Presenter Disclosure Information

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Interference with Smooth Muscle Peroxisome Proliferator-Activated Receptor-gamma (PPAR\(\gamma\)) Exacerbates Hypertension and Vascular Dysfunction: Role of TIMP-4

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Interference with Smooth Muscle Peroxisome Proliferator-Activated Receptor-gamma (PPARγ) Exacerbates Hypertension and Vascular Dysfunction: Role of TIMP-4

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PPARγ

- Ligand-activated transcription factor
- Master regulator of adipogenesis, lipid metabolism and glucose homeostasis
- Unidentified endogenous ligand
- Synthetic ligand-Thiazolidinediones (TZD)

PPARγ and Cardiovascular System

1. PPARγ agonists: TZD

- Insulin sensitivity
- Glucose and fatty acid metabolism
- Atherosclerosis
- Blood pressure

Side effects: weight gain, sodium and fluid retention, heart failure and myocardial infarction
Patients with PPARγ mutation develop early-onset severe hypertension with insulin resistance, type 2 diabetes and lipodystrophy

- Ligand Binding Domain: P467L, V290M, L339X
- DNA Binding Domain: C114R, C131Y, C162W, R165T

Auclair M, et al. ATVB 2013
**Development of S-P467L Model**

- Impaired baroreflex *(Borges GR, et al. Hypertension 2014)*
- Severe aortic dysfunction that is dependent on an augmented RhoA/Rho kinase signaling *(Pelham CJ, et al. Cell Metabolism 2012)*
Hypothesis

Interference of smooth muscle PPAR$_\gamma$ exacerbates DOCA-salt-induced hypertension and resistance vessel dysfunction
Experimental Design

Male S-P467L mice and NT littermate controls

- subcutaneous pellet of 50 mg DOCA
- 0.15 M NaCl solution in addition to regular chow and water

- Blood pressure by radiotelemetry
- Mesenteric arterial function by pressure myograph
- Morphometry studies with aorta and 2nd order of mesenteric artery

Gene Expression Profiling: Microarray obtained from aorta and mesenteric arteries from untreated mice

Cell Culture Studies: Rat aortic smooth muscle cells
Exaggerated Hypertensive Response to DOCA-salt in S-P467L

* p<0.05 vs. NT  Two-way repeated-measures ANOVA interaction p = 0.039

NT (n=16 for baseline; n=13 for DOCA-salt)
● S-P467L (n=13 for baseline; n=11 for DOCA-salt)
Impaired ACh-induced Relaxation in S-P467L Mesenteric Arteries after DOCA-salt

○ NT (n=5 for baseline; n=7 for DOCA-salt)
● S-P467L (n=6 for baseline; n=9 for DOCA-salt)

* p<0.05 vs. NT
Augmented Vascular Remodeling in S-P467L after DOCA-salt

- Morphometry of mesenteric artery at P75 mmHg after DOCA-salt

- No difference at baseline

* p<0.05 vs. NT; NT=12, S-P467L=8
Augmented Vascular Remodeling in S-P467L after DOCA-salt

- Morphometry of aorta stained with Verhoeff–Van Gieson

* p<0.05 vs. NT; NT=6, S-P467L=6

- No difference at baseline
Potential Mechanisms

Unbiased Approach Criteria:

1. Positive expression in the blood vessel

2. Significant change in S-P467L artery

3. Change in both aorta and mesenteric artery microarray data

4. PPARγ binding sites near by

(Keen HL)
Potential Mechanisms

- Gene expression profiling of aorta and mesenteric arteries with Affymetrix microarrays

TIMP-4 = Tissue Inhibitor of Metalloproteinases-4

PPAR\(\gamma\) Binding (ChIP-Seq)

TIMP-4 = Tissue Inhibitor of Metalloproteinases-4
What is TIMP-4?

- Endogenous inhibitor of matrix metalloproteinases (MMPs), providing a tight control of extracellular matrix degradation


Specifically Reduced TIMP-4 Expression in S-P467L Mesenteric Arteries after DOCA-salt

n=6, * p<0.05 vs. NT, ** p<0.05 vs. NT+DOCA
Increased MMP-9 Expression in S-P467L Mesenteric Arteries after DOCA-salt

DOCA-salt

MMP-2

Fold change from NT

NT  S-P467L

MMP-9

Fold change from NT

NT  S-P467L

n=5, * p<0.05 vs. NT
Interference of PPARγ Activity Suppressed TIMP-4 Expression in Smooth Muscle Cells

(n=3)  
(n=4)
Increased total MMP Activity in Smooth Muscle Cells by PPARγ Inhibitor

In situ zymography

Vehicle

GW9662

n=4, * p<0.05 vs. vehicle

MMP activity normalized to vehicle-treated group
Conclusions

• Interference with PPARγ function in smooth muscle resulted in exacerbated DOCA-salt-induced hypertension and vascular dysfunction.

• Augmented vascular remodeling in S-P467L mice after DOCA-salt treatment was associated with a specific down-regulation of TIMP-4.

• Smooth muscle TIMP-4 expression could be at least, in part, regulated by PPARγ.

• GW9662, a PPARγ antagonist, led to an increased MMP activity.
Working Model

Vascular Remodeling
Exacerbated Hypertension

PPARγ
RXR

TIMP-4

Active MMPs

MMP/TIMP

DN-PPARγ

PPRE

TIMP-4
Clinical Relevance and Perspective

- TZDs are potent and effective insulin sensitizers previously used frequently to treat patients with type 2 diabetes.

- Due to serious potential side effects (i.e. congestive heart failure and myocardial infarction) in select patients, the use of TZDs has been restricted.

- Identifying PPARγ targets in the vasculature will help explain the adverse events of TZDs and design a new class of therapies that regulates PPARγ function more selectively.
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