Therapeutic Strategies in the treatment of diabetic nephropathy

• Impact of current therapeutic strategy for diabetic kidney disease
  Remaining risk for progression of diabetic nephropathy

• Pathogenesis of diabetic kidney disease
  Polyol Pathway, PKC, ROS, AGEs, Hexamine Pathway

• New therapeutic target for diabetic kidney disease
  PKC beta inhibitor-Oral
  Calorie Restriction

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Current therapeutic strategies for diabetic kidney disease

1. Life style modification
   - BW loss, Physical activity, Sodium restriction
   - stop smoking

2. Strict control of Blood glucose (HbA1c<6.5%)

3. Renin-angiotensin system blockade
   - BP<130/80 mmHg
     if not achieved
   - CCB、Diuretics、β blockade、α blockade

4. Statin/Fibrates

5. Low protein diet, Kremezin (AST-120)
Benefit of Blood Sugar Control in Diabetes: Important Findings of 4 Major Clinical Trials

DCCT/EDIC, UKPDS, Kumamoto Study, ADVANCE Trial

Normoalbuminuria to microalbuminuria

Microalbuminuria to overt proteinuria

conventional insulin injection treatment

multiple insulin injection treatment
Benefit of Angiotensin Receptor Blockers in Diabetes: Important Findings of 4 Major Clinical Trials

Normoalbuminuria to microalbuminuria

- **BENEDICT (2004)**
  - The angiotensin converting enzyme inhibitor trandrapril compared to the calcium channel blocker verapamil provided better renal protection in hypertensive type 2 diabetics, reducing the development of microalbuminuria.

Microalbuminuria to overtproteinuria

- **IRMA II (2001)**
  - Higher doses of the angiotensin receptor blocker irbesartan reduced the risk of progression of renal insufficiency.

Overtproteinuria to ESKD

- **RENAAL (2001)**
  - The angiotensin receptor blocker losartan compared to placebo reduced the risk of diabetic nephropathy developing to renal failure.

- **IDNT (2001)**
  - The angiotensin receptor blocker irbesartan compared to the calcium channel blocker amlodipine provided better renal protection in hypertensive type 2 diabetics, reducing the chance of diabetic nephropathy developing to renal failure.
RENAAL STUDY
ESDK (Dialysis, Transplantation)

Placebo

Losartan

RRR: 28%
p = 0.002

Potential molecular and biochemical mechanisms by which the diabetes causes diabetic nephropathy

Diabetes (Hyperglycemia)

- Polyol pathway
- Heoamine pathway
- PKC-MAPK

Outflows:
- ROS
- RAS Endothelin
- Altered adipokines dyslipidemia
- AGEs-RAGE

Inflows:
- growth factors, chemokines, cytokines (TGF-β, CTGF, PDGF, VEGF, MCP-1)

Consequences:
- Mesangial expansion, inflammation, interstitial fibrosis
- Development and progression of diabetic nephropathy
PKC-TGFβ activation and diabetic kidney disease

PKC

PKC-β inhibitor
Vitamin E
Pioglitazone

TGF-beta
ROS- NF-kB
Osteopontin
Inflammation
MCP-1
ICAM-1

thiazolidinedione (TZD)
Ac-SDKP
Estrogen & Raloxifen
Sirt1

Diabetic Nephropathy

Ishii et al. Science 1996
Koya et al. FASEB J 2000
Kitada et al. Diabetes 2003
Kelly et al. Diabetes 2003
Yu et al. J Pharmacol Sci. 2006

Kanasaki K et al. J Am Soc Nephrol 2004
Guo B et al. Diabetes. 2004
ShibuyaK et al. Diabetes. 2005
Chin M et al. Am J Pathol. 2005
Guo B et al. Kidney Int. 2005
Chin M et al. Am J Path 2005
**PKC beta inhibitor**

**Ruboxistaurin (RBX)**

<table>
<thead>
<tr>
<th>PKC isoform (nM)</th>
<th>LY333531</th>
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<tr>
<td>α 360</td>
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<tr>
<td>β1 4.7</td>
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<tr>
<td>β2 5.9</td>
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</tr>
<tr>
<td>γ 300</td>
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<tr>
<td>δ 250</td>
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<tr>
<td>ε 600</td>
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<tr>
<td>ζ &gt;10⁵</td>
<td></td>
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<tr>
<td>η 52</td>
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**Other kinase (nM)**

- cAMP kinase: >10⁵
- Ca²⁺-Calm kinase: 8000
- Casein kinase: >10⁵
- src Tyr kinase: >10⁵

Ishii et al. Science 1996
**Effect of PKCβ inhibitor on diabetes-induced renal dysfunction**

Ishii H et al. Science 1996
Effect of PKCβ inhibitor on diabetes-induced overexpression of TGF-β and ECM

Effect of PKC β inhibitor on diabetes–induced mesangial expansion

FASEB J 2000 KOYA D et al.

db/m  db/db

PKC β inhibitor

PKC β inhibitor

Mesangial area (μm²)

Urinary albumin excretion (μg/day)

<table>
<thead>
<tr>
<th></th>
<th>db/m</th>
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<tr>
<td>PKC β inhibitor</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Number</td>
<td>9</td>
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</tbody>
</table>
8-OHdG immunohistochemistry

PKC i inhibits membrane translocation of p67 and p47phox

Kitada M et al. Diabetes 2003

* p<0.01 vs. control
** p<0.05 vs. DM (N=5)
ROS(A) and PKC(B) mediate HG-induced NF-kB activation

Ha H et al. JASN 13:894-902, 2002
ROS and PKC mediate HG-induced, NF-kB-dependent MCP-1 expression.

Ha H et al. JASN 13:894-902, 2002
Effect of PKC β inhibition on diabetes-induced TI injury

Kelly et al. Diabetes 2003

RBX addition to ACEi/ARB reduced ACR in type 2 diabetic patients with overt proteinuria

RBX addition to ACEi/ARB ameliorated the annual change of GFR decline in type 2 diabetic patients with overt proteinuria.

Urinary TGF-beta/Cre ratio

PKC beta inhibitor

Unadjusted

Adjusted

by baseline TC and ACR

HYPERGLYCEMIA

↓

AGEs

de novo synthesis

↓

OXIDANTS

DAG

↓

PKC β Isoforms

PKC δ Isoforms

PKC β Inhibitor

Functional Enzymes
NADPH Oxidases
Na⁺K⁺ATPases
PLA2, eNOS
COR 2 Transferase

Signaling Proteins
Receptors
PI3K, MAPK
GSK-3β, IKKα
SOCS2, SHP-1

Cytokine Expression
VEGF, PDGF-B
ET-1, CTGF, MCP-1
TGF-β, Mø, ICAM1

Cell Cycle Factors and Transcription Factors
Rb, Egr-1, SPI1

Oxidative Stress, Fibrosis, Podocyte Loss, TI injury, Albuminuria, GFR decline
Silent Information Regulator 2 (Sir2 / SIRT1)

- is an oxidized NAD-dependent histone deacetylase (Class III HDAC).
- is activated in response to metabolic conditions, such as starvation.
- deacetylates histone protein, resulting in gene silencing and cell survival.
- also interacts with some transcriptional factors (p53, FOXO).

(Min et al., Cell, 2001)
Diabetes

TGFβ / AII

CR

NAD/NADH ↓

ROS

Ac-p53

SIRT1

apoptosis

Diabetic Kidney Disease
EFFECT OF SIRT1 ACTIVATOR ON DIABETIC KIDNEY DISEASE

- **Calorie Restriction**


- **Resveratrol**


- **Visfatin/Nampt**


- **Small molecule activators of SIRT1**

30% CR for 20 yrs keeps the monkey younger and younger

A little BW reduction
Big fat reduction
Keep Muscle mass
Mortality reduction
DM incidence 0%
Cancer incidence -50%
CVD -50%
Keep brain mass

Colaman RJ et al. Science 2009

Wistar fatty Rats

Wistar rat as a control

6 weeks old RAT, male

0 week

24 weeks

Every 1 months

BW, BG, BP, Food intake

T-CHO, TG, FFA

Urinary Albumin, PAS

Kidney → EM

real time PCR

Groups.

1) WT (normal chow) n=4
2) WT (CR 40% cut) n=4
3) WFR (normal chow) n=4
4) WFR (CR 40% cut) n=4

The fa-gene was transferred from the Zucker rat (13 M strain) to the Wistar Kyoto (WKY) rat. Wistar fatty rats (fa/fa), a congenic strain of WKY, developed obesity and obesity-related features, such as hyperinsulinemia and hyperlipemia
real time PCR

Col4a1

Fn1

TGFβ1
Wild

Wild-CR

Homo

Homo-CR

ED-1 staining
What is the underlying mechanism by which diabetes induces inflammation?
swelling and disintegration of cristae
Real time PCR

PGC1α

WT       WT-CR     Homo     Homo-CR

0.00     0.20      0.40      0.60      0.80      1.00      1.20      1.40

0.00     0.20      0.40      0.60      0.80      1.00      1.20      1.40

Sirt1

WT       WT-CR     Homo     Homo-CR

Western blotting

Sirt1

Ace NFkB

beta actin

Wild       Wild-CR     Homo     Homo-CR
Potential molecular and biochemical mechanisms by which the diabetes causes diabetic nephropathy

Diabetes (Hyperglycemia)

- Aldose reductase inhibitor
- PKCβ inhibitor
- GFAT inhibitor (azaserine)
- Polyol pathway
- PKC-MAPK
- Heoamine pathway
- AGEs-RAGE
- ROS
- Resveratrolol
- Bardoxolone methyl (AIM)
- PARP inhibitor
- Apocynin
- Benfotiamine
- N-acetylcysteine
- α-lipoic acid
- RAS Endothelin
- RAS blockade
  - Vitamin D
  - Avosentan
- Altered adipokines (dyslipidemia)
- Insulin sensitizer
- Statin, fibrates
- TTP488 (RAGE inhibitor)
- GLY-230
- ALT-711
- FG-3019
- Prifenidone
- Calorie restriction
- Growth factors, chemokines, cytokines (TGF-β, CTGF, PDGF, VEGF, MCP-1)
- Mesangial expansion, inflammation, tubuli-interstitial fibrosis
- Development and progression of diabetic nephropathy

Mt dysfunction
Summary

• PKC beta inhibitor
• Calorie Restriction

may be new therapeutic strategies for diabetic kidney disease

Final Answers To detect New Molecules and Drug Discovery For Diabetic Kidney Disease Lie in Conducting Research More!!

KOYA D