Preventing Mitochondrial Dysfunction and Cell Death in the Lipid Exposed Cardiomyocyte

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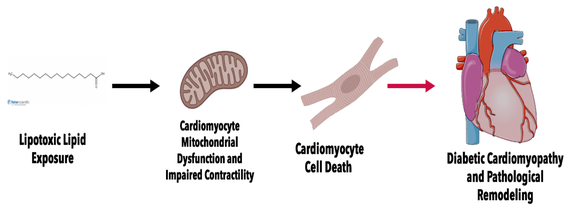
University of Manitoba and The Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Theme of the Children's Hospital Research Institute of Manitoba

Background

- Diabetic cardiomyopathy is a form of heart disease that occurs independent of other cardiac risk factors
- Lipotoxicity, an intracellular cell stressor caused by lipid accumulation, ultimately leads to diabetic cardiomyopathy and pathological remodelling of the heart.



BioRender

Fig 1. Lipotoxicity leads to diabetic cardiomyopathy and pathological remodeling of the heart

- Roflumilast (an FDA approved drug) is a phosphodiesterase 4 inhibitor
- Phosphodiesterase 4D has been shown to be induced in rat hearts following high fat feeding and diabetic human hearts that have undergone heart failure (Circulation (2016), Wang et al.)

Hypothesis

Roflumilast Prevents Mitochondrial Dysfunction and Cell Death in the Lipid Exposed Cardiomyocyte

Materials and Methods

Models
Immortalized cardiac myoblast cell line (H9C2)
isolated primary ventricular neonatal cardiomyocytes (PVNC)

Drug Treatment:
200uM Palmitate (lipid) ± 10uM Roflumilast, (18 hours)

Epifluorescent Imaging
Stain-Based Assays:
Mitochondrial Membrane Potential: TMRM
Live/Dead Assay: Calcein-AM/Ethidium Homodimer-1
Mitochondrial Calcium: Dihydrorhod-2 AM
Mitochondrial Superoxide Production: MitoSOX
Permeability Transition: Calcein-AM

Immunofluoresnce using the Cell Signaling Technology Nix antibody, performed according to manufacturer's instructions

Results

Roflumilast Prevents Lipid-Induced Mitochondrial Dysfunction and Cell Death

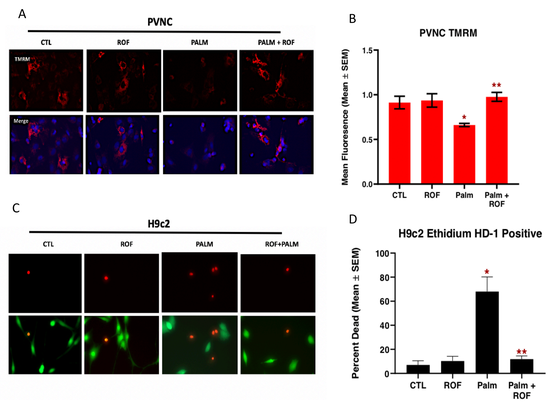


Fig 2. Assays for mitochondrial function and cell death. Quantification and epifluorescence of mitochondrial membrane potential in PVNC's **(A)** and **(B),** and quantification and epifluorescence of cellular viability (Live/Dead) in H9C2's **(C)** and **(D)**. Data are Mean ± SEM, (*) indicates p<0.05 compared to control (CTL) , (**) indicates p<0.05 compared to palmitate (PALM)

Lipotoxicity Induces Expression of Cell Death Gene

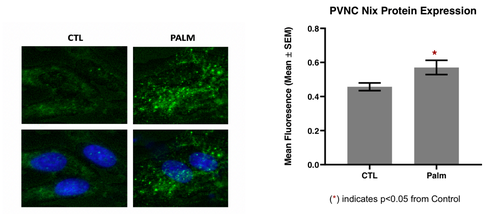


Fig 3. Assay for Nix Protein Expression Quantification and immunofluorescence of Nix protein expression in PVNC's. Data are Mean ± SEM, (*) indicates p<0.05 compared to control (CTL)

Roflumilast Prevents Mitochondrial Permeability Transition

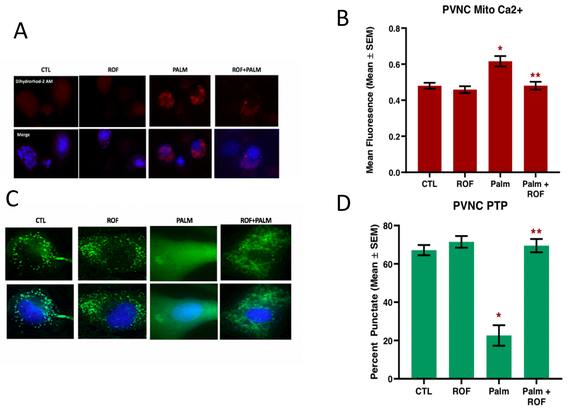


Fig 4. Assays for calcium homeostasis and mitochondrial permeability transition. Quantification and epifluorescence of mitochondrial calcium in PVNC's **(A)** and **(B)**, and quantification and epifluorescence of mitochondrial permeability transition pore opening in PVNC's **(C)** and **(D)**. Data are Mean ± SEM,, (*) indicates p<0.05 compared to control (CTL) , (**) indicates p<0.05 compared to palmitate (PALM)

Roflumilast Prevents Mitochondrial Superoxide Production

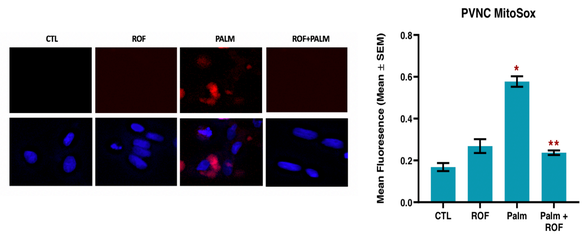


Fig 5 Assay for mitochondrial superoxide production. Quantification and epifluorescence of mitochondrial superoxide in PVNC's. Data are Mean ± SEM,, (*) indicates p<0.05 compared to control (CTL), (**) indicates p<0.05 compared to palmitate (PALM)

Proposed Mechanism

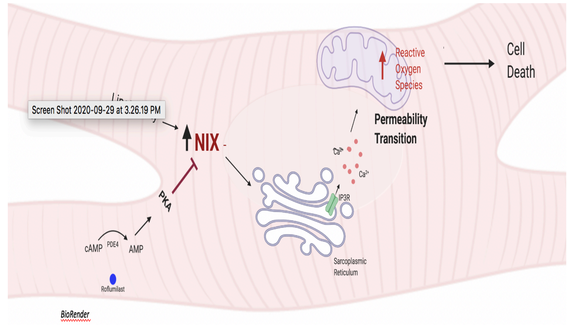


Fig 6 Roflumilast Inhibits Nix through PKA. Lipotoxicity drives Nix expression leading to altered calcium homeostasis and opening of mitochondrial permeability transition pore. Mitochondrial permeability transition pore opening drives an increase of reactive oxygen species (ROS) leading to cardiomyocyte death. Roflumilast inhibits breakdown of cyclic adenosine monophosphate (cAMP) increasing protein kinase A and leads to the phosphorylation of Nix.

Conclusions

1. Roflumilast prevents lipid-induced mitochondrial dysfunction and cell death in cultured cardiomyocytes
2. Roflumilast likely follows a a specific molecular mechanism

Roflumilast could treat and/or prevent lipid-induced mitochondrial dysfunction and cell death in the heart

Contact Information/Acknowledgements

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