Induction of Glyoxalase 1 as a Strategy to Prevent Methylglyoxal-Induced Insulin Resistance in Rat Cardiomyocytes: Implications for Diabetic Cardiomyopathy

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Background

- Diabetes Mellitus (DM) is one of the major challenges to human health in the 21st century.
- Diabetic cardiomyopathy: a long-term complication of DM
- Insulin resistance is associated with diabetic cardiomyopathy
- DM is associated with an increased in methylglyoxal (MG) a highly reactive compound
- MG induces insulin resistance
- MG is detoxified through the glyoxalase system by glyoxalase-1 (Glo-1) enzyme using reduced glutathione (GSH) as co-factor
- Glo-1 expression is increased by trans-Resveratrol (tRES, R) and Hesperetin (HES, H) which are Nrf2 activators (Figure 1)

Study Objectives

- Investigate the effect of tRES-HES co-treatment in preventing MG-induced cardiac insulin resistance by evaluating:
  a) Glo-1 activity
  b) Phosphorylation of Akt and GSK3b

Methods

- Design
  In vitro laboratory experimental study using H9C2 rat cardiomyocyte cell line
- Treatment Protocol
  Cells were seeded onto 6-well plates and treated with MG (100uM) + t-RES-HES (RH 10uM) for 24 hours. Some cells were stimulated with insulin (100 nM) for 10 minutes
- Study Experiments
  - Cell viability using Alamar Blue reagent
  - Glo1 activity assay
  - Protein expression (Akt, GSK3b) via Western blotting
- Statistical Analysis
  - One-way ANOVA and post hoc analysis
  - Values represent mean ± SEM; P<0.05

Results

Cell Viability

- MG did not affect the cell viability significantly after 24 hours of treatment (P=0.05). Doses beyond 100 uM MG decreased the cell viability below 80%.

Glo-1 Activity

- MG reduced Glo-1 activity (P<0.05)
- RES-HES combination restored Glo-1 activity compared to MG100 uM (0.97±0.05 vs. 0.53±0.06; P<0.05)

Akt Phosphorylation

- RES-HES treatment of cells incubated with MG 100 increased the phosphorylation of Akt in response to insulin compared to cells treated with MG 100 in response to insulin (2.2±0.21 vs. 0.90±0.13; P<0.01)

GSK3b Phosphorylation

- GSK3b phosphorylation in response to MG and tRES-HES co-treatment for 24 hours.
  - GSK3b phosphorylation was increased in control cells with insulin (P<0.01)
  - RES-HES treatment of cells incubated with MG 100 insulin increased the phosphorylation of GSK3b in response to insulin compared to cells treated with MG 100 + insulin (4.1±0.3 vs. 2.06±0.14; P<0.01)

Conclusions

MG induced cardiac-insulin resistance and a decrease in Glo1 activity was prevented by tRES-HES co-treatment

Future Steps

- Use Glo-1 inhibitors to specifically study the role of Glo1 in improving insulin signaling in cardiomyocytes

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