Digoxin Sensitivity in Amyloid Cardiomyopathy

ALAN RUBINOW, M.D., MARTHA SKINNER, M.D., AND ALAN S. COHEN, M.D.

SUMMARY Digoxin (5 mg/ml) was added to 10-mg and 20-mg pellets of purified primary and secondary amyloid fibrils, a normal human liver and heart homogenate and a homogenate from the heart of a patient with amyloid cardiomyopathy who had not received digitalis. After centrifugation, the supernatants were recovered and assayed for digoxin concentrations. Aliquots from the sediments were studied for the presence of digoxin, using rabbit antidigoxin antiserum and an indirect immunofluorescent technique. The results showed that 0.11–0.13 ng/ml of digoxin bound per milligram of fibrils and could not be separated by repeated washings. Elution with citrate or changes in the pH of the buffer. Immunofluorescent studies demonstrated diffusely bright immunofluorescence with the fibril preparation and amyloid heart homogenate when reacted with digoxin and digoxin-specific antiserum. These studies demonstrate that isolated amyloid fibrils bind digoxin and suggest that this interaction may play some role in the sensitivity to digitalis that has been observed in some patients with amyloid cardiomyopathy.

THE PATHOPHYSIOLOGIC ABNORMALITIES that result from amyloid deposition are thought to be caused by replacement of functional tissue by the amyloid fibrils, which have been considered to be inert. However, the affinity of the amyloid fibril for various dyes, including Congo red and Alcian blue, and the recent studies that show selective binding to amyloid fibrils of certain proteins, i.e., P-component, blood clotting factors and the bone-seeking radio-nuclide technetium methylene diphosphonate, suggest that the fibrils may play a more complex role in various organs.

All clinical forms of amyloidosis can involve the heart, which may cause abrupt changes in cardiac rhythm or sudden death. In an effort to understand why digitalis preparations could affect the amyloid-laden heart, an in vitro study was carried out to assess the interaction of isolated amyloid fibrils and digoxin, and in particular, to determine whether isolated amyloid fibrils bind digoxin.

Methods

Fibril Preparation

Amyloid fibrils were isolated from the spleens of two patients with primary amyloidosis and one with secondary amyloidosis as described elsewhere. The fibrils were washed three times in Tris-buffered saline (TBS) 0.01 M, pH 8.0, containing 10 mM EDTA to free them from any calcium-bound proteins. They were then dialyzed exhaustively against distilled water and lyophilized.

Digoxin Preparations and Assays

Digoxin, 1 mg/ml (Lanoxin, Burroughs Wellcome) was diluted in deionized water to a concentration of
2.5 ng/ml as measured by a radioimmunoassay (RIA) using 125I digoxin (New England Nuclear) and a competitive binding technique.\textsuperscript{10} Antidigoxin antibody prepared in rabbits with digoxin-protein conjugates\textsuperscript{11} was obtained from Behring Laboratories.

**Binding Experiments**

Digoxin (5 ng/ml) was mixed with 10-mg and 20-mg pellets of lyophilized primary or secondary amyloid fibrils in TBS or TBS containing calcium (0.002 \( M \)) and left at room temperature for 90 minutes. The mixtures were centrifuged and the first supernatants removed as completely as possible. The sediments were washed in TBS, centrifuged and the second supernatant was separated. The sediments were then eluted for 15 minutes at room temperature with 1.0 ml of TBS containing 0.05 \( M \) trisodium citrate, centrifuged and the supernatants recovered. These experiments were repeated using TBS at pH of 5.0, 6.0, 6.5, 7.2, 8.0 and 9.0. As control experiments, digoxin, 5.0 ng/ml, was incubated with normal human liver and heart homogenates. EDTA-washed fibrils were carried through the experimental procedure without the addition of digoxin.

In additional experiments, 5.0 ng/ml of digoxin was offered to 10-mg pellets of amyloid fibrils, a human heart homogenate and a homogenate of the heart of a patient with amyloid cardiomyopathy who had not received digitalis. The pellets and the digoxin were left at room temperature for 15, 30, 60, 90 and 120 minutes, 6 hours and 24 hours, and were then eluted as above. The concentration of digoxin in all supernatants and eluates was determined by RIA.

**Immunofluorescent Studies**

An indirect immunofluorescent study was performed. Aliquots from each sediment of amyloid fibrils and the normal liver and heart homogenates were washed with phosphate-buffered saline (PBS), pH 7.2, air dried and mounted on glycerol-treated microscopic slides. Rabbit antidigoxin antiserum was diluted 1:4 in PBS, applied to the preparations and left at room temperature for 20 minutes. Each slide was washed three times with PBS for 5 minutes and partially dried. Goat antirabbit IgG conjugated with fluorescein (Atlantic Antibodies) diluted 1:4 in PBS was applied to each specimen and allowed to stand at room temperature for 20 minutes. Slides were again washed three times with PBS, dried and viewed for positive immunofluorescence using a Zeiss epi-fluorescent microscope. Controls included preparations with normal rabbit serum substituted for digoxin antiserum.

**Results**

In repeated experiments, digoxin was found to bind to isolated primary and secondary amyloid fibrils in vitro (table 1). Of the 5.0 ng/ml of digoxin added to 10 mg of primary amyloid fibril pellets, only 3.6–3.8 ng/ml were recovered. There was a twofold increase in the amount of unrecovered digoxin when 20 mg fibril pellets were used. Similar findings were observed with secondary amyloid fibril preparations. Approximately 0.11–0.13 ng/ml of digoxin bound per milligram of fibrils and could not be separated by repeated washings with TBS. The addition of calcium ions to the buffer did not enhance binding, and digoxin could not be displaced from the fibrils by elution with trisodium citrate. The degree of digoxin binding was not affected by changes in the pH of the buffer. No significant binding of digoxin to the liver and heart homogenates could be demonstrated.

In additional experiments, almost no uptake of digoxin by the heart homogenate was demonstrated over the 24-hour study period (fig. 1). However, a marked affinity of isolated amyloid fibrils for digoxin was noted: 0.13 ng/ml of digoxin were bound per milligram of fibrils after 15 minutes. Thereafter, the amount of bound digoxin increased only to 0.17 ng/ml per milligram of fibrils after 24 hours. The binding of digoxin to the homogenate of cardiac muscle with amyloid deposits was negligible during the first 90

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Weight/fibrils (mg)</th>
<th>Digoxin offered (ng/ml)</th>
<th>Digoxin recovered after 90 min. (ng/ml)</th>
<th>Digoxin bound (ng/sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1st supernatant</td>
<td>2nd supernatant</td>
</tr>
<tr>
<td>Primary fibrils</td>
<td>10</td>
<td>5.0</td>
<td>3.6–3.8</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Primary fibrils</td>
<td>20</td>
<td>5.0</td>
<td>2.4–2.5</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Secondary fibrils</td>
<td>10</td>
<td>5.0</td>
<td>3.9</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Secondary fibrils</td>
<td>20</td>
<td>5.0</td>
<td>2.9</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Amyloid heart</td>
<td>10</td>
<td>5.0</td>
<td>4.5</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal liver</td>
<td>10</td>
<td>5.0</td>
<td>4.9</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Normal heart</td>
<td>10</td>
<td>5.0</td>
<td>4.7</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Primary fibrils</td>
<td>10</td>
<td>None</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Secondary fibrils</td>
<td>10</td>
<td>None</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
</tr>
</tbody>
</table>
minutes, but a steady increase in binding was observed, reaching 1.0 ng/ml bound digoxin per milligram of homogenate after 24 hours.

Immunofluorescent studies demonstrated a diffusely bright immunofluorescence pattern throughout the fibril preparation and the amyloid heart homogenate reacted with digoxin and digoxin-specific antiserum (table 2). Preparations containing only amyloid fibrils and antidigoxin antibody were negative. In addition, specimens that contained fibrils and digoxin to which normal rabbit serum was applied instead of rabbit antidigoxin revealed no immunofluorescence. A normal heart homogenate reacted with digoxin revealed trace amounts of fluorescence with rabbit antidigoxin serum.

**Discussion**

The binding to amyloid fibrils of serum proteins, drugs or metabolites and its significance in causing functional disturbances in amyloidosis have not been addressed. The affinity of the amyloid fibril for Congo red and other cotton dyes is well recognized, and recent studies indicate that certain proteins, i.e., P-component and blood clotting factors, do bind specifically to amyloid fibrils in a calcium-dependent fashion.

We have now demonstrated that a significant amount of digoxin binds to isolated amyloid fibrils in proportion to their dry weights. The binding did not show pH dependence in the range of 5.0–9.0, was not enhanced by a calcium buffer and the digoxin could not be eluted from the fibrils by citrate as has been demonstrated for other proteins. The affinity of the fibrils for digoxin was very striking, the majority being bound after 15 minutes. In contrast, a homogenate of cardiac muscle that contained amyloid deposits was shown to bind digoxin, albeit with less affinity than isolated fibrils, but significantly more than a normal cardiac muscle homogenate. The presence of digoxin on the amyloid fibril and in the amyloid heart homogenate was confirmed by positive indirect immunofluorescence, as shown with antibody to digoxin. There was no difference in all these experiments between the binding of digoxin to primary and secondary fibrils.

Amyloid consists of fine, rigid, nonbranching fibrils that aggregate laterally and have a cross beta configuration as revealed by x-ray diffraction. In primary amyloidosis, the protein moiety consists of portions of, or whole, immunoglobulin light chains. A new nonimmunoglobulin protein, AA, is the major component of secondary amyloidosis. These proteins are large polyanions, with aspartic acid and glutamic acid making up 20% of the total amino acid content. In addition, mucopolysaccharides originating from the adjacent connective tissue may be associated with the amyloid protein. Our data indicate no difference between the binding of primary and secondary fibrils with digoxin and suggests that their polyanionic configuration rather than primary structure may be a factor in this interaction. Clearly, potential binding sites are located on the protein and mucopolysaccharide moieties of the amyloid fibril.

The receptors for digitalis are located on the outer surface of the cell membranes of myocardial cells. The therapeutic activity of digitalis depends on its concentration at these active binding sites rather than in the plasma and only a minute quantity of glycoside is required for an inotropic effect. Consequently, any factor that influences the affinity of binding sites for glycosides, be it increased myocardial uptake or impaired excretion, will result in greater cardiac glycoside-receptor interaction.

Amyloid is deposited extracellularly in the heart and occurs initially in the interstitial areas closely

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**Table 2. Fluorescent Staining with Rabbit Antidigoxin Serum and Normal Rabbit Serum**

<table>
<thead>
<tr>
<th></th>
<th>RAD</th>
<th>NRS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With digoxin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary fibrils</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Secondary fibrils</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Amyloid heart homogenate</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Normal heart homogenate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal liver homogenate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Without digoxin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary fibrils</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Secondary fibrils</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amyloid heart homogenate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal heart homogenate</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: RAD = rabbit antidigoxin serum; NRS = normal rabbit serum; - = negative; ± = trace; ++ = moderate staining; +++ = heavy staining.
adherent to the myocardial cells. Consequently, the binding of digoxin to the amyloid fibril may increase local tissue levels in close proximity to the specific receptors located on the outer surface of the cell. Such binding would not necessarily destroy those radicals on the digoxin molecules, i.e., the lactone rings essential for its pharmacologic activity. On the other hand, binding may impair excretion and prolong the exposure of the glycoside to the receptor.

All clinical forms of amyloidosis can involve the heart. Deposits are particularly prominent in primary and in myeloma-associated amyloidosis as well as in heredofamilial types. Focal deposits are extremely common in the aged. Cardiac arrhythmias, conduction disturbances and indeed sudden death have been attributed to amyloid infiltration of the heart. Although a recent study did not correlate the incidence and severity of electrocardiographic abnormalities with amyloid deposits in the conducting system, 95% of the patients described in that study were in congestive heart failure and presumably on digitalis therapy. However, the deleterious effects of digoxin on the amyloid-laden heart were not mentioned.

We postulate that selective binding of digoxin to amyloid fibrils may enhance the severity of those disturbances of cardiac muscle previously attributed to amyloid per se. However, an opposite effect should be considered: Digoxin, when bound to the amyloid fibrils, may be pharmacologically impotent or unable to activate the myocardial receptors. Indeed, similar binding may occur in the gastrointestinal tract and effect the absorption of orally administered digoxin. Therapeutic judgment regarding the cautious administration of digoxin in patients with cardiac amyloidosis still rests on clinical grounds. Clearly, further studies defining the kinetics of parenteral and oral digoxin administration in experimental amyloid models and in patients with systemic amyloidosis are indicated to define more precisely digoxin-amyloid interaction.

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References

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