Is there a need of new RAAS blockers in diabetic hypertension?

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The Renin-Angiotensin System involves the conversion of angiotensinogen to angiotensin I (Ang I) by renin. Ang I is then converted to angiotensin II (Ang II) by ACE (angiotensin-converting enzyme) or chymase. Ang II binds to the angiotensin receptor subtypes AT₁ and AT₂. The conversion of Ang I to Ang II can also be achieved by ACE2. Ang-(1-7) is converted to Ang IV by ACE2.
Ang II levels are much higher in tissue than in plasma

Tissue RAS leads to damage in many organs especially in patients with metabolic disorders

Brain

Pituitary Gland

Eye

↑ in diabetes

Heart

↑ in diabetes, post-MI, failing ventricle

Aorta

↑ with atherosclerosis

Kidney

↑ in diabetes, membranous nephropathy

Adrenal Glands

Blood Vessels

↑ with atherosclerosis

Adipose Tissue

↑ in obesity, hypertension

Pancreas

↑ in nephropathy

The Cardiovascular Continuum

*Development and Progression of CV Disease*

Maladaptive remodelling

**Endothelial dysfunction**

**Vascular disease**
- Constriction, inflammation,
- hypertrophy, hyperplasia,
- atherogenesis, thrombosis

**Tissue injury**
- MI, stroke,
- glomerular
- ischaemia

**Pathologic remodelling**
- LVH, LV dilation,
- glomerulosclerosis

**Target-organ dysfunction**
- HF, nephropathy

**End-stage organ failure**

**Death**

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PRA Predicts the Incidence of MI
Interrelation Between PRA and CV Risk Factors

For every 2-unit increase in PRA, there is an overall 25% increase in MI incidence*

*Association strongest in white men.
†Risk status: high, ≥2 risk factors (smoking, cholesterol, LVH); moderate, 1 risk factor; low, no risk factors.
PRA, plasma renin activity.
Renin inhibition
Current Renin System blockers & diuretics raise plasma renin activity (PRA)*

*Increased peptide levels have not been shown to overcome the BP lowering effect of these agents
Renin inhibition acts at the Renin System’s point of activation and neutralizes the PRA rise.
Aliskiren binds to the active site of renin

Aliskiren binds to a pocket in the renin molecule, blocking cleavage of angiotensinogen to angiotensin I

Adapted from Wood JM, et al. 2003
Renin inhibition

Experimental studies
Renin inhibition vs. anti-hypertensive treatment

Hypertension 2000;35:587-94
Albuminuria
Aliskiren provides renoprotective effects in a rat model of hypertensive diabetic nephropathy

Feldman D, et al. 2005

![Graph showing urinary albumin levels over time with different treatments. The x-axis represents days, and the y-axis represents urinary albumin levels (mg/24h). The graph compares Vehicle, Aliskiren (10 mg/kg/d), and Aliskiren (30 mg/kg/d). Treatment starts at day 0.](image-url)
Aliskiren preclinical data
Summary

Aliskiren demonstrates organ protective effects in animal models
  – renoprotection comparable with ACEIs and ARBs
  – LVH reductions comparable with ARBs
  – suppresses markers of renal damage in diabetic nephropathy
  – atherogenesis prevention
Aliskiren is under evaluation in clinical studies for the treatment of hypertension. To date, no clinical trials have assessed the effect of aliskiren on morbidity and mortality.
Aliskiren compared with ramipril and combination therapy in patients with diabetes and hypertension – Study design

Study design:
- Single-blind
- Washout: 2 weeks
- Washout: 2–4 weeks
- Washout: 4 weeks
- Placebo
- Aliskiren 150 mg
- Aliskiren 300 mg
- Ramipril 5 mg
- Ramipril 10 mg
- Aliskiren 150 mg + ramipril 5 mg
- Aliskiren 300 mg + ramipril 10 mg

n=282  
n=278  
n=277

Uresin Y, et al. 2006 (Study 2307)
Aliskiren/ramipril combination provides significantly greater reductions in BP than component monotherapies

Mean change from baseline in BP (mmHg)

- DBP
  - Ramipril mono: $-10.7 \pm 2.0$ (n=275)
  - Aliskiren mono: $-11.3 \pm 2.1$ (n=279)
  - Aliskiren/ramipril combination: $-12.8 \pm 2.1$ (n=274)

- SBP
  - Ramipril mono: $-12.0 \pm 2.0$ (n=275)
  - Aliskiren mono: $-14.7 \pm 2.1$ (n=279)
  - Aliskiren/ramipril combination: $-16.6 \pm 2.1$ (n=274)

$p<0.05$ for superiority vs ramipril monotherapy; †$p<0.05$ for superiority vs aliskiren monotherapy;
‡$p<0.05$ for non-inferiority for aliskiren monotherapy vs ramipril monotherapy.

Error bars indicate standard error from the mean.

Uresin Y, et al. 2006 (Study 2307)
Aliskiren in combination with valsartan

Study design (2101)

Double-blind 4-period crossover study in healthy male subjects with mild salt depletion

14-day washout

Aliskiren 300 mg single dose

14-day washout

Valsartan 160 mg single dose

14-day washout

Aliskiren 150 mg/valsartan 80 mg single dose

14-day washout

Placebo single dose

Azizi M, et al. 2004
Aliskiren neutralizes ARB-induced increase in PRA

PRA at 24 hours after dosing (ng/mL/h)

- Aliskiren 300 mg: 0.83
- Valsartan 160 mg: 4.4
- Aliskiren + Valsartan 150/80 mg: 2.1
- Placebo: 2.0

*p<0.05 vs aliskiren 150 mg/valsartan 80 mg
†p<0.05 vs aliskiren 300 mg
‡p<0.05 vs valsartan 160 mg
n=12

Azizi M, et al. 2004
Aliskiren neutralizes ARB-induced rises in Ang II

Plasma Ang II (pg/mL)

Time (hours)

* p<0.05 vs aliskiren 150 mg/valsartan 80 mg
† p<0.05 vs aliskiren 300 mg
‡ p<0.05 vs valsartan 160 mg
n=12

Azizi M, et al. 2004
Aliskiren significantly decreases urinary aldosterone excretion in healthy volunteers.

*Urinary aldosterone excretion (µg/24h)*

- Placebo
- Aliskiren 40 mg
- Aliskiren 80 mg
- Aliskiren 160 mg
- Aliskiren 640 mg
- Enalapril 20 mg

- Pretreatment (Day 1)
- Day 8

*p<0.05 vs pretreatment (Day 1)*

Nussberger J, et al. 2002
Aliskiren demonstrates persistence of effect after discontinuation

Herron J, et al. 2006 (Study 2308)
Suppression of PRA is maintained following discontinuation of aliskiren treatment

Herron J, et al. 2006 (Study 2308)
Aliskiren in hypertension
Clinical summary

- Aliskiren provides long-term suppression of PRA
- Aliskiren effectively reduces PRA from baseline as monotherapy, and blocks the rise in PRA seen during treatment with other antihypertensives such as ARB
- Aliskiren monotherapy provides dose-dependent reductions in DBP and SBP
- Additional BP lowering when combined with other antihypertensives
- Sustained 24-hour BP control with prolonged effect after discontinuation

Increased peptide levels have not been shown to overcome the BP lowering effect of these agents
Prorenin and the (Pro)renin Receptor: Functional Significance
- A significant % of plasma prorenin is of extrarenal origin, whereas renin is of renal origin only.
- Prorenin-renin conversion appears to occur exclusively in the kidney.

Expression of prorenin:

- **High levels**
  - Kidneys
  - Adrenal glands

- **Low levels**
  - Eye

Reproductive system:
- Testes
- Ovaries
- Placenta
(Pro)Renin Disappearance after Bilateral Nephrectomy comparison with Ang I
Renin–Prorenin Relationship

Prorenin as a marker of microvascular complications in diabetes

Luetscher et al., NEJM 1985

▲ patient with microvascular complications
Diabetes: increased prorenin levels are associated with the development of retinopathy

- Prorenin levels were significantly (p ≤ 0.01) higher in diabetic patients with retinopathy compared with those without retinopathy ¹-³
  - Prorenin levels may be correlated with the degree of retinopathy ⁴

![Bar chart showing prorenin concentration (mU/L) for different types of retinopathy](chart.png)

**p < 0.01

Prorenin concentration (mU/L)

- Prorenin is elevated in patients with diabetes compared with non-diabetic siblings.¹
  - Prorenin can constitute up to 95% of total renin in these patients.²
- Prorenin levels are significantly elevated in diabetes patients with microalbuminuria ($p<0.0001$) compared with those without microalbuminuria.¹⁻⁴

¹ Deinum et al. 1999a; ² Deinum et al. 1999b; ³ Daneman et al. 1994; Chiarelli et al. 2001.
Diabetes: elevated levels of prorenin may predict the development of nephropathy

- Increase in prorenin levels precedes the development of nephropathy in patients with diabetes \(^{1,2}\)
  - Prorenin may identify diabetes patients at risk of developing nephropathy

\* \(^{p<0.05}\)

1 Chiarelli et al. 2001; 2 Deinum et al. 1999.
Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin

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Renin is an aspartyl protease essential for the control of blood pressure and was long suspected to be a cell surface protein. We report the cloning, cloning, cloning, and cloning of the human renin receptor and...
Receptor-bound prorenin is enzymatically active

Prorenin

Prosegment

Handle region

Nonproteolytic activation

Proteolytic cleavage

Angiotensinogen pocket

Active renin

Cell membrane

(Pro)renin receptor

Human prorenin: two crucial regions $T^{7}\text{FKR}^{10}$ (gate) and $I^{11}\text{FLKR}^{15}$ (handle) for non-proteolytic activation.
Sequence of the prosegment serves as blocker

**Prorenin prosegment**

**Handle region**

Renin - LPTDTASFORILLKKMPSVREILEERGVDTRISAEWGEKI - Proenin

$\text{NH}_2$-RILLKKMPSV-COOH

HRP
Decoy decapeptide
Inhibition of diabetic nephropathy by a decoy peptide corresponding to the “handle” region for nonproteolytic activation of prorenin

Research article

Prorenin Receptor Blockade Inhibits Development of Glomerulosclerosis in Diabetic Angiotensin II Type 1a Receptor–Deficient Mice

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We found that when a site-specific binding protein interacts with the “handle” region of the prorenin precursor, the prorenin molecule undergoes a conformational change to its enzymatically active state. This nonproteolytic activation is completely blocked by a decoy peptide with the handle region structure, which competitively binds to such a binding protein. Given increased plasma renin in diabetes, we examined the hypothesis that the nonproteolytic activation of prorenin plays a significant role in diabetic organ damage. Streptozotocin-induced diabetic rats were treated with subcutaneous administration of handle region peptide. Metabolic and renal histological changes and the renin-ang system components in the plasma and kidneys were determined at 8, 16, and 24 weeks following streptozotocin treatment. Knees of diabetic rats contained increased Ang I and II without any changes in renin, Ang-converting enzyme, or angiotensinogen synthesis. Treatment with the handle region peptide decreased the renal content of Ang I and II, however, and completely inhibited the development of diabetic nephropathy without affecting hyperglycemia. We propose that the nonproteolytic activation of prorenin may be a significant mechanism of diabetic nephropathy. The mechanism and substance causing nonproteolytic activation of prorenin may serve as important therapeutic targets for the prevention of diabetic organ damage.

Role of the Renin Receptor Blockade in Diabetic Nephropathy

WT mice
Role of the Renin Receptor Blockade in Diabetic Nephropathy

**WT mice**

- **A** Plasm Angiotensin II (fmol/L)
  - Untreated +ACEi +HRP +ACEi +HRP
- **B** Kidney Angiotensin II (fmol/g)
  - Untreated +ACEi +HRP +ACEi +HRP
- **C** Urinary TP/Cr
  - Untreated +ACEi +HRP +ACEi +HRP
- **D** Glomerulosclerosis index
  - Untreated +ACEi +HRP +ACEi +HRP

**AT1 receptor KO mice**

- **A** Plasm Angiotensin II (fmol/L)
  - Untreated +ACEi +HRP +ACEi +HRP
- **B** Kidney Angiotensin II (fmol/g)
  - Untreated +ACEi +HRP +ACEi +HRP
- **C** Urinary TP/Cr
  - Untreated +ACEi +HRP +ACEi +HRP
- **D** Glomerulosclerosis index
  - Untreated +ACEi +HRP +ACEi +HRP
Renin increases mesangial cell transforming growth factor-β1 and matrix proteins through receptor-mediated, angiotensin II-independent mechanisms

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Human Renin-induced TGF-β mRNA Expression

Huang Y. et al. Kidney Int. 2006;69:105-13
Renin-induced PAI-1 is mediated via TGF-β

PAI-1 mRNA  anti-TGF-β ab

Fibronectin and Collagen I

Huang Y. et al. Kidney Int. 2006;69:105-13
Renin-induced TGF-β is mediated via the Renin Receptor

Huang Y. et al. Kidney Int. 2006;69:105-13
Signaling Pathway

prorenin
renin receptor

HRP?

PLZF?

Th. Unger (Charite, Berlin, Germany, GRC 2006)

NOT via EGFR

MEK 1/2 | PD98059

p38, JNK?

ERK1/2

Cellular effects?
Summary

- (Pro)Renin receptor is most likely expressed in all cell types.
- (Pro)Renin induces ERK 1/2 phosphorylation independent of Ang II.
- Renin signaling depends on MEK-1/2 kinase, not on EGF receptor. In mesangial cells, TGF-β is involved leading to PAI-1, collagen and fibronectin expression.
- Location is on the surface of the cells, but also intracellular (endosomes??, lysosomes?? or ER??). Where does signaling take place?
- Affymetrix genes expression and signaling in (pro)renin receptor-deficient cells might help us to elucidate the function of the renin receptor.
Open Questions

- Does renin and prorenin activate the same signaling pathways?
- Is renin and prorenin signaling cell type-specific? Where does it exist (all cells)?
- Why is the time course different from other known ERK 1/2 activators (e.g. Ang II)?
- Are other MAP kinases (JNK or p38) involved?
- Does homodimerization affect signaling?
- Does renin signaling regulate its own receptor?
- Does a soluble truncated renin receptor exist?
We still have to elucidate whether the major function of the renin receptor is related to cardiovascular disease and whether the blockade of the renin receptor would ameliorate diabetic nephropathy.
Collaborators

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Aliskiren suppresses key markers of renal damage in a rat model of hypertensive diabetic nephropathy

- Urinary excretion and glomerular gene expression of TGF-β are elevated in diabetes
  - TGF-β is an important mediator of renal damage

- Aliskiren suppressed glomerular gene expression of TGF-β in a rat model of hypertensive diabetic neuropathy
  - Aliskiren showed a trend towards reducing urinary TGF-β excretion compared with vehicle

- Aliskiren showed a trend towards reducing renal collagen III and IV expression compared with vehicle

Feldman D, et al. 2005
Cardiac Ang II Depends on Renal Renin


Nx, nephrectomy.

Control Nx

Angiotensin II

0 10 20 fmol/g

0 20 40 60 fmol Ang I/ min/g

Renin

0 20 40 60 fmol/g

Control Nx

0 10 20 fmol/g