Altered Distribution of Tritiated Digoxin in the Infarcted Canine Left Ventricle

By George A. Beller, M.D., Thomas W. Smith, M.D., and William B. Hood, Jr., M.D.

SUMMARY
In order to determine the distribution pattern of digoxin in infarcted and ischemic tissue, the early uptake of tritiated digoxin (3H-digoxin) in the acutely and chronically infarcted canine left ventricle (LV) was studied. Seven open-chest anesthetized dogs were given 3H-digoxin intravenously 1 hour following acute anterior-wall infarction produced by serial coronary arterial branch ligations. Central and peripheral zones of infarction, ischemic border zones, and nonischemic myocardium were demarcated by epicardial electrode mapping. Two hours after 3H-digoxin administration, animals were sacrificed and epicardial and endocardial samples from each zone were analyzed for 3H-digoxin uptake. Significantly lower digoxin concentrations (ng/g wet weight ± SEM) were found in both endocardial (endo) and epicardial (epi) regions of the center (endo 27 ± 11; epi 58 ± 10) and periphery (endo 51 ± 13; epi 112 ± 13) of the infarct as compared to the border (endo 217 ± 21; epi 242 ± 15) and nonischemic (endo 256 ± 22; epi 220 ± 20) zones. Significant transmural gradients of the glycoside observed in infarct zones (endo/epi ratio 0.46) were the reverse of those found in nonischemic portions of the myocardium (endo/epi ratio 1.16). Four dogs with chronic infarcts showed 3H-digoxin uptake patterns similar to acutely infarcted animals. In four sham-operated dogs, 3H-digoxin uptake was homogeneous in all areas of the LV. This marked alteration of early 3H-digoxin uptake by the infarcted LV may predispose to electrical instability and the genesis of ectopic rhythm disturbances. The pattern of LV digoxin uptake appears to reflect, at least in part, regional blood flow to infarcted, ischemic, and nonischemic tissue.

Additional Indexing Words:
Cardiac glycoside Ischemia
Serum digoxin concentration Regional myocardial blood flow

The use of digitalis glycosides in acute myocardial infarction remains controversial, as discussed in recent reviews of the subject.1,2 Experimental myocardial infarction studies have demonstrated a lower threshold for digitalis-toxic rhythm disturbances,3-8 and there is evidence suggesting that patients with coronary atherosclerotic heart disease may experience toxicity at lower serum concentrations of digoxin or digitoxin than patients without coronary artery disease.9,10 Although there is a substantial amount of information available defining the distribution of digoxin in the nonischemic heart,11-13 few data are available concerning alterations in

From the Thordhike Memorial Laboratory, Harvard Medical Unit, Boston City Hospital, The Cardiac Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

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Address for reprints: Dr. George A. Beller, Thordhike Memorial Laboratory, Boston City Hospital, 818 Harrison Avenue, Boston, Massachusetts 02118.

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this pattern in the acutely or chronically ischemic or infarcted heart. The purpose of the present investigation was to study the early uptake of tritiated digoxin in the acutely and chronically infarcted canine left ventricle, in order to determine the distribution pattern of the glycoside in infarcted and ischemic tissue.

Materials and Methods

Left ventricular myocardial uptake patterns of intravenously administered randomly labeled tritiated digoxin (3H-digoxin; specific activity 123 μCi/mg)* were investigated in a canine model. Thin-layer chromatography of the 3H-digoxin used in this study (silica gel G, using 49:2:49 acetone:glacial acetic acid:cyclohexane as the developing solvent) showed a single peak which contained more than 95% of the counts applied, and had an R f identical to that of native digoxin. Fifteen mongrel dogs (mean weight 23.4 kg ± 1.3 SEM) were divided into three study groups.

1. Acute Myocardial Infarction Group

This group comprised seven dogs which underwent left thoracotomy under pentobarbital anesthesia (30 mg/kg) on the day of the study. Respiration was maintained with a positive-pressure Bennett Respirometer (Bennett Respirometer Products, Inc., Santa Monica, California). All animals breathed 40% oxygen throughout the experiment in order to prevent the development of hypoxia which might have ensued during prolonged anesthesia. The heart was suspended in a pericardial cradle, after which 2-0 silk ligatures were placed upon multiple branches of the left anterior descending and circumflex coronary arteries supplying a confluent area of the apex and anterior wall of the left ventricle (fig. 1, A and B).

For each dog, 10–12 mapping areas were designated on the anterior and lateral surface of the left ventricle (fig. 1A). Control electrocardiographic epicardial surface mapping at each site was then performed utilizing a stainless-steel cylinder 8 cm long and 2.5 mm in diameter, the tip of which was smoothly ground and polished. The cylinder was attached to the exploring electrode of the conventional electrocardiographic lead system. This electrode was held lightly at a right angle to the epicardium during the surface mapping procedure. The technic utilized here was similar to that described by Maroko et al.14 with some minor modifications. The tip of the electrode was dipped into normal saline at room temperature prior to recording at each site. In no instance during these experiments was there evidence of myocardial injury demonstrable in the control epicardial electrocardiograms which might have resulted from dissection of the coronary artery branches or from trauma to the epicardial surface induced by the tip of the electrode. Epicardial electrograms were standardized at 1-mm/mv stylus deflection.

A femoral artery was isolated and a catheter was passed into the central aorta. Arterial pressure was monitored with a Statham P23Db pressure transducer. Both arterial pressure and electrocardiographic recordings were made on a two-channel Sanborn recorder at a paper speed of 50 mm/sec.

Following control recordings, serial coronary arterial branch ligations were performed at 10–15-min intervals until approximately one third of the anterior surface of the left ventricle exhibited S-T-segment elevation in the epicardial electrocardiograms. This required from three to six ligatures. After ligations were completed, surface mapping was again performed at the same sites mapped previously in the control period. Three distinct zones were delineated (fig. 1B): (1) an “infarct” zone of S-T-segment elevation (ST) greater than 1 mv; (2) a border zone of ischemic S-T-segment depression (ST) greater than 1 mv; and (3) a nonischemic zone within which S-T segments were isoelectric. One hour after the last ligation, 1 mg of 3H-digoxin diluted in 1.0–3.0 ml of 95% ethanol was administered to each dog over a 1-min period via a foreleg vein. Blood samples for serum 3H-digoxin concentration determination were obtained from the femoral artery before and 15, 30, 60, 90, and 120 min after the administration of the drug in five of the seven animals. Two hours after 3H-digoxin was given (3 hours after infarction), repeat epicardial electrograms and pressure recordings were made and central and peripheral infarct zones of ST, ischemic border zones of ST, and nonischemic zones were again delineated. The hearts were then rapidly excised, and multiple full-thickness myocardial samples weighing between 0.5 and 1.0 g from each of the three zones were obtained (fig. 1C). These samples were then each divided into endocardial and epicardial halves for myocardial 3H-digoxin determinations.

2. Chronic Infarction Group

This group comprised four dogs which had myocardial infarctions experimentally produced 1 week, 29 days, 4 months, and 9 months prior to study. In these animals infarction was produced

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Figure 1

(A) Diagrammatic representation of the canine heart. Twelve epicardial mapping areas are designated on the anterior and lateral surfaces of the left ventricle (B) Acutely infarcted canine left ventricle showing infarct zone of S-T-segment elevation (ST) and border zone of S-T-segment depression (ST). Arrows indicate sites of ligation of branches of the left coronary system in this example. (C) Myocardial sampling technic. Samples taken from the center and periphery of the infarct, from the contiguous border zone, and from nonischemic posterior wall were analyzed for 3H-digoxin concentration.

as previously described15 by inflating a balloon cuff device implanted on the left anterior descending coronary artery. On the day of study, a thoracotomy was performed after which epicardial surface mapping and arterial pressure recordings were done. One mg of 3H-digoxin was administered in a manner identical to that described for group 1 dogs. Serial arterial blood samples for serum 3H-digoxin were obtained before and 15, 30, 60, 90, and 120 min following the injection of the glycoside. One hundred twenty minutes after digoxin administration, the hearts were removed, and myocardial sampling was carried out as described above. Infarct zones were identified by inspection of gross anatomic changes as well as by epicardial electrocardiograms. Epicardial complexes from the center of chronic infarcts revealed wide Q waves, prolonged QRS duration, and decreased amplitude of the R wave. Persistence of ST in the center of the infarct was seen only in the dog with an infarct of 1-week duration. Border zones adjacent to the chronic infarct regions did not show ST comparable to that seen in the acutely infarcted hearts.

3. Sham-Operated Control Group

This group was composed of four dogs that received 1 mg of 3H-digoxin intravenously in a foreleg vein following a left thoracotomy performed under pentobarbital anesthesia. As done in groups 1 and 2, serial femoral arterial blood samples were drawn for serum 3H-digoxin concentrations, and 120 min after 3H-digoxin administration the hearts were removed. Endocardial and epicardial specimens were obtained from the apex, base, and posterior wall of the left ventricle for myocardial 3H-digoxin concentration measurements.

Myocardial and Serum 3H-Digoxin Measurements

Left ventricular endocardial and epicardial samples (fig. 1C) were gently blotted and
immediately refrigerated at 4°C. Soon afterward the specimens were weighed, cut into small slivers 1–2 mm in length, and placed into vials containing 15 ml of the liquid scintillation medium described by Bray.16 Following vigorous shaking for 24 hours in a water bath at 37°C C to elute 3H-digoxin from the samples, the vials were counted in a liquid scintillation spectrometer. Efficiency of extraction of tritiated digoxin was 96–99% as determined in parallel experiments comparing this technic with total solubilization of the tissue using NCS.* These values were also confirmed by counting the extracted myocardial fragments following complete solubilization in NCS. Samples solubilized in NCS were counted using a toluene-based scintillation fluid (5.5 g 2.5 diphenyloxazole, 0.1 g p-bis [2-(5-phenoxyazo- lyl)]-benzene, 667 ml toluene, 333 ml Triton X-100) and were recounted with 3H-digoxin internal standards for quenching correction. 3H-digoxin concentrations of samples extracted and recounted in Bray’s solution were also quantitated by recounting after the addition of internal standards of 3H-digoxin. 3H-digoxin concentrations in serum samples were determined by counting 1-ml serum samples in 15 ml Bray’s solution after separation of precipitated protein by centrifugation. Quenching correction was accomplished by the use of internal standards of 3H-digoxin.

**Statistical Analysis**

All data are presented as mean values ± SEM; significance of differences was assessed by the paired t test.17

**Results**

1. Acute Myocardial Infarction Group

Endocardial and epicardial digoxin concentrations (ng/g wet weight ± SEM) in the center and periphery of the ST zone of infarction, in the ST ischemic border zone, and in the nonischemic posterior-wall region are shown in table 1. There was a significant decrease in digoxin uptake in the endocardial and epicardial halves of the center and periphery of the infarct as compared to the border and nonischemic zones (P < 0.05). There was also a significant gradient of digoxin concentration in both endocardium and epicardium from the center of the infarct to the periphery of the infarct zone with a greater uptake in the latter (P < 0.05). A significant transmural gradient of digoxin was demonstrable between endocardial and epicardial layers of both infarct zones, with less uptake in endocardium (P < 0.05).

<table>
<thead>
<tr>
<th>Site</th>
<th>Nonischemic (N = 7)</th>
<th>Border (N = 7)</th>
<th>Periphery infarct (N = 6)</th>
<th>Center infarct (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocardial</td>
<td>256 ± 22*</td>
<td>217 ± 21</td>
<td>51 ± 13*†‡</td>
<td>27 ± 11*†‡§</td>
</tr>
<tr>
<td>Epicardial</td>
<td>220 ± 20</td>
<td>242 ± 15</td>
<td>112 ± 13†‡</td>
<td>38 ± 10†‡§</td>
</tr>
<tr>
<td>Endocardial/epicardial ratio</td>
<td>1.16</td>
<td>0.89</td>
<td>0.46</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*Compared to epicardium.
†Compared to nonischemic.
‡Compared to border.
§Compared to periphery.

There was no statistically significant difference in digoxin concentrations of either endocardial or epicardial layers of the border zone as compared to the nonischemic region; however, the endocardium of the border zone showed a lower mean digoxin concentration as compared to the epicardium of this zone which was of borderline significance (0.05 < P < 0.10). In nonischemic myocardium, endocardial digoxin concentration was slightly but significantly greater than epicardial concentration with an endocardial/epicardial ratio of 1.16.

Thus, in the acutely infarcted canine left ventricle there was a decreasing concentration of digoxin from nonischemic to border to infarct zones, which was more pronounced in endocardial layers. Of particular interest was an abrupt change in 3H-digoxin concentration.


*Circulation, Volume XLVI, September 1972
Table 2

Myocardial $^3$H-Digoxin Concentrations (ng/g ± SEM) in Dogs with Chronic Infarction

<table>
<thead>
<tr>
<th>Site</th>
<th>Nonischemic (N = 4)</th>
<th>Border (N = 4)</th>
<th>Infarct (N = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocardial</td>
<td>260 ± 13*</td>
<td>185 ± 30</td>
<td>70 ± 38†‡</td>
</tr>
<tr>
<td>Epicardial</td>
<td>225 ± 12</td>
<td>174 ± 23</td>
<td>102 ± 32†</td>
</tr>
<tr>
<td>Endocardial/epicardial ratio</td>
<td>1.16</td>
<td>1.06</td>
<td>0.68</td>
</tr>
</tbody>
</table>

$P < 0.05$:
*Compared to epicardium.
†Compared to nonischemic.
‡Compared to border.

between infarct and border zones, with concentration differences up to two- or threefold in magnitude frequently observed in adjacent 1-cm cubes of myocardium. The transmural gradients of the glycoside observed in border and infarct zones were the reverse of those found in nonischemic portions of the myocardium.

2. Chronic Infarct Group

Digoxin uptake patterns in the infarct, border, and nonischemic areas of the four chronically infarcted animals are shown in table 2, and are similar to those observed in the acutely infarcted dogs except for an endocardial/epicardial ratio of $^3$H-digoxin concentration of 1.06 in the border zone, which is similar to that seen in nonischemic regions of the myocardium. This is in contrast to $^3$H-digoxin concentration in the border zone of acutely infarcted hearts in which the normal ratio was reversed.

3. Sham-Operated Control Group

Digoxin concentrations in the apex, base, and posterior wall for the four control animals are shown in table 3. There was no significant difference in digoxin uptake in these three regions indicating a homogeneous distribution of the glycoside in the intact canine left ventricle. There was, however, significantly greater digoxin uptake in the endocardial layers of these regions with an average endocardial/epicardial ratio of 1.08 ($P < 0.05$) which was quite similar to that observed in the nonischemic zones of the acute and chronic infarction groups, and in the border zone of chronically infarcted hearts.

Serum Digoxin Concentrations

Serum $^3$H-digoxin concentrations 15, 30, 60, 90, and 120 min after administration of the drug to dogs in the three groups are shown in figure 2 (mean ± SEM). There was no significant difference in the mean serum $^3$H-digoxin levels at any of the times sampled between the infarct (acute and chronic) and control groups.

Arterial Blood Pressure

There was no significant difference in the mean aortic pressure at the time of sacrifice in the acute and chronic infarct groups (107 mm

Table 3

Left Ventricular Myocardial $^3$H-Digoxin Concentrations (ng/g ± SEM) in Sham-Operated Dogs

<table>
<thead>
<tr>
<th>Site</th>
<th>Apex (N = 4)</th>
<th>Base (N = 4)</th>
<th>Posterior wall (N = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocardial*</td>
<td>246 ± 31</td>
<td>249 ± 31</td>
<td>257 ± 30</td>
</tr>
<tr>
<td>Epicardial</td>
<td>227 ± 21</td>
<td>231 ± 27</td>
<td>236 ± 28</td>
</tr>
<tr>
<td>Endocardial/epicardial ratio</td>
<td>1.08</td>
<td>1.08</td>
<td>1.09</td>
</tr>
</tbody>
</table>

*P < 0.05 for mean endocardial concentration from apex, base, and posterior wall (N = 12) compared with epicardial mean for all three regions.
DISTRIBUTION OF DIGOXIN

Figure 2

Serum ³H-digoxin concentrations (mean ± SEM) over a 2-hour period following intravenous administration of 1.0 mg of the drug to acutely and chronically infarcted, and sham-operated dogs (N = 13).

Hg ± SEM, as compared to the predigitalization values (104 mm Hg ± SEM).

Discussion

The study reported here showed a marked alteration of ³H-digoxin uptake by the acutely and chronically infarcted left ventricle of dogs. ³H-digoxin uptake in all regions of the normal left ventricle was homogeneous, with a slightly but significantly greater uptake by the endocardial layers. Very similar endocardial/epicardial ratios of digoxin concentration were also found in the nonischemic myocardial specimens from both acute and chronic infarct groups, ranging from 1.08 to 1.16. In the center and periphery of the infarct zones in the acutely infarcted hearts the endocardial/epicardial digoxin gradient was reversed, with a ratio of 0.46. Although the mechanism for this distribution pattern cannot be stated with certainty, left ventricular myocardial digoxin uptake appears to reflect, at least in part, the regional blood flow to infarcted, ischemic, and nonischemic tissue. Other studies utilizing radioisotope-labeled carbonized microspheres21 or ¹³¹I-iodoantipyrine21 have shown similar patterns of marker distribution in the normal and acutely ischemic canine left ventricle. These latter studies demonstrated that there was a greater distribution of radioactivity per gram of normal left ventricular subendocardial muscle than that of subepicardial muscle with endocardial/epicardial ratios varying from 1.08 to 1.3. Following acute occlusion of the left circumflex artery, endocardial/epicardial radioactivity decreased from 1.17 to 0.76 in the ischemic zone.¹⁸ The present study showed an even greater reduction in subendocardial radioactivity in an infarcted area when ³H-digoxin was given with an endocardial/epicardial ratio of digoxin down to 0.46 from 1.16 in the nonischemic zone. This may have been a result of larger infarcts produced in the present study.

Additional factors which would tend to minimize ³H-digoxin uptake by acutely ischemic tissue include high local extracellular potassium concentration²² resulting from cellular injury and loss of intracellular potassium.²³, ²⁴ Also, limitation of availability of ATP might be a factor since ATP is known to be necessary for optimal binding of cardiac glycosides to Na⁺, K⁺-activated ATPase in the presence of physiologic concentrations of Na⁺, K⁺, and Mg²⁺.²⁵-²⁷

Of particular interest in this study, in addition to the marked transmural gradients of digoxin concentration in the infarcted myocardium, were pronounced gradients of digoxin from the center to the periphery of the infarct and from the infarct periphery to nonischemic tissue. It has been shown that in ischemic myocardial tissue, adjacent fibers or fiber groups may repolarize at disparate times which may promote reentrant activity responsible for ectopic rhythms.²⁸-³¹ In addition, during myocardial infarction the presence of hypoxia, catecholamine accumulation, and fiber stretch may increase the rate of spontaneous diastolic depolarization in His-Purkinje cells resulting in enhanced automaticity.²⁸ Since digitalis appears to facilitate ectopic activity by enhanced automaticity in Purkinje fibers,³² focal reexcitation due to increased asynchrony of recovery in ventricular muscle,³⁰ and local reentry resulting from depressed conductivity in the His-Purkinje system,³³ electrophysiologic effects similar to and perhaps additive to those associated with myocardial ischemia might be expected. It is conceivable that the marked gradients of
digoxin concentration observed may potentiate this already unstable electrophysiologic situation. Concentrations of $^3$H-digoxin in Purkinje fibers were not specifically determined, however, and cannot be assumed to follow a pattern identical to that of myocardial samples.

In this study, only the early uptake of $^3$H-digoxin in infarcted, ischemic, and nonischemic myocardium was assessed, at 2 hours following administration of the glycoside. Although it is not certain that the pattern of myocardial distribution would be the same after full equilibration with body tissues, Deutscher, Harrison, and Goldman have recently observed a plateau in $^3$H-digoxin concentration of nonischemic canine myocardium extending from 60 through 240 min after infusion of the drug. The proposed explanations for the mechanism of altered $^3$H-digoxin uptake in acutely infarcted myocardium may not be entirely applicable to the chronically infarcted heart. Diminished uptake of the glycoside in chronic infarcts may be related to the replacement of myocardial fibers by connective tissue which does not appreciably bind digitalis.

In conclusion, this study demonstrated marked alteration of early digoxin uptake in the acutely and chronically experimentally infarcted canine left ventricle compared with normally perfused controls. The differing magnitude of the electrophysiologic effects of digitalis in adjacent tissues which may result from the quantitative differences in digoxin concentrations in these regions, combined with the adverse effects of ischemia, may contribute to the reported increase in sensitivity to digitalis glycosides in myocardial infarction and coronary atherosclerotic heart disease in general. $^3$H-digoxin also appeared to be a good marker of regional blood flow. The early distribution of the glycoside in infarcted, ischemic, and intact myocardium observed in this study correlates well with prior observations of regional flow under similar conditions utilizing different techniques.

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