PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-Α AGONIST AS A NOVEL THERAPEUTIC TO PREVENT OXIDATIVE STRESS AND ATRIAL FIBROSIS

Farhan Rizvi, Ph.D.1, Larisa Emelyanova, Ph.D.1, Stacie Edwards, B.S.1, Breanna Aldred, B.S.1, Peter Homar BS.1, Sean Ryan BS.1, Francis X Downey, M.D.1, Gracious Ross, Ph.D.1, A. Jamil Tajik M.D.1, and Arshad Jahangir, M.D.1,2

1 Center for Integrated Research on Cardiovascular Aging, 2 Aurora Research Institute, Aurora Cardiovascular Sciences, Milwaukee WI

BACKGROUND
Atrial Fibrillation (AF), the most common heart rhythm disorder that predisposes stroke and heart failure is a major public health burden. Prevalence of AF associated with cardiac fibrosis and oxidative stress increases with aging. A common denominator for aging-related AF is excessive extracellular matrix deposition, yet, the molecular mechanisms regulating fibrosis are still obscure. Peroxisome proliferator-activated receptor-α (PPAR-α), an important molecule for reactive oxygen species (ROS) and lipid degradation also involved in the development of cardiovascular disease. PPAR-α reported to negatively correlate with kidney, liver, and lungs fibrosis, but its role in cardiac fibrosis is less clear.

OBJECTIVE
We hypothesized that decreased expression of PPAR-α in AF contributes to oxidative stress and interfere with TGF-β signaling, a cytokine implicated in inflammation and cardiac fibrosis.

RESULTS
Cell culture: Human atrial fibroblasts (HAf) isolated from AF and non AF patients right atrial appendages were grown in cardiac fibroblasts culture media. Cells were plated in media with or without 5ng/ml TGF-β1 in presence or absence of PPAR-α agonist ciprofibrate.

Transfection: HAf were transfected with miR-21 mimic or inhibitor using INTERFEKIN (PolyPlus Inc.)

Histology of Atrial Appendage: Right atrial tissue pieces, washed in PBS, fixed overnight in 4% paraformaldehyde, and embedded in paraffin. 5-μm sections were stained with Masson trichrome and fibrosis within sections as blue-stained areas was determined.

Immunohistochemistry: Immunostaining was performed on PFA fixed sections from atrial appendages and HAf for colocalization of α-SMA and Vimentin for determining the presence of myofibroblast (myofib). HAf treated with TGF for 72 hours were fixed, permeabilized and then treated with α-SMA antibody and anti Vimentin, stained with AlexaFluor 594 and 488-conjugated secondary and counterstained in Hoechst 33342.

Determination of 4-hydroxyenononal (4-HNE) protein adduct: Frozen atrial samples were homogenized and lysates were prepared. Supernatant obtained by centrifuging the lysates was used for measuring the levels of 4-HNE by ELISA using commercially available kit and superoxide production (MitoSOX Red) were assayed in patients’ atrial tissue homogenates and permeabilized cardiac myofibers respectively.

Western blotting: Cell lysates were prepared in RIPA lysis buffer. Protein concentrations were determined and SDS-PAGE were run on NuPAGE NOVEX Bis-Tris 4-12%. Protein expressions were detected on the immunoblots with either Clarity (BioRad) or Femto West Chemiluminescent Substrate Kit (Pierce).

CONCLUSION
Reduced expression of PPAR-α in AF patients is associated with impaired cardiac mitochondrial metabolism and promotes TGF-β1 induced atrial fibrosis. Preliminary studies suggest PPAR-α agonist might offer therapeutic benefits for patients in reducing oxidative stress and cardiac fibrosis and therefore predisposition to AF.