Infusion of Light Chains From Patients With Cardiac Amyloidosis Causes Diastolic Dysfunction in Isolated Mouse Hearts

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Background—Primary (AL) amyloidosis is a plasma cell dyscrasia characterized by clonal production of immunoglobulin light chains (LC) resulting in the subsequent systemic deposition of extracellular amyloid fibrils. Cardiac involvement is marked by the hemodynamic pattern of impaired diastolic filling and restrictive cardiomyopathy. Although cardiac death in patients with AL amyloidosis is usually associated with extensive myocardial infiltration, the infiltration alone does not correlate with the degree of heart failure or survival. We hypothesized that circulating monoclonal LC may directly impair cardiac function, in addition to any mechanical effects of amyloid fibril deposition. Therefore, we examined the effects of amyloid LC proteins on diastolic and systolic cardiac function, as measured in an isolated mouse heart model.

Methods and Results—LC were obtained from patients with nonamyloid disease or from those with noncardiac, mild cardiac, and severe cardiac involved AL amyloidosis. Saline or LC (100 μg/mL) was infused into a Langendorff-perfused, isovolumically contracting mouse heart. Saline and control, noncardiac, and mild-cardiac LC infusions did not alter ex vivo cardiac function. In contrast, infusion of sever cardiac LC resulted in marked impairment of ventricular relaxation with preservation of contractile function.

Conclusion—These results demonstrate that infusion of LC from patients with AL amyloidosis result in diastolic dysfunction similar to that observed in patients with cardiac involved AL amyloidosis, and they suggest that amyloid LC proteins may contribute directly to the pathogenesis and the rapid progression of amyloid cardiomyopathy, independent of extracellular fibril deposition. (Circulation. 2001;104:1594-1597.)

Key Words: amyloidosis ■ physiology ■ mice ■ heart ■ diastole

Primary (AL) amyloidosis is a plasma cell dyscrasia characterized by clonal production of immunoglobulin light chains (LC), resulting in the subsequent systemic deposition of extracellular amyloid fibrils. Prognosis is highly dependent on the organs involved, and the worst outcome is associated with cardiac involvement, a feature that is present in >50% of all patients with AL amyloidosis. Cardiac involvement is marked by the hemodynamic pattern of impaired diastolic filling and restrictive cardiomyopathy, which often rapidly progresses to congestive heart failure, with a median survival of 6 months and a 5-year survival of ~2%. Although this disease was identified in the mid-19th century, the mechanisms by which amyloidosis results in the rapid development of cardiac dysfunction remain unknown. Cardiac death in patients with amyloidosis is usually associated with extensive myocardial infiltration, although the infiltration alone does not correlate with the degree of heart failure or survival. Furthermore, clinical observations show that with similar degrees of myocardial infiltration, patients with AL amyloidosis have a worse prognosis than those with non-AL amyloidosis. We hypothesized that certain circulating monoclonal LC may directly impair cardiac function, in addition to any mechanical effects of amyloid fibril deposition. Therefore, we examined the effects of amyloid LC proteins on diastolic and systolic cardiac function measured in an isolated mouse heart model. LC were obtained from patients with nonamyloid disease or noncardiac, mild cardiac, and severe cardiac AL amyloidosis.

Methods

Sources of LC
Monoclonal immunoglobulin LC were isolated from 24-hour urine specimens collected from one patient with multiple myeloma, one with nonamyloid disease, and 5 with AL amyloidosis who were referred to the Amyloidosis Treatment and Research Program at Boston Medical Center. One AL amyloidosis patient had no evidence of cardiac involvement (noncardiac), two AL amyloidosis patients had echocardiographic abnormalities (slightly impaired ventricular filling and mild ventricular hypertrophy with no ECG...
changes) in the absence of heart failure (mild cardiac), and two AL amyloidosis patients had echocardiographic abnormalities (greatly impaired ventricular filling and severe hypertrophy) and NYHA class III or IV heart failure (severe cardiac). All procedures were performed with Institutional Review Board approval and informed consent from the patients.

**Purification of LC Proteins**

Urine samples were dialyzed against deionized water, lyophilized, and reconstituted in 0.02 mol/L sodium-phosphate buffer (pH 7.1) with Affigel blue (Bio-Rad) to remove albumin. The entire filtrate was again dialyzed and lyophilized. The affinity-purified sample was fractionated on Sephacryl S-200 columns (Amersham-Pharmacia) in 0.02 mol/L Tris (pH 7.5).

The purity of fractionated samples was assessed by SDS-PAGE and Western blotting using the Pharmacia Phast System. Samples were run under reducing conditions and stained with Coomassie blue. Immunodetection was performed with human Igκ or Igλ LC antibody (Atlantic Antibodies/DiaSarin), and results were visualized by incubation with alkaline phosphatase–conjugated secondary antibody (Sigma Chemical) using BCIP/NBT phosphatase substrate (Promega).

**Isolated Mouse Heart Preparation**

All procedures strictly adhered to the regulations of the Institutional Animal Care and Use Committee at Boston University. Hearts were isolated from C57/Bl6 mice weighing 25 to 30 g and were perfused in the Langendorff mode, as previously described. Briefly, mice were heparinized (10 000 U/kg IP) and anesthetized with ketamine (150 mg/kg IP) and xylazine (15 mg/kg IP). Hearts were isolated and perfused with oxygenated Krebs-Henseleit buffer (pH 7.4). A polyvinyl-chloride balloon was inserted into the left ventricle and perfused with oxygenated Krebs-Henseleit buffer (pH 7.4). A polyvinyl-chloride balloon was inserted into the left ventricle and connected to a pressure transducer (Gould Statham) to record left ventricular pressures. Hearts were paced through epicardial platinum wires. An ultrasonic flow probe (Transonics Systems) measured coronary flow. End-diastolic and end-systolic pressures were recorded using a physiology recorder.

**Experimental Protocol**

All hearts were stabilized for 15 minutes at 37°C at a coronary perfusion pressure of 80 mm Hg and paced at 7 Hz. After stabilization, the balloon was inflated with saline to adjust the isovolumic end-diastolic pressure to 5 to 7 mm Hg, and the balloon volume was held constant for the duration of the experiment. Coronary flow was then recorded and also held constant. Hearts were infused for 30 minutes with 0.09% NaCl (saline; 5 mouse hearts) or LC protein (100 μg/mL) that was isolated from a nonamyloid patient (control; 1 patient, 7 mouse hearts) or AL amyloidosis patients with either noncardiac (1 patient, 5 mouse hearts), mild cardiac (2 patients, 11 mouse hearts), or severe cardiac (2 patients, 10 mouse hearts) involvement, followed by a 15-minute washout period. The concentration of LC protein was chosen to represent a circulating level comparable to that found in patients with AL amyloidosis. Investigators performing heart experiments were blinded regarding the protein group.

**Statistical Analysis**

Data are reported as mean±SEM. Group differences were tested using repeated-measures 2-factor ANOVA with least significant difference post hoc examination. P<0.05 was considered statistically significant.

**Results**

**Amyloid LC Proteins**

Figure 1A illustrates a representative elution profile of 80 mg of urinary protein collected from a patient that was fractionated on Sephacryl 200 after albumin was removed by Affigel blue purification. Four separate peaks were observed with their corresponding SDS-PAGE and Western blots (Figure 1B). The major protein in peak C had a molecular weight of ≈30 kDa and was immunoreactive to anti-human Igκ LC antibody. The LC in peak C had >90% purity, which was confirmed by mass spectroscopic analysis, and was isolated for subsequently use in heart perfusion studies.

**LC Proteins and Cardiac Function**

Before the infusion of saline or protein, all hearts were set to an end-diastolic pressure between 5 and 7 mm Hg, and the balloon volume was then held constant for the duration of the experiment. Coronary flows were comparable among groups (saline, control, noncardiac, mild cardiac, and severe cardiac flows were 2.3±0.2, 2.5±0.1, 2.5±0.2, 2.2±0.4, and 2.7±0.2 mL/min; P=NS). Infusion of saline did not alter isovolumic end-diastolic pressures over the 30-minute infusion period or the subsequent 15-minute washout period (Figure 2A). Similarly, control, noncardiac, and mild cardiac proteins did not significantly alter diastolic function, suggesting a lack of acute cardiac toxicity from these LC proteins. In contrast, severe cardiac LC proteins, obtained from patients with severe cardiac involvement, resulted in a progressive increase in end-diastolic pressure, peaking at 18.7 mm Hg at the end of the 30-minute infusion period, indicative of severe diastolic dysfunction. Elevated diastolic pressures persisted in these hearts throughout the washout period. Interestingly, systolic pressure generation was unaltered with the infusion of...
of saline, control, noncardiac, mild cardiac, or severe cardiac LC proteins (Figure 2B). Congo Red staining and histological analysis of isolated hearts after LC protein infusion revealed the absence of any amyloid fibril deposits (data not shown).

**Discussion**

Cardiac-involved AL amyloidosis is a fatal disease associated with the extensive deposition of amyloid β-pleated fibrils within the heart. Although cardiac death in patients with AL amyloidosis is usually associated with extensive myocardial amyloid infiltration, the infiltration alone does not explain the rapid progression of heart failure. Our results suggest, for the first time, that circulating LC isolated from patients with nonamyloid disease (LC-Control) or noncardiac (AL-NC), mild cardiac (AL-MC), or severe cardiac (AL-SC) AL amyloidosis, *P* < 0.05 vs all groups.

Patients with heart failure due to cardiac-involved AL amyloidosis are often distinguished by a “stiff heart” syndrome, which is echocardiographically characterized by impaired ventricular relaxation and near-normal ejection fractions. Reduction of ejection fraction is usually associated with advanced stages of disease. The dysfunction observed with infusion of severe cardiac LC, namely impaired ventricular relaxation with preservation of contractile function, is very similar to the hemodynamic abnormalities reported in cardiac AL amyloidosis, further supporting a direct role for LC in the pathogenesis of cardiac AL amyloidosis.

AL amyloidosis involving the heart results in the rapid progression of cardiac failure with a median survival of only several months. Currently, pharmacological therapy to treat this condition is limited, because traditional medications such as β-blockers, calcium channel blockers, and digoxin are contraindicated. Furthermore, the particularly short survival associated with cardiac AL amyloidosis prevents patients from receiving the multiple cycles of chemotherapy needed to slow this disease. Our data demonstrate that circulating monoclonal LC may contribute substantially to the cardiac dysfunction observed in AL amyloid patients. The particular characteristics of severe cardiac LC proteins that promote cardiac dysfunction remain to be elucidated, and they may include unique protein sequences and/or post-translational modifications. Furthermore, amyloid protein–induced cardiac dysfunction may involve alterations in cardiomyocyte metabolism, cellular edema, intracellular calcium handling, and/or direct activation of cellular receptors. Understanding the mechanisms responsible for the swift decline in cardiac function may allow for the development of new therapies aimed at preventing or reversing the dysfunction and consequent mortality associated with cardiac AL amyloidosis.

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**References**

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In the articles by De Leon et al, “Adventitial Cells Do Not Contribute to Neointimal Mass After Balloon Angioplasty of the Rat Common Carotid Artery” (Circulation. 2001;104:1591–1593) and Liao et al, “Infusion of Light Chains From Patients With Cardiac Amyloidosis Causes Diastolic Dysfunction in Isolated Mouse Hearts” (Circulation. 2001;104:1594–1597), which published in the October 2, 2001 issue of the journal, the DOIs were missing for both articles. The DOIs are as follows:


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The current online versions of the manuscripts have been corrected.