N-acetyl-seryl-aspartyl-lysyl-proline prevents cardiac remodeling and dysfunction induced by galectin-3, a mammalian adhesion/growth-regulatory lectin

Yun-He Liu,1 Martin D’Ambrosio,1 Tang-dong Liao,1 Hongmei Peng,1 Nour-Eddine Rhaleb,1 Umesh Sharma,1 Sabine André,2 Hans-J. Gabius,2 and Oscar A. Carretero1

1Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan; and 2Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians University, Munich, Germany

Submitted 17 July 2008; accepted in final form 16 December 2008

Liu YH, D’Ambrosio M, Liao T, Peng H, Rhaleb NE, Sharma U, André S, Gabius HJ, Carretero OA. N-acetyl-seryl-aspartyl-lysyl-proline prevents cardiac remodeling and dysfunction induced by galectin-3, a mammalian adhesion/growth-regulatory lectin. Am J Physiol Heart Circ Physiol 296: H404–H412, 2009. First published December 19, 2008; doi:10.1152/ajpheart.00747.2008.—Galectin-3 (Gal-3) is a member of a large family of β-galactoside-binding adhesion/growth-regulatory endogenous lectins. This lectin is a multifunctional factor and binds to distinct glycan and protein ligands (13). Gal-3 is expressed and released by the epithelium and by inflammatory cells, including macrophages, mast cells, and neutrophils, which are involved in different physiological and pathological conditions. Evidence links macrophage activation and inflammation to cardiac remodeling and to pathogenesis of heart failure (HF) (12, 38). In a model of renin-dependent hypertension with HF (transgenic Ren-2 rats), cardiac Gal-3 is one of the most strongly overexpressed genes. In this model of hypertension, at early stages of cardiac hypertrophy (before HF development), myocardial Gal-3 expression was increased to higher levels in those rats that later progressed to HF, compared with those that remained compensated (34, 35). Recently, clinical studies have shown that serum Gal-3 levels were elevated in patients with acute HF, and they were prognostic of adverse outcome (19, 39). Increased Gal-3 secretion stimulates release of various mediators, such as transforming growth factor (TGF)-β and interleukins-1 or -2, and promote cardiac fibroblast proliferation, collagen deposition, and ventricular dysfunction (1, 35, 40).

N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) is a naturally occurring anti-inflammatory and antifibrotic peptide. Ac-SDKP is hydrolyzed almost exclusively by angiotensin-converting enzyme (ACE), and its plasma concentration is increased substantially by ACE inhibitors (3). In fact, Ac-SDKP mediates the anti-inflammatory and antifibrotic effects of ACE inhibitors (22, 24). Rats overexpressing cardiac ACE have decreased Ac-SDKP concentration and increased fibrosis in the heart (26). Also, inhibition of Ac-SDKP release from thymosin-β4 promotes cardiac and renal perivascular fibrosis (PVF) and nephrosclerosis (7). Our laboratory and others have shown previously that in vitro Ac-SDKP inhibited cardiac fibroblast proliferation and collagen synthesis (25, 29). Treatment with Ac-SDKP reduces inflammation and collagen deposition in the heart and kidney in various hypertensive models and in HF post-myocardial infarction (MI) (22, 23, 30, 42).

We have evidence that, in the left ventricle (LV), Ac-SDKP inhibits Gal-3 expression caused by ANG II infusion. Also, Ac-SDKP, in vitro, inhibits macrophage activation and migration induced by Gal-3 (33), suggesting that this tetrapeptide may inhibit not only Gal-3 expression, but also its effects. In the present study, we investigated the hypothesis that Ac-SDKP prevents Gal-3-induced cardiac inflammation, remodeling, and dysfunction, and these effects are mediated by the TGF-β/Smad3 signaling pathway. We used intrapericardial administration of Gal-3 and/or Ac-SDKP in rats. This method...
allows us to target the heart and obtain site-selective drug efficiency with low-level systemic effects.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 275–300 g, were housed in an air-conditioned room with a 12:12-h light-dark cycle, where they received standard laboratory rat chow (0.4% sodium) and drank tap water. They were given 7 days to adjust to their new environment. Before all surgical procedures, they were given analgesia with butorphanol (2 mg/kg sc) and anesthetized with pentobarbital sodium (50 mg/kg ip). This study was approved by the Henry Ford Hospital Institutional Animal Care and Use Committee.

Systolic blood pressure (SBP) was measured by tail cuff before surgery and by the end of the experiment.

**Surgical procedure for installing an intrapericardial catheter.** MRE 010 Micro-Renathane tubing (inner diameter 0.005 mm, outer diameter 0.01 mm) is made into a loop (10 mm in diameter), which is attached to a silicone disk with silicone adhesive. One end of the loop is closed, and the other is opened and connected to a Tygon tubing (inner diameter 0.015 mm, outer diameter 0.03 mm) and perforated through the disk. The Tygon tubing is, in turn, connected to a PE-60 tubing, which is fitted to the flow modulator of an osmotic minipump (Alzet 2004, Durect, Cupertino, CA). The loop was punctured with ~10 holes for perfusing drug solution. We modified the technique of inserting a catheter into the pericardial sac reported previously (14) by not cutting the midline sternum, thus lessening bleeding. Rats were intubated and ventilated with room air using a positive-pressure respirator (model 680, Harvard, South Natick, MA). A left thoracotomy was performed via the third intercostal space, and the lungs retracted to expose the thymus lobes and the heart. The thymus lobes were carefully separated, and the upper part of the pericardial sac was punctured (1- to 2-mm opening). The loop catheter was inserted into the pericardial sac, which was then closed by sealing it to the thymus with tissue adhesive glue (vetbond, 3M, St. Paul, MN). The minipump was implanted subcutaneously between the shoulder blades. The open end of the catheter was guided to the place where the osmotic minipump was implanted and connected to it. The lungs were inflated by increasing positive end-expiratory pressure, and then the thoracotomy site was closed.

**Experimental protocols.** In a preliminary study, we infused Gal-3 intrapericardially for 2 wk (n = 5 each). Although we found that Gal-3 increased interstitial collagen fraction (ICF) (P < 0.05) and PVF (P < 0.05), the heart and lung weights were not increased. In addition, echocardiographic study showed that Gal-3 tended to reduce ratio of early left ventricular filling phase to atrial contraction phase (E/A ratio), but did not reach statistical significance (P = 0.07), and LV ejection fraction (LVEF) was not changed. Hence, in the present study, we extended the infusion of Gal-3 to 4 wk. The following intrapericardial treatment groups were studied: 1) vehicle; received saline; 2) Ac-SDKP, 800 μg·kg⁻¹·day⁻¹; 3) Gal-3, 12 μg/day [the dose was calculated on the basis of reported bioactivity, adjusting for the local advantage of pericardial delivery (14)]; and 4) Gal-3 plus Ac-SDKP. The perfusion period was 4 wk. Gal-3 was prepared by recombinant production and purified using affinity chromatography and checked for purity by one- and two-dimensional gel electrophoresis and mass spectrometry (32). To exclude the possibility that Ac-SDKP binds Gal-3 and directly interferes with its effects, we performed binding assays with biotinylated Gal-3, both in solid phase (with asialofetuin as matrix) (2) and in a cell system with the natural glycan profile (5). Biotinylated Gal-3 was quantitated spectrophotometrically or by flow cytometry, in the presence of lactose (a positive control) or Ac-SDKP. Lactose inhibited Gal-3 binding nearly completely at 1 mM, while Ac-SDKP caused no inhibition up to 16 mM in the solid-phase assay and up to 8 mM in the cell system. These data indicate that Ac-SDKP does not interfere with Gal-3 binding to glycans.

**Echocardiography.** Echocardiography and Doppler sonography (Acuson, Sequoia C 256 with 15-MHz transducer) were performed while simultaneously recording ECG. M-mode echocardiography was performed in the parasternal long-axis view for the measurement of LV dimensions, and then in the anterior short-axis view to evaluate LVEF. Transmural Doppler inflow waves were used to measure peak early diastolic filling velocity (E wave), peak filling velocity at atrial contraction (A wave), and their ratio (E/A), assessing diastolic function as described previously (27). Aortic systolic velocity-time integral (VTI) and aortic root dimension (AoD) were determined, and stroke volume calculated according to the formula: stroke volume (VTVI)/(AoD²). Cardiac output (CO) = stroke volume × heart rate (HR). All Doppler spectra were recorded for five to seven cardiac cycles at a sweep speed of 200 mm/s.

**Cardiac hemodynamics.** Following echocardiography, rats were intubated. Cardiac contractility was assessed following previously described methods for LV catheterizations (44) and stimulation of cardiac reserve (31). Briefly, a 2-French Mikro-Tip catheter (Millar, Houston, TX) was introduced in the LV through the right carotid artery. A jugular vein was also cannulated, and isoproterenol (0.02 μg·kg⁻¹·min⁻¹) was infused for 4 min. LV pressure and positive and negative change in pressure over time (±dP/dt) were visualized and recorded with Chart 5.5 software (AD Instruments, Colorado Springs, CO).

**Heart and lung weights.** Following the hemodynamic studies, the rats were killed and the chest opened. The heart was stopped during diastole by injecting 15% potassium chloride solution, then excised, weighed, and expressed as heart weight-to-body weight (BW) ratio. The LV of the heart was sectioned transversely into five slices from apex to base. One midventricular slice was fixed in 10% formalin and embedded in paraffin. Others were rapidly frozen and kept at −80°C until the assay. The lungs were also excised and weighed.

**Hydroxyproline assay.** Collagen content of myocardial tissue was determined by hydroxyproline assay, as described previously (23). Briefly, samples were dried, homogenized, and hydrolyzed with 6 N HCl for 16 h at 110°C. A standard curve of 0–5 μg of hydroxyproline was used. Data were expressed as micrograms of collagen per milli-

---

**Table 1. Body weight, systolic blood pressure, heart rate, and heart and lung weight**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Ac-SDKP</th>
<th>Galectin-3</th>
<th>Galectin-3 + Ac-SDKP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>447±12</td>
<td>456±9</td>
<td>487±12</td>
<td>457±7</td>
</tr>
<tr>
<td>SBP</td>
<td>116±5</td>
<td>107±3</td>
<td>110±2</td>
<td>109±3</td>
</tr>
<tr>
<td>HR</td>
<td>357±11</td>
<td>333±14</td>
<td>364±12</td>
<td>351±14</td>
</tr>
<tr>
<td>Heart weight, mg/100 g</td>
<td>291±15</td>
<td>294±8.2</td>
<td>330±5.5*</td>
<td>287±10†</td>
</tr>
<tr>
<td>Lung weight, mg/100 g</td>
<td>392±15</td>
<td>335±27</td>
<td>440±17.5*</td>
<td>373±20†</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>7.68±0.17</td>
<td>7.55±0.16</td>
<td>7.77±0.19</td>
<td>7.46±0.21</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>3.9±0.7</td>
<td>3.7±0.2</td>
<td>4.3±0.25</td>
<td>3.8±0.29</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ac-SDKP, N-acetyl-seryl-aspartyl-lysyl-proline; SBP, systolic blood pressure; HR, heart rate; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension. *P < 0.05, vehicle vs. galectin-3; †P < 0.05 and ‡P < 0.01, galectin-3 vs. galectin-3 + Ac-SDKP.
gram of dry weight, assuming that collagen contains an average of 13.5% hydroxyproline (8).

**Histology.** A transmural section of LV was taken from the mid-ventricle. Sequential 5-μm paraffin-embedded sections were stained with Toluidine blue (Sigma) (11) to visualize mast cells. Mast cell density was determined as the total number of mast cells/LV cross-sectional area. Picrosirius red was used to quantify myocardial interstitial and perivascular collagen deposition and myocyte cross-sec-

---

**Fig. 1.** Effect of N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) on myocardial macrophage and mast cell infiltration induced by intrapericardial infusion of galectin-3 (Gal-3). **Top left:** four panels are representative images showing macrophages (brown color with immunohistochemical staining, ×400; black bar = 100 μm). **Bottom left:** four panels are representative images showing mast cell infiltration (dark blue with toluidine blue staining, ×400; black bar = 100 μm) in the myocardium 4 wk after treatment with vehicle, Ac-SDKP, Gal-3, or Gal-3 + Ac-SDKP. **Right:** quantitative results of myocardial macrophage (top) and mast cell density (bottom) in 4 treatment groups. *P < 0.05, **P < 0.01.
tional area (MCSA) (37). Microphotographs of each slide were taken at \( \times 400 \) magnification using a microscope (IX81, Olympus America, Center Valley, PA) with a digital camera (DP70, Olympus America). Image analysis was performed with a computerized image analysis system (Microsuite Biological imaging software, Olympus America). ICF was detected by calculating the ratio of the collagen area to the entire area of an individual section. Perivascular collagen deposition was measured as the ratio of the fibrosis area surrounding the vessel to total vessel area (43). MCSA was measured separately in the outer half area (close to epicardium) and inner half area (close to endocardium).

**Immunohistochemical staining.** Frozen sections (6 \( \mu \)m) were immunostained with ED-1 antibody, which recognizes monocytes/macrophages. Negative controls were processed in a similar fashion, except for the incubation step with primary antibody. Positive cells (with dark brown staining) were counted in high-power fields in each section and expressed as cells per millimeter squared (10).

TGF-\( \beta_1 \), -\( \beta_2 \), and phosphorylated Smad3 were determined using Western blot. Protein (50 \( \mu \)g) from the LV extracts was subjected to 12% SDS-PAGE under nonreducing conditions for TGF-\( \beta_1 \), -\( \beta_2 \), and -\( \beta_3 \), or reducing conditions for phosphorylated Smad3 (p-Smad3) and electrotransferred to a nitrocellulose membrane. Membranes were incubated with primary antibody overnight at 4°C. The primary antibodies were as follows: a monoclonal antibody against TGF-\( \beta_1 \), -\( \beta_2 \), and -\( \beta_3 \) (2 \( \mu \)g/ml; R&D Systems, Minneapolis, MN), a rabbit polyclonal antibody against p-Smad3 (1:1,000, Cell Signaling Technology), and a goat polyclonal antibody against actin (1:1,000; Santa Cruz Technology, Santa Cruz, CA). Bound antibodies were visualized by using secondary antibodies with conjugated horseradish peroxidase (Cell Signaling Technology, Danvers, MA) and ECL-plus chemiluminescence detection system reagent (Amersham Biosciences, Piscataway, NJ) with a digital camera (DP70, Olympus America). Image analysis was performed with a computerized image analysis system (Microsuite Biological imaging software, Olympus America).

**Results**

**BW, SBP, HR, and autopsy findings.** There were no differences in BW, SBP, and HR among groups. In autopsy, we did not observe thickened, rigid pericardium, or adhesions to the surrounding structures. There was no fluid effusion in all rats, including Gal-3-treated rats. Gal-3 significantly increased heart and lung weight compared with vehicle; these changes were blocked by Ac-SDKP (Table 1).

**Effect of Ac-SDKP on myocardial inflammation induced by Gal-3.** Macrophages (ED-1-positive cells) in the myocardium increased significantly in the Gal-3 treatment group compared with vehicle (\( P < 0.01 \)). Ac-SDKP significantly reduced macrophage count (\( P < 0.01 \), Gal-3 vs. Gal-3 + Ac-SDKP) (Fig. 1). There was also a significant increase in mast cell density in the Gal-3 treatment group compared with vehicle (\( P < 0.01 \)), which was blocked by Ac-SDKP (\( P < 0.05 \)). Figure 1 also shows representative microphotographs of mast cell infiltration into the myocardium from rats treated with vehicle, Ac-SDKP, Gal-3, and Gal-3 + Ac-SDKP. The increased mast cell density was more marked in the epicardial myocardium.

**Effect of Ac-SDKP on cardiac fibrosis and MCSA induced by Gal-3.** LV collagen content (hydroxyproline assay) increased significantly in the Gal-3 group compared with the vehicle group (\( P < 0.01 \)). This increase was prevented by treatment with Ac-SDKP (\( P < 0.05 \)) (Table 2). Figure 2, left, shows representative microphotographs of interstitial and perivascular collagen deposition estimated by staining with picrosirius red. The average value of myocardial interstitial and perivascular collagen deposition (Fig. 2) increased significantly in the Gal-3 group compared with vehicle (ICF: \( P < 0.01 \), PVF: \( P < 0.001 \); vehicle vs. Gal-3). The increases in interstitial and perivascular collagen were partially and completely prevented, respectively, by Ac-SDKP (ICF: \( P < 0.01 \), PVF: \( P < 0.001 \); Gal-3 vs. Gal-3 + Ac-SDKP). MCSA was increased in Gal-3-treated rats; this change was prevented by Ac-SDKP. The increase in MCSA was greater in the epicardial than in the endocardial area (Fig. 3).

**Effect of Ac-SDKP on TGF-\( \beta_1 \), -\( \beta_2 \), and -\( \beta_3 \) expression in LV of rats treated with Gal-3.** Gal-3 increased TGF-\( \beta_1 \), -\( \beta_2 \), and -\( \beta_3 \) expression in LV compared with vehicle group; Ac-SDKP inhibited this effect. Gal-3 also increased Smad3 phosphorylation compared with vehicle, and Ac-SDKP prevented this effect (Fig. 4).

**Effect of Ac-SDKP on hemodynamics and cardiac function.** The percent change of amplitude of the \( \Delta P/\Delta t \) from baseline after isoproterenol challenge was decreased in Gal-3-treated rats compared with vehicle (\( P < 0.05 \)). Ac-SDKP significantly improved this response (\( P < 0.05 \), Gal-3 vs. Gal-3 + Ac-SDKP) (Fig. 5). The percent change of amplitude of the \( \Delta P/\Delta t \) from the baseline after isoproterenol challenge was also decreased in the Gal-3-treated rats (vehicle 0.76 ± 0.09, Gal-3 0.53 ± 0.09, \( P < 0.05 \)). However, Ac-SDKP treatment did not prevent this decrease (0.46 ± 0.01, not significant).

---

**Table 2. Cardiac collagen content, Ac-SDKP level, and left ventricle dimensions**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Ac-SDKP</th>
<th>Galectin-3</th>
<th>Galectin-3 + Ac-SDKP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac tissue Ac-SDKP, pg/mg tissue</td>
<td>76.1 ± 7.4</td>
<td>391 ± 128*</td>
<td>63.5 ± 6.7</td>
<td>339.4 ± 129†</td>
</tr>
<tr>
<td>Plasma Ac-SDKP level, pg/μl</td>
<td>1.58 ± 0.2</td>
<td>2.82 ± 0.6</td>
<td>1.65 ± 0.2</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>Cardiac collagen content, μg/mg dry tissue</td>
<td>20 ± 2</td>
<td>15.1 ± 1.2</td>
<td>33.3 ± 3.8†</td>
<td>22.2 ± 2.3§</td>
</tr>
</tbody>
</table>

Values are means ± SE. *\( p < 0.01 \), Ac-SDKP vs. vehicle. †\( p < 0.05 \), galectin-3 vs. vehicle. ‡\( p = 0.056 \) and §\( p < 0.05 \), galectin-3 + Ac-SDKP vs. galectin-3.

---

AJP-Heart Circ Physiol • VOL 296 • FEBRUARY 2009 • www.ajpheart.org
Transmitral Doppler E- (early LV filling phase) to A-wave (atrial contraction phase) ratio was significantly decreased in Gal-3 rats ($P < 0.001$, vehicle vs. Gal-3), which was prevented by Ac-SDKP ($P = 0.01$, Gal-3 vs. Gal-3 + Ac-SDKP) (Fig. 6). Intrapericardial delivery of Gal-3 reduced LVEF ($P < 0.01$) and CO ($P < 0.01$, vehicle vs. Gal-3); these changes were prevented by Ac-SDKP (LVEF $P < 0.05$, and CO $P < 0.01$; Gal-3 vs. Gal-3 + Ac-SDKP) (Fig. 7). There were no differences among groups in LV end-diastolic diameter, although LV end-systolic diameter in Gal-3 group was slightly higher, it

Fig. 2. Effect of Ac-SDKP on myocardial interstitial collagen fraction (ICF) and perivascular fibrosis (PVF) induced by intrapericardial infusion of Gal-3. Left: representative images of ICF and PVF following vehicle, Ac-SDKP, Gal-3, or Gal-3 + Ac-SDKP infusion (Picrosirius red staining, $\times 400$, black bar = 100 $\mu$m). Right: quantitative results of ICF and PVF in the 4 groups. **$P < 0.01$. ***$P < 0.001$. 

H408 Ac-SDKP PREVENTS CARDIAC REMODELING AND DYSFUNCTION AJP-Heart Circ Physiol • VOL 296 • FEBRUARY 2009 • www.ajpheart.org

Downloaded from http://ajpheart.physiology.org.org by 10.220.33.6 on June 28, 2017
Ac-SDKP content in cardiac tissue and plasma. Ac-SDKP content in cardiac tissue was about fivefold higher in intrapericardial Ac-SDKP infusion groups compared with their counterpart vehicle and Gal-3 groups (P < 0.05, vehicle vs. Ac-SDKP; P = 0.056, Gal-3 vs. Gal-3 + Ac-SDKP). The plasma Ac-SDKP levels were elevated in Ac-SDKP and combined Gal-3 plus Ac-SDKP groups compared with their corresponding vehicle and Gal-3 groups; however, the differences are not statistically significant (Table 2).

Discussion

Anti-inflammatory and antifibrotic properties of Ac-SDKP are documented in the heart and kidney in the rat model of hypertension and MI (22, 28, 42). In the present study, we further confirmed these protective effects in a different model of cardiac remodeling and dysfunction induced by intrapericardial Gal-3 infusion. We found that Gal-3 enhanced macrophage and mast cell infiltration, as well as collagen deposition in the heart; these were prevented by Ac-SDKP. Ac-SDKP also prevented cardiac hypertrophy and pulmonary congestion, as demonstrated by decreased heart and lung weights. Furthermore, Ac-SDKP improved cardiac function, as shown by increases in LVEF, CO, E/A ratio, and −dP/dt response to isoproterenol. The beneficial effects of Ac-SDKP may be via inhibition of the TGF-β/Smad3 signaling pathway, since we found that Gal-3 markedly increased TGF-β expression, and Smad3 activity in the LV and Ac-SDKP prevents these effects. The presented evidence clearly supports our hypothesis that Ac-SDKP prevents harmful effects of Gal-3 in an inflammatory model of cardiac remodeling and dysfunction.
Ac-SDKP PREVENTS CARDIAC REMODELING AND DYSFUNCTION

It is well known that immunological and inflammatory processes play an important role in HF (18). Proinflammatory cytokines, especially the lectin Gal-3 (MAC-2 antigen), not only activates macrophages, but also recently have been shown to drive “alternative” macrophage activation (17), which is involved in organ fibrosis, which is an important component of several cardiovascular diseases. In unstable regions of atherosclerotic plaques, Gal-3 expression is upregulated, and it exacerbates vascular inflammation by stimulating macrophages to express a range of chemokines, including CC chemokines (20). Sharma et al. (35) previously reported that intrapericardial Gal-3 infusion caused macrophage infiltration in the heart and resulted in both cardiac structural changes and dysfunction. Consistent with these findings, we demonstrate here that Gal-3 enhances macrophage and mast cell infiltration, thus generating a microenvironment rich in proinflammatory cytokines, which promote fibrosis. Hence, our data identify Gal-3 as a target for development of new strategies for therapeutic intervention preventing Gal-3’s harmful effects. In the present study, we define Ac-SDKP as a viable option. We have shown in vitro that the anti-inflammation mechanism of Ac-SDKP is due to its direct effect on bone marrow stem cells and macrophages, inhibiting their differentiation, activation, and cytokine release (33). Furthermore, Ac-SDKP inhibits DNA and collagen synthesis in cardiac fibroblasts, which suggests that it may be an important endogenous regulator of fibroblast proliferation and collagen synthesis in the heart (29). Not only did we observe that Ac-SDKP prevents cardiac inflammation and fibrosis, but it also inhibits Gal-3-induced cardiac hypertrophy. More importantly, it improves cardiac systolic and diastolic function. The view is generally held that increased collagen content in the heart increases diastolic stiffness and impairs LV relaxation, resulting in LV diastolic dysfunction. Ac-SDKP improved –dP/dt response to isoproterenol and transmural Doppler E/A ratio, indicating improved diastolic function. In addition, Gal-3 caused a decrease in systolic function, which was prevented by Ac-SDKP. The decrease in systolic function caused by Gal-3 may be due to release of proinflammatory cytokines and superoxides. Ac-SDKP may prevent the decrease in systolic function by its anti-inflammatory effects. We previously reported that, in models of hypertension and HF induced by MI, Ac-SDKP decreased macrophage infiltration and fibrosis in the heart. However, these changes were not associated with improvement of either LVEF or diastolic dysfunction (9, 42). A possible explanation of this discrepancy could be the use of a different model of cardiac dysfunction. In the hypertensive model, the main reason for cardiac dysfunction is probably not inflammation but rather increased afterload that causes hypertrophy, fibrosis, and diastolic dysfunction. While in the post-MI model, the cause of HF is due to a significant decrease of viable myocardium, which results in a major reduction of the ejection fraction. In the Gal-3 pericardial sac infusion model, Gal-3 directly triggers a cardiac inflammatory response, fibrosis, and hypertrophy, with little changes in viable myocardium. In this model, cardiac remodeling and dysfunction are probably a consequence of the inflammatory process caused by Gal-3. Ac-SDKP, likely by blocking inflammatory effects of Gal-3, prevents the lectin’s detrimental effects on both cardiac remodeling and dysfunction. Ac-SDKP also promotes angiogenesis (16). Our laboratory previously found that Ac-SDKP stimulated endothelial cell proliferation, migration, and tube formation in vitro and increased myocardial capillary density in vivo (41). This angiogenic property of Ac-SDKP may favorably provide blood flow and oxygen supply to the damaged myocardium, thus improving cardiac function.

The antifibrotic effects of Ac-SDKP appear to involve multiple signaling pathways, including inhibition of expression and effects of TGF-β/Smad signaling and connective tissue growth factor-p42/p44 MAPK (22, 25, 29), which play essential roles in fibrotic and hypertrophic remodeling of the heart (6). We have reported that Ac-SDKP reduces TGF-β expression and Smad3 activity, Ac-SDKP prevented these effects, suggesting that Ac-SDKP prevents Gal-3-induced cardiac inflammation, fibrosis, and dysfunction, possibly via inhibition of TGF-β/Smad3 signaling pathway. However, we did not find antihypertrophic effects of Ac-SDKP in our laboratory’s previous studies on various hypertensive models (21, 24, 30), suggesting that, in the present study, this effect may be involved in a different signaling pathway induced by Gal-3. We found that Gal-3 induced mast cell infiltration mostly in the epicardial area (Fig. 1). Gal-3 increased cross-sectional area of both epicardial and endocardial myocytes, although changes in the epicardium were larger than in the endocardium. Ac-SDKP prevented this hypertrophic effect, suggesting that it worked via either inhibition of mast cell infiltration and/or blocking a direct effect of Gal-3. The involvement of mast cells in cardiac hypertrophy and HF is well documented (4, 36). Mast cells are an important source of cytokines, growth factors, and chemokines, including histamine, TNF-α/INF-κB/IL-6 and renin (4), which are released upon mast cell degranulation. Mast cell-derived chymase appears to be an important source of intracardiac angiotensin II formation. In addition, products released by mast cells, such as TNF-α and ANG II, increase production of reactive oxygen species, which are involved in induction of cardiac hypertrophy.

Finally, to evaluate the therapeutic efficacy of Ac-SDKP by local delivery into the pericardial sac, we measured both
Ac-SDKP PREVENTS CARDIAC REMODELING AND DYSFUNCTION

cardiac tissue and plasma Ac-SDKP levels and found that myocardial Ac-SDKP levels were fivefold higher in Ac-SDKP groups compared with vehicle or Gal-3 groups (Table 2). However, plasma Ac-SDKP levels were not significantly different among the four groups. These results suggest that this technique provided mainly a local Ac-SDKP therapeutic effect, while limiting systemic side effects of Gal-3.

In summary, this study demonstrated that intrapericardial Gal-3 delivery causes cardiac inflammation, fibrosis, and dysfunction. Simultaneous Ac-SDKP administration prevents detrimental Gal-3-induced effects. The improvements in cardiac function are likely due to decreased cardiac inflammation, fibrosis, and hypertrophy. Also, these preventive effects of Ac-SDKP may be via inhibition of TGF-β/Smad3 signaling pathway. Thus Ac-SDKP treatment may represent a potential therapeutic strategy for halting progression of HF induced by immunological or inflammatory reactions, such as viral myocarditis, rejection posttransplant, or pericarditis.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL28982 (O. A. Carretero) and HL071806 (N.-E. Rhaleb), the Verein zur Förderung des biologisch-technologischen Fortschritts in der Medizin e.V. Grants HL28982 (O. A. Carretero) and HL071806 (N.-E. Rhaleb), the Verein zur Förderung des biologisch-technologischen Fortschritts in der Medizin e.V.(Heidelberg, Germany), and the research initiative LMU.

REFERENCES


Downloaded from http://ajpheart.physiology.org/ on June 28, 2017


