Background

- Poor glycemic control is an important contributor to the development of heart failure (HF).
- Type 2 diabetes mellitus (T2DM) has almost doubled the risk of developing heart failure.
- Increased levels of intracellular Na+ lead to cardiac hypertrophy.

Empagliflozin (EMPA), a sodium-glucose cotransporter-2 (SGLT-2) inhibitor, regulates glucose levels and reduces myocardial intracellular Na+ levels.

- The Na+/H+ exchanger isofrom 1 (NHE1) is located in the heart and functions by regulating the cardiomycocytes pH.
- Enhanced expression of NHE1 has been implicated in the progression of heart failure by promoting cardiac hypertrophy.
- Activation of NHE1 is directly coupled to the activation of sodium/calcium exchanger (NCX).

Elevated levels of cytoplasmic Na+ leads to increased levels of cytoplasmatic Ca2+ levels, which induces cardiomyopathy and injury to the heart.

Methods

- Study design: In-vitro experimental study
- Cell culture: H9c2 cardiomyoblasts were plated at 35 mm dish and cultured in DMEM/F12 1:1 culture media containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37° C for 24 hours, were starved in FBS free DMEM medium for additional 24 hours and then treated with ANG II (100nM), EMPA (500nM), and ANG II + EMPA for 18 hours.
- Crystal violet staining: After treatment, cells were fixed and stained with crystal violet stain. Measurement of the cell surface area was done using AxiolView Imaging software. An average of 50-70 images was taken from each treatment group and represented as one n value for each group.
- Protein assay: Fresh Bovine serum albumin (BSA) stock solution and 6 dilutions of protein standards were prepared. BSA dilutions and samples were loaded into wells. After 15 minutes, the absorbance was read at 750 nm using a spectrophotometer located in Dr. Asnat lab (corridor C).
- Western blot: Cells were lysed with RIPA and transferred into microcentrifuge tubes. Protein samples were loaded into gels. The gels were run and then transferred onto nitrocellulose membrane. The membrane was then blocked, primary antibodies were added, and incubated overnight. Secondary antibodies were incubated for 1.5 hours. Proteins were visualized using chemiluminescence and imaged using the Alpha Innotech FluorChem® Imager (R&D Systems).

Results

- Figure 1: EMPA reduced SGLT-1 expression following stimulation with ANG II in H9c2 cardiomyoblasts
  - Representative western blot of SGLT-1 (73 kDa), SGLT-2 (73 kDa) and NHE1 (150 kDa) was shown. Protein expression was tested in non-treated (control), 100nM ANG II, 500nM EMPA, and EMPA + ANG II treated cells for 18 hours in 3 sets.

- Figure 2: ANG II induced cardiomyocyte hypertrophy in H9c2 cardiomyoblasts
  - Table 1: Protein concentrations of H9c2 cardiomyoblasts treated with ANG II in the presence and absence of EMPA
  - Table 1: Protein concentrations of H9c2 cardiomyoblasts treated with ANG II in the presence and absence of EMPA
    - Set # | Samples | Conc. (mg/mL)
    - ------|---------|------------------
    - Control | 1.386089425
    - Ang II | 1.591199432
    - EMPA | 1.375916726
    - EMPA + ANG II | 1.304944405
    - Control | 1.387863733
    - Ang II | 1.407381121
    - EMPA | 1.310622191
    - EMPA + ANG II | 1.400757038

Conclusions

- Despite that EMPA reduced ANG II-induced cardiomyocytes hypertrophy, we can not conclude whether EMPA significantly reduces the risk of hypertrophy.
- The study was not able to identify the effect of NHE1 protein expression in the cardiovascular benefits of EMPA.

Limitations

- Low protein expression in WB may have contributed to the difficulties in quantifying the bands.
- Low concentration of EMPA used compared to clinical dosage regimens (500nM vs. 10 or 25 mg) limited our abilities to generalize our results to humans.
- The cell line used is H9c2 cardiomyoblasts (immature cells) are not cardiomyocytes.