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Glycosaminoglycan Changes in Healing Myocardial Infarction[1] (40173)

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Many studies have been made of damaged heart tissue of dogs immediately following injury produced by ligation of a coronary artery. However, there have been few studies of the myocardial lesion during the healing phase following myocardial injury. Judd and Wexler (1–3) have studied the healing phase of myocardial injury produced in rats by the injection of isoproterenol. These investigations showed alterations in the glycoproteins and glycosaminoglycans of healing heart tissue analogous to those found in an experimental dermal wound healing model by White, Shetlar and Schilling (4). As studies of the healing pattern resulting from the injury produced in dogs by ligation of a coronary artery have not been made, we have recently undertaken a study of the glycosaminoglycans in the healing areas of dog heart following myocardial infarction caused by the ligation of the circumflex coronary artery.

Material and methods. Myocardial damage was produced in 32 mongrel dogs weighing 10–26 kg by ligation of the left circumflex coronary artery. All animals were anesthetized with sodium pentobarbital and operated under sterile conditions. Respiration was maintained with a Harvard respirator and electrocardiographic monitoring was done throughout the procedure. Lidocaine was injected to prevent ventricular fibrillation. After recovery, the dogs were fed Purina Dog Chow and water, *ad libitum*, and maintained in pens sufficiently large to allow for adequate exercise. Two control dogs were subjected to the same procedure except the artery was only exposed, but not ligated; one of these dogs was sacrificed after 4 days and one after 12 days.

Animals were killed at various times postoperative after anesthetization with sodium pentobarbital. The heart was removed and examined for damage. Samples of injured and uninjured tissue were minced with scissors. These samples were placed in dialysis bags with 5 ml of water and dialyzed against deionized water for 48 hr with several changes of water. The tissue samples were removed from the dialysis bags and freeze dried. Tissue samples of suitable size were digested with a papain solution by the method of Mier and Wood (5). After digestion, aliquots were taken for cellulose acetate electrophoresis using Beckman Microzone equipment. Standards containing equimolar amounts of hyaluronic acid, dermatan sulfate and chondroitin-4-sulfate were applied to each electrophoretic pattern. A zinc sulfate electrolyte (0.2 *M*, pH 5.1) was used for quantitative work. Chondroitin-6-sulfate and heparin sulfate were utilized with the other glycosaminoglycans for identification purposes. As the electrophoresis with the zinc sulfate electrolyte does not allow separation of chondroitin-4-sulfate from chondroitin-6-sulfate, most samples were also run with calcium acetate electrolyte (0.3 *M*, pH 7.25) which does allow this separation. After electrophoresis on the cellulose acetate the resulting patterns were stained with Alcian Blue (6). The electrophoretic patterns were quantitated with a Beckman Microzone Densitometer using the 600 nanometer interference filter. The densitometer reading for the two standard chondroitin sulfates were quantitatively the same, but those for hyaluronic acid were 60–70% of the chondroitin sulfate readings. Consequently, the hyaluronic acid values ob-

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tained from the samples were corrected according to the values obtained from the standard on the appropriate pattern. Selected samples were assayed for uronic acid using the carbazole method as modified by Bitter and Muir (7).

Results and discussion. Ligation of the circumflex coronary artery usually resulted in ischemic necrosis in the posterior papillary muscle and adjacent ventricular wall.

Evidence of myocardial ischemia was identified at 4.5 hr after ligation. Such injury was expressed in the form of dark, pyknotic myocardial nuclei set in clumped, deeply eosinophilic cytoplasm. The change was much more pronounced at 9 hr. During these early stages, the interstitial tissue remained negative for the presence of mucopolysaccharide by the Periodic-Acid-Schiff (P.A.S.) stain. At 2 days the infarction was associated with considerable edema and extensive leucocytic infiltration at the periphery. The edematous infiltration at the edge of the infarct was faintly positive with the P.A.S. stain. The most striking P.A.S. positive reaction however, was observed in the healing infarct 10 days after ligation. The granulation tissue was so strongly P.A.S. positive that the stain was readily identified in the sections with the naked eye. At this time there was also brisk activity of the fibroblasts associated with abundant fibrils in the extracellular matrix. These fibrils stained with a reticulin stain. The well developed collagen scar of the healed infarct at 90 days showed scant P.A.S. positive material. Detailed morphological aspects will be published elsewhere.

Typical densitometer patterns of the glycosaminoglycans at various times are shown for injured and corresponding uninjured tissues from the same heart (Fig. 1). With some allowance for variation between animals it appeared that the electrophoretic patterns from injured tissue indicated a progressive increase in chondroitin-4-sulfate fractions relative to other glycosaminoglycans after the third day with the maximum elevation occurring by the 10th to 16th day. There was a slight decrease in this fraction on the 28th and 30th days, and a striking decrease in the 90-day sample.

The results clearly indicated a striking increase in the amount of glycosaminoglycans, as indicated by the uronic acid content, and

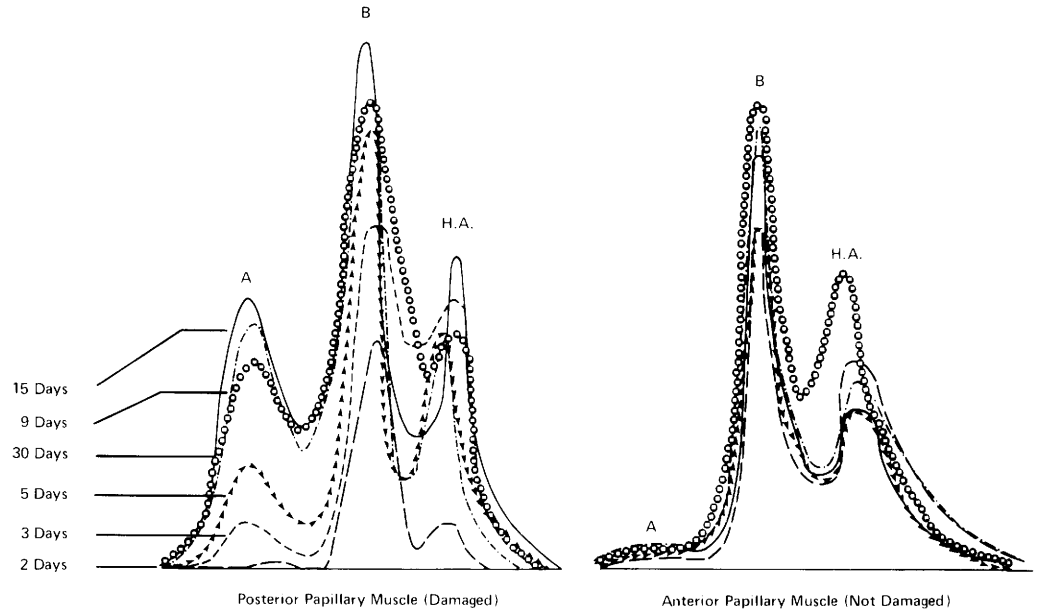

FIG. 1. Densitometer tracings of electrophoretic patterns of glycosaminoglycans from damaged and undamaged heart muscle. H.A.-hyaluronic acid area; B-Dermatan Sulfate area; A-chondroitin-4-sulfate area. Curves for the damaged series are labeled as to the number of days after injury. The same type of line was used for the corresponding times for the undamaged series on the left. All curves were made from cellulose acetate patterns; zinc sulfate electrolyte (0.2 *M*, pH 5.1) was used. The progressive increase of chondroitin-4-sulfate with time until the 15th day is apparent.

the distribution of types of glycosaminoglycans after injury. The major glycosaminoglycans found in the normal tissue in this study by electrophoresis were in the hyaluronic acid and dermatan sulfate areas. There were indications of the presence of other minor components staining with Alcian Blue. No comparative data on dog heart has been found in the literature. However, Berenson *et al.* (8) reported that the major glycosaminoglycans of normal human myocardium were hyaluronic acid and heparan sulfate with small amounts of dermatan sulfate and chondroitin-4-sulfate or chondroitin-6-sulfate. Ohkawa *et al.* (9) also reported hyaluronic acid and heparan sulfate as the major glycosaminoglycans of the human heart. In two cases of tissue from old myocardial infarctions, they reported increased amounts of dermatan sulfate and chondroitin sulfates. In our electrophoretic procedures, heparan sulfate had a mobility slightly slower than dermatan sulfate and could not be distinguished from this fraction in the usual procedure. It is also possible that heparan sulfate was lost in our dialysis procedure. In some samples, we utilized chondroitin ABC lyase (4.2.2.5) from *Arthrobacter aurescens* which degrades chondroitin-4-sulfate, chondroitin-6-sulfate and hyaluronic acid, but leaves dermatan sulfate, heparan sulfate and heparin (10). Typical densitometer tracings of the cellulose acetate electrophoretic patterns of glycosaminoglycan preparations from a dog 28 days after injury are shown in Fig. 2. The chondroitin ABC lyase removed essentially all of the material with mobility faster than hyaluronic acid. Similarly the chondroitin AC lyase removed the first and third peaks which are considered to be due to hyaluronic acid and chondroitin-4-sulfate. There is some lack of symmetry in both of the residual peaks (ahead of the hyaluronic acid peak and behind the dermatan sulfate peak) which may have indicated the presence of several other components. One component could have been heparan sulfate. In addition, there is a flat peak with a slower mobility than hyaluronic acid (indicated by the arrows in Fig. 2). This peak may have been due to undersulfated glycosaminoglycans. Judd and Wexler (2), using isoproterenol injured rat hearts, reported an increase of the hyaluronic acid fraction two days after injury and a later more sustained elevation of the chondroitin sulfate as indicated by an increase of galactosamine. Our data (derived from the percentage distribution and the uronic acid content) indicated an increase of hyaluronic acid in the 3- to 5.5-day group. The dermatan sulfate and chondroitin-4-sulfate fractions were also elevated in this group. Our data indicated sustained elevations in injured tissue of all glycosaminoglycan fractions after 3 days until at least 30 days after injury. Electrophoretic patterns obtained with the use of calcium acetate electrolyte failed to indicate the presence of chondroitin-6-sulfate. However, it is possible that the glycosaminoglycans obtained after the papain digestion may contain chondroitin-6-sulfate chains still linked by small peptide residues to the chondroitin-4-sulfate chains. In this situation this complex may have the electrophoretic mobility of chondroitin-4-sulfate. The identification of glycosaminoglycans in the fraction with the mobility of chondroitin-4-sulfate is under further investigation.

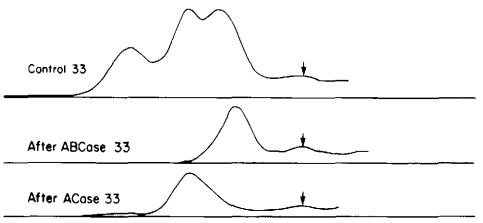

FIG. 2. Densitometer tracings of electrophoretic patterns of an extract from damaged myocardial tissue, 15 days after injury. The top curve is from untreated extract. The large peak to the right is hyaluronic acid, the middle peak is dermatan sulfate and the peak to the left is chondroitin-4-sulfate. The middle curve is from the same extract after treatment with chondroitin ABC lyase (ABCase), while the lower curve is from extract treated with chondroitin AC lyase (ACase). All curves were made of cellulose acetate electrophoresis patterns; calcium acetate electrolyte (0.3 *M*, pH 7.25) was used. A small unidentified peak (indicated by arrow) was not affected by the enzymes. The chondroitin ABCase treatment has essentially left only hyaluronic acid and the chondroitin-ACase has left largely dermatan sulfate.

The major change in glycosaminoglycans following injury to the myocardial tissue is clearly an increase in the chondroitin-4-sulfate fraction. The increase in the 10- to 15-day group, as calculated from the uronic acid level and percentage distribution, was more than 30-fold greater than the corresponding uninjured tissue. At the same time, hyalu-

TABLE I. SUMMARY OF GLYCOSAMINOGLYCANS IN INJURED AND UNINJURED TISSUES OF DOG HEARTS AT VARIOUS TIMES AFTER LIGATION OF A CORONARY ARTERY.

Time		Uronic[a] acid	Hyaluronic acid area[b]	Dermatan sulfate area[b]	Chondroitin-4 sulfate area[b]
Controls (2)		2.13	52.9	45.0	2.1
0 hr (7)	NI[c]	1.69	58.1 ± 3.1	39.2 ± 3.0	2.7 ± .2
1–48 hr (6)	I	3.16	41.4 ± 4.1[d]	55.8 ± 4.3[d]	2.8 ± .7
	NI	2.02	59.1 ± 2.6	38.6 ± 1.9	2.3 ± .3
3–5.5 days (8)	I	3.25	50.4 ± 2.1	36.2 ± 2.8	13.4 ± 1.7[d]
	NI	1.43	53.1 ± 1.8	43.6 ± 2.1	3.3 ± .5
6–9 days (4)	I	5.91	43.7 ± 6.5[d]	33.7 ± 7.4	22.6 ± 1.2[d]
	NI	2.20	63.4 ± 3.9	33.0 ± 4.3	3.5 ± .7
10–15 days (4)	I	6.92	38.1 ± 2.9[d]	34.6 ± 2.4	27.3 ± 2.8[d]
	NI	2.00	61.0 ± 4.9	35.6 ± 4.6	3.4 ± .4
28–30 days (2)	I	8.62	33.4	41.3	25.3
	NI	1.98	42.6	52.1	5.3
90 days (1)	I	4.29	31.6	55.9	12.5
	NI	1.84	40.7	56.0	3.3

[a] Expressed as mg uronic acid/g dry tissue.
[b] Expressed as a percentage of total glycosaminoglycan.
[c] I, injured; NI, noninjured tissues.
[d] Significantly different from corresponding noninjured group at 1% level of significance. Figures following ± sign are SE of the mean. Figures in parentheses ( ), indicate number of animals.

ronic acid and dermatan sulfate levels were approximately doubled in the injured group. The mechanism by which this was accomplished is of great interest and should be investigated as this may provide an important key for the control of wound healing in the heart and other tissues as well. A similar elevation of chondroitin-4-sulfate has been noted in human hypertrophic scars (6) and in human granulation tissue (unpublished). The glycosaminoglycan alterations in the healing myocardial infarction is of interest as one of the current suggested treatments of patients with myocardial infarction involved the use of hyaluronidase (11); this treatment has recently been evaluated using dogs with coronary artery occlusions and was shown to reduce the size of the infarcted area (12). Hyaluronidase may influence healing by affecting glycosaminoglycan metabolism.

The lidocaine used during the surgical part of this work may have influenced the size of the injury as suggested by Gould *et al.* (13), however, it seems unlikely that it had an effect on the healing following the injury. Furthermore, structure and composition of injured areas were compared with uninjured areas from the same heart, consequently, the differences found cannot be due to the effects of lidocaine.

Summary. Experimental myocardial infarcts were produced in mongrel dogs by ligation of the circumflex coronary artery. Surviving animals were killed at times varying from one hour to 90 days after injury. Damaged and undamaged areas of the heart were immediately removed, minced and freeze dried. Samples were digested with papain and the glycosaminoglycans were separated by cellulose acetate electrophoresis and further identified by use of chondroitin AC lyase and chondroitin ABC lyase. The most striking change was a 30-fold increase of the chondroitin-4-sulfate fraction in the injured tissue after 10 days with significant elevations of this component three days postinjury (see Table I). The increased uronic acid together with the electrophoretic distribution of glycosaminoglycans also indicated increased hyaluronic acid and dermatan sulfate fractions in injured tissue. These dramatic changes in glycosaminoglycan composition were supported by morphological studies which demonstrated a brisk fibroblastic activity and a large increase of P.A.S. staining material in the injured tissue at 10 days post injury. By

90 days, the chondroitin-4-sulfate was much decreased but was still elevated as compared with uninjured tissue.

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