Comparison between somatostatin analogues and ACE inhibitor in the NOD mouse model of diabetic kidney disease

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Abstract

Background. The growth hormone (GH)-insulin-like growth factor (IGF)-SST (SST) axis is involved in diabetic nephropathy (DN). We have recently shown a beneficial effect on diabetic kidney disease markers by the use of a novel somatostatin (SST) analogue (PTR-3173) (S). The purpose of this study is to compare the effects of S with a previously used SST analogue (octreotide) and an ACE inhibitor (ACEi), a standard of care in DN.

Methods. Non-obese diabetic mice (a model of type I diabetes) were treated with either S (DS), octreotide (DO), enalapril (DA), or PTR-3173 and enalapril (DAS group) for 3 weeks.

Results. Diabetic renal hypertrophy was blunted in the DS and DO groups only. Serum GH and IGF-I were markedly increased and decreased, respectively, in the D group, a change significantly blunted in DO and DS. Diabetic hyperfiltration and albuminuria were blunted in all the four treated diabetic groups. The marked deposition of type IV collagen and PAS material were mildly decreased in DA, but more markedly reduced in DS as well as DO. Diabetic renal laminin accumulation was suppressed in all treated animal groups. No synergistic effect was observed for any parameter in the combination group DAS.

Conclusion. SST analogues exert beneficial effects in most parameters of diabetic kidney disease to the same extent as the ACEi. Enalapril treatment had no effect on renal hypertrophy and did not cause a significant decrease in mesangial type IV collagen deposition. A synergistic effect of combined SST-ACEi therapy could not be shown in this study.

Keywords: diabetic nephropathies; growth hormone; insulin-like growth factor I; immunohistochemistry

Introduction

Nephropathy (DN) is the most severe complication of diabetes mellitus (DM). It is characterized by early renal hypertrophy followed by an increased deposition of extracellular matrix (ECM), leading to glomerulosclerosis and renal failure [1]. ACE inhibitors are recognized as the standard of care in DN. However, this therapy does not cause a full reversal or even halting of renal function deterioration [2]. Therefore, a search for additional therapeutic avenues continues.

Several studies have shown that the growth hormone (GH)-insulin-like growth factor (IGF)-somatostatin (SST) system may play a significant role in diabetic and other nephropathies [3]. Kidney tissue expresses receptors not only for IGF-I but also for GH. Thus, even though most of the biologic effects of GH are IGF-I mediated, it may also act independently of IGF-I. We have recently reported an increase in serum GH levels in non-obese diabetic (NOD) mice [4], a model of spontaneous insulin-dependent DM. This is similar to the changes described in humans. Suppressing circulating GH levels with octreotide, a non-selective long-term SST analogue also exerts beneficial effects in experimental DN [3]. SST, a 14 amino acid polypeptide, is a natural cyclic peptide inhibitor of pituitary, pancreatic and gastrointestinal secretion. The administration of octreotide, a long-acting SST analogue, to insulin treated patients with
type 1 DM may impair anti-hypoglycemic counter regulatory mechanisms through suppression of glucagon and GH responses. This may have negative actions in diabetic patients. However, as SST has potent inhibitory effects on the secretion of a large number of other hormones, including insulin, glucagon, gastrin, cholecystokinin, and other mediators secreted by the pituitary, pancreas and the gastrointestinal tract [5], it may adversely affect the metabolic balance. Octreotide is a non-specific SST analogue and decreases the secretion of both GH and other hormones, such as insulin and glucagon. The regular use of octreotide has been curtailed because of its side effects, mainly the appearance of bile duct problems, including cholelithiasis [6]. Therefore, a more specific antagonist of GH secretion is needed to prevent diabetic complications. Recently, Afarag et al. [7] developed a novel SST derivative (PTR-3173), which has no inhibitory effects on glucagon and insulin but exerts a major inhibition on pituitary GH secretion. We have shown beneficial effects on DN markers by the use of this novel SST analogue, using the NOD mouse model of type I DM. These findings may have a significant preventive effect on the development of diabetic complications [8]. The established beneficial effects of an ACE inhibitor (ACEi) in DN have not been so far compared with this new therapeutic approach. The purpose of this study is to compare the effects of the new SST analogue (PTR-3173) with a previously used SST analogue (octreotide) and an ACE inhibitor.

**Methods**

**Animal experimentation**

Twelve-week-old female NOD/Alt mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Animal breeding complied with the NIH Guide for the Care and Use of Laboratory Animals. The local institutional review committee approved the study protocol. The cumulative incidence of overt DM in these animals is over 70% by 100 days, in females. Animals were housed in standard laboratory cages and fed normal mouse chow ad libitum with free access to unlimited amount of tap water. The onset of DM was determined by the appearance and persistence of glycosuria, checked twice a week with chemstrips (Ketostix, Bayer-Ames, UK). When the urine glucose test was positive, tail capillary glucose blood samples were examined with a glucometer (Elite, Bayer Diagnostics, Puteaux Cedex, France). DM was diagnosed when blood glucose levels were above the normal values for NOD mice (99% confidence levels of 3.0–9.9 mmol/l) on two consecutive days. The second day of persistent hyperglycaemia was considered day 1 of DM. Only mice with elevated serum glucose levels and no ketonuria were defined as diabetic. Diabetic NOD mice can be maintained alive without insulin supplementation for up to 1 month.

Mice were divided into six groups (eight animals per group): control non-diabetic mice treated with saline (C); diabetic mice (D); diabetic mice injected with daily octreotide (1 mg/kg/day) (DO); diabetic mice treated with the ACE inhibitor enalapril (5 mg/150 ml drinking water), as previously described [9]. The sixth experimental group (DAS) of diabetic animals was treated with ACEi and PTR-3173, at the same dosages. All animals were killed 3 weeks after DM onset. Body weight was measured twice weekly. Water intake was recorded every other day. Urine was collected for 24 h, using metabolic cages, on the day prior to death. A standardized method of GH stimulation and secretion was applied, wherein mice were anaesthetized with intraperitoneal pentobarbital (50 mg/kg), and blood was drawn from the retrobulbar plexus at least 5 min after injection. The serum was separated and frozen at −20°C for later measurements of glucose, IGF-I and GH. The right kidney was carefully removed and immediately frozen in liquid nitrogen and then at −70°C. A coronal 2 mm slice from the midportion of the left kidney was separated and fixed in a 4% paraformaldehyde solution for histomorphological assessment.

**Immunoassays**

**Serum GH.** Serum GH was measured by RIA. Mouse sera were diluted 1:4 or 1:20 in assay buffer (0.01M PBS, 0.02% NaN3, 0.33% EDTA, 0.5% BSA, pH 7.5); anti-rat GH antiserum (monkey) (donated by Dr A. F. Parlow) was used for iodination and standards. Samples and standards (100 μl), 125I mGH (200 μl) and antiserum (100 μl) were incubated for 22 h at room temperature. Bound and free antibodies were separated by incubation with anti monkey IgG diluted 4:6 (ICN Pharmaceuticals Inc., Aurora, OH, USA.) (100 μl) and the addition of ice-cold PEG 5% (1 ml) at 4°C for 2 h. The sensitivity of the method was 0.04 ng mGH/tube; the coefficient of variation for sera 13.9 and 201.1 ng/ml were 13.6 and 5.9%, respectively. All sera were run in the same assay.

**Serum IGF-I.** Serum IGF-I was measured by the functional separation method in which excess IGF-II blocks the interference of IGF binding proteins (IGFBPs). Serum (10 μl) was diluted 1:100 in acidic buffer (0.02 M NaH2PO4, 0.1 NaCl, 0.2% BSA, 0.02% NaN3, 0.1% Triton X-100, pH 2.8) at room temperature for 1 h. Anti-IGF-I antiserum (Fujsawa Pharmaceutical Co., Osaka, Japan) was diluted 1:70 000 in assay buffer (0.1 M PBS, 0.2% BSA, 0.02% NaN3, 0.1% Triton X-100, pH 7.8). Thereafter, 100 μl of samples and recombinant hIGF-I standards (Fujsawa Pharmaceutical Co.) were incubated with 50 μl of IGF-II (PeproTech Inc., Rocky Hill, NJ, USA) (100 ng/ml), 400 μl of antiserum and 125I IGF-I (25000 c.p.m./50 μl) at 4°C for 20 h. Bound and free antibodies were separated by incubation with a pre-precipitated suspension of an anti-rabbit IgG (Sigma, St Louis, MO)-normal rabbit serum mix (1%:0.1%) in 5% PEG (1 ml). IGF-II (5 ng/tube) completely reversed the interference of human recombinant IGFBP-3 (Upstate Biotechnology, Lake Placid, NY, USA) (5 ng/tube). The sensitivity was 0.02 ng IGF-I/tube; the intra-assay coefficient of variation for sera with IGF-I levels 80, 212 and 412 ng/ml were 8.9, 2.9, and 9.6%, respectively. All sera were run in the same assay.
Kidney IGF-I. Kidney protein extraction was performed as follows: 80–100 mg of tissue was homogenized on ice in 1 M acetic acid (5 ml/g tissue) with an Ultra Turrax TD 25 and further disrupted with a Potter Elvehjem homogenizer. With this procedure, all IGFBPs are removed from kidney tissue. After lyophilization the samples were redissolved in phosphate buffer (pH 8.0) and kept at −80°C until the IGF-I assay was performed in diluted extracts. Serum and kidney IGF-I levels were measured by RIA using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistrano, CA, USA) and recombinant human IGF-I as standard (Amersham International). The tissue IGF-I concentrations were corrected for the contribution of entrapped serum IGF-I. Mono-iodinated IGF-I [125I-(Tyr31)-IGF-I] was obtained from Novo-Nordisk A/S (Bag-Svaerd, Denmark). Intra- and inter-assay coefficients of variation were less than 5 and 10% for both assays.

Urine albumin excretion
The urine samples were stored at −20°C until assayed. A modified ELISA determined the urinary albumin concentration in the urine samples collected prior to death. Ninety-six well-immunoplates were coated with goat anti-mouse albumin (Bethyl Labs, Montgomery, TX, USA), diluted with 0.1 M carbonate buffer (pH 9.6) and incubated over-night at 4°C. The plates were washed four times with 0.01 M phosphate buffer saline (PBS) (pH 7.4), 0.05% Tween-20, and incubated for 1 h with 5% milk to avoid non-specific binding. Standard dilutions of mouse albumin (Bethyl Labs, Montgomery, TX, USA), diluted with 0.1 M carbonate buffer (pH 9.6) and incubated overnight at 4°C, were then incubated for 1 h at room temperature and then washed again three times. The band antibody was visualized by enhanced chemiluminiscense (ECL; Amersham Life Sciences Inc.) and the supernatants collected and frozen. For the detection of kidney laminin homogenates were mixed with 5× sample buffer and boiled for 5 min, then 150 µg portions of sample protein were loaded in each gel lane and subjected to 7.5% SDS–polyacrylamide gel, and electroblotted into nitrocellulose membranes. Blots were blocked for 1 h in TBS buffer (10 mM Tris, pH 7.4, 138 mM NaCl) containing 5% non-fat dehydrated milk, followed by overnight incubation with polyclonal antibody against laminin (Santa Cruz Biotechnology, CA, USA) diluted in TBS containing 5% dry milk. After washing three times for 15 min in TBST (0.05% Tween-20), the blots were incubated with secondary anti-goat antibody conjugated to horseradish peroxidase for 1 h at room temperature and then washed again three times. The band antibody was visualized by enhanced chemiluminiscense (ECL; Amersham Life Sciences Inc.) and exposed to Kodak-BioMax film (Eastman Kodak, Rochester, NY, USA). Protein expression was quantitated densitometrically using Fluorchem software (Alpha-Innotech, CA, USA).

Western immunoblot analysis
Kidney tissue was homogenized on ice with a polytron (Kinetica, Littau, Switzerland) in lysis buffer (50 mM Tris, pH 7.4, 0.2% Triton X-100) containing 20 mM sodium pyrophosphate, 100 mM NaF, 4 mM EGTA, 4 mM Na3VO4, 2 mM PMSF, 0.25% aprotinin and 0.02 mg/ml leupeptine. Extracts were centrifuged for 20 min at 17,000 g at 4°C and the supernatants collected and frozen. For the detection of kidney laminin homogenates were mixed with 5× sample buffer and boiled for 5 min, then 150 µg portions of sample protein were loaded in each gel lane and subjected to 7.5% SDS–polyacrylamide gel, and electroblotted into nitrocellulose membranes. Blots were blocked for 1 h in TBS buffer (10 mM Tris, pH 7.4, 138 mM NaCl) containing 5% non-fat dehydrated milk, followed by overnight incubation with polyclonal antibody against laminin (Santa Cruz Biotechnology, CA, USA) diluted in TBS containing 5% dry milk. After washing three times for 15 min in TBST (0.05% Tween-20), the blots were incubated with secondary anti-goat antibody conjugated to horseradish peroxidase for 1 h at room temperature and then washed again three times. The band antibody was visualized by enhanced chemiluminiscense (ECL; Amersham Life Sciences Inc.) and exposed to Kodak-BioMax film (Eastman Kodak, Rochester, NY, USA). Protein expression was quantitated densitometrically using Fluorchem software (Alpha-Innotech, CA, USA).

Statistical analysis

One-way analysis of variance was used to evaluate differences between groups for multiple comparisons. The Kruskal–Wallis modification for non-parametric data was used as a first step, and the Mann-Whitney test for differences between the groups was used subsequently. A P-value of < 0.05 was considered as significant. Means are given as ±SEM.
Results

Growth and metabolism

Serum glucose levels were elevated but not significantly different between the diabetic groups upon death (479±53, 477±23, 488±27, 483±27 and 446±44 mg/dl in D, DS, DO, DA and DAS, respectively, vs 90±7 mg/dl in C). Body weight at death was also similar between the groups. Mean enalapril intake (according to water consumption) in the DA group was 50±18 mg/kg/day. Kidney weight (KW) (Figure 1) was significantly increased in the D animals in comparison to controls (211.6±8 vs 148.5±5 mg; P<0.001). No change in KW was observed in the DA group in comparison to D animals (211±10.2 mg; P<0.01 vs C only). However, this increase in KW was blunted in the two groups given SST analogues (DS: 187±4 mg; DO: 190±4 mg; p<0.05 vs D using the Mann–Whitney test). The combination group (DAS) showed no added blunting effect on renal hypertrophy (175±4 mg).

Endocrinological parameters

Serum GH (Figure 2a) was markedly increased in the D group in comparison to C (133.1±35.1 vs 18.7±3.8 ng/ml; P<0.001). This increase in serum GH in DM was significantly blunted in the SST treated animals, DS, DO and DAS (54.3±7.5, 44.5±4.6 and 48.7±6 ng/ml, respectively; p<0.05 vs D). No significant change in serum GH levels was observed in the DA group in comparison to D animals (79.5±21.3 ng/ml; P<0.01 vs C only using the Mann–Whitney test).

Serum IGF-I levels (Figure 2b) were significantly decreased in the D group (165.5±28.9 vs 355.9±11.5 ng/ml in the C group; P<0.001). This decrease in serum IGF-I in DM was blunted in the SST analogue treated animals, DS and DO (246.3±20.7 and 262.3±23.2 ng/ml, respectively; P=0.06 vs D using the Mann–Whitney test). In the DA animals a similar trend of serum IGF-I decrease blunting was seen (251.1±30.3 ng/ml; P=0.06 vs D using the Mann–Whitney test). There was no difference in serum IGF-I levels in the DAS group in comparison to D (175±30 ng/ml; P=NS).

Renal functional studies

Creatinine clearance (CrCl) was calculated from 24 h urine collection in all animals, using metabolic cages prior to death. It was used as a marker of glomerular filtration rate (Figure 3a). CrCl was markedly elevated in the D animals in comparison to controls [246±28 (or 374.5±43.3% of C) vs 65.7±16.9 ml/min in C; P<0.001]. This diabetic hyperfiltration was blunted in all the four treated diabetic animals, DS, DO, DA and DAS (265±14, 273±16, 277±11 and 278±11% of C, respectively; P<0.05 vs D). Albuminuria was also measured from the 24 h urine collections (Figure 3b). A similar trend was observed for this parameter as the one described for CrCl, namely, a marked increase in D animals (27.5±2.9 vs 1.7±0.3 mg/24 h in D and C, respectively; P<0.001), but a blunted effect in the four treated diabetic groups [19.2±1.5, 18.6±1.8, 13.8±1.7 and 18.5±0.8 μg/24 h

![Fig. 1. Mean absolute kidney weight (KW) (mg) on day 21 in the control untreated (C), untreated diabetic (D), PTR-3173 treated diabetic (DS), octreotide treated diabetic (DO), enalapril treated diabetic (DA) and PTR-3173+enalapril treated mice (DAS). Values are mean±SEM (n=8 in each group). Differences between groups are significant using the Kruskal–Wallis test. KW is significantly lower in the DS, DO and DAS groups (and not in the DA) in comparison to DS using the Mann–Whitney test.](image1)

![Fig. 2. Mean serum growth hormone (GH) (ng/ml) (a) and insulin-like growth factor-I (IGF-I) (ng/ml) (b) levels on day 21 in the control (C), untreated diabetic (D), PTR-3173 treated diabetic (DS), octreotide treated diabetic (DO), enalapril treated diabetic (DA) mice and PTR-3173+enalapril treated mice (DAS). Values are mean±SEM (n=8 in each group). Differences between groups are significant using the Kruskal–Wallis test.](image2)
in DS, DO, DA and DAS, respectively ($P < 0.001$ vs C and $P < 0.05$ vs D using the Mann–Whitney test). Renal IGF-I protein (Figure 4) was markedly elevated in the D animals (218 ± 22% of C; $P < 0.001$). There was a less prominent increase in renal IGF-I concentration in the four experimental groups (182 ± 29, 182 ± 19, 193 ± 10, 193 ± 14 and 179 ± 14% of C in DS, DO, DA and DAS, respectively; $P < 0.05$ vs D using the Mann–Whitney test). The glomeruli of the D animals showed more extensive matrix expansion and thickening of capillary walls, in comparison to control animals. Glomerular volume (GV) increased significantly in the D group (4.2 ± 0.3 × 10^5 in the D group vs 2.4 ± 0.1 × 10^5 μm^3 in the C group; $P < 0.05$). In the DS, DO and DA groups this increase in GV was suppressed (2.4 ± 0.3, 2.8 ± 0.3 and 3.3 ± 0.3 × 10^5 μm^3, respectively; $P < 0.05$ vs D using the Mann–Whitney test). In the combination therapy group (DAS) there was no added difference to GV in comparison to the DA group (3.4 ± 0.2 × 10^5 μm^3) (Figure 5). However, an intermediate value of glomerular volume (4.1 ± 0.2 × 10^5 μm^3) between the C and D groups was observed.

Immunostainable type IV collagen was used as a marker of extracellular matrix deposition in diabetic kidney disease (Figure 6a). Type IV collagen is normally detected at a low level in the mesangial area, and also delineates the glomerular capillary loops. Semiquantitative analysis of the staining intensity showed a marked deposition of type IV collagen in the D animals, mostly in the mesangium (663 ± 50% of C) and the capillary membranes (275 ± 19% of C) (Figure 6b). Mesangial immunostainable type IV collagen was mildly decreased in the DA animals (469 ± 56% of C; $P = 0.06$ vs D), but more markedly decreased in the DS as well as the DO mice (281 ± 31 and 275 ± 50% of C; $P < 0.05$ vs D using the Mann–Whitney test) (Figure 6b). Type IV collagen deposition in the DAS group was similar to that of the DA and no synergistic effect could be observed. Kidney laminin accumulation was analysed by western blots and found to be elevated in D animals (140 ± 18% of C; $P < 0.001$), but totally blunted by the use of SST analogues or enalapril (103 ± 7, 88 ± 18, 105 ± 14 and 86 ± 19% of C in DS, DO, DA and DAS, respectively) (Figure 7).
Discussion

SST analogues exert beneficial effects in this model of DN: they blunt the increase in circulating GH and the decrease in serum IGF-I observed in DM. Diabetic-induced renal hypertrophy is also blunted by these agents, in association with a decrease in the accumulation of renal IGF-I, a normalization of hyperfiltration and albuminuria and a marked decrease in type IV collagen accumulation. No significant changes were observed in the effect of PTR-3173 in comparison to the non-selective SST analogue octreotide. SST biological functions are mediated via high affinity, non-selective SST binding to specific cell surface receptors (SSTR-1, -2A, -2B, -3, -4 and -5), which are encoded by five genes and belong to the G protein-coupled receptor family. According to in vitro studies, PTR-3173 is bound with nanomolar affinity to three
of the six identified human SSTRs (SSTR-2, -4 and -5). SST inhibition of glucagon release in mouse islets is primarily mediated via SSTR-2, whereas insulin secretion is regulated primarily via SSTR-5 [11]. These receptors have variable tissue distribution, and metabolic effects, including kidney tissue, where they localize in a tubular distribution and vasa recta [12]. SST exerts direct renal vasoconstriction in addition to the demonstrated modulation of other vasoactive systems [13] and tubular functions. In humans, SST receptors revealed binding over cortical and medullary areas. In the cortex, the receptors were located in the proximal tubules but not in the glomeruli. Human SSTR-2 mRNA is expressed at highest levels in cerebrum and kidney medulla. SST prevented in a dose-dependent manner the reduction in mesangial cell planar surface area induced by angiotensin II. This effect was not inhibited by the use of other vasorelaxing agents [14].

The beneficial effects of the SST analogues were equivalent in most parameters as the effect of the ACE inhibitor enalapril, which is the commonly used therapeutic intervention in DN. Glomerular angiotensin II concentrations are markedly elevated in DM in comparison to the circulation and vascular ACE activity, including glomerular vasculature, is increased [15]. In our study, enalapril treatment had no effect on renal hypertrophy and did not cause a significant decrease in mesangial type IV collagen deposition. However, capillary membrane type IV collagen deposition was significantly blunted in both enalapril treated groups. The presence of such differences between ACEi and SST analogues on DN parameters exemplifies the fact that these agents exert their effects through different pathways, which are both deranged in type I DM. GH and IGF-I may play a pathogenic role in diabetic as well as other nephropathies. Poorly controlled DM in man is characterized by GH hypersecretion. Transgenic mice for the bovine GH gene show a disproportionate increase in glomerular volume followed by severe glomerulosclerosis, uraemia and death. In addition, kidneys of transgenic diabetic mice that express GH antagonists had less glomerular hypertrophy and damage, no proteinuria and no increase in glomerular zl type IV collagen mRNA levels compared with kidneys of transgenic diabetic mice that expressed wild-type bGH [16]. However, mice transgenic to GH antagonist may be a priori less susceptible to diabetic renal changes owing to an inborn effect of GHR blockade and low circulating IGF-I levels (resulting in a dwarf phenotype) before the induction of DM. In contrast, our data are based on a model of spontaneously acquired insulin dependent DM, which closely resembles human disease. Like humans, NOD mice develop proteinuria and glomerular lesions, including an increase in glomerular surface area and in mesangial sclerosis [17]. We have previously described an increase in circulating GH levels [4] associated with a persistent increase in kidney-extractable IGF-I levels and renal hypertrophy, up to 4 weeks after DM onset in NOD mice [3], imitating human type I DM. The renal renin-angiotensin system (RAS) is upregulated in DM and mediates the late glomerulosclerosis changes seen in this disease. ACE inhibitors and angiotensin II (AII) receptor antagonists (AIIra) have been shown to have significant protective effects on DN, secondary to both type I and type II DM [2]. These medications have significant blood pressure lowering effects, since AII is a potent vasoconstrictor. AII has many potential deleterious effects on the kidney that could contribute to DN, including: systemic hypertension, systemic and renal vasoconstriction, increased glomerular capillary pressure and mesangial cell contraction, the latter leading to a reduction in glomerular filtration surface area. The potential links between the RAS and the GH-IGF system in the kidney tissue have not been well established. Infusion of AII to rats causes weight loss and a decrease in serum IGF-I. This change could be reversed by the administration of an AIIra (and not by another antihypertensive, such as hydralazine) [18]. Therefore, an AT1 receptor mediated link between the RAS and the IGF system was postulated. Both IGF-I and AII are involved in smooth muscle hypertrophy in a synergistic way [19]. There are few data on the effects of the modulation of either GHR on the expression of the RAS and vice versa. In a model of STZ DM, IGF-I overexpression interferes with the development of diabetic cardiomyopathy by attenuating p53 function and AII production and thus AT receptor activation [20]. There have been no previous reports on animal studies which examined the potential benefit of a combined SST-ACEi therapy for diabetic kidney disease. We here show that the administration of SST analogues is as efficient as enalapril in the prevention of markers of nephropathy in diabetic NOD mice. However, the combination of enalapril and SST showed no additional effects in this experiment. Possible reasons for this finding
may include the relative short period of diabetes and the possibility that no additional effect may be achieved at this stage.

Thus, the interrelation between the RAS and the renal GH-IGF system needs to be further clarified and the combination of ACEi and SST needs to be examined in a model of DM extended to a longer time. In future studies it would be also interesting to have additional information on long-term outcome of such interventions in DM, with additional electron microscopy-based morphology data analysis.

This suggests that PTR-3173, a SST analogue with potential additional beneficial effects in DM is as efficient as octreotide and shows similar effects as the established therapy with ACE inhibitors in this animal model of diabetic kidney disease.

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