Pharmacological inhibition of galectin-3 protects against hypertensive nephropathy

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1Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 2Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 3Department of Radiation Therapy, Harbin Medical University Cancer Hospital, Harbin, China; and 4Cardiovascular Translational Research, Navarra Biomed (Miguel Servet Foundation), Pamplona, Spain

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Frenay AS, Yu L, van der Velde AR, Vreeswijk-Baudoin I, López-Andrés N, van Goor H, Silljé HH, Ruifrok WP, de Boer RA. Pharmacological inhibition of galectin-3 protects against hypertensive nephropathy. Am J Physiol Renal Physiol 308:F500–F509, 2015. First published December 19, 2014; doi:10.1152/ajprenal.00461.2014.—Galectin-3 activation is involved in the pathogenesis of renal damage and fibrogenesis. Limited data are available to suggest that galectin-3-targeted intervention is a potential therapeutic candidate for the prevention of chronic kidney disease. Homozygous TGR(mREN27) (REN2) rats develop severe high blood pressure (BP) and hypertensive end-organ damage, including nephropathy and heart failure. Male REN2 rats were treated with N-acetyllactosamine [galectin-3 inhibitor (Gal3i)] for 6 wk; untreated REN2 and Sprague-Dawley rats served as controls. We measured cardiac function with echocardiogram and invasive hemodynamics before termination. BP and proteinuria were measured at baseline and at 3 and 6 wk. Plasma creatinine was determined at 6 wk. Renal damage was assessed for focal glomerular sclerosis, glomerular desmin expression, glomerular and interstitial macrophages, kidney injury molecule-1 expression, and α-smooth muscle actin expression. Inflammatory cytokines and extracellular matrix proteinases were quantified by quantitative real-time PCR. Systolic BP was higher in control REN2 rats, with no effect of Gal3i treatment. Plasma creatinine and proteinuria were significantly increased in control REN2 rats; Gal3i treatment reduced both. Renal damage (focal glomerular sclerosis, desmin, interstitial macrophages, kidney injury molecule-1 expression, and α-smooth muscle actin expression, collagen type I, and collagen type III) was also improved by Gal3i. All inflammatory markers (CD68, IL-6, galectin-3, and monocyte chemoattractant protein-1) were elevated in control REN2 rats and attenuated by Gal3i. Markers of extracellular matrix turnover were marginally altered in untreated REN2 rats compared with Sprague-Dawley rats. In conclusion, galectin-3 inhibition attenuated hypertensive nephropathy, as indicated by reduced proteinuria, improved renal function, and decreased renal damage. Drugs binding to galectin-3 may be therapeutic candidates for the prevention of chronic kidney disease.

chronic kidney disease; fibrosis; galectin-3; hypertension; renin-angiotensin system; TGR(mREN27)

RENAI IMPAIRMENT is frequently observed in cardiovascular disease (17, 50). Signs of glomerular damage include increased renal clearance and increased proteinuria. Together, they can be defined as chronic kidney disease (CKD), which represents a significant global health problem (22, 23). Early detection and prevention of CKD could improve both renal and cardiovascular morbidity and mortality.

Progressive impaired renal function results from a triad of glomerular sclerosis, tubular interstitial fibrosis, and vascular sclerosis (2). Over the past decade, glomerular sclerosis has been found to be an important factor in the progression of chronic kidney injury. Accordingly, drugs that aim to protect against glomerular injury can be of great value.

Galectin-3 belongs to the galectin-3 family of mammalian lectins and is characterized by a carbohydrate recognition domain (CRD) that has an affinity for β-galactosides. Galectin-3 mediates cell-cell and cell-matrix interactions by binding to lactosamine-containing glycoconjugates via its CRD (34). Accumulating evidence demonstrates that galectin-3 plays an important role in inflammatory and fibrotic processes (7, 28, 32, 51, 56, 58). Upregulation of galectin-3 has been shown to be involved in various types of organ fibrosis. Macrophage-derived galectin-3 induces myofibroblast activation, promotes collagen synthesis and collagen deposition, and subsequently leads to fibrosis. A role for galectin-3 has also been demonstrated in the development of tubular damage and fibrosis in a mice model of chronic allograft injury (7). Moreover, previous research has shown that binding of pectins, such as modified citrus pectin, to the galectin-3 CRD exerts beneficial effects in experimental acute kidney injury (24). Recently, O’Seaghdha et al. (36) also reported the clinical involvement of galectin-3 in kidney disease, since they found that elevated levels of plasma galectin-3 were associated with increased risks of rapid glomerular filtration rate decline and incident CKD (36). We hypothesized that galectin-3-targeting therapy may be of use in CKD, particularly when fibrosis is concerned.

Transgenic TGR(mREN27) (REN2) rats exhibit persistent high blood pressure (BP), progressive proteinuria, and nephropathy that strongly resembles the human situation: injury and dysfunction of glomerular endothelial cells, microinflammation, and excessive production of the extracellular matrix (ECM), which eventually results in glomerular sclerosis (9, 31). This collective of inflammation, glomerular sclerosis, tubular interstitial fibrosis, and proteinuria are all early markers for progressive clinical renal dysfunction in CKD (12, 13). Previous experimental and clinical studies (5, 20) have demonstrated that the proteinuria caused by hypertension could be almost totally reversed by angiotensin-converting enzyme inhibitor, whereas other experimental studies (15, 32, 39) found that the variance that reduced proteinuria was partially independent of BP levels. We (57) have previously reported that targeted therapy aimed at galectin-3, using N-acetyllacto-
MATERIALS AND METHODS

Animals. We studied male homozygous REN2 rats (age: 6 wk). These rats show a phenotype of severe hypertension and left ventricular (LV) hypertrophy, leading to heart failure (HF) at 13–15 wk of age (43). Rats were bred at the Max Delbrück Center for Molecular Medicine (Berlin, Germany). Male age-matched Sprague-Dawley (SD) rats were used as controls (Harlan). Animals were housed under standard conditions. The present study was approved by the Animal Ethical Committee of the University of Groningen and was conducted in accordance with existing guidelines for the care and use of laboratory animals. The general characteristics and myocardial phenotype have been reported elsewhere (57).

Experimental design. After 1 wk of adaption and 2 wk of daily BP measurement training, the experiment was started. To study the effects of galectin-3 inhibition on renal protection, we allocated SD rats and REN2 rats to control treatment or galectin-3 inhibitor. Rats were treated with an established inhibitor of galectin-3, N-Lac (Gal3i; Carboxynt, Berkshire, UK). Based on the known affinity of N-Lac, we selected a final dose of 5 mg·kg⁻¹·day⁻¹. Injections were administered intraperitoneally, every other day, 3 times/wk in total. Untreated REN2 and SD rats were used as controls. Here, we report results from the following three groups: control SD rats (SD-con group; n = 5), control REN2 rats (REN2-con group; n = 5), and REN2 rats treated with Gal3i (REN2-Gal3i group; n = 10); results of SD rats treated with Gal3i are not presented, as Gal3i affected none of the measured parameters in SD rats. Rats were placed in metabolic cages for 24 h at baseline and at 3 and 6 wk after treatment, and urine samples were collected for the determination of urinary protein. Systolic BP (SBP) was measured at serial time points at baseline and at 3 and 6 wk after treatment by a noninvasive tail-cuff method using a computer-assisted oscillatory detection device (Millar Instruments, Houston, TX). Between official BP measurements, rats were handled daily and trained for BP measurement so that the effect of gradual adaptation was ruled out. Rats were terminated 6 wk after the start of the experiment.

Measurement of cardiac and hemodynamic function. Cardiac function was assessed by echocardiography at baseline and before termination [Vivid 7, GE Healthcare, Chalfont St Giles, UK, equipped with a 10-MHz (rats) phase array linear transducer], as previously described (25). Hemodynamic function was assessed invasively, as previously described (37), by introducing a 1.4-Fr microtip pressure-volume transducer (Millar Instruments, Houston, TX) via the right carotid artery into the aorta. A 3-min period was allowed for stabilization before SBP, diastolic BP, and heart rate were recorded (average of 20 heart cycles). Other parameters that were measured include peak SBP and LV end-diastolic pressure.

Tissue and plasma processing. After hemodynamics had been measured, arterial blood was drawn and collected. Samples were centrifuged at 3,000 rpm for 15 min at 4°C, and plasma was frozen for creatinine analysis. Kidneys were removed, and their weights were measured, arterial blood was drawn and collected. Samples were frozen for SBP and LV end-diastolic pressure.

Immunohistochemistry. Paraffin sections were dewaxed and subjected to antigen retrieval procedures by overnight incubation at 80°C in 0.1 M Tris·HCl (pH 9.0). Sections were subsequently washed three times with PBS, endogenous peroxidase was blocked with 0.075% H₂O₂ in PBS (pH 7.4) for 30 min, and sections were incubated with the following primary antibodies: the myofibroblast marker α-smooth muscle actin (α-SMA; clone 1A4, Sigma-Aldrich, St. Louis, MO), the macrophage marker ED1 (MCA341R, AbDSerotec, Oxford, UK), the tubular damage marker kidney injury molecule (KIM)-1 (a kind gift from Veronique Bailly, BIOGEN-IDEC, Cambridge, MA), and the glomerular epithelial damage marker desmin (Dako, Dako, Glostrup, Denmark). All incubations with primary antibodies were in 1% BSA-PBS for 1 h at room temperature. Binding was detected using sequential incubation with the appropriate peroxidase-labeled secondary and tertiary antibodies diluted in 1% BSA-PBS buffer and 1% normal rat serum for 30 min. Peroxidase activity was developed using 3,3′-diaminobenzidine tetrachloride for 10 min containing 0.03% H₂O₂. Ultimately, countering staining was performed using Mayer’s hematoxylin. All sections were digitalized using a scanning system (Nanoozoomer 2.0-HT, Hamamatsu, Japan). α-SMA protein expression was determined in the renal cortex, including vessels, and glomerular desmin expression was measured as well, with both at ×20 magnification with ScanScope software (Aperio Technologies version 9, Vista, CA). KIM-1 expression was similarly determined in the entire renal cortex. Numbers of both glomerular (50 glomeruli) and interstitial macrophages (30 interstitial fields) were manually counted by a blinded observer. Additionally, kidney sections were stained with periodic acid-Schiff and scored for focal glomerular sclerosis (FGS). FGS was scored positive if all of the following features were present: collapse of capillaries, mesangial matrix expansion, and adhesion of the glomerular visceral epithelium to Bowman’s capsule. A score for the degree of affected glomeruli was applied as follows: 0 = unaffected glomeruli, 1 = 25% affected glomeruli, 2 = 50% affected glomeruli, 3 = 75% affected glomeruli, and 4 = all glomeruli were positive for FGS. The ultimate score was obtained by multiplying the degree of change by the percentage of glomeruli with the same degree of injury and adding the two score (14). Histopathological analysis was performed in a blinded fashion.

Quantitative real-time PCR. cDNA synthesis was performed using 0.5 μg total RNA (Quantitect Reverse Transcripase kit, Qiagen, Venlo, The Netherlands) as previously described (25, 29). Quantitative real-time PCR was performed using SYBR green mix (Absolute SYBR Green ROX mix, Thermo Scientific, Breda, The Netherlands) on a CFX384 Real-Time PCR Detection System (Bio-Rad Laboratories, Veenendaal, The Netherlands). All target gene expression levels under the same reaction conditions: 95°C for 15 min and then 36 cycles of 95°C for 15 s and 60°C for 30 s. The following primers were used for quantitative real-time PCR: α-SMA, forward 5′-CATCATCGGCTGTTGCATTTGG-3′ and reverse 5′-TCAGCGTCACAGTAGTCAC-3′; transforming growth factor (TGF)-β, forward 5′-CGTTGAAATCAATGGTAGATG-3′ and reverse 5′-GGAAAGGCTGGTTCTATGCA-3′; proliferating cell nuclear antigen (PcnA), forward 5′-GGCTCCCAAGATCGAGATG-3′ and reverse 5′-TCAGAAGCGATCGTCAAAG-3′; matrix metalloproteinase (Mmp)2, forward 5′-CCGGCCGCCCATCATCA-3′ and reverse 5′-TTGCACTGGCAACTTCTTGTCT-3′; Mmp9, forward 5′-GGGAAGCTATCTGGAAATCTG-3′ and reverse 5′-ATGGCCAAGTAAATGGCC-3′; tissue inhibitor of metalloproteinase (Timp)1, forward 5′-AGAGCTCTGTTGATCTGTC-3′ and reverse 5′-TCAGATTAGTTGCAAGGAC-3′; Timp2, forward 5′-TGAGAGGTGGAGAAGAAG-3′ and reverse 5′-TGTCCTCGGCCCAAATAA-3′; collagen type I (Col1), forward 5′-ACAGGCTGAGGCTCACAG-3′ and reverse 5′-AATCTGCTGGATGATGAC-3′; and reverse 5′-CTGTGACAGGACCACCAC-3′. Samples were analyzed with quantification software (Bio-Rad CFX Manager 1.6). mRNA levels were expressed in relative units based on a standard curve obtained with serial dilutions of a calibration cDNA mixture. To normalize expression data, GAPDH was used as a reference gene, since it showed little sample-to-sample variability. The following primers were used for GAPDH: forward 5′-CATCAAGAAGGTTGCGGAACTG-3′ and reverse 5′-ACCACCCCTTGTCCGAT-3′.
**RESULTS**

An overview of the experimental design is shown in Fig. 1A. **Characteristics of REN2 rats.** Due to their genetic genotype, REN2 rats developed severe hypertension (SBP > 200 mmHg) at an early age. SBP was significantly increased in the REN2-con group compared with the SD-con group at all time points (baseline, 3 wk, and 6 wk; Table 1 and Fig. 1B). There was no difference in SBP between REN2-con and REN2-Gal3 rats (217 ± 7 vs. 236 ± 6 mmHg, P = not significant; Fig. 1B). REN2-con rats had significantly lower body weights compared with SD-con rats. Cardiac and hemodynamic measurements before termination showed a significant decrease in fractional shortening and a significant increase in LV end-diastolic pressure in REN2-con rats compared with SD-con rats (fractional shortening: 34 ± 1% vs. 44 ± 2% and LV end-diastolic pressure: 9 ± 1 vs. 4 ± 1 mmHg, P < 0.05; Table 1). Untreated REN2 rats developed LV dysfunction. Treatment with Gal3i attenuated LV dysfunction by interfering with myocardial fibrogenesis (as previously published) (57). Renal function was impaired in REN2-con rats, as shown by significant increases in proteinuria and plasma creatinine and a significant decrease in renal clearance after 6 wk (proteinuria: 81 ± 19 vs. 26 ± 1 mg/24 h, P < 0.05; plasma creatinine: 45 ± 5 vs. 26 ± 2 μmol/l, P < 0.05; and renal clearance: 2.2 ± 0.1 vs. 4.0 ± 0.3 ml/min, P < 0.05 for REN2-con vs. SD-con rats; Table 1 and Fig. 1, C–E).

**Galectin-3 inhibition protects against renal damage.** Proteinuria was significantly increased in REN2-con rats compared with SD-con rats, but this increase was significantly reduced by treatment with Gal3i (Fig. 1D, E).

### Table 1. Characteristics of rats

<table>
<thead>
<tr>
<th></th>
<th>SD-con Group</th>
<th>REN2-con Group</th>
<th>REN2-Gal3i Group</th>
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<tr>
<td>Number of rats/group</td>
<td>5</td>
<td>5</td>
<td>10</td>
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<tr>
<td>Body weight, g</td>
<td>377 ± 6*</td>
<td>329 ± 14</td>
<td>347 ± 26</td>
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<tr>
<td>Kidney weight, mg/g</td>
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<td>9.0 ± 0.3</td>
<td>8.3 ± 0.3</td>
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<td>Systolic blood pressure, mmHg</td>
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<td>Week 0 (baseline)</td>
<td>146 ± 8*</td>
<td>229 ± 13</td>
<td>220 ± 5</td>
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<td>Week 3</td>
<td>151 ± 9*</td>
<td>227 ± 7</td>
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<td>Week 6</td>
<td>140 ± 11*</td>
<td>216 ± 8</td>
<td>235 ± 19</td>
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<tr>
<td>Proteinuria, mg/24 h</td>
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</tr>
<tr>
<td>Week 0 (baseline)</td>
<td>14.3 ± 0.9*</td>
<td>37.2 ± 8.1</td>
<td>21.6 ± 3.5</td>
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<tr>
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<td>59.3 ± 8.2</td>
<td>22.7 ± 2.8*</td>
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<tr>
<td>Week 6</td>
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<td>80.6 ± 19.0</td>
<td>16.4 ± 1.6*</td>
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<td>41 ± 3*</td>
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<td>LV end-diastolic pressure, mmHg</td>
<td>4 ± 1*</td>
<td>9 ± 1</td>
<td>5 ± 2*</td>
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Values are means ± SE. N-acetyllactosamine was used as a galectin-3 inhibitor (Gal3i). Results from the following three groups are shown: control Sprague-Dawley rats (SD-con group), control TGR(mREN)27 (REN2) rats (REN2-con group), and REN2 rats treated with Gal3i (REN2-Gal3i group). *P < 0.05 vs. the REN2-con group.
attenuated in REN2-Gal3i rats (16 ± 2 vs. 81 ± 19 mg/24 h in REN2-con rats, P < 0.05; Fig. 1C). In addition, a significant increase in the level of plasma creatinine was found in REN2-con rats, and this was also significantly attenuated with Gal3i treatment (27 ± 2 vs. 45 ± 5 μmol/l in REN2-con rats, P < 0.05; Fig. 1D). Creatinine clearance was significantly decreased in the REN2-con group compared with the SD-con group, which was slightly improved in the REN2-Gal3i group (2.8 ± 0.4 vs. 2.2 ± 0.1 ml/min in the REN2-con group, P = not significant; Fig. 1E).

In REN2-con rats, FGS was significantly higher compared with SD-con rats (15 ± 4% vs. 0%, P < 0.05). Gal3i treatment resulted in a significant decrease of FGS (3 ± 1% vs. 15 ± 4% in REN2-con rats, P < 0.05; Fig. 2, A–C and G). Additionally, glomerular desmin expression was significantly increased in REN2-con rats (21 ± 1 vs. 5 ± 1 in SD-con rats, P < 0.05), which was significantly attenuated after Gal3i treatment (14 ± 2 vs. 21 ± 1 in REN2-con rats, P < 0.05; Fig. 2, D–F and H).

Persistent systemic hypertension induces glomerular microinflammation (31). We observed that the number of interstitial macrophages was significantly increased in REN2-con rats compared with SD-con rats, whereas the number of glomerular macrophages was not changed in REN2-con rats (Fig. 3, A–H). Gal3i treatment significantly attenuated macrophage influx in both glomerular and interstitial compartments in REN2 rats (Fig. 3, C and F–H). The macrophage marker CD68 was not significantly increased in the REN2-con group compared with the SD-con group, but Gal3i significantly reduced CD68 mRNA expression (Fig. 3J). IL-6 and other relevant inflammatory markers, such as galectin-3 and monocyte chemoattractant protein (MCP)-1, were subsequently determined by quantitative real-time PCR (Fig. 3, I, K, and L). Galectin-3, IL-6, and MCP-1 were all significantly increased in REN2-con rats, and treatment with Gal3i significantly reduced galectin-3 and IL-6 levels, whereas MCP-1 levels showed only a downward trend.

α-SMA expression, studied with both immunohistochemistry and PCR, was significantly increased in REN2-con rats and significantly reduced in REN2 rats after treatment with Gal3i (Figs. 4, A–C and G, and 5A). KIM-1 expression, as assessed by immunohistochemistry, was also significantly increased in REN2-con rats, and Gal3i treatment was able to significantly reduce expression of this tubular damage marker (Fig. 4, D–F and H). Additionally, mRNA levels of TGF-β, PcnA, Mmp2, and Timp2 were not altered in the REN2-con group compared with the SD-con group (Fig. 5, B–D and G). Mmp9 tended to be expressed less expressed in...
REN2-con and REN2-Gal3i rats; however, this was not significant (Fig. 5E). Furthermore, Timp1 was significantly increased in REN2-con rats and significantly decreased in REN2 rats treated with Gal3i (Fig. 5F). Finally, we found that Col1a1 and Col3a1 were not changed in kidneys from REN2-con rats compared with SD-con rats. However, these were significantly decreased in REN2 rats treated with Gal3i (Fig. 5, H and I).

Fig. 3. Inflammatory cells and markers in the rat kidney. A–C: representative pictures of glomerular macrophages in a SD-con rat (A), REN2-con rat (B), and REN2-Gal3i rat (C). D–F: representative pictures of macrophages in the tubulointerstitial space of a SD-con rat (D), REN2-con rat (E), and REN2-Gal3i rat (F). Arrowheads indicate ED1-positive cells. Numbers of glomerular macrophages (G) and macrophages in the tubulointerstitial space (H) were quantified. Inflammatory markers were quantified by quantitative real-time PCR; galectin-3 (I), Cd68 (J), IL-6 (K), and monocyte chemoattractant protein (Mcp)-1 (L). *P < 0.05 vs. REN2-con rats.
DISCUSSION

The present study shows that targeted inhibition of galectin-3 attenuates renal structural and functional deterioration in REN2 rats with hypertensive end-organ damage and increased galectin-3 levels. Over the course of 6 wk, REN2-con rats developed substantial proteinuria, which is associated with glomerulosclerosis. Treatment with Gal3i almost completely prevented the development of proteinuria and associated histological markers of kidney damage in REN2 rats. Interestingly, this protection seems to be BP independent, as BP was equally elevated in REN2 rats treated with Gal3i.

In recent years, the role of galectin-3 in fibrosis and inflammation has been elucidated. It has been shown that galectin-3 is expressed in various organs, including the heart, lung, liver, and kidney (56). In a mouse model of unilateral ureter obstruction, which is characterized by severe hydronephrosis, inflammation, and fibrosis, galectin-3 knockdown [using galectin-3 knockout (KO) mice] resulted in decreased renal inflammation. This was associated with a reduced profibrotic response, as evidenced by a decrease in collagen production and deposition (16). Furthermore, it has been showed that inhibition of galectin-3 with modified citrus pectin, a pectin derivative that binds to the CRD of galectin-3, reduced kidney fibrosis and attenuated macrophage influx (24).

The affinity of galectin-3 toward its ligands is often in the range of 10-6 M, which is lower than typical protein-protein interactions (10-8 M) (11). Despite this low affinity, the interaction between galectin-3 and its ligands remains stable because of its multivalency: binding to multiple ligands increases their avidity (55). Whereas many lectins are oligomeric and thus the most potent inhibitors are targeted against more than one subunit (3, 10), galectin-3 is monomeric in solution and multivalent compounds do not seem to exert an increased inhibitory effect (1). N-Lac, a lectin inhibitory polysaccharide, is recognized by the CRD of galectins, which is folded into a sandwich structure of five to six stranded β-sheets (42). N-Lac has an established affinity for galectin-3 ($K_d = 67 \mu M$) (52). N-Lac has been used to inhibit galectin-3, as shown in ex vivo (30) and in vivo (57) experiments. Furthermore, intraperitoneal injections with N-Lac have been shown to exert cardioprotective effects (57). Additionally, intraperitoneal injections of a related modified polysaccharide (pectin-derived GCS-100) in tumor-bearing mice resulted in the rejection of tumors, and in control mice, GCS-100 showed no side effects (8). Intravenous

![Fig. 4. Renal tubular and interstitial changes in the rat kidney. A–C: representative pictures of tubulointerstitial α-smooth muscle actin (α-SMA) expression in a SD-con rat (A), REN2-con rat (B), and REN2-Gal3i rat (C). D–F: representative pictures of the tubular damage marker kidney injury molecule (KIM)-1 in a SD-con rat (D), REN2-con rat (E), and REN2-Gal3i rat (F). G and H: quantification of α-SMA expression (G) and KIM-1 expression (H). *P < 0.05 vs. REN2-con rats.](http://ajprenal.physiology.org/)

AJP-Renal Physiol • doi:10.1152/ajprenal.00461.2014 • www.ajprenal.org
administration of GCS-100 has been well tolerated in a trial with 24 human patients (8).

Our data are in line with these observations showing that inhibition of galectin-3 results in an attenuated inflammatory response (CD68, IL-6, and MCP-1) and less renal fibrosis. However, our findings are in contrast with the findings of Okamura et al. (35). They concluded that galectin-3 preserves renal tubules during chronic kidney injury in galectin-3 deficient (KO) mice (35). They observed that the severity of fibrosis in galectin-3 KO mice was increased at day 14 and this persisted through day 21 compared with wild-type mice. This contradictory observation may be explained by the fact that Okamura et al. particularly focused on the later phase of unilateral ureter obstruction-induced kidney injury. Moreover, it has been shown that galectin-3 KO mice have unfavorable glucose homeostasis (38, 40), and it has been suggested that disruption of galectin-3 may not always be protective. In fact, it has been shown that galectin-3 is protective in advanced glycation end product (AGE)-induced nephropathy (18). AGEs are highly expressed in prooxidant states, like diabetes and aging (19), and induce a dysregulation in tissue remodeling but also play a role in the removal of irreversibly glycated molecules (53). In galectin-3 KO mice, it was observed that lack of galectin-3 expression accelerated diabetic (AGE-induced) glomerulopathy (18). An explanation for this discrepancy in the effects of galectin-3 could be that galectin-3 disruption (galectin-3 KO mice) may exhibit another phenotype than that of pharmacological inhibition. Nevertheless, studies using pharmacological inhibition of galectin-3 have, almost without exception, pointed to a protective effect of galectin-3 inhibition, with less fibrosis and a decrease in myofibroblast activation (24, 26, 27, 57).

Renal dysfunction is frequently observed in cardiovascular disease (17, 50) and is one of the most powerful predictors in chronic HF prognosis. It also plays an important role in the pathophysiologic process (49). The main reasons for renal dysfunction in chronic HF include hypertension and inflammation (33, 48). Phenotypic changes may involve decreased glomerular clearance and persistently increased proteinuria, as a sign of ongoing glomerular damage. The Prevention of Renal and Vascular Endstage Disease Intervention Trial and the Lifestyle Interventions and Independence for Elders study also demonstrated that proteinuria is associated with higher risk of mortality and morbidity (5, 20). Numerous studies have confirmed the concept that consequent antihypertensive therapy slows down the decline of renal function by reduction of BP and decreasing proteinuria (41, 54). It has been suggested that the reduction of proteinuria is, at least in part, independent from BP. Mifsud and colleagues (32) investigated an intervention with tranilast, an inhibitor of TGF-β; they found that tranilast resulted in beneficial effects of a reduction of tubulo-interstitial pathology and proteinuria, independent of BP, in diabetic REN2 rat. Furthermore, statin treatment reduces glomerular inflammation and podocyte damage in experimental DOCA-salt hypertension rats (15). Moreover, it was observed that growth arrest-specific protein 6 is involved in cardiac and

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**Fig. 5. Gene expression of extracellular matrix proteins in the rat kidney.**

A–I: α-smooth muscle actin (α-SMA) (A), transforming growth factor (TGF)-β (B), proliferating cell nuclear antigen (Pcna; C), matrix metalloproteinase (Mmp)2 (D), Mmp9 (E), tissue inhibitor of metalloproteinase (Timp)1 (F), Timp2 (G), collagen type IA1 (Col1a1; H), and collagen type IIIA1 (Col3a1; I). *P < 0.05 vs. REN2-con rats.
renal injury; growth arrest-specific protein 6 deficiency reduced renal inflammation, fibrosis, and cardiac remodeling independent of BP (39). Collectively, although hypertension and proteinuria are both markers and pathophysiological mechanisms of renal disease development, the above evidence suggests that renal protection can be reached not only by lowering BP levels but also by specific antiproteinuric and anti-inflammatory actions.

In the present study, treatment with Gal3i almost completely prevented the development of proteinuria and attenuated relevant histological damage markers (FGS, desmin, α-SMA, and KIM-1). Interestingly, this pathological conversion without apparent effects on BP in REN2 rats treated with Gal3i. Therefore, we conclude that galactin-3 inhibition exerts its protective effects by direct acting on renal glomeruli, parenchyma, and tubuli. This new finding offers us a novel target in the therapy of chronic HF associated with CKD.

Previous studies (16, 47) have demonstrated that macrophage-derived galactin-3 directly modulates myofibroblast activation followed by an upregulation in collagen synthesis. Loss of galactin-3 leads to reduced myofibroblast activation and a subsequent reduction in collagen synthesis. Furthermore, TGF-β has been implicated as an important mediator of kidney fibrosis (44, 46). TGF-β mRNA expression is markedly elevated in unilateral ureter obstruction-induced kidney injury and induces myofibroblast activation via increased galactin-3 expression (16). Henderson et al. (16) also observed that loss of galactin-3 does not affect TGF-β expression, which implies that the mechanism of renal fibrosis is TGF-β independent. These consistent results were also found in the present study, where quantitative real-time PCR for TGF-β showed no differences between REN2-Gal3i and REN2-con groups.

Mmps and TimpS are involved in ECM remodeling and are crucial for tissue development and homeostasis; however, the role and sequence of activation of Mmps and TimpS in the kidney are complex and have not been completely defined. Some previous investigations have provided insights into the role of Mmps (e.g., Mmp2 and Mmp9) and TimpS (e.g., Timp1 and Timp2), which are involved in the development of renal fibrosis (4, 6, 21, 45). In our study, PCR for these matrix proteins (TGF-β, Mmp2, Timp2, Col1a1, and Col3a1) and Pcn showed no differences between untreated REN2 and 2D rats. This fact may be explained by the observation that the studied animals exhibit only mild (prefibrotic) renal damage.

As a multifunction biomarker, galactin-3 promotes macrophage migration, myofibroblast activation, and collagen synthesis, which is involved in the fibrogenesis process and further develops into organ fibrosis. The relationship among galactin-3, cardiovascular risk factors, and renal function suggests a role of galactin-3 in integrating these multiple mechanisms of progressed chronic HF and CKD. These findings require further studies to fully elucidate the role of galactin-3 in chronic HF and CKD management and study the exact mechanism of how galactin-3 exerts its effects.

In conclusion, pharmacological inhibition of galactin-3 attenuates progressive hypertensive nephropathy. Galactin-3 inhibition may improve renal function in the early stages of nephropathy by attenuating myofibroblast activation and reducing proteinuria and subsequently improving glomerular filtration function and tubular regeneration. Taken together, our findings suggest a role for galactin-3 in CKD. Galactin-3-targeted intervention may be a potential therapeutic strategy in the early stages of hypertensive nephropathy.

**DISCUSSIONS**

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**AUTHOR CONTRIBUTIONS**


**REFERENCES**


15. Hartner A, Klanne B, Cordasic N, Amann K, Schmidt RE, Veelken R, Hilgers KF. Statin treatment reduces glomerular inflammation and


