfate; N = number.

or fibroblastlike cells dispersed in large amounts of an amorphous, metachromatic ground substance. An excellent review by Kelly and Bhagwat (19) of the histologic features of cardiac myxomas suggests that individual tumors may be differentiated by cellular composition (at least three types of cells were found (21)). Heath's histochemical studies (22) have suggested that a Ch 6-SO_4 is the predominant GAG in cardiac myxoma tissue. Our present studies indicate that in some myxomas the major GAG is Ch 6-SO_4, although significant proportions of other GAG are present, and in others the major GAG is HA. Studies of myxomas in other parts of the body, such as the mandible and salivary glands, suggest that these myxomas could form from cells that are morphologically similar (23–25). These observations are consistent with the occurence of myxomas as actual tumors and not organized thrombi.

Malignant mesenchymoma is a soft-tissue tumor of mesenchymal origin that is composed of tumor cells differentiating into two or more unrelated malignant forms. This tumor has areas of large vesicular cells and many tumor giant cells. These hypercellular areas blend with areas composed of interlacing bundles of spindle-shaped cells with an occasional focus of myxomatous stroma. These spindle-shaped enlongated cells are poorly differentiated and with azocarmine stain show collagen production. An occasional small focus of asteroid tissue with bony spicules is seen in the viable fibrous portions of the tumor. Most mesenchymal cells have been shown to have the ability to produce both collagen and GAG.

Rhabdomyoma is found mostly in the newborn or infant but may occur at any age. The neoplastic tissue, which is usually gathered into multiple small nodules, may comprise a solitary tumor or involve the entire heart. Microscopically, the "spider cells" are greatly enlarged, having a central nucleus and a cytoplasmic mass connected to the cell wall by cytoplasmic strands separated by large glycogen-filled vacuoles.

Angiosarcomas are true malignant neoplasms that appear to arise from angioblasts, usually from the interatrial septum. The tumor consists of ill-defined, anastomic vascular channels lined by atypical endothelial cells amassed in the lumen. Previously, neither histochemical nor chemical analyses have revealed the GAG composition of angiosarcomas, but our studies clearly demonstrate that GAG are present.

The observation that the angiosarcomas of the left ventricle and right atrial cavity in the same heart had the same relative proportions of GAG suggests both that the tumor's site of origin is not associated with any particular change in the characteristics of the GAG or their relative proportions, and that a cell similar to that of a multipotential cell, perhaps a less differentiated form of "normal" myocardial cells, make up tumors at both sites.

Unfortunately, there were not enough tumors available in this study to precisely associate their GAG makeup with cellular morphology. The present studies, however, suggest that a knowledge of GAG composition of the cardiac tumors can contribute to a better understanding of the nature of the tumors.

Summary. The glycosaminoglycan composition of six human cardiac tumors (three myxomas, a malignant mesenchymoma, a rhabdomyoma, and an angiosarcoma) and five control hearts were studied. Found in two different relative proportions, hyaluronic acid and chondroitin 6-sulfate were the major glycosaminoglycans in the myxomas, suggesting the possible existence of two biochemically different myxomas, one high in hyaluronic acid, the other high in chondroitin 6-sulfate. The glycosaminoglycans of angiosarcoma tissues were mainly hyaluronic acid, heparan sulfate and chondroitin 4-sulfate. Tissue of the left and right ventricles of the same heart had the same relative proportions of glycosaminoglycans. Biochemical studies on the connective tissue components may be helpful in understanding the nature of the tumors.

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