Prevention of diabetic nephropathy by compound 21, selective agonist of angiotensin type 2 receptors, in Zucker diabetic fatty rats

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Castoldi G, di Gioia CR, Bombardi C, Maestroni S, Carletti R, Steckelings UM, Dahlöf B, Unger T, Zerbini G, Stella A. Prevention of diabetic nephropathy by compound 21, selective agonist of angiotensin type 2 receptors, in Zucker diabetic fatty rats. Am J Physiol Renal Physiol 307: F1123–F1131, 2014. First published September 3, 2014; doi:10.1152/ajprenal.00247.2014.—The aim of this study was to evaluate the effect of compound 21 (C21), a selective AT2 receptor agonist, on diabetic nephropathy and the potential additive effect of C21, when associated with losartan treatment, on the development of albuminuria and renal fibrosis in Zucker diabetic fatty (ZDF) rats. The experiments lasted 15 wk (from 5 to 20 wk of age) and were performed in 40 ZDF rats and 12 control lean rats. ZDF rats were divided into 4 groups: 1) 9 rats were treated with losartan; 2) 10 rats were treated with C21; 3) 9 rats were treated with losartan plus C21; and 4) 12 rats were maintained without any treatment. ZDF rats showed an increase in blood glucose level, albuminuria, renal fibrosis, macrophage infiltration, and TNF-α expression and a reduction of glomerular nephrin expression compared with control lean rats. C21 treatment reduced renal glomerular, tubulointerstitial, and perivascular fibrosis, and macrophage infiltration and TNF-α expression in ZDF rats. C21 treatment caused a decrease in albuminuria in ZDF rats up to 11 wk of age. Losartan decreased macrophage infiltration, TNF-α expression, and renal glomerular and perivascular fibrosis, restored glomerular nephrin expression, but did not affect tubulointerstitial fibrosis. Losartan treatment caused a decrease in albuminuria in ZDF rats up to 15 wk of age. At the end of the protocol, only the combination of C21 plus losartan significantly reduced albuminuria in ZDF rats. These data demonstrate that C21 has beneficial effects on diabetic nephropathy, suggesting the combination of C21 and losartan as a novel pharmacological tool to slow the progression of nephropathy in type II diabetes.

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masked by angiotensin-converting enzyme inhibition, is mediated by nitric oxide (6).

Stimulation of AT2 receptors by C21 blunted early renal inflammatory cell infiltration in renovascular hypertension (17) and in stroke-prone SHR (9, 24). In obese Zucker rats, the C21 anti-inflammatory effect in the kidney is mediated by an increase in IL-10 production by proximal tubule epithelial cells (8).

As of today, it is not known whether the direct stimulation of the AT2 receptor in diabetes has nephroprotective effects. Here, we investigate in Zucker diabetic fatty (ZDF) rats, a well-known experimental model of type 2 diabetes (14, 21, 22), the effects of C21 on the progression of diabetic nephropathy as measured by changes in urinary albumin excretion and in the degree of renal fibrosis.

METHODS

Experimental protocol. Animal husbandry was in conformity with institutional guidelines and in compliance with national laws and policies (D.L.n. 116, Gazzetta Ufficiale della Repubblica Italiana, suppl. 40, Feb. 18, 1992), and experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

 Conscious male ZDF and control lean rats (5–6 wk old, Charles River, Calco, Italy) were individually housed in a temperature-controlled room (22°C) with a 12:12 light-dark cycle and allowed to become accustomed to the experimental procedures. Animals had free access to a high-protein diet (Purina 2008) and tap water.

At 5–6, 10, 14, and 19 wk of age, rats were individually housed in metabolic cages for 1 wk to collect 24-h urine samples. Body weight (BW, g) and blood glucose (mg/dl, OneTouch Ultra System, LifeScan, Milpitas CA) were measured once a week.

At 6 wk of age, immediately after the 24-h urine collection, losartan (10 mg·kg⁻¹·day⁻¹, in drinking water; n = 9), C21 (0.3 mg·kg⁻¹·day⁻¹, intraperitoneal injection; n = 10; provided by Dr. A. Ljunggren and Dr. P. Jansson, Vicore Pharma, Mölnäld, Sweden), and losartan plus C21 (n = 9) were administered to ZDF rats for 15 wk. A group of ZDF (n = 12) and control lean rats (n = 12) was maintained without any treatment. At the end of the experimental protocol, a blood sample was collected to measure total cholesterol (mg/dl), HDL cholesterol (mg/dl), LDL cholesterol (mg/dl), triglycerides (mg/dl), and uric acid (mg/dl; Cobas Mira Plus, Roche, Basel, Switzerland), and then rats were euthanized with an overdose of anesthesia (pentobarbital sodium). Plasma cholesterol, HDL, LDL, triglycerides, and uric acid were not measured in two ZDF rats and in one ZDF+losartan-treated rat for insufficient amount of blood sampling. Systolic blood pressure (SBP, mmHg) was measured at the beginning and at the end of the experimental protocol by the tail cuff method (average of 6 recordings. BP Recorder, Ugo Basile Instruments) by an investigator who was unaware of the specific treatments. Blood and urinary creatinine (mg/dl) were measured by the colorimetric technique on a Cobas Mira Plus, and the glomerular filtration rate (GFR, ml/min) was calculated by creatinine clearance. Urinary albumin excretion (mg) was measured on 24-h urine collection by ELISA (Nephrat II ELISA, Exocell, Philadelphia, PA) and expressed as albumin excretion (mg) was measured on 24-h urine collection by ELISA (Nephrat II ELISA, Exocell, Philadelphia, PA) and expressed as

Histological analysis and morphometric evaluation of renal fibrosis. For each group, renal transmural sections (4 μm) were deparaffinized, rehydrated, and stained with hematoxylin–eosin (H&E) using normal light, and 5-Mp (24-bit color depth) images, captured by a computerized digital camera (Olympus Summit 5050, Olympus, Tokyo, Japan), were analyzed for collagen volume fraction in glomerular, tubulointerstitial, and perivascular areas with a computerized imaging software (Image J, NIH, Bethesda, MD). Image analysis was performed by two pathologists, blinded to the experimental protocol. The glomerular and tubulointerstitial collagen volume fractions were evaluated at ×200 magnification, respectively, in semiautomated fashion in 20 traced glomeruli (considered as matrix, cells, capillary loops, and space surrounding glomerular segments) and in automated fashion in 20 fields without vessels or glomeruli, randomly selected from each kidney section. The glomerular collagen fraction was expressed as the ratio of the red-stained collagen area to glomerular area; the tubulointerstitial collagen fraction was expressed as the ratio of collagen area to total area (7).

For analysis of perivascular fibrosis, 10 vessels were randomly selected at ×400 magnification from each kidney section. The image analysis of intraparenchymal vessels was performed in semiautomatic fashion. Only the collagen immediately surrounding each intraparenchymal vessel was considered to represent perivascular collagen deposition. The perivascular collagen volume fraction was expressed as the ratio of collagen area surrounding the traced vessel to total cross-sectional area, to correct differences in vessel size (7).

Collagen analysis (polarized light microscope). For each sample, the sirius red-stained transmural section was analyzed using a light microscope under polarized light to evaluate the different types of collagen content in glomerular, tubulointerstitial, and perivascular areas, as previously described (7). By polarized light microscopy, type I collagen appears yellow/orange, while type III collagen appears green (12). For each sample, randomly selected microsections fields of glomerular, tubulointerstitial, and perivascular areas were analyzed with a polarized light microscope (Leitz Camera) using a ×20 objective. Images were captured by a computerized digital camera (Olympus Camedia 5050) using SPOT (Diagnostic Instruments, Sterling Heights, MI), and analyzed for different type of collagens (7).

Immunohistochemical evaluation of monocyte/macrophage renal infiltration. The evaluation of monocyte/macrophage infiltration was performed on formalin-fixed and paraffin-embedded renal sections (4 μm), using, respectively, mouse anti-rat monocyte/macrophage (CD68) monoclonal antibody (clone ED1, MAB 1435, Chemicon, Temecula, CA), anti-TNF-α polyclonal antibody (NBPl–19532, Novus Biologicals, Littleton, CO), and rabbit anti-IL-10 polyclonal antibody (bs-0698R, Bioss, Woburn, MA). The sections were deparaffinized and rehydrated, treated by boiling in citrate buffer (0.01 mol/l, pH 6) in a microwave (750 W), and incubated with primary antibody (monocye/macrophage CD68 antibody: overnight at 4°C, dilution 1:300; TNF-α antibody: 1 h at room temperature, dilution 1:100; IL-10 antibody: overnight at 4°C, dilution 1:100). Ultra Tek horseradish peroxidase anti-mouse (ScyTek Laboratories, Logan, UT) was used to label the primary antibody. The reaction product was visualized with diaminobenzidine (DAB; Dako, Glostrup, Denmark). Negative control was obtained by omitting the primary antibody. Sections were viewed using a Leica microscope (Leitz Camera), and digital images were taken using a computerized digital camera (Olympus Camedia 5050).

Two independent investigators blinded to the treatment counted the number of interstitial positive cells in 15 randomly selected non-
Table 1. Blood glucose levels, systolic blood pressure, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, uric acid, body weight, and kidney wt/body wt ratio in control lean, ZDF, ZDF+C21, ZDF+losartan, and ZDF+losartan+C21 rats at the end of the experimental period

<table>
<thead>
<tr>
<th></th>
<th>Lean (n = 12)</th>
<th>ZDF (n = 12)</th>
<th>ZDF+C21 (n = 10)</th>
<th>ZDF+Los (n = 9)</th>
<th>ZDF+Losartan+C21 (n = 9)</th>
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<tbody>
<tr>
<td>Blood glucose, mg/dl</td>
<td>144.23 ± 6.51</td>
<td>555.82 ± 20.30*</td>
<td>573.70 ± 16.44*</td>
<td>596.62 ± 2.73*</td>
<td>565.62 ± 26.94*</td>
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<tr>
<td>SBP, mmHg</td>
<td>147.0 ± 1.7</td>
<td>150.9 ± 3.0</td>
<td>144.1 ± 3.1</td>
<td>134.6 ± 2.4‡</td>
<td>139.4 ± 5.6‡</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>99.17 ± 4.85</td>
<td>119.29 ± 23.47</td>
<td>125.00 ± 10.90</td>
<td>133.83 ± 19.26</td>
<td>136.00 ± 10.23</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>13.08 ± 1.75</td>
<td>47.43 ± 7.78*</td>
<td>38.40 ± 5.09*</td>
<td>26.33 ± 3.17*</td>
<td>36.75 ± 8.02*</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>28.75 ± 1.88</td>
<td>64.29 ± 12.22*</td>
<td>62.80 ± 6.70*</td>
<td>82.00 ± 1.97*</td>
<td>58.75 ± 7.36*</td>
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<tr>
<td>Triglycerides, mg/dl</td>
<td>79.17 ± 10.13</td>
<td>701.43 ± 139.0*</td>
<td>492.80 ± 39.39*†</td>
<td>555.33 ± 126.4*</td>
<td>464.50 ± 106.2*</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>1.72 ± 0.17</td>
<td>13.23 ± 3.23*</td>
<td>7.16 ± 0.83*†‡</td>
<td>9.24 ± 2.43*</td>
<td>9.55 ± 2.41*</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>405.08 ± 4.78</td>
<td>418.00 ± 10.28</td>
<td>412.00 ± 7.30</td>
<td>430.87 ± 13.48*</td>
<td>441.62 ± 11.95‡</td>
</tr>
<tr>
<td>Kidney wt/body wt, mg/g</td>
<td>3.620 ± 0.258</td>
<td>5.277 ± 0.180*</td>
<td>5.165 ± 0.149*</td>
<td>4.979 ± 0.247*</td>
<td>4.476 ± 0.152‡†</td>
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Values are means ± SE; n = no. of rats. ZDF, Zucker diabetic fatty rats; C21, compound 21; SBP, systolic blood pressure. For technical reasons (blood sampling insufficient to perform all the measurements), plasma cholesterol, LDL, HDL, triglycerides, and uric acid were not measured in 2 ZDF rats and 1 ZDF+losartan rat. *P < 0.01 vs. lean rats. †P < 0.05 vs. ZDF rats. ‡P < 0.01 vs. ZDF+C21 rats.

RESULTS

Effects of C21 and losartan on systolic blood pressure and metabolic parameters. At the end of the experimental period, blood glucose levels were significantly increased in diabetic rats (Table 1). C21 or losartan treatment, alone or in combination, did not modify blood glucose levels (Table 1). Blood pressure was similar in control lean and ZDF rats and was not significantly modified by C21 treatment (Table 1). Losartan treatment, alone or in combination with C21, caused a decrease in blood pressure (Table 1). ZDF rats, treated or not with C21 and losartan, did not show a significant modification in total plasma cholesterol. Triglycerides were significantly increased in ZDF rats compared with control lean rats. In ZDF rats treated with C21, but not with losartan, triglycerides were significantly lower than in untreated ZDF rats. Plasma uric acid level was higher in ZDF rats compared with control lean rats. Losartan administration did not significantly modify plasma uric acid compared with ZDF rats. C21 alone or in combination with losartan significantly reduced plasma uric acid level in ZDF rats compared with ZDF rats without treatment (Table 1). Body weight in ZDF rats and ZDF rats treated with C21 was similar to control lean rats, while it was significantly increased in ZDF rats treated with losartan alone or in combination with C21. The kidney weight/body weight ratio was significantly increased in ZDF rats and in ZDF rats treated with C21 or losartan compared with control lean rats. The kidney weight/body weight ratio was significantly decreased in ZDF rats treated with C21 plus losartan compared with ZDF rats without any treatment (Table 1).

Effects of C21 and losartan on GFR and urinary albumin excretion. GFR was significantly higher in ZDF rats compared with control lean rats, indicating the presence of hyperfiltration (Fig. 1). C21 caused a significant decrease in GFR compared with untreated ZDF rats. GFR in ZDF rats treated with losartan...
alone or in combination with C21 was similar to control lean rats (Fig. 1). As expected, all groups of ZDF rats showed an increase in urinary albumin excretion compared with control lean rats (Fig. 2, δ P < 0.0001). At 5–6 wk of age, just before the onset of hyperglycemia and before the beginning of the different treatments, urinary albumin excretion was higher in ZDF rats compared with control lean rats. In control lean rats, urinary albumin excretion did not change during the time of the experimental protocol, while a marked increase in urinary albumin excretion was observed in ZDF rats (P < 0.01). C21 blunted the increase in urinary albumin excretion at 15 wk of age in ZDF rats, but lost this effect in 20-wk-old ZDF rats, along with the progression of nephropathy. The anti-albuminuric effect of losartan was greater and longer lasting than the one observed for C21, but also in this case there was a loss of efficacy during the time. At 20 wk of age, only the combination C21 plus losartan was able to significantly blunt urinary albumin excretion (Fig. 2). Similar results were obtained using 24-h albumin excretion, expressed as milligrams per 24 hours (data not shown).

**Effects of C21 and losartan on renal fibrosis.** At the end of the experimental period, glomerular, tubulointerstitial, and perivascular fibrosis was significantly increased in ZDF rats (Fig. 3, A and B). Type I collagen fibers, which appear yellow-orange under polarized light microscopy, characterized glomerular, tubulointerstitial, and perivascular fibrosis (Fig. 3A).

Administration of C21 to ZDF rats significantly prevented the development of glomerular, tubulointerstitial, and perivascular fibrosis and kept it down to values observed in control lean rats (Fig. 3, A and B). In ZDF rats, treatment with losartan caused a significant reduction in glomerular and perivascular fibrosis compared with ZDF rats without any treatment, but there was no effect on tubulointerstitial fibrosis (Fig. 3, A and B). C21 administration was more effective than losartan in reducing tubulointerstitial fibrosis (P = 0.057. Fig. 3, A and B). Combination of C21 plus losartan reduced tubulointerstitial fibrosis as observed with C21 treatment alone.

**Effects of C21 and losartan on renal inflammatory cell infiltration, TNF-α, IL-10, and glomerular nephrin expression.** ZDF rats showed a significant increase in monocyte/macrophage infiltration and TNF-α in renal tissue compared with control lean (Fig. 4, A and B). C21 administration in ZDF rats caused a significant reduction in monocyte/macrophage infiltration and TNF-α expression compared with ZDF rats without any treatment (Fig. 4, A and B).

Renal monocyte/macrophage infiltration, but not TNF-α expression, was significantly higher in ZDF rats compared with control lean rats (Fig. 4, A and B). Losartan treatment, alone or in combination with C21, completely blocked inflammatory cell infiltration (Fig. 4, A and B) and the increase in TNF-α expression (Fig. 4, A and B). As shown in Fig. 4, the presence of monocytes/macrophages and TNF-α expression in ZDF rats treated with losartan or losartan plus C21 were similar to that observed in control lean.

Renal IL-10 expression was significantly higher in ZDF rats and ZDF rats treated with C21 compared with control lean rats (Fig. 4, A and B). No significant differences in renal IL-10 expression was observed among the different groups of ZDF rats, regardless of treatment.

At 20 wk of age, ZDF rats and ZDF rats treated with C21 showed a significant decrease in glomerular nephrin expression compared with control lean rats (Fig. 5, A and B). Losartan administration, alone or in combination with C21, restored glomerular nephrin expression (Fig. 5, A and B).

**DISCUSSION**

The results of this study demonstrate that C21 treatment promotes nephroprotection in diabetes. In fact, C21 administration is effective in reducing renal fibrosis, in the absence of a decrease in blood pressure or blood glucose level. In addition, C21 treatment blunts the increase in albuminuria in the early stage of the disease and improves the antiproteinuric effects of losartan during the progression of diabetic nephropathy.

While increasing evidences demonstrate that direct stimulation of AT₂ receptors by C21 reduces tissue fibrosis in the cardiovascular system in different experimental models of hypertension (20, 24), heart failure, and after myocardial infarction (15, 16), the effect of C21 on the kidney is less investigated. C21 treatment at a high dose (10 mg·kg⁻¹·day⁻¹)
reduced proteinuria, renal inflammation, and collagen deposition in stroke-prone SHR fed a high-salt diet (9). In renovascular hypertensive rats, C21 administration reduced inflammatory cell infiltration and transforming growth factor-β1 expression in the clipped kidney (17). In obese rats, C21 treatment reduced renal inflammation, not only decreasing the expression of TNF-α and IL-6 and monocyte/macrophage infiltration, but also inducing the expression of IL-10, a potent anti-inflammatory cytokine (8).

The present data demonstrate that in ZDF rats, a well-known experimental model of type 2 diabetes (14, 21, 22), the administration of C21 has beneficial effects on kidney tissue. In fact, in our experimental condition, C21 showed a potent antifibrotic effect on renal tissue, reducing glomerular, tubulointerstitial, and perivascular fibrosis.

Fig. 3. Effect of C21 and losartan administration on renal fibrosis in ZDF rats. A: representative photomicrographs of glomerular, tubulointerstitial, and perivascular fibrosis in control lean rats (a, f, k), ZDF rats (b, g, l), ZDF+C21 rats (c, h, m), ZDF+losartan rats (d, i, n), and ZDF+losartan+C21 rats (e, j, o)-treated rats (sirius red staining: glomerular and tubulointerstitial fields, original magnification ×200; perivascular fields: original magnification ×400). Staining shows an increase in glomerular, tubulointerstitial, and perivascular fibrosis in ZDF rats (b, g, l) vs. control lean rats (a, f, k). C21 treatment (c, h, m) and coadministration of losartan plus C21 (e, j, o) significantly reduced glomerular, tubulointerstitial, and perivascular fibrosis compared with ZDF rats (b, g, l). Losartan treatment caused a significant decrease in glomerular and perivascular fibrosis (d, n) vs. control lean rats (a, f, k). C21 treatment (c, h, m) was more effective than losartan treatment (i) in reducing tubulointerstitial fibrosis. A combination of C21+losartan (j) reduced tubulointerstitial fibrosis as observed with C21 treatment alone (h). Glomerular, tubulointerstitial, and perivascular fibrosis is caused by an increase in type I collagen fibers, which appear orange/yellow under polarized light microscopy. B: quantification of glomerular, tubulointerstitial, and perivascular fibrosis in the different groups of rats. Control lean rats (n = 8); ZDF rats (n = 9); ZDF+C21 rats (n = 9); ZDF+losartan rats (n = 8); ZDF+losartan+C21 rats (n = 8). Values are means ± SE. *P < 0.01.
and perivascular fibrosis. Losartan treatment reduced renal fibrosis at the glomerular and perivascular level, but it was unable to significantly reduce tubulointerstitial fibrosis. The combination of losartan and C21 reduced tubulointerstitial fibrosis as observed with C21 treatment alone.

Taken together, these data show a potent antifibrotic effect of C21, which is mediated at least partially, by its anti-inflammatory action, through the reduction of monocyte/macrophage infiltration and TNF-α expression in renal tissue. Even if the antifibrotic effect of losartan is certainly mediated by its effect on monocyte/macrophage infiltration, by the decrease in blood pressure and albuminuria, losartan treatment is less effective in reducing tubulointerstitial fibrosis than C21. Since tubulointerstitial fibrosis shows the best correlation with the progression of renal disease (4), these evidences strongly suggest that direct stimulation of the AT2 receptor could counteract an important hallmark of progression of nephropathy. In this regard, we can speculate that the beneficial effects of C21 on the kidney tissue of ZDF rats might depend on the renal upregulation of AT2 receptor expression, as previously described for obese Zucker rats (1, 8, 28).

In addition, after the onset of hyperglycemia, which occurs at ~7 wk of age, and until 11 wk of age, C21 is able to blunt the increase in urinary albumin excretion, counteracting the decrease in glomerular nephrin expression (data not shown). This antialbuminuric effect is lost at 15 wk of age, along with
the progression of diabetic nephropathy. Losartan treatment is more effective in reducing albuminuria compared with C21, preserving glomerular nephrin expression longer, also at 20 wk of age. However, during the progression of nephropathy also in the losartan-treated ZDF rats, an escape phenomenon in albuminuria is evident, with a progressive loss of the antialbuminuric effect of losartan. Even if an escape phenomenon is present also in ZDF rats treated with the combination of losartan plus C21, at 20 wk of age, only this combination was able to significantly reduce urinary albumin excretion compared with ZDF rats without any treatment. In addition, we can reasonably hypothesize that the administration of losartan and C21 at higher doses will result in a greater effect, slowing the progression of proteinuria. However, on the basis of our results we can also speculate that, since losartan loses its effect over time in the case of diabetic nephropathy, C21 could represent a valuable supplement to diabetic patients to protect against further deterioration of renal function and to prevent end-stage renal disease. In this regard, clinical studies testing the effect and long-time efficacy of C21 in humans (and of its association with losartan) are needed.

As it is reasonable to assume that plasma concentration of angiotensin II is increased in ZDF rats treated with losartan, we interpreted the finding that the C21 and losartan combination still exerts an additive effect as evidence that the AT$_2$ receptor was not fully activated by circulating levels of angiotensin II. The same conclusion could be drawn by hypothesizing that C21 might activate the AT$_2$ receptor in a way independently of the one followed by angiotensin II.

Furthermore, in ZDF rats C21 treatment alone or in combination with losartan induced a significant reduction in triglycerides and plasma levels of uric acid, which are two important factors involved in renal disease progression. The beneficial effects of AT$_2$ stimulation on lipid metabolism has been described (27), while the effect on uric acid is an unexpected finding. It has been described that the blockade of the AT$_1$ receptor interferes with the metabolism of uric acid, resulting in a reduction of serum values (19), or in the stimulation of renal uric acid excretion (23). Even though a direct relationship between uric acid and AT$_2$ receptors is not known, we can nonetheless speculate that the stimulation of AT$_2$ receptors might result in a reduction of levels of uric acid.

However, the reduction of plasma levels of uric acid by C21 treatment could potentiate the positive effects of C21 on renal tissue, because elevated plasma levels of uric acid increase the risk of kidney diseases and contribute to the worsening of renal diseases, as demonstrated in clinical studies in humans (11, 29, 38, 39), through an increase in oxidative stress and inflammatory processes (13).

In addition, it has been recently demonstrated that acute C21 administration improves renal functions in female, but not in male, SHR rats (10). AT$_2$ receptors are expressed to a greater extent in the kidney of female SHR rats, suggesting sex-specific effects of AT$_2$ stimulation by C21 on the kidney (10). In this regard, we can speculate that the effects of C21 in the prevention of diabetic nephropathy, which we are showing here in male ZDF rats, could be similar or even larger in female rats.

In summary, these data indicate that C21 should be considered an antifibrotic drug potentially of use to counteract the development of nephropathy in type II diabetes and suggest a
novel pharmacological combination with AT1 receptor blockade to slow the progression of diabetic nephropathy.

DISCLOSURES

B. Dahlöf and T. Unger received speaker fees from Vicore Pharma. T. Unger has a modest interest in Vicore Pharma. U. M. Steckelings received modest research support from Vicore Pharma. B. Dahlöf has a significant interest in Mintage Scientific, the owner of Vicore Pharma.

AUTHOR CONTRIBUTIONS


REFERENCES


