Vascular Inflammation and Peroxisome Proliferator-Activated Receptors (PPAR)

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  action mechanisms
  vascular inflammation

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PPAR

- originally cloned during identifying peroxisome proliferation in the liver of rodents. (*Nature*. 1990)
- activate transcription factors
- belong to the nuclear receptor superfamily
Classification of PPAR

• PPAR family
  – α, γ and β/δ
  – 60~80% homology
  – mostly activated by fatty acid

• PPAR α
  – agonist: oxidized phospholipid, fibrate, gemfibrozil…

• PPAR γ
  – agonist: 15d-PGJ₂, thiazolidinedione…

• PPAR β/δ
  – involved in lipid metabolism, obesity, insulin resistance…
Action Mechanism (1)
- binding as heterodimer with RXR
Action Mechanism (2)
- effects of PPARγ agonist on glucose metabolism

Lee et al. Endocrinology 2004
Action Mechanism (3) lipid metabolism

Lee et al. Endocrinology 2004
Action Mechanism (4)
– transactivation & transrepression

Hannele Yki-Jarvinen. NEJM 2004
PPAR = strong anti-inflammatory molecule blocking various inflammatory signaling pathways

Atherosclerosis = Vascular Inflammation mechanism

Effect of LDL infiltration
Role of macrophage
Effect of T cell activation

Vascular Inflammation; significance

Coronary inflammation
→ plaque rupture
→ sudden death

PPAR γ
Classical **metabolic** effect of PPAR-γ activation

- Induce **fat cell** differentiation
  (large, insulin resistant → small, sensitive)

- Reduce free fatty acids and **adipocytokines**
  (TNF-α, leptin, resistin)

- Increase anti-atherogenic **adiponectin**
PPAR-γ expression in neointima after injury to rat aorta

PPAR are expressed upon differentiation of human monocytes to macrophage

Western blot analysis

RT-PCR

PPAR γ express in macrophage and atherosclerotic lesion of human coronary a.

PPAR-\(\gamma\) activators also induce apoptosis in macrophages

PPAR γ agonist decreased inflammatory cytokine (TNF-α, IL-1β, IL-6) released from human monocytes

Rosiglitazone reduces oxidative stress in hyper-cholesterolemic rabbit

Ling et al. Circulation. 2003
PPAR\(\gamma\) - cellular actions

• in ECs, VSMCs, Macrophages and T cell.

- Apoptosis
- P27 (CDKI)

- endothelin-1
- IFN-\(\gamma\), TNF-\(\alpha\)
- Angiogenesis
- ICAM-1, VCAM-1
- iNOS, MMP-9
Mechanism of PPAR-γ to prevent cardiovascular ds.

| Direct                                      | ↓ Vascular smooth muscle cell growth and migration |
|                                            | ↓ Endothelial cell growth and migration (antiangiogenic) |
|                                            | ↓ Monocyte attachment and movement |
|                                            | ↓ Inflammatory changes in all vascular cell types |
|                                            | ↑ Reverse cholesterol transport |

| Metabolic                                  | ↑ HDLC |
|                                            | ↓ Triglycerides |
|                                            | ↓ Small, dense LDLc |

| More common to PPARγ                      | ↓ Proinflammatory, proinsulin resistant adipokines (fat) |
|                                            | ↑ Adiponectin |
|                                            | ↑ Insulin sensitivity (fat, liver, skeletal muscle) |
|                                            | ↓ Circulating insulin |
|                                            | ↓ Blood pressure |
PPAR-γ – clinical data

• decrease inflammatory biomarkers CRP, MMP-9, sCD40L, TNF- α, serum amyloid A, E-selectin.

• improve endothelial function

• decrease human carotid IMT

• decrease human aortic atheroma

• decrease late loss at 6 month after stenting
Rosiglitazone reduces inflammatory markers

- type 2 DM
- N= 353
- RSG vs placebo
- 6m F/U

Haffner et al. Circulation. 2002
Rosiglitazone reduce inflammatory marker; 
MMP9, SAA, TNF-α

CAD & DM patients, N=39, RSG vs placebo, 3m F/U

Marx et al, Arterioscler Thromb Vasc Biol. 2003
Troglitazone reduce carotid atheroma

- type 2 DM
- N= 132
- troglitazone vs control
- 6m F/U
- carotid IMT by US

Minamikawa et al. J Clin Endocrinol Metab. 1998
Troglitazone reduce late loss after stenting at 6m F/U

Takagi et al. J Am Coll Cardiol. 2000
What is the underlying mechanism of PPAR-\(\gamma\) to inhibit neointimal hyperplasia other than VSMC-cycle arrest through p27 & Rb?

- in balloon injury model
- *Min et al, Metabolism 2002*

- after stenting, 6m F/U MLD
- *Takagi et al, JACC 2000*
GSK3β is a key molecule in neointimal hyperplasia [SNUH data]

- GSK is phosphorylated and inactivated in the vessel wall after balloon injury
- Its activation by celecoxib reduced neointimal hyperplasia.

Park et al. ATVB 2003
Yang et al. Circulation 2004
MMP-9 involves VSMC migration

MMP-9 & TIMP-1 are involved smooth muscle cell migration which is a key mechanism of restenosis after balloon injury.

- in balloon injury model
- *Dolley et al. Circulation 1999*
Hypothesis
mechanism of PPAR-γ to inhibit neointimal hyperplasia
Rosiglitazone reduces NIH via GSK3β and MMP-9 pathway?
Rosiglitazone inhibits proliferation & induces apoptosis of rat VSMC

Figure 1

A  BrdU incorporation

<table>
<thead>
<tr>
<th>Condition</th>
<th>% of RSG 0uM</th>
<th>RSG 0uM</th>
<th>RSG 5uM</th>
<th>RSG 15uM</th>
<th>RSG 25uM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 4.6</td>
<td>100 ± 4.6</td>
<td>100 ± 4.6</td>
<td>100 ± 4.6</td>
<td>100 ± 4.6</td>
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<tr>
<td>RSG 0uM</td>
<td>100 ± 4.6</td>
<td>100 ± 4.6</td>
<td>100 ± 4.6</td>
<td>100 ± 4.6</td>
<td>100 ± 4.6</td>
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<tr>
<td>RSG 5uM</td>
<td>90 ± 3.5</td>
<td>90 ± 3.5</td>
<td>90 ± 3.5</td>
<td>90 ± 3.5</td>
<td>90 ± 3.5</td>
</tr>
<tr>
<td>RSG 15uM</td>
<td>80 ± 2.4</td>
<td>80 ± 2.4</td>
<td>80 ± 2.4</td>
<td>80 ± 2.4</td>
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<tr>
<td>RSG 25uM</td>
<td>70 ± 1.5</td>
<td>70 ± 1.5</td>
<td>70 ± 1.5</td>
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</table>

B  Apoptosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>RSG 0uM</th>
<th>RSG 0uM + FBS</th>
<th>RSG 15uM + FBS</th>
<th>RSG 25uM + FBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Cells</td>
<td>8.74±2.4</td>
<td>4.88±1.5</td>
<td>42.18±5.5</td>
<td>44.69±7.39</td>
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</table>

C  PDGF RSG

<table>
<thead>
<tr>
<th>PDGF</th>
<th>RSG 0uM</th>
<th>RSG 0uM</th>
<th>RSG 15uM</th>
<th>RSG 25uM</th>
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</tbody>
</table>

- Akt
- ERK
- GSK-3β
- α-tubulin
Rosiglitazone reduces VSMC viability by activation of GSK3β

**Figure 2**

**A**

![Graph showing cell viability (%) relative to vehicle for different treatments.](image)

- **Vehicle**
- **RSG 5µM**
- **RSG 15µM**
- **RSG 25µM**
- **RSG 25µM and GSK-KM**

**B**

**PDGF(-)**
- **Vehicle**
- **RSG 25µM**

**PDGF(+)**
- **Vehicle**
- **RSG 25µM**
- **RSG 25µM with GSK-KM**

**Western Blot Analysis**

- **p-GSK-3β**
- **α-tubulin**
Hypothesis
Rosiglitazone reduces NIH via GSK3β and MMP-9 pathway?

Moon et al. JBC 2004

Desbois-Mouthon et al. Hepatology 2003

Park et al. ATVB 2003

Dolley et al. Circulation 1999

↑ proliferation ↓
↑ apoptosis ↓
↓ migration
Figure 4

A

<table>
<thead>
<tr>
<th></th>
<th>TNF-α (-)</th>
<th>+</th>
<th>+</th>
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<tbody>
<tr>
<td>RSG</td>
<td>0</td>
<td>0</td>
<td>25</td>
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</table>

Non-specific Competitor

Specific Competitor

β-gal

GSK-KM

EMSA for NF-κB

B

<table>
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<tr>
<th></th>
<th>TNF-α (-)</th>
<th>+</th>
<th>+</th>
<th>+</th>
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</thead>
<tbody>
<tr>
<td>RSG</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

Non-specific Competitor

Specific Competitor

β-gal

GSK-KM

EMSA for AP-1

C

<table>
<thead>
<tr>
<th></th>
<th>TNF-α (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>vehicle</td>
</tr>
</tbody>
</table>

vehicle

RSG 25uM

RSG 25uM +GSK-3β

ICC for p65 of NF-κB

D

<table>
<thead>
<tr>
<th></th>
<th>TNF-α (+)</th>
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<tbody>
<tr>
<td>vehicle</td>
<td>vehicle</td>
</tr>
</tbody>
</table>

vehicle

RSG 25uM

RSG 25uM +GSK-3β

ICC for c-Jun of AP-1
Rosiglitazone suppresses MMP-9 and induces TIMP-1
Rosiglitazone suppresses MMP-9 and induces TIMP-1, which results in inhibition of VSMC migration.
Rosiglitazone inhibits VSMC migration by activation of GSK3β

**Figure 3**

**A** Migration

![Bar chart showing the effect of Rosiglitazone (RSG) on VSMC migration compared to vehicle control.](chart.png)

- **Pro MMP-9** and **MMP-9** levels are monitored.

**B** Zymography

![Zymogram showing MMP-9 protein levels.](zymogram.png)

- **Control**, **+vehicle**, **RSG 5uM**, **RSG 25uM** treatments are compared.

**C** TNF-α

![Western blot showing MMP-9 protein levels.](western_blot.png)

- **Vehicle** and **RSG 25µM** treatments are compared under **TNF-α(-)** and **TNF-α(+)** conditions.

* * *
Hypothesis
Rosiglitazone reduces NIH via GSK3β and MMP-9 pathway?

MMP-9 gene

AP-1

NF-kB

PI-3K

p-AKT

p-ERK↓

p-GSK↓

RSG

apoptosis↑

proliferation↓

migration↓

Dolley et al. Circulation 1999

Desbois-Mouthon et al. Hepatology 2003

Moon et al. JBC 2004

Park et al. ATVB 2003

True in in-vivo situation?
Animal experiment to confirm that rosiglitazone inhibits neointimal hyperplasia

• in rat carotid injury model
Balloon injury activate ERK and inactive GSK3β resulting in MMP-9 induction, which was suppressed by Rosiglitazone through GSK3β activation.
Rosiglitazone inhibits neointimal hyperplasia, which is partially reversed by GSK3β inactivation.
Rosiglitazone inhibits proliferation & induces apoptosis of VSMC after vascular injury, which is partially reversed by GSK3β inactivation.
Rosiglitazone reduces neointimal formation via activating GSK3β and inhibiting MMP-9
PPAR $\alpha$
PPAR α polymorphism influence CAD progression

L162V polymorphism & G to C transversion in intron 7 of PPAR-alpha gene

IHD patients
N= 3309
11yr F/U (retrospective)
survival

David et al. Circulation. 2002
PPAR α - cellular actions

- endothelial cell,
- VSMC,
- macrophage
- T cell.

- MCP-1,
- IL-8
- eNOS
- IL-6,
- prostanoglandin
- HDL Rc

- glycated LDL uptake
- TG-rich remnant Lp.
- VCAM-1
- tissue factor
- MMP
- IFN-γ
- TNF-α
- IL-2
Fibrate reduces CV event

- Effect of **bezafibrate** treatment over five years on **coronary plaques** causing 20% to 50% diameter narrowing

- Effect of **fenofibrate** on **progression of coronary-artery disease** in type 2 diabetes.
  - DAIS Investigators. Lancet. 2001

- Prevention of the **angiographic progression** of coronary and vein-graft atherosclerosis by **gemfibrozil** after coronary bypass surgery in men with low levels of HDL cholesterol

- **Primary-prevention trial** with **gemfibrozil** in middle-aged men with dyslipidemia: safety of treatment, changes in risk factors, and incidence of coronary heart disease.

- **Gemfibrozil** for the **secondary prevention** of coronary heart disease in men with low levels of high-density lipoprotein cholesterol
Fibrate reduces CV event

male CHD patients with low HDL
N= 2531
6yr F/U
CV event

RR 22% reduction (p=0.006)

Fibrate improves endothelial function

- **Ciprofibrate** therapy improves *endothelial function* and reduces *postprandial lipemia and oxidative stress* in type 2 diabetes mellitus.

- **Gemfibrozil** improves *insulin sensitivity and FMD* in type 2 diabetic patients.

- Effect of **fenofibrate** on brachial artery *FMD* in type 2 diabetes mellitus.

- Short-term TG lowering with **fenofibrate** improves *vasodilator function* in subjects with hypertriglyceridemia.

- **Exercise-induced vasomotion** of angiographically normal and stenotic coronary arteries improves after cholesterol-lowering drug therapy with **bezafibrate**.
Fibrate improves endothelial function

FMD

PPAR α – clinical data summary

• PPAR α polymorphism ↔ CAD progression
• fibrate intervention
  – reduce atherosclerosis and CAD event
  – improve endothelial function

.. well correlated with animal studies

PPAR-α
improves lipid metabolism
reduces vascular inflammation
improves vascular tone
PPAR & Cardiovascular disease
Metabolic to Vascular actions of PPARα, PPARγ, PPARβ/δ.
Overall actions of PPARs
Ongoing cardiovascular endpoint trials with PPARα and γ agonists

- FIELD: *Fenofibrate* Intervention and *Event* Lowering in *Diabetes*

- RECORD: *Rosiglitazone* Evaluated for *Cardiac* Outcomes and for Regulation of glycaemia in *Diabetes*

- PROACTIVE: PROspective *pioglitAzone* Clinical Trial In *macroVascular* Events

- DREAM: *Diabetes* REduction Assessment with ramipril and *rosiglitazone* Medication

- CHICAGO: A Study Evaluating *Carotid* Intima-Media Thickness in Atherosclerosis Using *Pioglitazone*
Ongoing cardiovascular endpoint trials with PPARα and γ agonists

- **PERISCOPE**: Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation

- **STARR**: Study of Atherosclerosis with Ramipril and Rosiglitazone

- **BARI-2D**: Bypass Angioplasty Revascularization Investigation – Type 2 diabetes

- **RECORD**: Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes

- **ACCORD**: Action to Control Cardiovascular Risk in diabetes

- **ACT NOW**: Act Now for the Prevention of Type 2 Diabetes
Acknowledgement

• PPAR project members
  – PPAR-γ, α
  – PPAR-γ
  – PPAR-γ
  – PPAR-δ
  – PPAR-δ
Therapeutic Perspectives in PPAR

- selective PPAR modulator (SPPARM)
  different modification of 3-dimensional conformation of ligand
  $R_c \Rightarrow$ different interaction with cofactors
  ex.) effects on glucose metabolism without stimulating adipocyte
differentiation

- combined PPAR $\alpha$/PPAR $\gamma$
  - ragaglitazar, tesaglitazar
Summary: mechanism of PPAR-γ to inhibit NIH
[SNUH data]

• Rosiglitazone
  – Induction of VSMC apoptosis via ERK/GSK pathway (WST-1, FACS, western blot)
  – Attenuation of AP-1 activity (EMSA)
  – Inhibition of VSMC migration via suppression of MMP-9 and induction of TIMP-1 (western blot, zymography, scratch assay)
  – Reduction of VSMC proliferation (PCNA)
  – Induction of VSMC apoptosis (TUNEL)
  – Inhibition of neointimal hyperplasia (in rat)
Rosiglitazone inhibits neointimal hyperplasia, which is partially reversed by GSK3β inactivation.