PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **165**, 141–146 (1980)

Analysis of the Geometry of Myocardial Infarct Size Reduction with Hyaluronidase[1] (40949)

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Abstract. Although several drugs have been shown to reduce myocardial infarct size the exact location where myocardial salvage occurs remains controversial. In order to study this problem, anesthetized open-chest dogs were randomized into 10 control and 8 treated animals which received hyaluronidase (500 NF units/kg, iv) 15 min, 2 hr, and 24 hr after occlusion of the left anterior descending coronary artery. Twenty-one days after occlusion, the hearts were excised, divided into 1-cm-thick slices, and incubated in triphenyl tetrazolium chloride, and scar size was determined by planimetry. The distance along the circumference (DAC) of the endocardium, midmyocardium, and epicardium which was composed of scar tissue was measured in each slice below the site of occlusion and mean DACs were calculated for each dog. In untreated dogs DACs were composed of scar along the endo-, mid-, and epicardium which were 2.6 ± 0.3, 3.1 ± 0.4, and 3.6 ± 0.5 cm, respectively, compared to hyaluronidase-treated dogs in which the DACs along the endo-, mid-, and epicardium were 1.3 ± 0.3 cm ($P < 0.02$) compared to control; 1.6 ± 0.4 cm ($P < 0.02$); and 0.8 ± 0.4 cm ($P < 0.001$), respectively. Hence, although the infarct reduction was greatest in the epicardial region, there was also reduction in the endocardial and midmyocardial regions. Thus, scar size reduction with hyaluronidase occurs in both lateral and epicardial directions.

It has been shown by several investigators that significant amounts of ischemic myocardium can be protected from necrosis by various pharmacologic interventions (1–7). There has been controversy concerning the location of myocardial salvage. Reimer *et al.* (8) stated that myocardial salvage by reperfusion occurs only in the subepicardial myocardium lying over a nontransmural infarct and postulated that reduction of infarct size occurs only by converting a transmural myocardial infarction to a nontransmural infarction. On the other hand, Jugdutt *et al.* (9) showed that nitroglycerin reduced infarct size both in a lateral and epicardial direction.

The goal of the present investigation was to assess the extent of infarcted tissue in the epicardial, midmyocardial, and endocardial layers in treated and untreated dogs with long-term coronary artery occlusion by using a planimetric technique. Hyaluronidase, an intervention which has been shown to be effective in reducing infarct size in several experimental models of coronary artery occlusion (10–15) as well as in patients (16, 17) was used in the treated group.

Methods. Twenty-two mongrel dogs were anesthetized (sodium thiamylal, 25 mg/kg iv), intubated, and mechanically ventilated. Thoracotomies were performed and the left anterior descending coronary artery was ligated 2 to 2.5 cm from the aorta, just proximal to the first major diagonal branch of the left anterior descending artery. The area of epicardial cyanosis was measured by placing clear cellophane over the heart and tracing the area with a felt-tipped marker. Careful diagrams of the epicardial coronary anatomy were made and from these, eight sites within the distribution of the occluded coronary bed and two sites remote from this region were picked for epicardial electrocardiographic mapping as previously described (12, 15). Mapping was specifically performed prior to coronary artery occlusion and 3 weeks after occlusion for analysis of the QRS complex. The dogs

[1] Supported in part by Grant R01-HL-23140 under the National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland.

0037-9727/80/090141-06$01.00/0

were then randomized by the toss of a coin into untreated (14 dogs) or hyaluronidase-treated groups (eight dogs, 500 NF units/kg iv 15 min after occlusion with the chest open and 2 and 24 hr after occlusion with the chest closed). Their chests were closed and the dogs were allowed to survive for 3 weeks. At the end of this period, a second thoracotomy was performed and epicardial electrocardiographic mapping was performed at those sites selected prior to coronary occlusion, with the drawing as a guide. The amplitude of the R and Q waves at each site was measured before and 3 weeks after coronary artery occlusion.

The hearts were excised and "bread loafed" into 1-cm slices from apex to the site of coronary artery occlusion. The slices were incubated for 30 min in triphenyl tetrazolium chloride, a stain for dehydrogenase enzymes, in order to enhance the contrast between viable and necrotic tissue (18). Clear plastic sheets were placed over the slices and tracings of the infarcted and noninfarcted left ventricle were made on the basal surface of each slice under a magnifying light. The following measurements were performed on each slice of the left ventricle: distance along the circumference (DAC), in centimeters, of infarcted and normal tissue along the endocardium, midmyocardium, and epicardium. The mean DAC of infarcted tissue in the endocardium, midmyocardium, and epicardium for each dog was determined by averaging the slices. In addition, the area of the endocardial half of the slice which was infarcted and noninfarcted was planimetered, as well as the area of the epicardial half of the slice which was infarcted and noninfarcted. The mean area of the endocardial and epicardial halves which were infarcted for each dog was determined by averaging the slices. The overall infarct size was calculated on the basis of the volume of infarct size where gram infarction of each slice was calculated by multiplying the percentage area of infarction of the slice by the weight of the slice. As a second method of measuring volume of infarct size, the weight of the infarcted myocardium also was calculated by cutting each slice into infarcted and noninfarcted myocardium and weighing the tissue.

Results. Of the initial dogs entered into the protocol, four dogs in the untreated group died during the first 24 hr, presumably secondary to arrhythmia. All eight dogs in the hyaluronidase-treated group survived the 3-week period.

The mean weight of dogs in the untreated group was 23 ± 3 kg (±SEM) and in the hyaluronidase-treated group was 25 ± 1 kg (NS). The mean left ventricular weight in the untreated group was 96 ± 6 g, and the mean left ventricular weight in the hyaluronidase group was 98 ± 5 g (NS). The average distance from the aorta to the site of occlusion was similar between the two groups (2.5 ± 0.2 and 2.2 ± 0.2 cm in the control and hyaluronidase-treated dogs, respectively). The epicardial area of cyanosis was similar in control (15.2 ± 1.5 cm^2) and hyaluronidase-treated (15.5 ± 1.3 cm^2) dogs. The mean number of 1-cm heart slices below occlusion was identical in untreated and treated dogs, 5.5 slices per heart.

At 3 weeks the infarcts appeared as pale yellow areas. Histology of some of the slices revealed a mixture of fibrous tissue and necrotic myocardial cells within the infarcts. Hence, the infarction will be referred to as the scar.

In hyaluronidase-treated dogs, the mean DAC which was scar tissue (expressed both in centimeters and as a percentage of the total circumference) of the endocardial, the midmyocardial, and the epicardial layers was significantly smaller than in untreated dogs (Table I and Fig. 1). This effect was most prominent on the epicardial surface where hyaluronidase decreased the percentage of the circumference which was scarred from an average of 26 to 6%. However, the reduction by hyaluronidase of the extent of the scar along the endocardial circumference was also marked, since it decreased scar size from 44 to 19% (Figs. 2 and 3).

The mean area of the subendocardial half of myocardium which was scar tissue in the untreated dogs was 2.1 ± 0.3 cm^2 per slice compared to only 1.2 ± 0.4 cm^2 per slice in

TABLE I

	Infarcted circumference (cm)			Infarcted circumference (%)		
	Endocardium	Midmyocardium	Epicardium	Endocardium	Midmyocardium	Epicardium
Untreated						
A	2.9	3.7	4.3	46	36	29
B	3.5	4.9	6.2	54	47	42
C	2.3	3.3	2.8	44	34	26
D	1.9	1.5	1.7	25	15	12
E	2.3	2.8	3.4	49	37	32
F	1.6	1.9	1.3	29	22	11
G	5.1	5.4	6.1	83	49	38
H	2.5	3.0	4.1	48	34	29
I	2.0	3.0	4.3	30	30	27
J	1.5	1.9	2.2	32	20	17
Mean ± SE	2.6 ± 0.3	3.1 ± 0.4	3.6 ± 0.5	44 ± 5	32 ± 3	26 ± 3
Hyaluronidase-treated						
AA	1.4	1.9	1.1	25	21	9
BB	0.7	1.2	0.9	12	13	7
CC	2.3	3.1	3.3	34	26	18
DD	2.5	3.0	1.3	30	29	11
EE	0.7	0	0	5	0	0
FF	0	0	0	0	0	0
GG	1.1	2.2	0	15	21	0
HH	1.5	1.4	0	28	11	0
Mean ± SE	1.3 ± 0.3	1.6 ± 0.4	0.8 ± 0.4	19 ± 4	15 ± 4	6 ± 2
p	<0.02	<0.02	<0.001	<0.005	<0.005	<0.001

the hyaluronidase-treated dogs ($P < 0.05$). The mean area of the subepicardial half of myocardium which was scar tissue was 2.2 ± 0.3 cm² per slice in the untreated dogs compared to only 0.7 ± 0.4 cm² per slice in the hyaluronidase-treated dogs ($P < 0.01$). Thus, while 35 ± 1% of the subendocardial area became scarred in the untreated dogs, only 16 ± 1% of the subendocardial area became scarred in the hyaluronidase group ($P < 0.01$); and while 26 ± 3% of the subepicardial myocardium became scarred in untreated animals, only 7 ± 3% of the subepicardial myocardium became scarred in hyaluronidase-treated animals ($P < 0.001$).

The volume of scar size was significantly less in the untreated (23 ± 2% of the left ventricles; 22 ± 2 g of scar) compared to the treated group (9 ± 3% of the left ventricle; 9 ± 2 g of scar) when calculated from planimetry and weight of the tissue slices ($P < 0.001$). Scar size was similar when calculated by simply dissecting the scar out

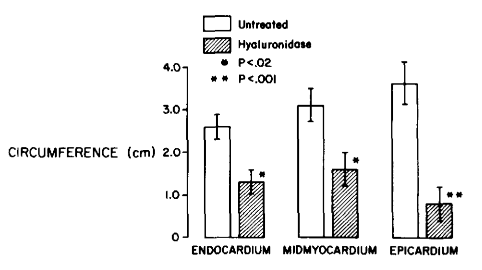

FIG. 1. Bar graft showing the mean centimeters of infarcted circumference along the endocardial surface, along the midmyocardium, and along the epicardial surface. There were significantly fewer centimeters of infarcted tissue along all three circumferences in hyaluronidase-treated animals.

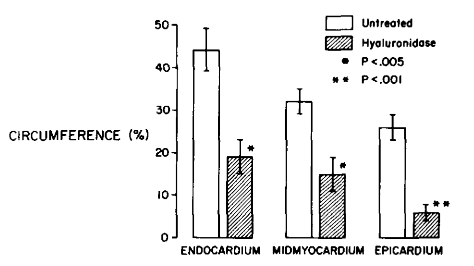

FIG. 2. Bar graft showing the mean percentage of infarcted circumference along the endocardial surface, along the midmyocardium, and along the epicardial surface. The percentage of infarcted circumferences in all three layers was significantly smaller in the hyaluronidase-treated animals.

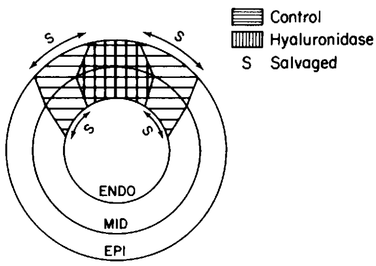

FIG. 3. Composite drawing constructed from the data in Table I showing the mean representation of the untreated and hyaluronidase-treated infarcts along the endocardium (ENDO), the midmyocardium (MID), and the epicardium (EPI). The horizontally hatched area represents the mean untreated and the vertically hatched area represents the mean hyaluronidase-treated infarcts. The subtraction of the two areas marked S is the salvageable border zone. The figure shows that reduction of infarct size as represented by S was greater in the outer wall but that infarction was reduced on the endocardial surface as well.

and weighing it (22 ± 3% of the left ventricle in untreated and 9 ± 3% in treated dogs, $P < 0.002$).

Electrocardiographic results. Epicardial electrocardiographic mapping revealed normal R waves and no pathologic Q waves prior to coronary artery occlusion from the eight sites within the distribution of the occluded left anterior descending (LAD) coronary artery and from the two sites remote from the LAD. At 3 weeks the two sites remote from the LAD were unchanged while sites from the LAD region demonstrated pathologic Q waves and loss of R waves in both groups. However, within the occluded LAD bed the mean depth of the Q waves was 4.7 ± 0.8 mV in control dogs and 1.6 ± 0.5 mV ($P < 0.01$) in hyaluronidase-treated animals; while the average height of the R waves was 3.2 ± 0.8 in the control and 6.6 ± 1.4 mV ($P < 0.05$) in animals which received hyaluronidase.

Discussion. There now is general agreement that the amount of ischemic necrosis which occurs following experimental coronary occlusion can be reduced by a number of interventions (1–7). However, there continues to be debate concerning the question of where the salvage occurs (8, 9).

The purpose of this study was to define the anatomic nature of infarct size reduction after a long-term occlusion by planimetering the extent of myocardial infarction along the endocardium, midmyocardium, and epicardium of untreated and treated animals. The principal finding was that dogs treated with hyaluronidase had scars which were smaller along the endocardial, midmyocardial, and epicardial circumference compared to untreated dogs.

There are several potential possibilities to explain the smaller circumferential dimensions of the scars in the hyaluronidase group. One possibility is that lateral salvage of myocardial tissue may have occurred; another is that infarcts in the untreated group expanded to occupy a larger circumference while more subendocardial infarcts in the hyaluronidase group did not undergo expansion. We favor the former explanation. There is no evidence of infarct expansion or aneurysmal dilitation of the left ventricle in our model of infarction. In fact, when we compared DACs in 14 control dogs with 24 hr of occlusion to control dogs with 3 weeks of occlusion there was evidence of some shrinking of the infarctions rather than expansion. DACs along the endocardium, midmyocardium, and epicardium which were infacted at 24 hr were 2.7 ± .5, 4.8 ± 0.2, and 4.9 ± 0.5 cm, respectively, compared to 2.6 ± 0.3 (P = NS); 3.1 ± 0.4 ($P < 0.001$); and 3.6 ± 0.5 cm ($P < 0.01$) at 3 weeks.

The fact that treated scars were smaller in all three myocardial layers suggests that the infarcts were salvaged to some extent in a lateral direction, but the fact that the salvage was greatest along the epicardium suggests that salvage also occurred in an epicardial direction. This latter finding may be related to the transmural gradient of flow which occurs following coronary occlusion in which the subendocardial flow is most severely depressed and subepicardial flow is least depressed (19–21). Similar results were found recently when other pharmacologic agents were given to dogs with high left anterior descending coronary artery occlusions in our laboratory. The drugs verapamil and ibuprofen both reduced the extent of infarction following a 24-hr occlusion along the endocardium, midmyocardium, and epicardium (22). Again, salvage was most prominent in the epicardium. Jugdutt *et al.* also observed that nitroglycerin decreased infarct size in both a lateral and subepicardial direction (9), while indomethacin increased infarct size in both a lateral and subepicardial direction (23) in the conscious dog model. Jugdutt *et al.* (24) used postmortem angiography and examination of gross pathology to define the anatomic region of risk for developing infarction. Although in our study we did not use this more complex technique, our results of reduction in scar tissue with pharmacologic interventions yielded very similar results in the anesthetized dog model.

On the other hand, Reimer *et al.* (8), using temporary circumflex coronary occlusions followed by reperfusion, found that salvageable myocardium produced by reperfusion occurred only in the epicardial half of the myocardium overlying a nontransmural infarction and not along the lateral edge of the myocardium. In a recent study we found that coronary reperfusion following temporary left anterior descending coronary artery occlusions also resulted in subepicardial salvage compared to permanent coronary ligation, but there was no apparent salvage along the lateral edge of the infarction (25). Thus, it appears that beneficial pharmacologic interventions may result in a decrease in infarct size both in a lateral and epicardial direction. On the other hand, reperfusion appears to result in a decrease in infarct size only in an epicardial direction. The location where myocardial salvage occurs may not only be of theoretical but also of practical importance. It is possible that when a scar is reduced in a lateral direction it results in a different ventricular function and long-term prognosis than when a scar is converted from a transmural to a nontransmural location.

The epicardial measurement of infarction in the control group appeared greater than the endocardial measurement when expressed as centimeters alone; (Table I) although this was not statistically significant. However, when examined as a percentage of the total circumference, the opposite was true—the percentage of the endocardial circumference which was infarcted was greater than the percentage of the epicardium which was infarcted ($P < 0.01$). This may seem somewhat paradoxical but can be explained by the circular geometry of left ventricular slices (Fig. 3) where the infarct does appear to occupy a large percentage of the endocardial half and a smaller percentage of the epicardial half, largely because the endocardial aspect of the slice composes a smaller circumference than the epicardial aspect of the slice, just as the circumference of the inside lane of a racetrack is always less than the outside lane.

Although no area at risk measurement by injections of dyes or barium down the coronaries was performed in this particular study the extent of epicardial cyanosis was measured shortly after occlusion and prior to drug therapy. The extent of epicardial cyanosis corresponds to the area of epicardial ST segment elevation (15) and also to the extent of low blood flow as manifested by nonfluorescence along the epicardial surface of the heart when fluorescein is injected into the left atrium of dogs with coronary artery occlusions (26). Recently, we have used dye injections to measure areas at risk of developing infarction during shorter-term occlusions and have found results similar to this study, that is, myocardial salvage with beneficial pharmacologic agents occurred in both lateral and subepicardial directions (26).

We gratefully acknowledge the technical assistance of Sharon Hale and Joseph Gannon and the secretarial assistance of Nancy Watterson and Charlotte Ferguson.

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Received December 27, 1979. P.S.E.B.M. 1980, Vol. 165.