DISTRIBUTION OF GLYCOSAMINOGLYCANS IN THE HUMAN HEART

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The glycosaminoglycan was studied in the heart of normal human subject. The heart was separated into six different parts including the right ventricle, right atrium, tricuspidal valve, left ventricle, left atrium and mitral valve. The glycosaminoglycan preparation was made by digesting the different parts of the heart with proteolytic enzymes followed by removal of enzymes and undigested protein, and final freeze-drying of the supernatant and the content of uronic acid was determined. Both values per gram of wet and dry tissues were highest in the mitral valve and were lowest in the left ventricle. Cellulose acetate strip-electrophoresis of materials from both the tricuspidal and mitral valves in veronal buffer revealed the presence of two alcian blue positive fractions which corresponded in mobility to hyaluronic acid and chondroitin sulfates, respectively. In each preparation from other four parts of the heart, another component was found in the region between these two fractions which stained with alcian blue and corresponded in mobility to heparan sulfate.

INTRODUCTION

The glycosaminoglycans have been isolated from many kinds of human organs and tissues, and have well been studied by many investigators (Tanaka, 1961; Mathews, 1965; Shetlar et al, 1972; Constantopoulos et al, 1973). At present, however, little is known these substances in the human heart muscle. The glycosaminoglycans in the human kidney, liver and spleen have been recently reported by a few authors (Constantopoulos et al, 1973; Masuda et al, 1974).

This work was carried out to examine the distribution of these substances in the different parts of the human heart.

MATERIALS AND METHODS

Five intact human hearts were obtained at autopsy from male subjects with ages ranging from 25 to 64 years. The heart was separated into six different regions including the right ventricle, right atrium, tricuspidal valve, left ventricle, left atrium and mitral valve. They were used for the present

experiments after histological examination. The samples were cut into cherry-sized pieces, put into several changes of acetone for several days and dried over CaCl_2 in a desiccator. They were ground in a Wiley mill, sufficiently small to pass through a 60 mesh sieve.

Isolation and determination of the glycosaminoglycan from the heart

The glycosaminoglycan was extracted from these dry tissues according to the procedure of Schiller et al. (1961). These were digested with 1/50 weights of papain (EC 3.4.4.10) in the presence of EDTA and cysteine (5:9:4, w/w/w) at 65°C for 48 hr. The mixture was adjusted to pH 7.8 and then digested with 1/50 weights of trypsin (EC 3.4.4.4) at 37°C for 48 hr with simultaneous dialysis against 0.1 M phosphate buffer of pH 7.8. The digested solution was deproteinized with 5 % trichloroacetic acid in the cold and the supernatant was dialyzed against several changes of the cold deionized water for 72 hr. Then 4 volumes of alcohol containing 1 % acetic acid and 1 % potassium acetate were added in order to precipitate the glycosaminoglycan. The precipitate was washed with alcohol, dried in a desiccator over CaCl_2 and then redissolved

in 5 ml of deionized water. An aliquot was used for uronic acid analysis and for cellulose acetate strip-electrophoresis. Uronic acid was determined by the carbazole reaction of Dische (1962).

Electrophoresis of the glycosaminoglycan on a cellulose acetate-strip

Cellulose acetate strip-electrophoresis of the material was carried out in a horizontal type apparatus at a constant current of 1.0 mA/cm on an OXOID strip (9×1.2 cm) using veronal buffer, pH 8.6, $\mu = 0.06$ for 30 min. Aqueous suspensions of preparations, 5 to 10 μ l of 1.0 mg per ml preparations, were used in this study. After electrophoresis, the strip was stained with alcian blue, and scanned in an Atago densitometer (Tokyo, Japan). Marker samples containing hyaluronic acid, heparan sulfate, chondroitin-4-sulfate, dermatan sulfate and chondroitin-6-sulfate were applied alongside each samples; all of them were purchased from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). Hyaluronic acid was derived from human umbilical cord, heparan sulfate from bovine liver, dermatan sulfate from swine skin, and both chondroitin-4-sulfate and chondroitin-6-sulfate were derived from whale cartilage, respectively.

TABLE 1
Glycosaminoglycan content in the different parts of the heart^{a)}

Subject	$\mu\text{g/g}$ of wet tissue	Ratio	$\mu\text{g/g}$ of dry tissue	Ratio
Right ventricle	116 ± 37.3	1.0	707 ± 228.9	1.0
Right atrium	165 ± 23.4	1.6 ± 0.7	1,116 ± 147.6	1.8 ± 0.7
Tricuspidal valve	461 ± 146.3	4.1 ± 0.7	3,329 ± 1,045.1	4.8 ± 0.8
Left ventricle	112 ± 49.3	0.9 ± 0.2	693 ± 284.5	0.9 ± 0.2
Left atrium	159 ± 48.8	1.5 ± 0.5	1,038 ± 267.7	1.6 ± 0.4
Mitral valve	675 ± 191.5	6.9 ± 3.9	4,322 ± 828.0	7.1 ± 3.4

^{a)} Results given are average of 5 samples. Figures following ± sign are standard deviations of the mean.

RESULTS AND DISCUSSION

The average uronic acid content of the glycosaminoglycan preparation from the different parts of the individual hearts are summarized in Table 1. The results were compared with one of past reported figures of 5,410-7,400 $\mu\text{g/g}$ of dry tissue for the tricuspidal valve and 5,730-13,700 $\mu\text{g/g}$ for the mitral valve, respectively (Torii et al., 1965). The differences may be explainable on the basis of differences in procedures. In the present work, the heart tissues were treated with the proteolytic enzymes, both papain and trypsin and dialyzed, while their work treated the materials with only papain and dialyzed before analysis. It is of interest that the digestion of the protein with trypsin greatly reduced the uronic acid content. There are no comparative data concerning the uronic acid content in the heart muscle in the literature.

Cellulose acetate strip-electrophoresis of the concentrates in veronal buffer, as shown in Fig. 1, revealed the presence of two alcian blue positive fractions in both the tricuspidal valve and mitral valve. In the preparations from the other parts of the heart, another component was also found in the

region between these two alcian blue positive fractions stainable with alcian blue. They are numbered in order of increasing electrophoretic mobility, fraction 1, fraction 2 and fraction 3. The fraction 1 was corresponded in mobility with hyaluronic acid, the fraction 2 with heparan sulfate and the fraction 3 with chondroitin-4-sulfate, dermatan sulfate and chondroitin-6-sulfate, respectively.

Densitograms after cellulose acetate strip-electrophoresis of each material from several regions of the human heart are shown in Fig. 2. The relative proportions of fraction 1, fraction 2 and fraction 3 on cellulose acetate strip-electrophoresis in veronal buffer are summarized in Table 2. The different pattern of distribution of each of these glycosaminoglycan fractions was observed in the ventricle, atrium and valve of the heart, as seen in another organ and tissue like the kidney and abdominal walls (Kaku, 1978); the relative proportion of the fraction 2 was lowest, though that of fraction 3 was highest. Any differences in the proportions of these three fractions were not observed, however, between different sides of the ventricles, atria and valves of the heart. Only a little propor-

TABLE 2

Relative proportions of fractions 1-3 on cellulose acetate strip-electrophoresis using veronal buffer in the normal human heart (%)^{a)}

Subject	Fraction 1	Fraction 2	Fraction 3
Right ventricle	32 $\pm$ 5.0	24 $\pm$ 2.9	44 $\pm$ 2.2
Right atrium	30 $\pm$ 9.1	19 $\pm$ 1.6	51 $\pm$ 7.7
Tricuspidal valve	31 $\pm$ 2.0	5 $\pm$ 2.1	64 $\pm$ 2.1
Left ventricle	30 $\pm$ 4.3	29 $\pm$ 2.4	41 $\pm$ 2.1
Left atrium	34 $\pm$ 3.5	16 $\pm$ 1.3	50 $\pm$ 2.3
Mitral valve	31 $\pm$ 2.2	3 $\pm$ 1.0	66 $\pm$ 1.4

^{a)} Results given are average of 5 samples. Figures following $\pm$ sign are standard deviation of the mean.

Fig. 1 Cellulose acetate strip-electrophoretic patterns of heart glycosaminoglycans and marker samples in veronal buffer, pH 8.6, $\mu=0.06$.

(a) right ventricle, (b) right atrium, (c) tricuspidal valve, (d) left ventricle, (e) left atrium, (f) mitral valve, (g) standard containing hyaluronic acid, (h) standard containing heparan sulfate, (i) standard containing chondroitin-4-sulfate, (j) standard containing dermatan sulfate, (k) standard containing chondroitin-6-sulfate.

Fig. 2 Densitograms after cellulose acetate strip-electrophoresis of each glycosaminoglycan from several regions of the human heart.

(a) right ventricle, (b) right atrium, (c) tricuspidal valve, (d) left ventricle, (e) left atrium, (f) mitral valve.

tion of the fraction 2 was contained in both the tricuspidal valve and mitral valve. Torii et al. (1965) reported that three kinds of glycosaminoglycans, hyaluronic acid, dermatan sulfate and chondroitin-4-sulfate or chondroitin-6-sulfate were found in the tricuspidal valve and mitral valve. Concerning the relative proportions of these glycosaminoglycan fractions in the heart valves, our data are almost agreed with their data. The proportions of the preparations in both the ventricles and atriums, however, were not available for their report. Kaku (1978) reported that human striated muscle (musculus rectus abdominis) contained $21 \pm 5.6 \mu\text{g}$ (mean $\pm$ standard deviations) of uronic acid per gram of wet tissue and $111 \pm 27.1 \mu\text{g}$ of uronic acid per gram of dry tissue, respectively. And he also appointed that the relative proportions of fraction 1, 2 and 3, in this muscle were $46 \pm 14.3\%$, $20 \pm 7.1\%$ and $34 \pm 7.4\%$, respectively. Although comparison of the results obtained by cellulose acetate strip-electrophoresis using veronal buffer, pH 8.6, $\mu=0.06$ may probably show that the fraction 1 in veronal buffer corresponded to hyaluronic acid, the fraction 2 to heparan sulfate and the fraction 3 to a mixture of chondroitin-4-sulfate, dermatan sulfate and chondroitin-6-sulfate, respectively, thus a further study will be necessary to isolate and identify each of the glycosaminoglycans from the different regions of the heart.

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