It Takes Two to Tango: Drug abuse and HIV in the Development of PAH

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About 37 million people living with HIV/AIDS worldwide
Non-infectious complications on the rise

- **HIV-Related Cardio-pulmonary complications**
  - Chronic obstructive pulmonary disease (COPD)
  - Coronary artery disease
  - Pulmonary Arterial Hypertension (PAH)
HIV-Related Pulmonary Hypertension

- **1997** Swiss HIV Cohort – 0.5% Opravil et. al, AJRCCM
- **2006/2008** French HIV Registry – 0.5% Humbert et. al, AJRCCM /Sitbon O, AIDS
- **2008**, UCSF study – 15% (RVSP > 35mmHg), 6.7% (RVSP > 40mmHg) Hsue et al, AIDS
- **2012** Spain Cohort - 9.9% (RVSP>35mmHg), 2.8% 40-65mmHg) Quezada et al, AIDS
- **2015** German Cohort – 6.1% Schwarze-Zander et.al, HIV Medicine

- ~ 300-4000 fold higher prevalence in HIV patients compared with general population.
HIV-PH more common in IVDUs

Percentage of HIV-PH with IVDU Risk Factor

- Himelman et al. *Am J Cardiol.*, 1989: 50% (n=3/6)
- Speich et al. *Chest*, 1991: 83% (n=5/6)
- Petitpretz et al. *Circulation*, 1994: 60% (n=12/20)
- Opravil et al, *Am J Respir Crit Care Med.*, 1997: 84% (n=16/19)
- Aguilar & Farber, *Am J Respir Crit Care Med.*, 2000: 100% (n=6, prostacyclin therapy)
- Sitbon et al. *AIDS*, 2008: 51%
- Quezada et al. *AIDS*, 2012: 59% (n=17/39)
- Araujo et al. *World journal of cardiology*, 2014: 78% (n=14/18)
IVDU contributes to the enhanced HIV-1 related pulmonary vascular remodeling and PAH

HIV+IVDU (opioids+/-cocaine)

Endothelial dysfunction

Inflammation
Increased apoptosis
Proliferation

Aberrant vascular remodeling

Smooth muscle dysfunction

“First Hit”

HIV infection
HIV viral protein exposure
− Tat, Nef, Env
Inflammation
Immune system activation

“Second Hit”

Toxic insult
Morphine
Methamphetamine
Cocaine

SIV-infected macaques
Advanced stage lesions in SIV+Morphine macaque

Hyperactive proliferative endothelial cells within plexiform lesions
Main Question

How combination of HIV-1 infection and IVDU exposure exacerbates pulmonary arteriopathy ????
Additive increase in the proliferation of pulmonary SMCs in the presence of HIV- Tat and cocaine

Activation of proliferative PDGF receptor axis without increase in PDGF-BB ligand

Ligand independent activation of PDGFR

Dalvi et al, Am J Respir Cell Mol Biol. 2015
Overview

Smooth Muscle Dysfunction

- Anti-proliferative Bone Morphogenetic Protein receptor (BMPR) signaling in Cocaine & HIV-Tat mediated Smooth Muscle Hyperplasia

- Role of miRNAs in the regulation of BMPR-2 expression

- Role of macrophage derived extracellular vesicles on HIV-1 and /cocaine associated smooth muscle dysfunction
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TGF-β Superfamily of Receptors

Bone Morphogenetic Protein Receptors

Transforming growth factor β Receptors

BMPs signaling pathway

Humpath.com - Human pathology
Attenuation of Tat and/or cocaine mediated increased proliferation of HPASMCs on activation of BMP/BMPR axis.

Dalvi ... Dhillon, Arterioscler Thromb Vasc Biol. 2013
Combined treatment of pSMCs with Tat & cocaine results in increased BMPR mRNA expression
Down-modulation of BMP-Receptor protein expression on Tat and cocaine exposure

Dalvi ... Dhillon, Arterioscler Thromb Vasc Biol. 2013
Cocaine exposure results in the repression of BMPR mediated downstream signaling in HIV-Tat treated PASMCs.
Expression of BMPR-2 in human lung tissues from HIV infected IVDUs.

Dalvi ....Dhillon, Arterioscler Thromb Vasc Biol. 2013
HIV-Transgenic rats exposed to cocaine
Increase in mPAP and RVSP of HIV-transgenic rats on exposure to cocaine

Cocaine: IP, 14mg/kg body weight for 21 days
Enhanced pulmonary vascular remodeling in HIV-Tg rats treated with cocaine
Hyper-proliferation of pulmonary arterial smooth muscle cells isolated from HIV-Tg rats exposed to cocaine

Full media

Serum-starved
Apoptosis resistance in PASMCs isolated from HIV-Tg rats exposed to cocaine

Full media

Serum-starved

A. Oligonucleosomes (% control)

B. Oligonucleosomes (% control)

C. WT, WT+Coc., HIV, HIV+Coc.
Down-regulation of BMPR signaling in PASMCs from cocaine treated HIV-Tg rats.
Increased BMPR mRNA
Decreased BMPR protein

COCAIN + HIV-TAT → BMP 2,4,7 → BMPRII, 1A, 1B → Smad 1/5/8

Antiproliferative ID1 gene
Proliferative IL6 gene

Dalvi ... Dhillon, Arterioscler Thromb Vasc Biol. 2013
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Increased expression of BMPR2 targeting miRNAs in human PASMCs exposed to Cocaine & Tat

Chinnappan et al, JAHA, 2018, In press
*Inhibition of BMPR2 targeting miRNAs prevented the cocaine & Tat mediated decrease in BMPR2 protein levels*
**Overexpression of BMPR2 targeting miRNAs enhanced the cocaine & Tat mediated decrease in BMPR2 protein levels**

- **BMPR2 mRNA level**
- **BMPR2 Protein level**

![Graphs showing BMPR2 mRNA and protein levels](image-url)
Loss or gain of BMPR2 targeting miRNAs on smooth muscle proliferation

Inhibition of miRNAs using antagomir

overexpression of miRNAs using mimics
Increased expression of BMPR2 targeting miRNAs in pulmonary arterial smooth muscle cells from **HIV-transgenic rats** exposed to cocaine.
MiRNA 216a mediates inhibition of BMPR2 expression by directly binding to its 3’ UTR
Inhibition of BMPR2 translation by miR-216 without any effect on RNA

In-vitro translation assay

Firefly luciferase protein levels

Reportor RNA levels
Translation inhibition of BMPR-2 expression by miRNAs
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Extracellular vesicles: Nanoshuttles
Smooth Muscle dysfunction: Role of inflammation

Increased perivascular inflammation around remodeled vessels
Question

Do HIV-infection and cocaine mediated alterations in the macrophage-derived extracellular vesicles (EVs) promote hyper-proliferation of pulmonary arterial smooth muscle cells?
Human monocyte derived macrophages

- Un-treated HIV-1
- Cocaine (1µM)
- HIV-1 + cocaine

Collected supernatant day 2 or 4 post-infection

Isolated Extracellular vesicles: Differential ultracentrifugation

Effect of EVs on pulmonary smooth muscle proliferation
Nanosight Analyses of macrophage derived extracellular vesicles
Characterization of EVs by TEM and surface markers

TEM

WB for Exosomal markers

- Tsg-101 (45kDa)
- Alix (75kDa)
- CD9 (25kDa)
- LAMP-1
- HSP-60
HIV-1 p24 levels in EV fractions and cellular supernatant
Uptake of МΦ derived EVs by pulmonary smooth muscle cells

Control

+Exosomes

phalloidin (red)  
EVs labeled with PKH67 (green)
Increased SMC proliferation on uptake of HIV +/- cocaine treated Mφ-derived EVs.
Small RNA sequencing of RNA cargo revealed:

- Differentially regulated (>1.5 fold): 173-185 miRNA
- HIV+cocaine group: 17 (p<0.05)
- HIV group: 4 (p<0.05)
- Cocaine group: 5 (p<0.05)
Potential targeted pathways of miRNAs carried by HIV+cocaine EVs
Validation of alterations in miRNA cargo of macrophage-derived EVs

PTEN/Akt/mTOR signaling

Growth Factors
- VEGF, PDGF, TGF, EGF

Receptor tyrosine kinase
- ErbB3, ErbB2

PI3K
- PTEN
- PIP2
- PIP3
- PDK1
- Akt1
- TSC1/2
- GSK3β
- mTOR

miR-10a-5p
miR-10b-5p
miR-486-5p
miR-130a-3p
miR-27a-3p
miR-451a
miR-200b-3p
miR-181a-5p

Cell survival / Proliferation / Cell cycle

miRNA (fold change)
- miR-10a
- miR-130a
- miR-27a
- miR-181
- miR-200

Statistical significance:
- $\star\star\star$
- $\star\star$
- $\star$
- $\star\star\star$

Legend:
- control
- Coc
- HIV
- H+Coc
PTEN/AKT signaling in HPASMCs exposed to EVs from HIV+/-cocaine MDMs
PTEN/AKT signaling in HPASMCs exposed to EVs from HIV+/-cocaine MDMs
MiR130a levels in HPASMCs after the uptake of macrophage derived EVs

Uptake of Tx-Red SiRNA Exosomes by SMCs

![Image showing uptake of Tx-Red SiRNA Exosomes by SMCs with bar graphs showing pre-miRNA-130a and miR-130a fold change in different conditions: Cont, Coc, HIV, HIV+Coc.](image-url)
EV-associated miR130a promotes smooth muscle proliferation

Sharma et al. FASEB J. 2018 Apr 19
EVs from HIV-infected cocaine treated macrophages potentiate PAH in rats

RVSP

mm Hg

Con EVs  H+C EVs  Con EVs  H+C EVs

WT     HIV+cocaine
Acknowledgments

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