Renal injury in streptozotocin-diabetic Ren2-transgenic rats is mainly dependent on hypertension, not on diabetes

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Submitted 16 March 2006; accepted in final form 25 September 2006

Hartner A, Cordasic N, Klanke B, Wittmann M, Veelken R, Hilgers KF. Renal injury in streptozotocin-diabetic Ren2-transgenic rats is mainly dependent on hypertension, not on diabetes. Am J Physiol Renal Physiol 292: F820–F827, 2007. First published October 3, 2006; doi:10.1152/ajprenal.00088.2006.—Induction of streptozotocin (STZ) diabetes in hypertensive rats transgenic for the mouse ren-2 gene (TGR) has been described as a model of progressive diabetic nephropathy. We investigated the long-term course of STZ diabetes in TGR and appropriate Sprague-Dawley control rats (SD) and tested the role of angiotensin-dependent hypertension by treating rats with the angiotensin II type 1 receptor blocker losartan (1 mg·kg−1·day−1) via osmotic minipumps. Five weeks after STZ injection, diabetes developed in TGR and SD. Urinary albumin excretion was increased by diabetes and, to a much higher degree, by hypertension. The effects of hypertension and diabetes were not additive, and only the effects of hypertension were ameliorated by losartan. A similar pattern was observed for cell proliferation and macrophage infiltration in the kidney. In contrast, the effects of hypertension and diabetes on glomerular collagen IV accumulation were additive 5 wk after STZ injection. In a long-term study for 20 wk after STZ, survival was better in STZ-treated TGR than in normoglycemic TGR, whereas all SD survived. Impaired creatinine clearance and increased macrophage infiltration as well as glomerular and interstitial matrix deposition were prominent in TGR compared with SD, regardless of the presence or absence of diabetes. In conclusion, STZ diabetes in TGR may be useful to study glomerular and interstitial matrix deposition early in the course of diabetes. However, the long-term course of this animal model resembles severe hypertensive nephrosclerosis, rather than progressive diabetic nephropathy.

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glucose >250 mg/dl were included. Nineteen TGR-STZ and 17 SD-STZ rats, as well as 16 normoglycemic TGR and 8 normoglycemic SD rats, were followed for 5 wk. Of these, eight SD-STZ, eight TGR, and nine TGR-STZ received a low dose of the AT1 blocker losartan (1 mg·kg body wt·1·day−1) for the last 4 wk. The animals were implanted intraperitoneally with osmotic minipumps (Alzet model 2004; Alza Scientific Products, Palo Alto, CA), which delivered 0.25 μl/h for 28 days. In a second set of experiments, 6 SD, 7 SD-STZ, 12 TGR, and 9 TGR-STZ were followed for 20 wk. Blood glucose and systolic blood pressure (measured using tail-cuff plethysmography under light ether anesthesia) were monitored weekly (at 8:00 AM). At the end of the experiment, the rats were kept in metabolic cages for determination of urinary albumin excretion (enzyme immunoassay kit; CellTrend, Luckenwalde, Germany) for 24 h. Subsequently, the rats were equipped with a femoral artery catheter, and arterial blood pressure was measured via transducers (Grass Instruments, Quincy, MA) connected to a polygraph (Hellige, Freiburg, Germany) 4 h after termination of anesthesia. Protein, glucose, urea, and creatinine in serum or urine samples were analyzed with the automatic analyzer Integra 800 (Roche Diagnostics, Mannheim, Germany). Rats were killed, and kidneys were weighed and decapsulated. Renal tissue was fixed in methyl-Carnoy solution (60% methanol, 30% chloroform, and 10% glacial acetic acid) for histology and immunohistochemistry.

**Immunohistochemistry.** After overnight fixation in methyl-Carnoy solution, tissues were dehydrated by being bathed in increasing concentrations of methanol, followed by 100% isopropanol. After tissues were embedded in paraffin, 3-μm sections were cut with a Leitz SM 2000 R microtome (Leica Instruments, Nussloch, Germany). After deparaffinization, endogenous peroxidase activity was blocked with 3% H2O2 in methanol for 20 min at room temperature. A mouse monoclonal antibody detecting proliferating cells (proliferating cell nuclear antigen, PCNA) was purchased from Santa Cruz Biotechnologies (Heidelberg, Germany) and used at a dilution of 1:50. The mouse monoclonal antibody against the macrophage marker ED-1 was purchased from Serotec (Biozol, Eching, Germany) and used at a dilution of 1:250. Renal cortical collagen I was detected using a rabbit polyclonal antibody (Biogenesis, Poole, UK) at a dilution of 1:250. Renal cortical collagen IV was detected using a goat polyclonal antibody (Southern Biotechnology Associates, Birmingham, AL) at a dilution of 1:500. Each slide was counterstained with hematoxilin.

**Real-time RT-PCR detection of mRNA.** Renal cortical tissue extraction and real-time RT-PCR were carried out as described (35). Briefly, first-strand cDNA was synthesized with TaqMan reverse transcription reagents (Applied Biosystems, Darmstadt, Germany) using random hexamers as primers. Final RNA concentration in the reaction mixture was adjusted to 0.5 ng/μl. Reactions without M- 

**Analysis of data.** Intraglomerular PCNA- or ED-1-positive cells were counted in all glomeruli of a given kidney section (120–300 glomeruli, no selection) and expressed as cells per glomerular section. Interstitial PCNA- or ED-1-positive cells were counted in 30 medium-power (magnification ×250) cortical views per section and expressed as cells per square millimeter. Counting was begun in a random cortical field and in consecutive nonoverlapping cortical fields to the right of the previous view without selection; if necessary, counting was continued at the opposite (left) edge of the section. Expansion of interstitial collagen I was measured using Metaview software (Visitron Systems, Puchheim, Germany) in 10 nonoverlapping medium-power cortical views per section, excluding glomeruli, and was expressed as a percentage of stained area per cross section. Glomerular collagen IV staining was measured using Metaview in every third glomerulus per cross section, and the stained area was expressed as a percentage of the total area of the glomerular tuft.

Two-way analysis of variance, followed by the post hoc least significant difference test, was used to compare groups. A P value <0.05 was considered significant. The procedures were carried out using SPSS version 13.0 software (SPSS, Chicago, IL). Values are displayed as means ± SE.

**RESULTS**

Both SD and TGR developed diabetes mellitus following treatment with STZ (Fig. 1A). In untreated TGR, blood glucose did not differ from that in untreated SD rats (Fig. 1A). Losartan treatment did not significantly alter blood glucose levels (Fig. 1A). Diabetes led to increased water intake in both SD and TGR, which was not altered by losartan (Table 1). Systolic and mean arterial blood pressure were significantly higher in TGR compared with SD rats (Fig. 1B and Table 1). Hypertension in TGR was not significantly affected by STZ treatment (Fig. 1), although intra-arterial measurements showed a trend toward lower mean pressure in TGR-STZ than in TGR (Table 1). Chronic administration of a low dose of losartan did not significantly lower systolic blood pressure (Fig. 1B) but had a modest effect on mean arterial blood pressure in TGR and a marked effect on TGR-STZ (Table 1). Albumin excretion was increased in diabetic animals and even more in hypertensive rats, but a combination of both diseases did not further elevate albuminuria (Fig. 1C). In diabetic and nondiabetic TGR, but not in diabetic SD rats, losartan ameliorated albuminuria (Fig. 1C). Losartan reduced plasma creatinine levels in TGR and TGR-STZ (Table 1).

STZ diabetes led to reduced body weight gain and kidney hypertrophy. Kidney weight-to-body weight ratio was significantly increased in SD-STZ, TGR, and TGR-STZ compared with SD controls. Losartan reduced kidney hypertrophy in TGR and TGR-STZ but not in SD-STZ (Table 1). TGR hypertensive animals had significantly higher heart weight-to-body weight ratios than normotensive SD rats, and this was not affected by STZ diabetes (Table 1). Again, losartan reduced heart weight-to-body weight ratios in TGR and TGR-STZ. Plasma creatinine was significantly elevated only in TGR (Table 1).

Macrophage infiltration was significantly elevated in the kidney interstitium and in the glomerulus only in TGR (Table 2). Treatment with STZ did not further augment interstitial or glomerular macrophage infiltration in TGR (Table 2). Losartan significantly reduced renal interstitial and glomerular macrophage infiltration in all groups (Table 2). Cell proliferation, as counted after immunohistological detection of PCNA, was significantly increased in the kidney interstitium and in the glomerulus only in TGR, with a tendency toward more PCNA-positive cells in SD-STZ and TGR-STZ compared with SD rats.
Body weight, g 388.3

Body and organ weights, urine production, water intake, plasma creatinine, and mean arterial blood pressure after 5 wk of diabetes

<table>
<thead>
<tr>
<th>Group</th>
<th>SD</th>
<th>SD+STZ</th>
<th>SD-LOS</th>
<th>TGR</th>
<th>TGR-LOS</th>
<th>TGR+STZ</th>
<th>TGR-LOS</th>
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<tr>
<td>Body weight, g</td>
<td>388.3±9.6</td>
<td>269.1±5.9*</td>
<td>262.0±12.4*</td>
<td>337.9±12.0*</td>
<td>377.1±6.3†</td>
<td>265.6±16.1*</td>
<td>272.7±10.7*</td>
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<td>Heart weight/body weight ratio, mg/g</td>
<td>3.3±0.1</td>
<td>3.8±0.1</td>
<td>3.6±0.1</td>
<td>5.4±0.3*</td>
<td>4.6±0.2*†</td>
<td>4.7±0.2*</td>
<td>3.7±0.1†</td>
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<tr>
<td>Kidney weight/body weight ratio, mg/g</td>
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<td>4.4±0.1*</td>
<td>3.8±0.1†</td>
<td>5.7±0.2*</td>
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<tr>
<td>Water intake, ml/24 h</td>
<td>44.7±6.7</td>
<td>167.0±15.0*</td>
<td>136.5±14.3*</td>
<td>64.6±6.8</td>
<td>49.9±8.8</td>
<td>147.4±14.6*</td>
<td>147.2±9.6*</td>
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<td>Creatinine, mg/dl</td>
<td>0.31±0.01</td>
<td>0.28±0.01</td>
<td>0.24±0.01</td>
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<td>0.33±0.02†</td>
<td>0.34±0.07</td>
<td>0.24±0.01†</td>
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<td>MAP, mmHg</td>
<td>120.1±6.5</td>
<td>119.4±3.5</td>
<td>112.7±3.1</td>
<td>212.5±10.3*</td>
<td>171.6±10.4†</td>
<td>178.6±17.0*</td>
<td>122.5±5.7††</td>
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SD, normotensive normoglycemic Sprague-Dawley control rats; TGR, transgenic hypertensive rats; STZ, streptozotocin treatment; LOS, losartan treatment; MAP, mean arterial blood pressure. *P < 0.05 vs. SD, †P < 0.05 vs. respective non-losartan-treated group.
glomeruli (Fig. 8B) compared with control SD rats. In TGR, cortical and glomerular matrix expansion was even more prominent (Fig. 8B), but this was not further aggravated by 20 wk of diabetes (Fig. 8).

**DISCUSSION**

Our data confirm that both STZ diabetes and ren-2-induced hypertension cause some degree of kidney injury. The extent of the hypertension-induced damage was far more pronounced, however. The combination of both models of disease led to additive effects on a few parameters, especially glomerular matrix expansion. These effects were apparent in the early phase of diabetes, 5 wk after STZ injection. Over a prolonged period, the presence or absence of hypertension determined survival and kidney damage, whereas the additional presence or absence of diabetes mellitus had little effect. Thus the model of STZ diabetes in ren-2 transgenic rats may be useful to examine mechanisms of glomerular matrix expansion in early diabetes. However, our data do not support the notion that this model represents human progressive diabetic nephropathy, as suggested by Kelly et al. (16). Rather, severe hypertensive nephrosclerosis determines the outcome of diabetic as well as normoglycemic ren-2 transgenic rats.

Our findings are in agreement with the results of many authors who reported that STZ diabetes does not lead to progressive renal damage in rodents [for review, see O’Donnell et al. (29) for rats and Breyer et al. (4) for mice]. However, our results contrast with those of the Melbourne group reporting a far more pronounced renal damage in TGR-

### Table 2. Macrophage infiltration (ED-1-positive cells) in the tubulointerstitium or glomerulus of renal sections after 5 or 20 wk of diabetes

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>SD-STZ</th>
<th>SD-STZ-Los</th>
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<th>TGR-Los</th>
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<td>9</td>
</tr>
<tr>
<td>ED-1-positive cells/cortical view</td>
<td>10.3±0.7</td>
<td>13.4±1.8</td>
<td>6.4±1.0</td>
<td>44.7±5.9*</td>
<td>16.4±3.7†</td>
<td>46.2±10.6*</td>
<td>11.8±1.5†</td>
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<tr>
<td>ED-1-positive cells/glomerulus</td>
<td>0.40±0.04</td>
<td>0.59±0.06</td>
<td>0.57±0.11</td>
<td>0.73±0.06*</td>
<td>0.41±0.04†</td>
<td>0.68±0.09*</td>
<td>0.44±0.09†</td>
</tr>
<tr>
<td><strong>20 Wk</strong></td>
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<tr>
<td>ED-1-positive cells/cortical view</td>
<td>9.42±1.0</td>
<td>8.94±1.0</td>
<td>21.50±8.9*</td>
<td>13.75±5.0</td>
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<tr>
<td>ED-1-positive cells/glomerulus</td>
<td>0.46±0.04</td>
<td>0.51±0.07</td>
<td>1.61±0.75*</td>
<td>0.91±0.19</td>
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</table>

*P < 0.05 vs. SD, †P < 0.05 vs. respective non-losartan-treated group.

**Fig. 2.** Cell proliferation in interstitial space (A) and glomeruli (B) of the kidneys from SD and TGR after 5 wk of diabetes with and without losartan treatment, evaluated by staining for the proliferating cell nuclear antigen (PCNA). Glomerular proliferating cells are expressed as PCNA-positive cells per glomerular cross section; interstitial proliferating cells are expressed as PCNA-positive cells per square millimeter. Data are means ± SE. *P < 0.05 vs. normotensive, normoglycemic SD control rats. #P < 0.05 vs. respective non-losartan-treated group.

**Fig. 3.** Matrix expansion in renal cortex and glomerulus in the kidney of SD and TGR after 5 wk of diabetes with and without losartan treatment. A: measurement of cortical collagen I staining. B: measurement of glomerular collagen IV staining. Data are means ± SE. *P < 0.05 vs. normotensive, normoglycemic SD control rats. #P < 0.05 vs. respective non-losartan-treated group. §P < 0.05 vs. TGR.
STZ compared with normoglycemic TGR (16, 17, 27, 37). We cannot fully explain this discrepancy, but several factors may contribute. Kelly, Wilkinson-Berka, Mifsud, and other authors from the Melbourne group used female rats and injected STZ at a younger age (16, 17, 27, 37). Both factors could potentially lead to a less pronounced effect of hypertension on the kidney, because female TGR reportedly exhibit lower blood pressure than male TGR (21). On the other hand, the blood pressure levels reported by Kelly and colleagues (16, 17, 27, 37) for diabetic and normoglycemic TGR are not notably lower than
those measured in our study. Furthermore, Kelly and colleagues (16, 17, 27, 37) may have missed the effects of ren-2-induced hypertension, because these authors did not investigate the respective normotensive controls, neither normoglycemic nor hyperglycemic. Hannover SD rats were included in some studies of the Melbourne group (5, 23), but renal damage was not reported in those articles. Finally, Kelly et al. (16) used a small dose of insulin after STZ treatment, which we did not. The presence or absence of minimal insulin treatment may affect the type and extent of diabetic kidney injury in this model (26).

Our data confirm that both diabetes and ren-2 hypertension induce urinary albumin excretion. However, the effects of hypertension were much more pronounced, and STZ diabetes did not add to the hypertension-induced albuminuria, in contrast to the findings of Kelly et al. (16, 17, 27, 37). A similar pattern was observed for macrophage infiltration as well as cell proliferation in glomeruli and interstitium of the kidney. Losartan ameliorated albuminuria, infiltration, and proliferation in hypertensive rats. The effects of hypertension and diabetes on matrix accumulation were more complex. Interstitial collagen I was increased by diabetes and by hypertension; the effects of both diseases on glomerular collagen IV were additive. The gene expression of TGF-β and collagen I was increased in

Table 3. Body and organ weights, urine production, water intake, plasma creatinine, plasma urea, MAP, and renal macrophage infiltration after 20 wk of diabetes

<table>
<thead>
<tr>
<th>Group</th>
<th>SD</th>
<th>SD-STZ</th>
<th>TGR</th>
<th>TGR-STZ</th>
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<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>484.7±16.3</td>
<td>352.6±18.4</td>
<td>598.8±24.4</td>
<td>409.8±14.6</td>
</tr>
<tr>
<td>Heart weight/body weight ratio, mg/g</td>
<td>3.6±0.1</td>
<td>4.3±0.2</td>
<td>3.9±0.4</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>Kidney weight/body weight ratio, mg/g</td>
<td>3.1±0.1</td>
<td>5.2±0.3</td>
<td>3.0±0.3</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.35±0.01</td>
<td>0.19±0.10</td>
<td>0.45±0.02</td>
<td>0.31±0.01</td>
</tr>
<tr>
<td>Plasma urea, mg/dl</td>
<td>15.8±1.6</td>
<td>24.6±3.1</td>
<td>58.9±5.6</td>
<td>44.3±0.6</td>
</tr>
<tr>
<td>Water intake, ml/24 h</td>
<td>18.4±1.2</td>
<td>96.4±28.0*</td>
<td>33.0±9.6</td>
<td>119.3±20.7*</td>
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<tr>
<td>MAP, mmHg</td>
<td>109.3±2.2</td>
<td>120.4±2.1</td>
<td>128.0±8.6*</td>
<td>135.8±7.1*</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. SD.

![Fig. 6. Time course of plasma glucose (A), systolic blood pressure (B), and survival (C) of SD and TGR during 20 wk of diabetes. N, number of rats. Data are means ± SE. *P < 0.05 vs. normotensive, normoglycemic SD control rats.](image-url)

![Fig. 7. Albuminuria and creatinine clearance in SD and TGR after 20 wk of diabetes. A: albumin excretion. B: creatinine clearance. Data are means ± SE. *P < 0.05 vs. normotensive, normoglycemic SD control rats.](image-url)
This observation was somewhat unexpected. In many years of almost normalized mean arterial blood pressure, although tail-cuff blockade presumably contributed to nephroprotection in TGR. Thus nonhemodynamic effects of ANG II type 1 receptors are used (3, 22, 30, 38). On the other hand, we hesitate to interpret our observations in this way, because kidney inflammation and injury are determined by angiotensin-dependent hypertension for kidney injury in diabetic TGR (27). The reasons for the more pronounced blood pressure lowering induced by losartan in diabetic TGR remain unknown. It is tempting to speculate that the high urine excretion of streptozotocin diabetes may have induced a state of volume loss that could act synergistically with losartan to reduce blood pressure. A similar mechanism may explain the lower blood pressure observed in diabetic as opposed to normoglycemic TGR in the long-term experiment. Both mineralocorticoid as well as glucocorticoid hormones contribute to hypertension in TGR (7, 31) and may thus induce a volume-dependent component that might be alleviated by a tendency to fluid loss. We presume that the lower mortality of the diabetic TGR in the long-term experiment is most likely due to the somewhat lower blood pressure. We did not examine the precise reason for the high mortality of normoglycemic diabetic TGR. However, Whitworth et al. (36) previously investigated the long-term course of TGR rats and described a similar mortality rate. These authors reported that TGR died from malignant phase hypertension (36). The death of the most severely hypertensive animals could account for the apparently low extent of left ventricular hypertrophy in our 20-wk study.

In conclusion, our data do not support the notion that STZ diabetes in ren-2 transgenic, hypertensive TGR is a model of severe progressive diabetic nephropathy. The model may be useful to study the additive effects of hypertension and hyperglycemia on glomerular and interstitial fibrosis in the early phase of diabetes. In the long term, however, survival of the animals as well as the degree of kidney inflammation and injury are determined by angiotensin-dependent hypertension, irrespective of the presence or absence of STZ diabetes. In other words, this animal model resembles severe hypertensive nephrosclerosis, rather than diabetic nephropathy.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of Rainer Wachtveitl and Miroslava Kupraszewicz-Hutzler.

GRANTS

This study was part B12 of the Interdisziplinäres Zentrum für Klinische Forschung at the Hospital of the University of Erlangen-Nuremberg, funded by the Bundesministerium für Bildung und Forschung (01 KS 0002). In addition, the study was supported by a grant-in-aid (KFO 106, TP2) from the Deutsche Forschungsgemeinschaft. Parts of the data were presented in abstract form at the 31st Annual Meeting of the American Society of Nephrology in Philadelphia, PA, in 1998.
REFERENCES


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