Cell. Mol. Life Sci. 64 (2007) 3119 – 3128
1420-682X/07/233119-10
DOI 10.1007/s00018-007-7395-1

Cellular and Molecular Life Sciences

Research Article

Pancreatic islet β-cell deficit and glucose intolerance in rats with uninephrectomy

Y. Sui[a], H.-L. Zhao[a], R. C. W. Ma[a], C. S. Ho[b], A. P. S. Kong[a], F. M. M. Lai[c], H. K. Ng[c], D. K. Rowlands[d], J. C. N. Chan[a] and P. C. Y. Tong[a,*]

[a] Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (China), Fax: +852 2637 3852, e-mail: ptong@cuhk.edu.hk
[b] Department of Chemical Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong (China)
[c] Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong (China)
[d] Laboratory Animal Services Centre, The Chinese University of Hong Kong, Hong Kong (China)

Received 29 August 2007; received after revision 25 September 2007; accepted 27 September 2007
Online First 22 October 2007

Abstract. This study was performed to examine the effect of chronic renal impairment and renin-angiotensin system (RAS) activation induced by unilateral nephrectomy (UNX) on the development of pancreatic islet β-cell deficit and glucose intolerance. Sprague-Dawley rats were randomized into three groups: untreated UNX ($n = 10$), UNX treated with the angiotensin-converting enzyme inhibitor lisinopril ($n = 8$) and sham operation ($n = 10$). Blood glucose, serum insulin, renal function and histological changes of kidney and pancreas were examined 8 months post-operation. Compared with the sham rats, UNX rats developed renal impairment, insulin deficiency and glucose intolerance. Histological staining revealed an islet β-cell deficit associated with increased immunoreactivity for angiotensin and angiotensin type 1 receptor in UNX rats. Treatment with lisinopril significantly improved renal dysfunction, hyperglycemia, insulin secretion and islet RAS expression. These data suggest that chronic renal impairment and RAS activation may contribute to islet β-cell loss and glucose intolerance. RAS blockade may therefore prevent these disorders.

Keywords. Glucose intolerance, hyperglycemia, pancreas morphology, RAS blockade, rat, renin-angiotensin system, unilateral nephrectomy.

Introduction

The kidney is an important organ for glucose homeostasis. Approximately 20–30 % of the systemic glucose production [1] and glucose utilization is attributed to the kidney [2, 3]. Chronic renal disease compromises insulin degradation as well as glucose production and utilization [4]. Disturbed carbohydrate metabolism has been recognized as a common feature amongst human subjects with renal failure [4, 5] and animal models of uremia [6, 7], with the underling mechanism believed to be due to peripheral insulin resistance [6, 8, 9]. Whether renal impairment can cause islet β-cell damage is unknown; the relationship between the pancreatic islets and kidney is undoubtedly complex.

* Corresponding author.

The uninephrectomized (UNX) rat model has long been used for studies on the activation of the renin-angiotensin system (RAS) and renal impairment [10, 11]. Moreover, UNX rats demonstrate insulin resistance [6, 12] and elevated expression of angiotensin type 1 receptor (AT1R) [13]. Animal studies have revealed significantly beneficial effects of RAS inhibition with angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blockade (ARB) in the prevention and treatment of diabetes characterized by pancreatic β-cell dysfunction [14, 15]. Clinical trials have, however, provided conflicting results with regard to the impact of RAS inhibition on new onset of diabetes [16]. In addition, transforming growth factor-β1 (TGF-β1) has been recognized as a main factor in promoting fibrosis [17]. TGF-β1 is activated by RAS [18, 19] and induces expression of α-smooth muscle actin (α-SMA) [20], which in turn enhances extracellular matrix production and consequently leads to arteriosclerosis, glomerulosclerosis and fibrosis. Therefore, we hypothesize that chronic renal dysfunction and RAS activation lead to islet fibrosis associated with TGF-β overexpression and islet β-cell deficit, which subsequently contributes to glucose intolerance. In this study, we used UNX rats to investigate the role of chronic RAS activation and renal impairment in the development of islet β-cell deficit and glucose intolerance.

Materials and methods

Animals. Three-month-old male Sprague-Dawley rats weighing initially between 300 and 350 g were obtained from the Laboratory Animal Services Centre at the Chinese University of Hong Kong. The animals were caged in pairs, housed at $23 \pm 1^{\circ}$ C with a 12-h dark/light cycle, having free access to water and fed on a standard laboratory rat diet (5001 Rodent Diet, LabDiet, USA). The total duration of studies was 8 months.

Ethical approval for animal studies was according to the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong and in accordance with the Animals (Control of Experiments) Ordinance of the Department of Health of the Hong Kong SAR Government.

Unilateral nephrectomy. Rats were anesthetized with ketamine (75 mg/kg; Alfasan, Holland) and xalyzine (10 mg/kg; Alfasan) and subjected to a sham operation ($n = 10$), left nephrectomy (UNX, n=10) or UNX treated with ACEI-lisinopril (UNX+Lis, $n = 8$). Lisinopril was dissolved in distilled water and used for 8 months with a once daily dosage of 4 mg/kg body weight. All the sham and UNX rats were also gavaged with distilled water (3 ml) as a placebo control. The left kidney was exposed via a 1–1.5 cm incision in the flank and removed, leaving the adrenal gland intact. Sham-operated rats underwent anesthesia and ventral laparotomy without removal of the left kidney. The abdominal incisions were closed with 4–0 silk sutures, followed by anesthetic recovery of the animals under a heating lamp with food and water freely available.

Metabolic and biochemical studies. Body weight and average 24-h intake of water and food were monitored monthly. At 3, 6 and 8 months post-operation, fasting blood glucose (FBG) was monitored and oral glucose tolerance tests (OGTT) were conducted by gavage of dextrose (2.5 g/kg body weight) following 8 h of fasting. Blood glucose levels were measured with a blood glucose meter (Onetouch Ultra, LifeScan, Inc. USA) with blood collected from the tail vein, whereas 24-h urine samples were collected using metabolic cages (Su-Zhou, China).

Fasting blood samples were taken for measurement of glucose, insulin and renal functions. Fasting plasma glucose (hexokinase method), serum urea (enzymatic method) and serum/urine creatinine (Jaffe kinetic method) were measured using a Modular Analytics analyzer (Roche Diagnostics GmbH, Mannheim, Germany) and reagent kits supplied by the manufacturer. Analytical performances of these methods were within the manufacturer's specifications.

Fasting serum insulin, urine insulin and 2-h OGTT serum insulin concentrations were measured using enzyme immunoassay and a rat insulin ELISA kit (Enzyme immunoassay, MERCODIA, Sweden). Briefly, enzyme conjugate and substrate TMB were added to the calibrator 0, 0.15–5.5 μg/L and serum samples. After incubation and wash, the absorbance at 450 nm and insulin concentrations were read and demonstrated by an ELISA Analyzer (μQuant, Bio-Tek Instruments Inc., America). Serum insulin concentrations are expressed as mU/L. Insulin resistance (IR) was estimated using the homeostasis model assessment (HOMA) [6, 15]. HOMA-IR was calculated with the following formula:

$$\text{HOMA-IR} = \text{Fasting insulin (mU/L)} \times \text{Fasting blood glucose (mmol/L)} /22.5$$

Histological studies of pancreatic islets and kidneys. Rats were sacrificed at 8 months post-operation. Kidneys and pancreata were removed from all rats, weighed and processed for light microscopy. Half of the right kidneys and whole pancreata including both splenic and duodenal parts were fixed in 10% neutral

formaldehyde and embedded in paraffin. Serial cross-sections (4 μm) were cut perpendicular to the long axis of the pancreas, whereas serial longitudinal sections (4 μm) were spliced parallel to the longest axis of the kidney. Sections were stained with periodic acid-Schiff (PA) and examined with a Zeiss Axioplan 2 imaging microscope (Carl Zeiss, Inc, Germany), with representative images being automatically captured using a digital Spot Camera (Version 3.1 for Windows 95/98/NT; Diagnostic Instruments Inc, Sterling Heights, MI, USA).

Morphometry of pancreatic β-cells. Pancreatic β-cells were stained with a polyclonal guinea pig anti-porcine insulin antibody (Dako, cat. no. N1542, Denmark) at a dilution of 1 : 100. The antibody was specific for insulin and has no cross-reactivity with the other islet hormones, glucagon and somatostatin. Paraffin sections of rat pancreas were cut at a thickness of 4 μm and deparaffinized for immunofluorescence. Briefly, after blocking with bovine serum albumin, the pancreas sections were incubated with insulin antibody for 45 min, and nuclei were counterstained with DAPI. Sections of human pancreases were used as positive controls, whereas insulin antibody was replaced by 5% normal rabbit serum as a negative control.

Islet area and percentage of proportional islet β-cell area. For each pancreas, one section was cut and immunostained with insulin for image analysis. The entire section was viewed using a microscope, and each alternative field of view was captured. First, a field containing an insulin-positive β-cell or islet at 20× magnification was chosen at random, and then this field and the immediately adjacent fields containing insulin-positive β-cell clusters or islets were captured as images until 20–30 fields had been evaluated. Islets consisting of more than four insulin-immunoreactive cells [21] with blood vessels were studied. Each of these islets was evaluated to obtain the total β-cell area or islet cross-sectional area using image analysis software (MetaMorph 4.0 image acquisition program for Windows, 1999; Downingtown, PA, USA). A mean islet area and a mean percentage of proportional β-cell area within the islet area were therefore determined.

Immunofluorescence, immunohistochemistry and morphometry. Kidney tissue sections for immunofluorescence microscopy were stained using goat anti-rat renin antibody (Santa Cruz Biotechnology, Inc., CA; cat. no. E-17, dilution 1 : 400). Pancreatic tissue sections for immunofluorescence microscopy were stained using goat anti-rat angiotensin I/II (Angiotensin, Santa Cruz; cat. no. SC-7419, dilution 1 : 200), rabbit anti-rat TGF-β1 (Santa Cruz; cat. no. SC-146, dilution 1:200) and mouse anti-rat α-SMA (Dako; cat. no. N1584, dilution 1 : 10). Pancreatic tissue sections for immunohistochemistry were stained with rabbit anti-rat angiotensin type 1 receptor (AT1R, Santa Cruz; cat. no. SC-579, dilution 1 : 200). Briefly, paraffin pancreas sections were cut at a thickness of 4 μm and deparaffinized for immunostaining. After blocking with bovine serum albumin, the kidney and pancreas tissue sections were incubated with the primary antibodies for 45 min. Nuclei were counterstained with DAPI for immunofluorescence or with hematoxylin for immunohistochemistry. Sections of human kidney and pancreases were used as positive controls, while goat, rabbit and mouse serum were used to replace the primary antibodies as negative controls.
To evaluate the degree of renal renin expression, a total of 50 adjacent glomeruli per rat were randomly chosen. The degree of renin expression in each rat was graded as the percentage of renin-positive glomeruli in the 50 glomeruli. To evaluate the immunostaining of angiotensin and AT1R in pancreas, a total of 10–20 adjacent islets in each pancreas were randomly chosen. The degree of angiotensin and AT1R expression in each islet was based on staining intensity and graded as follows: 0, absent staining; 1, weak staining; 2, moderate staining; 3, strong staining. TGF-β1 expression in each pancreas was graded as follows: 0, 0–5%; 1, 5–15%; 2, 15–25%; 3, >25% staining area within each islet.

Statistical analysis. Data are shown as mean ± standard deviation unless otherwise specified. The statistical significance of differences noted in the biochemical parameters was evaluated using one-way ANOVA. Post hoc multiple comparisons were evaluated using the Bonfferoni test. A p value of less than 0.05 was taken as criterion for a statistically significant difference.

Results

Uninephrectomy-induced renal impairment. First we assessed the UNX-induced renal impairment with renin activation at 8 months post-operation. Compared with sham rats, UNX rats had significantly higher serum urea and creatinine, lower creatinine clearance, and lower serum total protein and albumin. Treatment with lisinopril prevented the development of renal dysfunction (Table 1). Body weight and daily food consumption at 0, 3, 6 and 8 months post-UNX were similar among the untreated UNX rats, UNX rats treated with lisinopril and sham rats. Daily water

Table 1. Metabolic and biochemical changes in rats 8 months post-operation.

	Sham	UNX	UNX+Lis
Number of rats	10	10	8
Body weight (g)	612 ± 54	620 ± 53	605 ± 43
Food intake (g/d)	32 ± 2	31 ± 2	32 ± 2
Water intake (g/d)	54 ± 11	69 ± 15†	53 ± 4*
24-h urine volume (ml)	31 ± 8	51 ± 14†	35 ± 5*
Right kidney weight (g)	2.1 ± 0.4	4.0 ± 0.5‡	3.4 ± 0.3*
Right kidney weight/body weight (%)	0.35 ± 0.03	0.64 ± 0.10‡	0.58 ± 0.03
Fasting serum urea (mmol/L)	5.8 ± 0.6	11.2 ± 3.7‡	6.8 ± 0.8$^{\#}$
Fasting serum creatinine (μmol/L)	35.0 ± 3.3	59.7 ± 12.6‡	42.1 ± 4.4$^{\#}$
Creatinine clearance rate (ml/Min)	5.7 ± 1.5	3.4 ± 1.2‡	4.9 ± 0.7*
Fasting serum total protein (g/L)	65.6 ± 1.5	61.3 ± 3.0‡	64.7 ± 2.6*
Fasting serum albumin (g/L)	17.8 ± 1.8	14.2 ± 1.6‡	17.9 ± 1.5$^{\#}$
Fasting blood glucose (mmol/L)	5.4 ± 0.5	6.0 ± 0.6†	5.4 ± 0.4*
Fasting serum insulin (mU/L)	9.3 ± 3.2	11.8 ± 4.6	11.7 ± 2.1
2-h OGTT serum insulin (mU/L)	26.5 ± 5.4	19.6 ± 7.1†	27.0 ± 4.8*
HOMA-IR	2.2 ± 0.8	3.3 ± 1.2†	2.7 ± 0.4
Urine insulin (mU/L)	0.067 ± 0.017	0.029 ± 0.007‡	0.048 ± 0.016*

Data are mean ± SD ($^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$ versus sham rats; $^{*}p < 0.05$, $^{\#}p < 0.01$ versus UNX rats).
HOMA-IR, homeostasis model assessment-insulin resistance; OGTT, oral glucose tolerance test.

Table 2. Renin, angiotensin, AT1R and TGF-β1 gradings semi-quantified by immunostaining.

	Sham	UNX	UNX+Lis
Renin-positive glomeruli (%)	8.6 ± 1.8	12.8 ± 5.1†	10.4 ± 2.6
Angiotensin grading	0.79 ± 0.28	1.23 ± 0.23†	0.72 ± 0.30$^{\#}$
AT1R grading	1.2 ± 0.2	1.8 ± 0.3†	1.3 ± 0.3*
TGF-β1 grading	0.49 ± 0.21	0.83 ± 0.23‡	0.54 ± 0.18*

Data are mean ± SD ($^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$ versus sham rats; $^{*}p < 0.05$, $^{\#}p < 0.01$ versus UNX rats).
AT1R, angiotensin receptor type 1; TGF-β1, transforming growth factor-β1.

intake and urine volume were higher in UNX rats than in both sham rats and UNX rats treated with lisinopril. The weight of the remnant kidney and the ratio of the remnant kidney weight to total body weight in UNX rats were approximately 2-fold greater of those of the corresponding right kidney in sham rats. Treatment with lisinopril significantly reduced the remnant kidney hypertrophy, whereas the increase in weight of the remnant kidney correlated with increased blood urea ($r = 0.732$, $p = 0.003$) and creatinine ($r = 0.713$, $p = 0.004$). Histopathological examination revealed glomerulosclerosis, glomerular hypertrophy, arteriolar hyalinosis, basement membrane thickening, chronic inflammatory infiltrates and tubular casts in the remnant kidneys (Fig. 1). Immunofluorescence microscopy revealed moderate to strong immunoreactivity of renin in arteriolar smooth muscle cells, juxtaglomerular apparatus cells and tubular epithelial cells in UNX rats. In contrast, only scattered juxtaglomerular apparatus cells were positive for renin in sham rats (Fig. 1). Quantitative analysis showed the percentage of renin-positive glomeruli was significantly higher in UNX rats compared with sham rats (Table 2). These findings suggest an increased expression of renin in remnant kidneys by 8 months post-uninephrectomy. Treatment with lisinopril largely attenuated these renal structural damages without significantly influencing cellular renin expression.

Uninephrectomy-induced glucose intolerance and insulin resistance. We then examined the UNX-induced glucose intolerance and insulin resistance during an oral glucose tolerance tests (OGTT). OGTT was performed at 0, 3, 6 and 8 months post-uninephrectomy (Fig. 2). At 3 months post-uninephrectomy, blood glucose levels were not significantly different between sham and UNX rats. By 6 months post-uninephrectomy, 2-h OGTT blood glucose was sig-

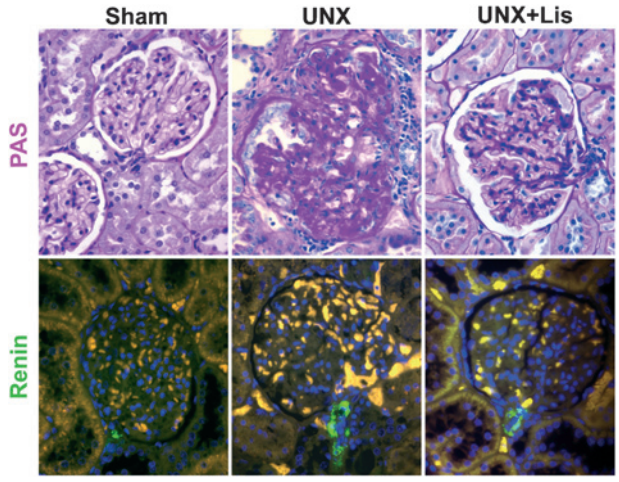

Figure 1. Renal structural changes and expression of renin. Renal tissue sections were obtained from sham, untreated uninephrectomy (UNX) and lisinopril-treated UNX rats (UNX+Lis) 8 months post-operation. Sections were stained with periodic acid Schiff (PAS) and renin. Compared with sham rats, UNX rats showed glomerulosclerosis and glomerular hypertrophy with periglomerular chronic inflammatory infiltrates. Strong immunoreactivity of renin in glomerular arteriolar walls was observed in UNX rats. Treatment with lisinopril attenuated glomerulosclerosis and chronic inflammatory infiltration. Original magnification ×400.

nificantly higher in UNX rats than in sham rats (7.7 ± 0.9 versus 6.7 ± 0.7 mmol/L, $p = 0.043$). At this time point, 2-h OGTT blood glucose (6.9 ± 1.1 mmol/L) in lisinopril-treated rats appeared to be reduced, but the reduction was not statistically significant ($p = 0.177$ versus UNX rats).

By 8 months post-uninephrectomy, UNX rats (compared to sham rats) had higher fasting blood glucose (FBG, 6.0 ± 0.6 versus 5.4 ± 0.5 mmol/L, $p = 0.038$), 30-min OGTT blood glucose (8.7 ± 1.2 versus 7.7 ± 0.8 mmol/L, $p = 0.061$) and 2-h OGTT blood glucose (8.2 ± 1.3 versus 6.5 ± 0.5 mmol/L, $p = 0.001$). UNX rats treated with lisinopril (versus untreated UNX rats) had significantly lower FBG (5.4 ± 0.4 mmol/L, $p = 0.043$ versus UNX) and 2-h OGTT blood glucose (6.7 ± 0.6 mmol/L, $p = 0.006$). UNX rats progressively developed hyperglycemia and glucose intolerance, and treatment with lisinopril significantly prevented the development of such glycemic elevation (Fig. 2).

By 8 months post-uninephrectomy, UNX rats were shown to have insulin resistance. Compared with sham rats, UNX rats had a higher homeostasis model assessment of insulin resistance index (HOMA-IR; 3.3 ± 1.2 versus 2.2 ± 0.8, $p = 0.043$). Treatment with lisinopril appeared to reduce HOMA-IR (2.7 ± 0.4), although the reduction was not significant ($p = 0.707$ versus UNX rats). Fasting serum insulin levels were similar among the three groups (UNX, 11.8 ± 4.6 mU/L; sham, 9.3 ± 3.2 mU/L; lisinopril, 11.7 ± 2.1 mU/L,

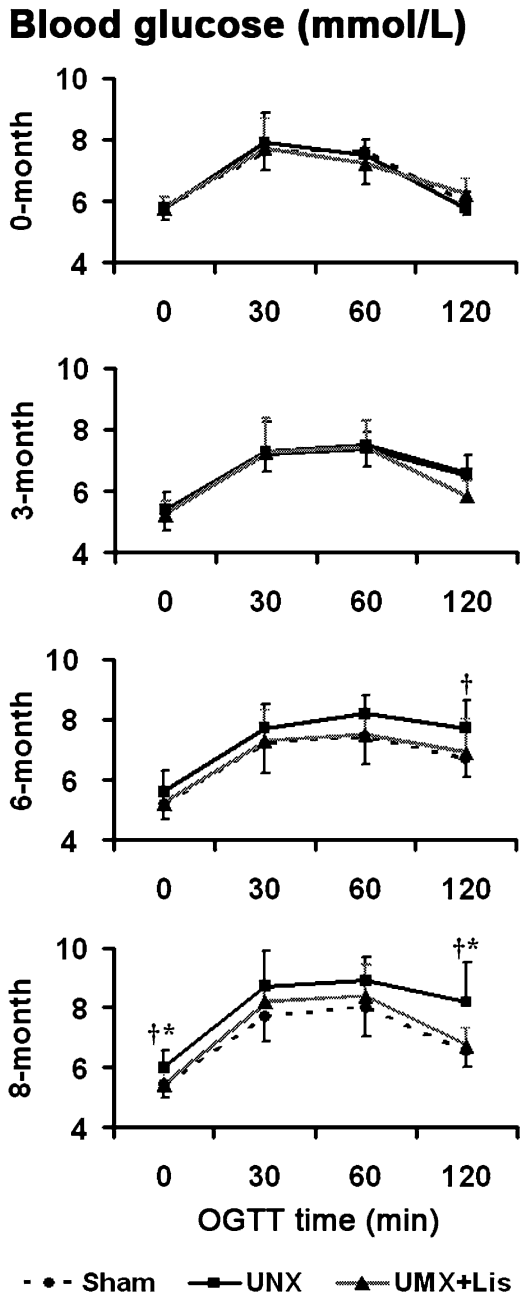

Figure 2. Blood glucose levels in relation to the time after uninephrectomy (UNX). Oral glucose tolerance test (OGTT) was performed in sham rats (circle, dashed), untreated UNX rats (square, dark solid) and lisinopril-treated UNX rats (UNX+Lis, triangle, light solid) at 0, 3, 6 and 8 months post-operation. The 2-h OGTT blood glucose was significant higher in UNX rats versus sham rats (†$p < 0.05$ UNX rats *versus* sham rats) by 6 months. Fasting blood glucose and 2-h OGTT blood glucose were significant higher in UNX rats versus sham and UNX+Lis rats by 8 months (†$p < 0.05$ UNX rats *versus* sham rats; *$p < 0.05$ UNX rats *versus* UNX+Lis rats).

$p = 0.161$). Due to renal impairment, the fasting urine insulin level was significantly decreased in UNX rats (0.029 ± 0.007 mU/L versus 0.067 ± 0.017 mU/L in sham rats, $p = 0.003$; lisinopril, 0.048 ± 0.016 mU/L,

Table 3. Pancreatic islet β-cells in rats 8 months after operation.

	Sham	UNX	UNX+Lis
Pancreas weight (g)	1.6 ± 0.3	1.8 ± 0.3	1.8 ± 0.4
Log_{10} mean islet area (μm^2)	4.7 ± 0.7	5.0 ± 0.3	5.0 ± 0.2
β-cell area/islet area (%)	64.2 ± 3.0	52.4 ± 8.5‡	64.3 ± 2.6#

Data are mean ± SE (‡$p < 0.01$ versus sham rats; #$p < 0.01$ versus UNX rats).

$p = 0.012$ versus UNX rats). The serum insulin level at 2-h post-glucose gavage was significantly lower in UNX rats compared with sham rats (19.6 ± 7.1 versus 26.5 ± 5.4 mU/L, $p = 0.030$), and the decrease in the serum insulin level in UNX rats was completely normalized by treatment with the ACEI lisinopril (27.0 ± 4.8 mU/L, $p = 0.023$).

Pancreatic RAS activation and islet β-cell deficit. Histological immunostaining and morphometry were used to investigate the UNX-induced pancreatic RAS activation and islet β-cell loss, which might contribute to hyperglycemia and glucose intolerance. In this study, islets were defined as a vascularized cluster of more than four insulin-secreting β-cells [21]. Islet β-cells were identified with immunostaining of insulin, and islet blood vessels were stained with periodic acid Schiff (PAS) to define the basement membrane and with α-smooth muscle actin (α-SMA) to demonstrate vascular smooth muscle cells. A total of 172 islets in sham rats, 343 islets in UNX rats and 140 islets in lisinopril-treated rats were analyzed. Since the islet areas were right skew distributed, we transformed the data to normal distribution by logarithmic transformation based on 10. Compared with sham rats, untreated UNX rats showed islet damage (Fig. 3) and an approximate 12 % reduction in the β-cell area fraction per islet ($p = 0.007$). Table 3 shows a significant deficit of islet β-cells in UNX rats versus sham rats. Treatment with lisinopril significantly improved islet structures as well as the islet β-cell fraction ($p = 0.006$ versus UNX). The difference in islet β-cell area correlated with fasting blood glucose levels ($r = -0.70$, $p = 0.017$), implying that elevated fasting blood glucose levels resulted from a reduced islet β-cell volume.

In parallel to the islet β-cell deficit, the islets in UNX rats showed capillary basement membrane thickening and diffuse fibrosis (Fig. 3). The severity of islet fibrosis increased progressively from sham rats, to UNX rats with normoglycemia, to UNX rats with impaired fasting blood glucose (data not shown). Normal islets usually had a diameter of 50 to 100 μm, whereas most of the islets with marked fibrosis were hypertrophic and had a diameter greater than 150 μm. Sequential sections revealed that bands of expanded fibrous tissues replaced the normally delicate islet stroma in UNX rats with impaired fasting blood glucose. Compared to untreated UNX rats, UNX rats treated with lisinopril were shown to have substantially less islet fibrosis.

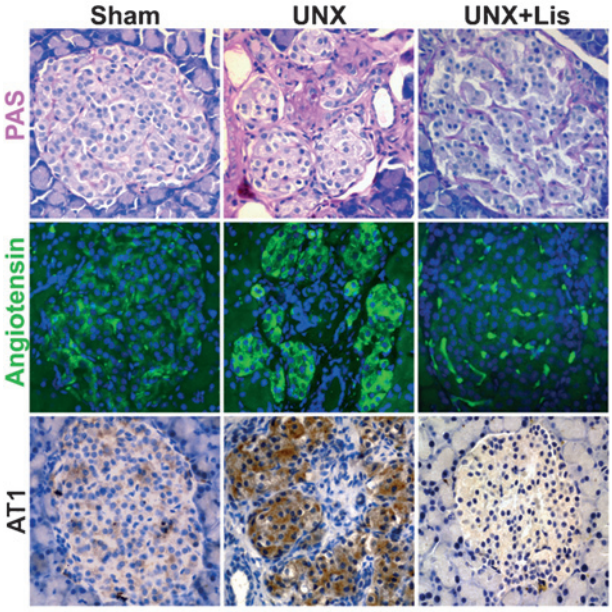

Figure 3. Pancreatic islet fibrosis and expression of angiotensin I/II (Angiotensin) and angiotensin type 1 receptor (AT1R). Pancreatic tissue sections were obtained from sham rats, untreated uninephrectomy (UNX) rats and lisinopril-treated UNX (UNX+Lis) rats 8 months post-operation. Sections were stained with periodic acid Schiff (PAS, pink), Angiotensin (green) and AT1R (brown). Compared with sham rats, UNX rats showed a marked diffuse islet fibrosis with diminished endocrine cells. Immunofluorescent and immunohistochemical staining revealed overexpression of angiotensin and AT1R in pancreatic islets of UNX rats. Such abnormalities were attenuated in UNX rats treated with lisinopril. Original magnification × 400.

Islet fibrosis and islet β-cell deficit were accompanied by overexpression of both angiotensin and AT1R (Table 2). Immunofluorescence of angiotensin showed strong immunoreactivity in islet cells surrounded by UNX-induced fibrotic tissues but weak immunoreactivity in normal islet cells from sham rats and from lisinopril-treated rats (Fig. 3). Fibrotic islets in UNX rats also exhibited strong expression of AT1R, whereas normal islets in sham rats and in lisinopril-treated rats were virtually negative for

AT1R. The UNX-induced overexpression of angiotensin and AT1R in pancreatic islet cells co-existed with islet fibrosis and islet β-cell deficit.

Since TGF-β is a potent pro-fibrotic factor linking islet RAS activation and islet fibrosis, sequential pancreatic tissue sections were double-stained with TGF-β1 and α-SMA (Fig. 4). Islet cells with overexpression of angiotensin and AT1R in UNX rats also showed a marked increase in the immunoreactivity for TGF-β1 (Table 2), whereas in normal islets of sham rats and lisinopril-treated rats, few islet cells were positive for TGF-β1. Additionally, expression of α-SMA in islets corresponded to the degree of islet fibrosis. Diminished expression of insulin and a reduced volume of insulin-positive cells within pancreatic islets were also in correlation with the overexpression of TGF-β1 and increased number of α-SMA-positive cells (Fig. 4).

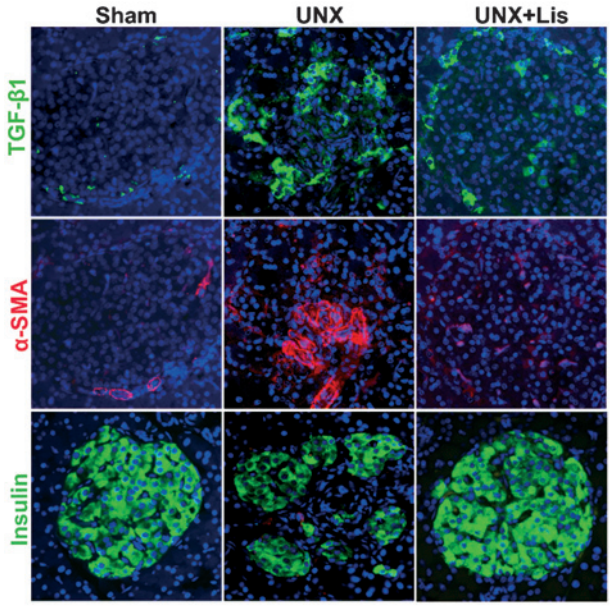

Figure 4. Expression of transforming growth factor-β1 (TGF-β1) and α-smooth muscle actin (α-SMA) in pancreatic islet β-cells. Pancreatic tissue sections were obtained from sham rats, untreated uninephrectomy rats (UNX) and lisinopril-treated UNX rats (UNX+Lis) 8 months post-operation. Sections were immunostained with TGF-β1 (green), α-SMA (red) and insulin (green). Double immunofluorescent staining revealed overexpression of TGF-β1 and α-SMA in islets from UNX rats with a diminished number of insulin-positive islet β-cells. In contrast, sham rats and lisinopril-treated rats showed weak expression of TGF-β1 and α-SMA in the peripheral region of islets and a normal number of insulin-positive islet β-cells. Original magnification ×400.

Discussion

This study demonstrates the overexpression of RAS in remnant kidneys and pancreatic islets of male adult uninephrectomized Sprague-Dawley rats. The activation of RAS accompanied by TGF-β1 overexpression causes islet fibrosis and islet β-cell deficit, which leads to insulin deficiency and results in hyperglycemia and glucose intolerance. Treatment with an ACEI such as lisinopril prevented the development of renal impairments associated with RAS activation, hyperglycemia and glucose intolerance in UNX rats. The beneficial effects of the ACE inhibition are mainly associated with islet β-cell protection due to RAS blockade and improved kidney function, which is central to glucose homeostasis.

The RAS activation in our study is strongly correlated with pancreatic fibrosis and islet damage. Pancreatic intrinsic RAS has been demonstrated in the endocrine pancreas, and the pathogenic functions of RAS in islet β-cell damage have been shown in several studies [22, 23]. During impaired status of renal function [24], RAS is activated and plays an important role in the mediation of physiological reactions such as stimulating and inhibiting cell proliferation, inducing apoptosis and participating in the increase of some risk factors including oxidative stress, inflammation and free fatty acid levels [25]. During a pathological status, TGF-β1 is activated by RAS [18, 19] and induces α-SMA expression [20]. Expression of α-SMA enables interstitial fibroblasts, vascular smooth muscle cells, glomerular mesangial cells and hepatic satellite cells to produce extracellular matrix, which eventually leads to fibrotic damage such as fibrosis, arteriosclerosis, glomerulosclerosis and cirrhosis. Moreover, the RAS may affect the function of pancreatic islets as a result of hemodynamic effects of decreased blood circulation and reduced insulin secretion. In an *in vivo* study, infusion of angiotensin II induced a dose-dependent reduction in both whole pancreatic and islet blood flow and delayed the first phase of insulin release in response to glucose [26]. In this study, both the structural damage and decreased blood flow in pancreatic islets are assumed to contribute to the insufficiency of β-cell function. These data are consistent with i*n vitro* studies of human pancreatic islets [27, 28] demonstrating that the AT1R and angiotensin II are expressed by islet β-cells and that locally produced angiotensin II may be important in regulating a coordinated insulin secretory response from islet β-cells. The effects of intrinsic pancreatic RAS on insulin release have been attributed to its vasoconstrictive actions on islet blood flow [26] and its direct regulation of insulin secretion [27, 28].

In our study, the decreased β-cell fraction and 2-h OGTT insulin secretion in addition to renal impairment and insulin resistance are assumed to be crucial factors that contribute to the development of hyperglycemia and glucose intolerance in UNX rats. This is similar to results in human studies in which β-cell mass

in patients with type 2 diabetes shows a 40–60% reduction in human autopsy studies [29–31]. The activation of RAS and the activated TGF-β1 and α-SMA are proposed to take part in intra-islet fibrosis formation and are thought to be related to the insufficient insulin secretion needed to compensate insulin resistance. It is clear that β-cell mass can be regulated to maintain euglycemia in various metabolic conditions, and β-cells change dynamically in mass and function in response to variations in demand for insulin [32]. Moreover, β-cell mass is known to be regulated by a balance between β-cell growth (β-cell replication and neogenesis) and β-cell death (apoptosis) [33, 34]. In this study, most of the islets with fibrosis were hypertrophic. This may imply that at an early stage of insulin resistance, islet mass self-expanded both morphometrically and functionally to compensate for the insulin resistance and maintain euglycemia. These compensative reactions were latterly disrupted by the intra-islet fibrosis formation, and eventually β-cells failed to compensate for insulin resistance.

Insulin uptake and degradation is a feature of all insulin-sensitive tissues and contributes partly to insulin clearance [35]. However, at higher concentrations, insulin removed by the liver and kidneys is assumed to have much importance in insulin clearance. The kidney is the major site of insulin clearance from the systemic circulation [36], removing approximately 50% of peripheral insulin [37]. During renal failure, insulin clearance is reduced, which may result in hypoglycemia in insulin-treated diabetic patients. Similarly, in this study, urine insulin concentration decreased in UNX rats. Moreover, suppressed insulin degradation in muscle and liver has been demonstrated in uremic subjects [37]. Taken together, decreased insulin excretion in urine and suppressed insulin degradation in muscle and liver are assumed to be the causes of insulin resistance observed in this study. The resulting insulin resistance in insulin target organs is contributable to the hyperglycemia and glucose intolerance.

The UNX rat model has long been used as an animal model to study nephropathy, high blood pressure and glucose metabolism. However, most studies involved models set up by 5/6 or 90% nephrectomy, hence the renal failure was relatively acute and severe, and observation periods were shorter (<4 months). In our experiment, we selected a unilateral nephrectomy rat model and went though an 8-month observation. This chronic model is closer to the real changes in pancreatic function and blood glucose metabolism in human kidney donors and subjects with renal dysfunction. Moreover, uninephrectomy is a severe procedure that produces a myriad of effects on many systems including the RAS and the endocrine pancreas.

Chronic kidney disease is a common public health problem that affects approximately 12% of adults in the United States [38]. The high prevalence of chronic renal failure and of living kidney donors for renal transplantation prompted this study of glucose disorder and pancreatic islet damage. Previous studies showed that patients with chronic renal failure have a high prevalence of pancreatic damage [39] and islet amyloid [40]. Reported pancreatic damage included peri-ductal fibrosis, interstitial fibrosis and inflammation, which were also observed in this study. Later studies found insulin resistance in renal failure patients [41]. All these data imply a higher incidence of diabetes in patients with renal dysfunction. In this disorder, the activated RAS is assumed to be the essential mediator. Although the mechanism of the effect of the RAS on the occurrence of diabetes was not illuminated previously, RAS blockades with ACEI or ARB have been observed to improve islet structure and function in experimental animal models of type 2 diabetes [14, 15, 42] and pancreatitis [43, 44]. Moreover, some clinical trials [45, 46] and a subsequent meta-analysis [47] also suggested that RAS blockade had a potential preventive effect on new-onset diabetes. In this study, treatment with ACEI prevented UNX rats from developing hyperglycemia and glucose intolerance through islet β-cell protection, possibly due to the blocking of RAS activation. In contrast, a recent clinical study did not show a protective effect (from progression to diabetes) of ACEI–ramipril in patients with impaired fasting glucose or impaired glucose tolerance [16]. One possible explanation is that pancreatic islet damage and insulin resistance may have already existed in subjects with impaired fasting glucose or impaired glucose tolerance. Once islet damage occurs, RAS blockade with an ACEI such as ramipril may not be effective. More studies are ongoing to test the effects of RAS and its blockades on diabetes [48].

There are some limitations in this study. The beneficial effects noted are not necessarily attributable to ACEI but may be related to blood pressure control. A control with another anti-hypertensive agent and measurement of blood pressure should be performed in future studies. Nonetheless, we report an observation of islet damage with β-cell deficit and glucose intolerance in rats with uninephrectomy. Clinical monitoring of blood glucose and islet function in kidney donors and in patients with chronic renal disease is therefore strongly recommended.

Acknowledgements. This study was partly funded by the Graduate School of the Chinese University of Hong Kong and by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (CUHK4462/06M). We gratefully acknowledge the technical assistance of Stanly Ho and Guan Jing. We thank Zhu Xun, Simon K. M. Lee and Laureen Kwok for their support and advice.

1 Kida, K., Nakajo, S., Kamiya, F., Toyama, Y., Nishio, T. and Nakagawa, H. (1978) Renal net glucose release *in vivo* and its contribution to blood glucose in rats. J. Clin. Invest. 62, 721–726.
2 McGuinness, O. P., Fugiwara, T., Murrell, S., Bracy, D., Neal, D., O'Connor, D. and Cherrington, A. D. (1993) Impact of chronic stress hormone infusion on hepatic carbohydrate metabolism in the conscious dog. Am. J. Physiol. 265, E314–E322.
3 Stumvoll, M., Chintalapudi, U., Perriello, G., Welle, S., Gutierrez, O. and Gerich, J. (1995) Uptake and release of glucose by the human kidney. Postabsorptive rates and responses to epinephrine. J. Clin. Invest. 96, 2528–2533.
4 Mak, R. H. and DeFronzo, R. A. (1992) Glucose and insulin metabolism in uremia. Nephron 61, 377–382.
5 Horton, E. S., Johnson, C. and Lebovitz, H. E. (1968) Carbohydrate metabolism in uremia. Ann. Intern. Med. 68, 63–74.
6 Stein, P. P., Hunt, W. A., Johnson, C. M., DeFronzo, R. A. and Smith, J. D. (1989) Insulin resistance in uremia: an *in vivo* and *in vitro* study. Metabolism 38, 562–567.
7 Saeed, A. M., El Munshid, H. A., Sukkar, M. Y. and Abdel Wahab, S. M. (1978) Insulin and glucose levels in acute and chronically uraemic rats. J. Endocrinol. 77, 151–152.
8 Cersosimo, E., Judd, R. L. and Miles, J. M. (1994) Insulin regulation of renal glucose metabolism in conscious dogs. J. Clin. Invest. 93, 2584–2589.
9 Perez, G., Carteni, G., Ungaro, B. and Sacca, L. (1980) Influence of nephrectomy on the glucoregulatory response to insulin administration in the rat. Can. J. Physiol. Pharmacol. 58, 301–305.
10 Mozaffari, M. S., Warren, B. K., Russell, C. M. and Schaffer, S. W. (1997) Renal function in the noninsulin-dependent diabetic rat: effects of unilateral nephrectomy. J. Pharmacol. Toxicol. Methods 37, 197–203.
11 Yokozawa, T., Nakagawa, T., Wakaki, K. and Koizumi, F. (2001) Animal model of diabetic nephropathy. Exp. Toxicol. Pathol. 53, 359–363.
12 Cecchin, F., Ittoop, O., Sinha, M. K. and Caro, J. F. (1988) Insulin resistance in uremia: insulin receptor kinase activity in liver and muscle from chronic uremic rats. Am. J. Physiol. 254, E394–401.
13 Mok, K. Y., Sandberg, K., Sweeny, J. M., Zheng, W., Lee, S. and Mulroney, S. E. (2003) Growth hormone regulation of glomerular AT1 angiotensin receptors in adult uninephrectomized male rats. Am. J. Physiol. Renal Physiol. 285, F1085–F1091.
14 Chu, K. Y., Lau, T., Carlsson, P. O. and Leung, P. S. (2006) Angiotensin II type 1 receptor blockade improves beta-cell function and glucose tolerance in a mouse model of type 2 diabetes. Diabetes 55, 367–374.
15 Tikellis, C., Wookey, P. J., Candido, R., Andrikopoulos, S., Thomas, M. C. and Cooper, M. E. (2004) Improved islet morphology after blockade of the renin-angiotensin system in the ZDF rat. Diabetes 53, 989–997.
16 Bosch, J., Yusuf, S., Gerstein, H. C., Pogue, J., Sheridan, P., Dagenais, G., Diaz, R., Avezum, A., Lanas, F., Probstfield, J., Fodor, G. and Holman, R. R. (2006) Effect of ramipril on the incidence of diabetes. N. Engl. J. Med. 355, 1551–1562.
17 Mauviel, A. (2005) Transforming growth factor-beta: a key mediator of fibrosis. Methods Mol. Med. 117, 69–80.
18 Bechara, R. I., Pelaez, A., Palacio, A., Joshi, P. C., Hart, C. M., Brown, L. A., Raynor, R. and Guidot, D. M. (2005) Angiotensin II mediates glutathione depletion, transforming growth factor-beta1 expression, and epithelial barrier dysfunction in the alcoholic rat lung. Am. J. Physiol. Lung Cell. Mol. Physiol. 289, L363–L370.
19 Huang, Z., Jansson, L. and Sjoholm, A. (2006) Pancreatic islet blood flow is selectively enhanced by captopril, irbesartan and pravastatin, and suppressed by palmitate. Biochem. Biophys. Res. Commun. 346, 26–32.
20 Desmouliere, A., Geinoz, A., Gabbiani, F. and Gabbiani, G. (1993) Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J. Cell Biol. 122, 103–111.
21 Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A. and Butler, P. C. (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 52, 102–110.
22 Lam, K. Y. and Leung, P. S. (2002) Regulation and expression of a renin-angiotensin system in human pancreas and pancreatic endocrine tumours. Eur. J. Endocrinol. 146, 567–572.
23 Leung, P. S., Chan, W. P., Wong, T. P. and Sernia, C. (1999) Expression and localization of the renin-angiotensin system in the rat pancreas. J. Endocrinol. 160, 13–19.
24 Wolf, G. (2000) Cell cycle regulation in diabetic nephropathy. Kidney Int. Suppl. 77, S59–S66.
25 Jandeleit-Dahm, K. A., Tikellis, C., Reid, C. M., Johnston, C. I. and Cooper, M. E. (2005) Why blockade of the renin-angiotensin system reduces the incidence of new-onset diabetes. J. Hypertens. 23, 463–473.
26 Carlsson, P. O., Berne, C. and Jansson, L. (1998) Angiotensin II and the endocrine pancreas: effects on islet blood flow and insulin secretion in rats. Diabetologia 41, 127–133.
27 Lau, T., Carlsson, P. O. and Leung, P. S. (2004) Evidence for a local angiotensin-generating system and dose-dependent inhibition of glucose-stimulated insulin release by angiotensin II in isolated pancreatic islets. Diabetologia 47, 240–248.
28 Ramracheya, R. D., Muller, D. S., Wu, Y., Whitehouse, B. J., Huang, G. C., Amiel, S. A., Karalliedde, J., Viberti, G., Jones, P. M. and Persaud, S. J. (2006) Direct regulation of insulin secretion by angiotensin II in human islets of Langerhans. Diabetologia 49, 321–331.
29 Clark, A., Wells, C. A., Buley, I. D., Cruickshank, J. K., Vanhegan, R. I., Matthews, D. R., Cooper, G. J., Holman, R. R. and Turner, R. C. (1988) Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Res. 9, 151–159.
30 Kloppel, G., Lohr, M., Habich, K., Oberholzer, M. and Heitz, P. U. (1985) Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Surv. Synth. Pathol. Res. 4, 110–125.
31 Rahier, J., Goebbels, R. M. and Henquin, J. C. (1983) Cellular composition of the human diabetic pancreas. Diabetologia 24, 366–371.
32 Bonner-Weir, S. (2000) Islet growth and development in the adult. J. Mol. Endocrinol. 24, 297–302.
33 Bernard-Kargar, C. and Ktorza, A. (2001) Endocrine pancreas plasticity under physiological and pathological conditions. Diabetes 50 Suppl 1, S30–S35.
34 Pick, A., Clark, J., Kubstrup, C., Levisetti, M., Pugh, W., Bonner-Weir, S. and Polonsky, K. S. (1998) Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. Diabetes 47, 358–364.
35 Canas, X., Fernandez-Lopez, J. A., Ardevol, A., Adan, C., Esteve, M., Rafecas, I., Remesar, X. and Alemany, M. (1995) Rat insulin turnover *in vivo.* Endocrinology 136, 3871–3876.
36 Rabkin, R. and Kitaji, J. (1983) Renal metabolism of peptide hormones. Miner. Electrolyte Metab. 9, 212–226.
37 Rabkin, R., Ryan, M. P. and Duckworth, W. C. (1984) The renal metabolism of insulin. Diabetologia 27, 351–357.
38 Coresh, J., Byrd-Holt, D., Astor, B. C., Briggs, J. P., Eggers, P. W., Lacher, D. A. and Hostetter, T. H. (2005) Chronic

kidney disease awareness, prevalence, and trends among, U. S. adults, 1999 to 2000. J. Am. Soc. Nephrol. 16, 180–188.
39 Avram, M. M. (1977) High prevalence of pancreatic disease in chronic renal failure. Nephron 18, 68–71.
40 de Koning, E. J., Fleming, K. A., Gray, D. W. and Clark, A. (1995) High prevalence of pancreatic islet amyloid in patients with end-stage renal failure on dialysis treatment. J. Pathol. 175, 253–258.
41 Fliser, D., Pacini, G., Engelleiter, R., Kautzky-Willer, A., Prager, R., Franek, E. and Ritz, E. (1998) Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. Kidney Int. 53, 1343–1347.
42 Kampf, C., Lau, T., Olsson, R., Leung, P. S. and Carlsson, P. O. (2005) Angiotensin II type 1 receptor inhibition markedly improves the blood perfusion, oxygen tension and first phase of glucose-stimulated insulin secretion in revascularised syngeneic mouse islet grafts. Diabetologia 48, 1159–1167.
43 Kuno, A., Yamada, T., Masuda, K., Ogawa, K., Sogawa, M., Nakamura, S., Nakazawa, T., Ohara, H., Nomura, T., Joh, T., Shirai, T. and Itoh, M. (2003) Angiotensin-converting enzyme inhibitor attenuates pancreatic inflammation and fibrosis in male Wistar Bonn/Kobori rats. Gastroenterology 124, 1010–1019.
44 Yamada, T., Kuno, A., Masuda, K., Ogawa, K., Sogawa, M., Nakamura, S., Ando, T., Sano, H., Nakazawa, T., Ohara, H., Nomura, T., Joh, T. and Itoh, M. (2003) Candesartan, an angiotensin II receptor antagonist, suppresses pancreatic inflammation and fibrosis in rats. J. Pharmacol. Exp. Ther. 307, 17–23.
45 Hansson, L., Lindholm, L. H., Niskanen, L., Lanke, J., Hedner, T., Niklason, A., Luomanmaki, K., Dahlof, B., de Faire, U., Morlin, C., Karlberg, B. E., Wester, P. O. and Bjorck, J. E. (1999) Effect of angiotensin-converting-enzyme inhibition compared with conventional therapy on cardiovascular morbidity and mortality in hypertension: the Captopril Prevention Project (CAPPP) randomised trial. Lancet 353, 611–616.
46 Wing, L. M., Reid, C. M., Ryan, P., Beilin, L. J., Brown, M. A., Jennings, G. L., Johnston, C. I., McNeil, J. J., Macdonald, G. J., Marley, J. E., Morgan, T. O. and West, M. J. (2003) A comparison of outcomes with angiotensin-converting–enzyme inhibitors and diuretics for hypertension in the elderly. N. Engl. J. Med. 348, 583–592.
47 Abuissa, H., Jones, P. G., Marso, S. P. and O'Keefe, J. H. Jr. (2005) Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for prevention of type 2 diabetes: a meta-analysis of randomized clinical trials. J. Am. Coll. Cardiol. 46, 821–826.
48 Teo, K., Yusuf, S., Sleight, P., Anderson, C., Mookadam, F., Ramos, B., Hilbrich, L., Pogue, J. and Schumacher, H. (2004) Rationale, design, and baseline characteristics of 2 large, simple, randomized trials evaluating telmisartan, ramipril, and their combination in high-risk patients: the ongoing telmisartan alone and in combination with ramipril global endpoint trial/telmisartan randomized assessment study in ACE intolerant subjects with cardiovascular disease (ONTARGET/TRANSCEND) trials. Am. Heart J. 148, 52–61.