Angiotensin II Type 1 Receptor Blockade Improves β-Cell Function and Glucose Tolerance in a Mouse Model of Type 2 Diabetes

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The prevalence of obesity is rising worldwide
61% of adults are now overweight or obese
(Wyatt 2003)

The accompanying epidemic of type 2 diabetes and its cardiovascular complications
(Zimmet et al 2001 & Bonow et al 2004)

Therapies aim
Increasing insulin sensitivity
offer only partial solutions
since β-cell dysfunction and β-cell loss may also contribute to disease progression

In this regard, the mechanisms that underlie islet failure have yet to be elucidated

Recently
Identified local renin-angiotensin system (RAS)
may play an important role in pancreatic physiology and pathophysiology

(Leung et al 2001 & 2003)
Systemic Renin-Angiotensin System (RAS)

- Angiotensinogen
- Renin
- Angiotensin I
- Angiotensin II

Angiotensin converting enzyme (ACE)
Sympathetic activity
Tubular Na+ & Cl- reabsorption and K+ excretion
Aldosterone
Vasoconstriction
ADH
Collecting duct: H2O reabsorption

Local Renin-Angiotensin System (RAS)

- Angiotensinogen
- Renin
- Angiotensin I
- Angiotensin II

Pancreatic islet:
- angiotensinogen
- ACE
- AT1R & AT2R

AT1R localized specifically to the β-cells
(Lau et al 2004)

Mechanism of angiotensin II and insulin secretion in pancreatic β cell

- Glucose
- Glut 2
- Glycolysis
- KATP channel
- Ca2+ channel
- Ca2+ depolarization
- Insulin

Angiotensin II
AT1R
pancreatic β cell
(pro)insulin biosynthesis
islet blood flow

The clinically observed benefits of RAS blockade in persons at risk for developing type 2 diabetes

- a reduced incidence of developing diabetes
(Scheen 2004)

Objective

- To study the role of local islet RAS especially AT1R in obesity-induced type 2 diabetes mice
- To study the effect of specific AT1R antagonist losartan in obesity-induced type 2 diabetes mice

Methods
Animal model of type 2 diabetes

The animals were obtained from the Laboratory Animal Services Centre of the Chinese University of Hong Kong. The experimental procedures were approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong.

control heterozygous db/db mice
genetically diabetic homozygous db/db mice

Measurements of blood glucose
Measurements of oral glucose tolerance test
Measurements of insulin tolerance test

Pancreatic islet isolation

Pancreatic islets were isolated from male control and diabetic mice aged 10 weeks. The pancreata were dissected and cut into small pieces of about 1 mm³. They were then placed in cold Hanks’ solution and transferred to vials containing collagenase solution.
The digest was then washed three times by filling the vial with Hank’s solution.

The islets were then selected under a light microscope and cultured free floating for 4–7 days in nonadherent culture dishes in RPMI 1640 medium supplemented with 10% (vol/vol) fetal bovine serum.

Statistical data analysis

• Results are expressed as means±SE
• Multiple comparisons between groups were performed using an ANOVA followed by Tukey’s post hoc test
• P<0.05 was considered statistically significant

Localization of AT1R in obese db/db mice

Experimental 1

Immunohistochemistry

Specific localization of AT1R in islets

Secondary antibody

Primary antibody

Anti-rabbit antibody labeled with rhodamine

Rabbit anti-AT1R serum

Pancreatic islet

Antigen

AT1R

Results
Immunohistochemistry

specific localization of insulin in islets

Secondary antibody

Primary antibody

anti-goat antibody labeled with aminocoumarin acetate

test

Pancreatic islet

Antigen insulin

Localization of AT1R & insulin in control and obese db/db mice

AT1R (red) in control

AT1R (red) in db/db islets

Insulin (blue) in control mice

Insulin (blue) in db/db islets

Negative control for AT1R

Negative control for insulin

AT1R-labeling: db/db islets mice > control islets mice

Insulin-labeling: db/db islets mice < control islets mice

Expression of AT1R in obese db/db mice

Real-time RT-PCR analysis

Detection & quantitation of mRNA

PCR = polymerase chain reaction

measured AT1R mRNA in control and db/db mice

Real-time RT-PCR analysis of AT1R mRNA expression in control and db/db islets

AT1R mRNA: db/db islets mice > control islets mice ~ 3 folds
Insulin release from isolated islets of obese db/db mice

Measurements of islet insulin release

Groups of six islets were transferred in duplicate to Falcon 24-well culture plates containing 0.25 ml Krebs-Ringer bicarbonate buffer (KRBB) supplemented with 10 mmol/l HEPES and 2 mg/ml of BSA.

Specific AT1R antagonist losartan 1 mol/l

10 min

0

1 h

1 mmol/l glucose

2 h

16.7 mmol/l glucose

Pretreatment with 1 mol/l losartan, a specific AT1R antagonist, before the addition of angiotensin II (100 nmol/l) completely rescued glucose-induced insulin secretion.

Effects of losartan (Los) and angiotensin II (AngII) on insulin release in 1.7 mmol/l (L) or 16.7 mmol/l (H) glucose from isolated islets of control mice (A) and db/db mice (B)

*P < 0.05 vs. islets exposed to 1.7 mmol/l glucose only †P < 0.05 vs. islets exposed to 16.7 mmol/l glucose only

n = 6–8

control mice
db/db mice

At the highest concentration of angiotensin II used (100 nmol/l), the glucose-induced insulin release was completely prevented.

Glucose

Insulin release

Effects of losartan and angiotensin II on insulin release in 1.7 mmol/l (L) or 16.7 mmol/l (H) glucose from isolated islets of control mice (A) and db/db mice (B)

*P < 0.05 vs. islets exposed to 1.7 mmol/l glucose only †P < 0.05 vs. islets exposed to 16.7 mmol/l glucose only

n = 6–8

control mice
db/db mice
Effects of losartan (Los) and angiotensin II (AngII) on insulin release in 1.7 mmol/l (L) or 16.7 mmol/l (H) glucose from isolated islets of control mice (A) and db/db mice (B).

*P < 0.05 vs. islets exposed to 1.7 mmol/l glucose only
†P < 0.05 vs. islets exposed to 16.7 mmol/l glucose only
n = 6–8

Losartan alone did not affect glucose-stimulated insulin secretion.

Losartan alone increased glucose-induced insulin release in the db/db mice.

Comparison of insulin release in control and diabetic mice.

*P < 0.05 vs. islets exposed to 1.7 mmol/l glucose only
†P < 0.05 vs. islets exposed to 16.7 mmol/l glucose only
n = 6–8

Level of glucose-induced insulin release from the db/db islets was about one-fifth of that from the control islets.

Measurements of (pro)insulin biosynthesis and total protein synthesis in islets of obese db/db mice.

(Pro)insulin and total protein biosynthesis in islets of obese db/db mice.

Measurements of (pro)insulin biosynthesis and total protein synthesis.

Measurements of (pro)insulin biosynthesis and total protein synthesis.

Measurements of (pro)insulin biosynthesis and total protein synthesis.
Effects of losartan (Los) and angiotensin II (AngII) on (pro)insulin biosynthesis in 1.7 mmol/l (L) or 16.7 mmol/l (H) glucose from isolated islets of control mice (A) and db/db mice (B)

- Pretreatment of isolated islets with 1 mol/l of losartan before the addition of angiotensin II (100 nmol/l) restored the glucose-induced (pro)insulin biosynthesis.

Comparison of (pro)insulin biosynthesis in control and db/db mice

Losartan treatment restored db/db islet glucose-stimulated (pro)insulin biosynthesis to a level similar to that of control islets.
Islet total protein synthesis was not affected by losartan treatment.

Effects of losartan-treatment on blood glucose concentrations in obese db/db mice

Chronic treatment with losartan

Four-week-old obese db/db mice

Measure blood glucose twice a week

Measurements of blood glucose concentration

Blood glucose

Blood from the mouse tail vein was withdrawn twice a week to measure plasma glucose levels using a glucometer.

Effects of losartan-treatment on blood glucose concentrations in obese db/db mice

Chronic treatment with losartan

Four-week-old mice

Measure blood glucose twice a week
Measurements of blood glucose concentration

Blood glucose

Blood from the mouse tail vein was withdrawn twice a week to measure plasma glucose levels using a glucometer.

*P < 0.05
**P < 0.01
***P < 0.001

Initiate: all four groups had similar blood glucose levels (n = 10-11).

Effects of losartan-treatment on blood glucose concentrations in control and obese db/db mice

The beneficial effect of losartan treatment on glucose homeostasis was more pronounced (12.6 ± 1.2 and 17.8 ± 1.3 mmol/l for losartan- and water-treated db/db animals, respectively).

Effects of losartan treatment on glucose tolerance and insulin tolerance in obese db/db mice

The plasma glucose levels of the losartan-treated db/db mice (17.6 ± 0.4 mmol/l) were lower than those of the water-treated db/db mice by nearly half (28.5 ± 0.8 mmol/l).
Chronic treatment with losartan

Four-week-old mice

- Water-treated db/db mice (n=10)
- Losartan-treated db/db mice (n=10)
- Losartan-treated control mice (n=11)
- Water-treated control mice (n=11)

Received losartan 10 mg·kg⁻¹·day⁻¹ dissolved in their drinking water for 8 wks

Measure oral glucose tolerance test

Measurements of oral glucose tolerance test (OGTT)

Oral glucose tolerance test (OGTT)

The mice were gavaged with glucose (1 g/kg, dissolved in water) 15 min after 16-h overnight fast.

Plasma glucose concentrations in mice undergoing an OGTT (1 g/kg) after treatment with losartan or water only for 8 weeks

Oral losartan treatment also reduced glucose intolerance in db/db mice compared with that in water-treated db/db mice, as evidenced by the OGTT data.

The 120-min areas under the curves (AUC) were expressed and compared

Plasma glucose concentrations in mice undergoing an OGTT (1 g/kg) after treatment with losartan or water only for 8 weeks

Oral losartan treatment also reduced glucose intolerance in db/db mice compared with that in water-treated db/db mice, as evidenced by the OGTT data.

Measurements of insulin tolerance test

Insulin tolerance test

Injected 2 units/kg human biosynthetic insulin

Measure insulin tolerance test

**Chronic treatment with losartan**

**Measurements of oral glucose tolerance test (OGTT)**

**Plasma glucose concentrations in mice undergoing an OGTT (1 g/kg) after treatment with losartan or water only for 8 weeks**

**Measurements of insulin tolerance test**
Effect of daily losartan treatment (10 mg/kg) on insulin tolerance in db/db mice

Insulin sensitivity of the peripheral tissue in db/db mice appeared not to be affected by chronic losartan treatment (10 mg.kg\(^{-1}\).day\(^{-1}\)) treatment.

Several clinical trials


In the present study

In vivo treatment of young diabetes-prone db/db mice with losartan did not ultimately prevent the development of diabetes but delayed the onset of hyperglycemia and ameliorated the hyperglycemia and glucose intolerance.

The clinical benefits of RAS inhibition

The clinical benefits of RAS inhibition include improved delivery of insulin and glucose to peripheral muscles and direct effects on peripheral glucose transport and insulin signaling pathways (Ferrannini et al 2003).
• AT1R blockers could improve insulin sensitivity (Olsen et al. 2005 & Pershadsingh et al. 2004)

• Meanwhile, losartan has been reported to abolish the insulin sensitivity—enhancing effects of angiotensin II in vitro and in vivo studies (Juan et al. 2005)

• The effects in this aspect remain controversial and varied (Scheen et al. 2004, Schupp et al. 2005 & Juan et al. 2005)

Conclusion

These findings provide a novel and at least partial explanation for the reduced incidence of type 2 diabetes