Vasopeptidase inhibition prevents nephropathy in Zucker diabetic fatty rats

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Received 20 May 2003; received in revised form 15 July 2003; accepted 31 July 2003

Abstract

Background: Blocking the renin–angiotensin system is an established therapeutic principle in diabetic nephropathy. We investigated whether inhibition of both neutral endopeptidase and ACE (vasopeptidase inhibition) can prevent functional and morphological features of nephropathy in the Zucker diabetic fatty (ZDF) rat, an animal model of type II diabetes.

Methods: Homozygous (fa/fa) ZDF rats (each \( n = 15 \)) aged 10 weeks were treated with placebo, ramipril (1 mg/kg/day in drinking water), or the vasopeptidase inhibitor AVE7688 (45 mg/kg/day in chow). Metabolic parameters and renal function (metabolic cages) were assessed at baseline (age 10 weeks), and at age 17, 27, and 37 weeks. Twenty heterozygous animals (fa/–) served as lean, nondiabetic controls. At age 37 weeks, the animals were sacrificed and the kidneys analyzed histopathologically.

Results: Overt diabetes mellitus (blood glucose >20 mmol/l) was established at age 17 weeks in all homozygous ZDF rats. In the placebo group, urinary protein excretion increased progressively from 8 mg/kg/day (baseline) to 342 mg/kg/day (week 37) whereas diabetes and proteinuria were absent in the lean control group. Ramipril tended to reduce albuminuria and morphological damage (metabolic cages) were assessed at baseline (age 10 weeks), and at age 17, 27, and 37 weeks. Twenty heterozygous animals (fa/–) served as lean, nondiabetic controls. At age 37 weeks, the animals were sacrificed and the kidneys analyzed histopathologically. Results: Overt diabetes mellitus (blood glucose >20 mmol/l) was established at age 17 weeks in all homozygous ZDF rats. In the placebo group, urinary protein excretion increased progressively from 8 ± 1 (baseline) to 342 ± 56 mg/kg/day (week 37) whereas diabetes and proteinuria were absent in the lean control group. Ramipril tended to reduce albuminuria and morphological damage (p = ns) but AVE7688 virtually prevented albuminuria (33 ± 12 mg/kg/day, \( p < 0.05 \) vs. ZDF placebo) and drastically reduced the incidence and severity of glomerulosclerosis and tubulointerstitial damage.

Conclusions: In ZDF rats, development of diabetes mellitus is accompanied by functional and morphological kidney damage that resembles human diabetic nephropathy. Diabetic nephropathy can be prevented by chronic vasopeptidase inhibition.

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Keywords: Zucker diabetic fatty rat; Diabetic nephropathy; Angiotensin-converting enzyme inhibitor; Vasopeptidase inhibitor

1. Introduction

Diabetic nephropathy is a disabling disease caused by longstanding diabetes mellitus, leading to progressive impairment of renal function and, ultimately, end-stage renal failure. Along with the dramatically increasing incidence of diabetes mellitus, the proportion of diabetics among patients with chronic renal failure is increasing, and type II diabetes has become the leading cause of end-stage renal disease in most industrialized countries [1]. While treatment of diabetic nephropathy has recently been studied successfully in the clinical setting, leading to novel therapeutic options [2–5], preclinical data on diabetic kidney disease are sparse. Particularly, animal models of type II diabetes have not been well characterized with regard to renal complications, a fact which has limited the preclinical evaluation of potentially beneficial novel compounds.

ACE inhibitors are well documented for their ability to delay diabetic nephropathy [2,6]. With the development of novel vasopeptidase inhibitors, it has been proposed that inhibition of neutral endopeptidase in addition to ACE may yield an increased benefit in chronic renal failure [7]. However, the experimental data have so far been equivocal. The vasopeptidase inhibitor omapatrilat has demonstrated superior nephroprotection over sole ACE inhibition in a subtotal rat nephrectomy model in one study [8], but not in another [9]. In addition, omapatrilat improved endothelial...
function more than the ACE inhibitor captopril in renal arteries of salt-sensitive hypertensive rats [10].

In the present study, we tested whether early and chronic treatment with the vasopeptidase inhibitor AVE7688 in comparison to ramipril, an established angiotensin enzyme inhibitor, can prevent diabetic nephropathy. As the Zucker diabetic fatty (ZDF) rat is frequently used in metabolic studies, we used this rat strain to characterize the effect of our interventions on functional and morphological features of nephropathy in this model of obesity-related, insulin-resistant type II diabetes.

2. Methods

2.1. In vitro characterization

AVE7688 (7-[(2S)-2-(acetylthio)-1-oxo-3-methylpropyl] amino)-1,2,3,4,6,7,8,12b-octahydro-6-oxo-,(4S,7S,12bR)-pyridol[2,1-a][2]benzazepin-4-carboxy-acid) is an acetylthio compound, which in vivo is rapidly deacetylated to its biologically active thiol analogue, M108048. For the in vitro assay, therefore, the major active metabolite M108048 was used. ACE was measured as previously described [11] by measuring the production of [3H]hippuric acid from [3H]hippuryl-glycyl-glycine (HGG) in endothelial cells isolated from the human umbilical vein (HUVEC). Confluent HUVEC in culture plates were pre-incubated for 30 min at 37 °C under a 5% CO2 air atmosphere in a buffer containing 50 mmol/l HEPES/Tris (pH 7.5) and 100 mmol/l NaCl in the absence (control) or presence of 1 μmol/l captopril (background) or M108048. Thereafter, 0.125 μCi of the substrate [3H]hippuryl-glycyl-glycine was added into each well and the mixture incubated in the same conditions for a further 5-h period, at the end of which the supernatant of each well was transferred into tubes containing 1 ml of 0.1 M HCl. After addition of 1 ml of ethyl acetate, the samples were centrifuged at 3000 g for 10 min at 4 °C. The supernatants were transferred into vials containing 4 ml of a scintillation cocktail (Formula 989, Packard) and radioactivity corresponding to [3H]hippuric acid was counted in a scintillation counter (LS series, Beckman).

The effect of M108048 on NEP was quantified [12] by measuring the formation of Dansyl-d-Ala-Gly from Dansyl-d-Ala-Gly-Phe(pNO2)-Gly (DAGNPG) using an enzyme isolated from the rat kidney. M108048, the reference compound (thiorphan), or water (for stimulated control) was pre-incubated for 10 min at 37 °C in a buffer containing 50 mmol/l Tris–HCl (pH 7.4) and the enzyme (1.8 μg) or buffer (for basal control). Thereafter, the substrate DAGNPG was added at 50 μmol/l final and the mixture incubated for 30 min at 37 °C. The fluorescence intensity corresponding to Dansyl-d-Ala-Gly produced was measured with a fluorimeter (Ultral, Tecan). The standard inhibitory reference compound was thiorphan, which was tested in each experiment at several concentrations to obtain an inhibition curve from which its IC50 value was calculated.

In addition, the affinity for various receptors of both AVE7688 and its metabolite, M108048, was measured by assessing displacement of radiolabeled ligands by scintillation counter (Cerep, Celle L’Evescault, France). No biologically relevant binding (less than 25% binding at 10 μmol/l) was observed for all receptors tested, including the α1a, β1a, angiotensin AT1, bradykinin B2, dopamine D1 and D2, muscarinic M2, histamine H1 and H2, and serotonin 5-HT2A receptors (details not shown).

2.2. Animals

Male Zucker diabetic fatty rats (ZDF/Gmi–fa/fa) and their heterozygous (ZDF/Gmi–+/fa) lean littermates were purchased from Charles River Germany (Sulzfeld, Germany) and housed in our local LASW facilities in Frankfurt-Hoechst. The animals were housed individually in standard cages and received a standard chow diet (standard diet #1320, Altromin, Lage, Germany) and tap water ad libitum. All animal experiments were performed in accordance with current Aventis Laboratory Animal Science and Welfare (LASW) guidelines and the German law for the protection of animals.

2.3. Pharmacokinetics

ZDF rats weighing approximately 380 g (n = 4) received AVE7688, 10 mg/kg (dissolved in hydroxyethyl cellulose), per gavage. After 1, 2, 4, 8, 12, and 24 h, the animals were anaesthetized lightly (3.5 vol.% isoflurane in 34:66 (v/v) N2O/O2) and blood was collected from retro-orbital plexus for determination of plasma levels. The vials were gently shaken and centrifuged for 15 min at 3000 × g at 4 °C within 10 min after sample collection. Two milliliters plasma for each was subsequently transferred into polypropylene vials containing 200 μl of 3 N hydrochloric acid cooled at 0 °C. After mixing, the samples were frozen in a dry ice/ethanol bath and stored at −20 °C. Time between blood collection and freezing did not exceed 45 min. As AVE7688 is rapidly metabolized to M108048, only this compound was determined. Samples were analyzed by LC-MS/MS with or without pretreatment with dithiothreitol for cleavage of disulfide bonds followed by extraction, in order to determine total and free plasma concentrations of M108048, respectively. After analysis of the samples, peak plasma concentrations, exposure (area under the curve, AUC), and half-life of M108048 were determined (WIN-NONLIN, Version 3.3, Pharsight, USA).

2.4. Effect on ACE activity

In order to assess the in vivo effectivity of AVE7688, plasma ACE activity was determined in ZDF rats weighing approximately 380 g at baseline (n = 8) and 2 and 8 h,
respectively, after a single dose of AVE7688, 10 mg/kg p.o. 

(n=4). In a separate set of experiments, plasma ACE activity was determined in ZDF rats after 10 weeks of chronic oral dosing (each n=10) of either AVE7688 (45 mg/kg/day in chow) or ramipril (1 mg/kg/day in drinking water).

2.5. Nephropathy study

After the baseline metabolic and renal functional characterization, the ZDF rats were randomly assigned to either of three groups (each n=15) at the end of their 10th week of age: The placebo group (ZDF Plac) did not receive specific treatment. Another group (ZDF Rami) received ramipril (1 mg/kg/day) in the drinking water. This dose was chosen because it normalizes blood pressure [13,14] and effectively inhibits plasma and tissue activity of ACE in the spontaneously hypertensive rat [15]. The concentration of ramipril in the drinking water was adjusted to the daily consumption as determined regularly throughout the treatment period (cf. Table 1). The third group (ZDF AVE) received AVE7688 in standard chow, at a concentration of 450 mg/kg. Taking into account an average daily food intake of 40 g/day/rat (cf. Table 1), a dose of approximately 45 mg/kg/day was achieved. The dose of AVE7688 was chosen based on data in the spontaneously hypertensive rat model. In that study, AVE7688 (450 mg/kg chow) had normalized blood pressure, depressed plasma ACE activity, and improved survival to a similar extent as ramipril (1 mg/kg/day in drinking water) [14]. Twenty heterozygous, lean rats (LEAN) received standard chow and served as nondiabetic controls.

At age 22 weeks, four animals of each group were sacrificed for an interim histopathological analysis.

2.6. Metabolism and renal function

Metabolic characterization was performed by collecting urine over 24 h in metabolic cages and by drawing blood samples from the retro-orbital plexus under light anaesthesia (3.5 vol.% isoflurane in 34:66 (v/v) N2O/O2) at age 10, 17, 27, and 37 weeks. Food and water intake were monitored frequently.

2.7. Laboratory studies

Glucose (in whole blood) and creatinine (in serum and urine) were quantified with standard kits (Roche Diagnostics) using a Hitachi 912 E analyzer. Urinary albumin was quantified using a fluorescence dye binding assay (Mikrofloral, Progen Biotechnik, Heidelberg, Germany).

The activity of ACE was measured in the plasma using a spectrophotometric assay. Hydrolysis of the tripeptide N-[3-(2-furyl)acryloyl]-L-phenylalanyl-glycyl-glycin (FAPPG) into furylacryloyl–phenylalanine and glycyl–glycin is catalyzed by ACE. The activity of the enzyme was determined by measuring the decrease in absorption at λ = 340 nm (Hitachi type 912 automatic analyzer) in comparison to known standards.

2.8. Terminal haemodynamic measurements

At age 37 weeks, after the final metabolic and renal functional measurements had been performed, the rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p., n=8–10 per group). Heart rate, left ventricular pressure and its derivative dP/dt were then measured using a catheter-tip micromanometer (Millar Instruments, Houston, TX). After completion of the measurements, the animals were killed by quick excision of the hearts and kidneys under ongoing deep anaesthesia.

2.9. Pathology

After removal, the kidneys were weighed and thereafter fixed in 10% buffered formalin. Tissues were processed by routine procedures. The specimens were stained with haematoxylin–eosin and periodic acid Schiff. A longitudinal section of one kidney per animal was examined of 12, 6, 9, and 8 animals per group of LEAN, ZDF Plac, ZDF Rami, and ZDF AVE rats, respectively. Absolute incidences of renal lesions were recorded and the extent of lesions was assessed semiquantitatively. Damage to individual glomeruli

Table 1

<table>
<thead>
<tr>
<th>Test compound</th>
<th>ACE IC&lt;sub&gt;50&lt;/sub&gt; (nmol/l)</th>
<th>NEP IC&lt;sub&gt;50&lt;/sub&gt; (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M108048</td>
<td>0.052</td>
<td>5.0</td>
</tr>
<tr>
<td>Captopril</td>
<td>0.50</td>
<td>n.d.</td>
</tr>
<tr>
<td>Thiorphan</td>
<td>n.d.</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Time course of fundamental metabolic parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Body weight, g</td>
</tr>
<tr>
<td>LEAN</td>
</tr>
<tr>
<td>ZDF Plac</td>
</tr>
<tr>
<td>ZDF Rami</td>
</tr>
<tr>
<td>ZDF AVE</td>
</tr>
<tr>
<td>Food intake, g/d</td>
</tr>
<tr>
<td>LEAN</td>
</tr>
<tr>
<td>ZDF Plac</td>
</tr>
<tr>
<td>ZDF Rami</td>
</tr>
<tr>
<td>ZDF AVE</td>
</tr>
<tr>
<td>Water intake, ml/day</td>
</tr>
<tr>
<td>LEAN</td>
</tr>
<tr>
<td>ZDF Plac</td>
</tr>
<tr>
<td>ZDF Rami</td>
</tr>
<tr>
<td>ZDF AVE</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. n=15 (ZDF groups), n=20 (LEAN).

*p<0.05 vs. ZDF Plac.
and the distribution of the damaged glomeruli were combined in a grading scheme of glomerulosclerosis according to the following definitions: grade 1 (minimal): single glomeruli affected, thickening of the mesangium; grade 2 (mild): multiple glomeruli affected, thickening of the mesangium; grade 3 (moderate): many glomeruli affected, segmental or nodular thickening of the mesangium and single glomeruli displaying synechia with Bowman’s capsule or complete sclerosis; grade 4 (marked): majority of glomeruli affected, many glomeruli with synechia with Bowman’s capsule or complete sclerosis, and presence of glomeruli with segmental or nodular thickening of the mesangium; grade 5 (severe): general sclerosis of glomeruli in all areas. A similar semiquantitative scheme was applied to assess tubulointerstitial damage.

2.10. Statistics

Differences were tested for significance for all groups against ZDF Plac by an unpaired two-sided \( t \)-test, corrected for multiple testing by the Bonferroni method. The incidences of tubular atrophy and glomerulosclerosis were tested by Fisher’s Exact test. Data are given as means \( \pm \) S.E.M. A \( p \) value of less than 5% was considered significant.

3. Results

3.1. In vitro assay

The inhibitory effect of M108048 on ACE and NEP was quantified using in vitro assays. Table 1 shows the concentration of M108.048 and reference compounds that inhibit 50% (IC\(_{50}\)) of ACE and NEP.

3.2. Pharmacokinetics

Peak plasma concentration of total M108048 was 201 ng/ml, measured at the first time point, i.e., 1 h after gavage. Area under the data curve (0–24 h) amounted to 1136 ng h/ml. Total M108048 was eliminated from plasma following a first-order kinetic with a terminal half-life of 12 h. In comparison, free M108048 was found at levels one order of magnitude lower (peak level 16.7 ng/ml).

3.3. ACE activity

Plasma ACE activity was 101 ± 2 U/l at baseline. After single dosing (10 mg/kg), plasma ACE activity was de-
creased to 7 ± 1 and 14 ± 1 U/l after 2 and 8 h, respectively. After 10 weeks of chronic oral dosing, plasma ACE activity was depressed to 10 ± 3 and 8 ± 1 U/l in the AVE7688 and ramipril groups, respectively.

3.4. Metabolic data

Basal metabolic and laboratory data over the time course from age 10 weeks to 37 weeks are given for each group in Tables 2 and 3. Obese animals developed progressive diabetes mellitus, as manifested by progressively increased water intake and blood glucose levels over time. There were no systematic effects of creatinine clearance in all groups except for a trend towards increased creatinine clearance at 27 weeks, which disappeared by week 37. Of note, creatinine clearance was not relevantly reduced in the ZDF groups compared to the LEAN groups.

Excretion of urinary albumin increased progressively in ZDF Plac rats, while that in LEAN was negligible. Chronic treatment with ramipril tended to decrease albuminuria over time, e.g., by 30% at 37 weeks, \( p = 0.20 \) vs. ZDF Plac. Treatment with AVE7688 practically prevented albuminuria at all time points. This anti-albuminuric effect in ZDF AVE was significantly greater than in ZDF Rami (\( p < 0.05 \)). These results were similar whether the urinary albumin/creatinine ratio (Table 3) or daily albumin excretion (Fig. 1) was taken as a measure of albuminuria.

3.5. Haemodynamics

Haemodynamic parameters at age 37 weeks are summarized in Table 4. Lean animals had a higher heart rate, and left ventricular \( dP/dt_{\text{min}} \) than their diabetic littermates. Peak systolic pressure and left ventricular \( dP/dt_{\text{max}} \) tended to be higher in LEAN as compared to ZDF Plac. There were no significant haemodynamic differences between ZDF Plac and either ZDF Rami or ZDF AVE. In particular, left ventricular peak systolic pressure was not altered by any treatment.

3.6. Pathology

In the interim analysis at age 22 weeks, there were no consistent pathological findings in the ZDF Plac kidneys. Armanni–Ebstein cells were noted in 50% of the homozygous animals, and there was no glomerulosclerosis.

In the final analysis, at age 37 weeks, kidneys weighed 0.26 ± 0.01 g/100 g body weight in the LEAN group. In the ZDF animals, the kidney weight was considerably higher, but without differences between treatment groups (ZDF Plac: 0.45 ± 0.02; ZDF Rami: 0.43 ± 0.04; ZDF AVE: 0.43 ± 0.07 g/100 g body weight). A moderate pelvic dilation was noted in about 50% of the animals in all groups including LEAN, but this did not impair determination of specific damage to glomerular or tubular structures.

Armanni–Ebstein cells, large clear tubular cells which sometimes contained remnants of PAS positive material, indicative of persistent diabetes and resembling human

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter unit</th>
<th>Heart rate (1/min)</th>
<th>LVSP (mm Hg)</th>
<th>LVdP/dt_{min} (mm Hg/s)</th>
<th>LVdP/dt_{max} (mm Hg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEAN</td>
<td></td>
<td>294 ± 11*</td>
<td>111 ± 6</td>
<td>6037 ± 485</td>
<td>5932 ± 504*</td>
</tr>
<tr>
<td>ZDF Plac</td>
<td></td>
<td>232 ± 14</td>
<td>103 ± 8</td>
<td>5123 ± 454</td>
<td>4383 ± 390</td>
</tr>
<tr>
<td>ZDF Rami</td>
<td></td>
<td>198 ± 14</td>
<td>101 ± 7</td>
<td>4949 ± 438</td>
<td>3858 ± 394</td>
</tr>
<tr>
<td>ZDF AVE</td>
<td></td>
<td>222 ± 14</td>
<td>100 ± 3</td>
<td>5162 ± 89</td>
<td>4388 ± 253</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. \( n = 8 – 10. \)

LVSP, left ventricular systolic pressure.

* \( p < 0.05 \) vs. ZDF Plac.

Fig. 2. Histopathological features of nephropathy in ZDF rats. (A) Glomerulosclerosis. A sclerotic glomerulum (left) and a normally appearing glomerulum (right) in close vicinity. (B) Tubular atrophy and Armanni–Ebstein cells. Atrophic tubuli (lower left) are outlined by a thickened basement membrane. Cells are small, nuclei are crowded and cytoplasm is scant. Armanni–Ebstein cells (central right) are characterized by large size and clear appearance, containing hardly any remnants of PAS positive material. Objective: \( \times 20 \).
Armanni–Ebstein lesions were regularly noted in all groups except LEAN. Glomerulosclerosis, varying from minimal to marked severity, was present in all ZDF Plac animals and in none of the LEAN controls. The sclerotic alterations within the glomerulum were characterized by collapse of capillaries and by focal, segmental, or nodular hyaline thickening of mesangium culminating in synechia and complete sclerosis. The lesions resembled those observed typically in human diabetic nephropathy. A typical example is presented in Fig. 2A. Tubulointerstitial lesions were generally mild in severity. Tubular atrophy (Fig. 2B) was present in all ZDF Plac animals and in none of the LEAN controls.

The incidences of glomerulosclerosis and tubular atrophy were reduced significantly in ZDF AVE to 13% (1/8) and 25% (2/8), respectively, compared to 100% (6/6) in ZDF Plac. The severity of glomerulosclerosis in the one animal affected in the ZDF AVE group was grade 1 (minimal damage). In ZDF Rami, the incidence of glomerulosclerosis was reduced to 67% (6/9), which was statistically not significant. The severity of glomerular and tubulointerstitial damage was similar in ZDF Plac and ZDF Rami kidneys.

Fig. 3 summarizes the semiquantitative scores of moderate glomerulosclerosis (A) or tubular atrophy (B). Glomerulosclerosis and tubular atrophy tended to be reduced by ramipril (not significantly). In ZDF AVE, both glomerulosclerosis and tubular atrophy were markedly reduced (each \( p < 0.05 \)).

4. Discussion

The present study shows that in ZDF rats, progressive nephropathy develops in parallel with diabetes mellitus, beginning at around age 10 to 17 weeks. Significant renal damage can be demonstrated both functionally and morphologically, thus resembling human diabetic nephropathy. Vasopeptidase inhibition can effectively prevent kidney damage in this animal model of type II diabetes.

4.1. Nephropathy in the ZDF rat

Diabetic nephropathy is a typical complication of long-standing diabetes mellitus, leading to progressive functional impairment and, ultimately, terminal renal failure. Considering the worldwide increasing incidence of diabetes mellitus, novel therapies for the prevention and treatment of diabetic nephropathy are urgently needed. Recently, landmark clinical trials investigating ACE inhibitors and angiotensin receptor blockers have demonstrated renal protection in patients with diabetes [2–5,16]. Interestingly, the profiling of these compounds in diabetic nephropathy was started long after these substances had entered the market in different indications. A reason for this delay may be that a commonly accepted animal model for type II diabetic nephropathy has not been available. Kidney damage has been described in streptozotocin-treated rodents, but this model is hypo-insulinaemic, thus reflecting more type I diabetes, which accounts for a relatively small proportion of all cases of diabetes. It seemed therefore worthwhile to characterize renal function and morphology in a model of non-insulin-dependent diabetes. The ZDF rat, which is frequently used in studies related to obesity and insulin resistance, is such a model. Our data show that along with the metabolic changes occurring over time in these rats, the nephropathy that develops functionally and morphologically resembles human diabetic nephropathy. In a previous study, the suitability of the Zucker rat has been questioned because of predominant pelvic dilation which precluded histopathologic analysis of diabetes related damage, such as glomerular damage [17,18]. In the present study, a moderate degree of pelvic dilation was noted, but nevertheless it was possible to assess glomerular and tubular morphology in all animals. We assume that the different substrains account for the differences in pelvic dilation observed by Vora et al. [18] and in our study (Drt/fa vs. fa/fa).

Progressive albuminuria, focal glomerulosclerosis, and tubular atrophy are common features in ZDF rat as well as in human diabetic nephropathy. It may therefore be justified to conclude that this animal model is suitable for preclinical evaluation of novel pharmacological compounds in human diabetic nephropathy. It should be noted, however, that the massive proteinuria in ZDF rat occurs in the absence of a decrease in glomerular filtration rate. While the extent of damaged glomeruli and tubuli can be severe, the absolute number of affected nephrons is obviously not sufficient to
produce a measurable impairment of renal filtration, at least until age 37 weeks. Thus, the ZDF rat resembles more the earlier stages of human diabetic nephropathy, which is characterized by an increase (rather than decrease) of creatinine clearance.

### 4.2. Effects of ACE and neutral endopeptidase inhibition in the ZDF rat

Data from the present study show that early intervention in the renin–angiotensin system can prevent the functional and morphological features of nephropathy in an animal model of type II diabetes. It is interesting to note that nephroprotection was unrelated to haemodynamic effects. While left ventricular contractility seems to be generally reduced in the obese animals as compared to their lean littermates, there were no relevant effects on heart rate, left ventricular contractility, or myocardial relaxation by chronic pretreatment with AVE7688 (cf. Table 4). Although the anaesthesia may have diminished potential differences between groups, the absence of a blood pressure effect in the ramipril and AVE7688 groups is not unexpected, because the ZDF rat is not a hypertensive animal model.

In addition to the absence of systemic haemodynamic effects, chronic treatment with AVE7688 had no effect on blood glucose levels after the first 7 weeks of treatment. This is an important finding, as it illustrates that the anti-albuminuric effect of AVE7688 is independent of, and probably additive to, control of blood pressure and glucose levels. Nephroprotection by AVE7688 in the ZDF rat likely represents a specific effect related to inhibition of the renin–angiotensin system and neutral endopeptidase locally in the kidney. The basic pharmacological characteristics of AVE7688 and its major metabolite, M108048, suggest that at the dose chosen in the present experiments, unspecific effects are unlikely.

The ACE inhibitor ramipril has proven clinical efficacy in diabetic and in nondiabetic nephropathy, independent of systemic blood pressure [2,19]. Interestingly, the relative decrease in albuminuria produced by ramipril in the present study (approximately 30%), albeit statistically not significant, is in the same range as that seen with ACE inhibition or angiotensin receptor blockade in recent clinical trials [16,20]. Data from previous studies indicate that chronic kidney damage may be ameliorated by improved intrarenal haemodynamics and/or reduced renal angiotensin II (for review, see Ref. [21]). In a rat double transgenic model overexpressing human renin and angiotensinogen, nephropathy is characterized by marked glomerulosclerosis and progressive albuminuria [22] illustrating the importance of the renin–angiotensin system in the pathogenesis of kidney related end organ damage. Based on theoretical considerations, the vasopeptidase inhibitors have been postulated to exert a more potent nephroprotection, because the bradykinin degradation is more effectively prevented, and tissue concentrations of the natriuretic peptides are increased.

Indeed, the vasopeptidase inhibitor omapatrilat was superior to enalapril with regard to long-term nephroprotection in a subtotal nephrectomy model [8] and superior to captopril with regard to endothelial function in a salt-sensitive hypertensive rat model [10]. In another study, though, both the ACE inhibitor fosinopril and the vasopeptidase inhibitor omapatrilat were equally effective in a nephrectomy model [9]. The present study is the first to demonstrate a superior effect of vasopeptidase over ACE inhibition in diabetic nephropathy at doses that are equally effective in controlling blood pressure, decreasing plasma ACE activity, preventing target organ damage, and prolonging survival in spontaneously hypertensive rats [14]. It has to be kept in mind, however, that the optimally effective doses in diabetic nephropathy may differ from those in hypertensive target organ damage. It remains therefore to be determined if higher doses of ramipril can exert a nephroprotection similar to AVE7688.

### 4.3. Summary

We have shown that the obese, insulin-resistant Zucker diabetic fatty rat can serve as a useful animal model of type II diabetic nephropathy. Nephropathy is characterized by a progressive albuminuria beginning between age 10 and 17 weeks, and by corresponding morphological alterations at age 37 weeks. The ZDF rat thus represents a feasible model for the preclinical study of therapeutic interventions in diabetic nephropathy. Simultaneous inhibition of both ACE and neutral endopeptidase with the vasopeptidase inhibitor AVE7688 can almost completely prevent diabetic nephropathy.

### Acknowledgements

The authors wish to thank Gerald Fischer for excellent technical support and Reiner Uhl for expert statistical advice and Dietmar Schmidt for the pharmacokinetic determinations. The present study is part of the “Cardiovascular and Renal Endpoints in Diabetes (CARED)” preclinical study program.

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