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

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BACKGROUND

Atrial Fibillation (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 (PPAR)-α, an important molecule for reactive oxygen species (ROS) and lipid degradation also involved in the development of cardiovascular disease. PPARα 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 PPARα in AF contributes to oxidative stress and interfere with TGF-β signaling, a cytokine implicated in inflammation and cardiac fibrosis.

METHODS

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 PPARα agonist, clofibracetate.

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

Histology of Atrial Appendage: Right atrial tissue pieces, washed in PBS, fixed overnight in 4% paraformaldehyde, and embedded in paraffin. 5-um 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 Alexa-Fluor 594 and 488-conjugated secondary and counterstained in Hoechst 3542.

Determination of 4-hydroxy nonenal (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.

qRT-PCR: Total RNA was isolated by lysing the fibroblast in Qiazol and cDNA was synthesized. PCR was performed on ABI7300 or Light Cycler484 at 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 seconds, annealing for 1 min at 60°C then extension at 72°C for 40 seconds. Relative expression was assessed by normalizing CT values of the tested gene or miRNA with CT values of the housekeeping gene/miRNA.

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).

RESULTS

Fig. 1. Significant fibrosis in AF patients. A: Representative photographs of α-SMA and Vimentin in AF and Non AF. B: Representative images of differentiating myofib (green) by alpha smooth muscle actin polymerization (green) in non AF (top) and AF (bottom) in right atrial tissue.

Fig. 2. Difference in 4-HNE protein adduct and mitochondrial superoxide level in right atrial of AF and non AF patients. A: the level of 4-HNE; B: changes in mitochondrial superoxide production in permeabilized myofibers from non AF and AF patients, 10μM Antimycin-A was added to stimulate superoxide production in mitochondria. C: difference in fluorescence intensity of MitoSAXO before and after Antimycin-A (15μM). Data are mean ± SE, n = 7 for non AF and n = 4 for AF

Fig. 3. Low Expression of PPARα in atrial fibroblasts of AF patients Bar graphs display the mRNA expression (n=20) of PPARα between hAF isolated from right atrial appendages of non AF and AF patients. Data are presented as mean ± S.E.M, analyzed by t-test (two-tailed), *p<0.05.

Fig. 4. PPARα agonist, clofibracetate reduced the expression of TGFβ1 induced expression of profibrotic genes and myofib differentiation. A: miRNA expression of ACT2 and COL1A1; B: protein levels of ACT2 and COL1A1 reduced by clofibracetate. C: bar graph showing quantified ratio of protein expression to GAPDH; D: Clofibracetate preventing the myofib differentiation induced by TGFβ1.

Fig. 5. TGFβ1 induced myofib is mediated by microRNA-21. A: miR-21 targets PPARα to regulate its expression and activation; B: TGFβ1 induced miR-21 expression; C-D: ACT2 and myofib expression; E: TGFβ1 induced ACT2 expression inhibited by Anti-miR21

CONCLUSION

Reduced expression of PPARα in AF patients is associated with impaired cardiac mitochondrial metabolism and promotes TGFβ1 induced atrial fibrosis. Preliminary studies suggest PPARα agonist might offer therapeutic benefits for patients in reducing oxidative stress and cardiac fibrosis and therefore predisposition to AF.