The Effect of Statins on Mitochondrial Respiration in Pancreatic Insulin Secreting MIN6 Clonal Cells

SC/BIOL 4000 - Maansi Malhotra, Christopher Perry, Robert Tsushima

Department of Biology and School of Kinesiology and Health Science, Muscle Health Research Centre, York University, Toronto, ON

Abstract

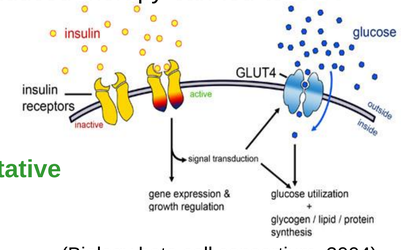
Background: Mitochondria are vital for synthesizing ATP through oxidative phosphorylation, which is required to maintain cell viability and cellular processes. In pancreatic beta cells, ATP is an important factor for regulating insulin secretion. Cholesterol synthesis can be reduced by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate limiting enzyme, with statins. Statins are effective in the treatment of cardiovascular disease, but they are associated with an increased risk of diabetes. Inhibition of cholesterol synthesis impairs ATP production in skeletal muscle, however less is known about how this pathway controls oxidative phosphorylation in beta cells. **Objective:** The purpose is to investigate the impact of inhibiting the cholesterol synthesis pathway via HMG CoA reductase inhibitors on mitochondrial ATP production in pancreatic beta cells. **Methods and Results:** A significant reduction in glucose-stimulated insulin secretion by statins (fluvastatin, pravastatin and atorvastatin) was observed compared to control in the clonal beta cell line, MIN6 cells. Mitochondrial O_2 respiration was measured using Oroboros high resolution respirometer from MIN6 clonal beta-cells treated in the absence or presence of statins (fluvastatin, pravastatin and atorvastatin) under direct, acute (2 h) or chronic (48 h) periods. There were no significant reductions in any of the treatment groups compared to the control when the drugs were added directly. However, during the chronic treatment, cells treated with atorvastatin increased mitochondrial respiration by ~ 45-51% when compared to cells treated with pravastatin and fluvastatin. Flow cytometry measurements showed no significant change in the viability of the treated cells. **Conclusion:** Statin induces decreased insulin secretion in pancreatic beta cells. However, in this study mitochondrial dysfunction did not appear to be implicated in this effect.

Introduction

- Expected type 2 diabetes mellitus (DM) to ↑ by **205 million** people by 2035 and 90% of people with type 2 DM are overweight/obesity. This ↑ risks such as cardiovascular disease (CVD), hypertension, stroke, cancer. (Ades et al, 2013)
- Every **7 minutes** in Canada, someone dies from heart disease or stroke and about **40%** of Canadians have high blood cholesterol.
- Statins are a class of drugs used to **lower** cholesterol levels by inhibiting the enzyme HMG-CoA reductase. They reduce low density lipoproteins levels by ~ 20-45%. (Lewington et al, 2007, 2010)
- Statins have been known to **reduce** the mortality and morbidity associated with CVD but statin induced therapy can led to **10% - 46%** ↑ risk of diabetes.

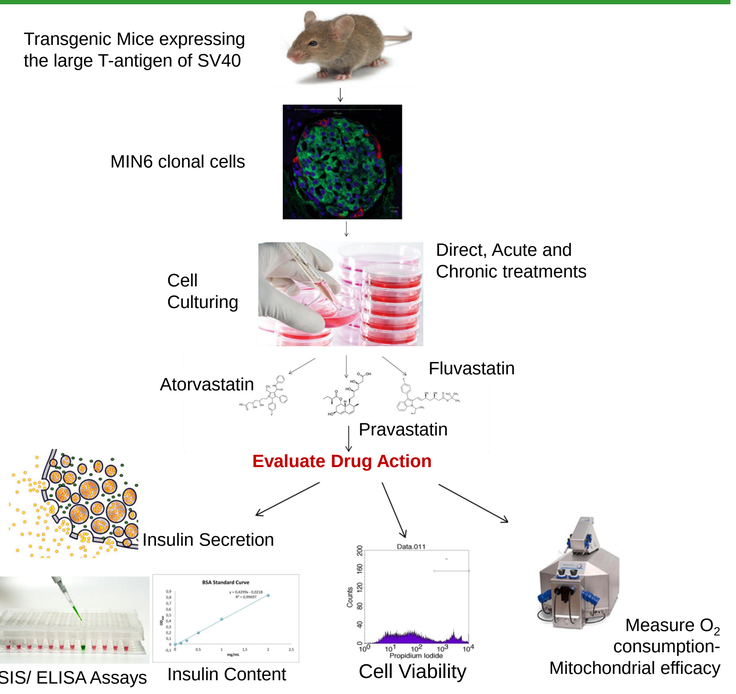
(Cederberg et al, 2015)

- **Hypothesis: Statins will impair mitochondrial respiration, a putative factor contributing to diabetes.**



(Biology beta cell consortium, 2004)

Methods



Results

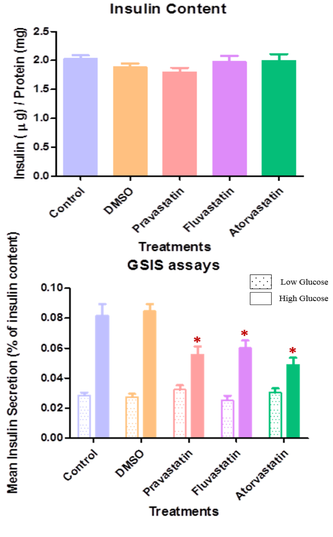


Figure 1: Insulin Content in treated MIN6 cells. The content of insulin for each drug treatment was standardized to the protein content of the samples collected. No significant reductions in insulin content were observed in any treated groups.

Figure 2: Glucose stimulated insulin secretion profiles of all treatment groups. Secretion was measured as a percent of total insulin content. Basal (1 mM glucose in KRB buffer, patterned columns) and high glucose stimulation (16.7 mM glucose in KRB buffer, filled columns) secretion is displayed. * p<0.0.5 from control high glucose treated group.

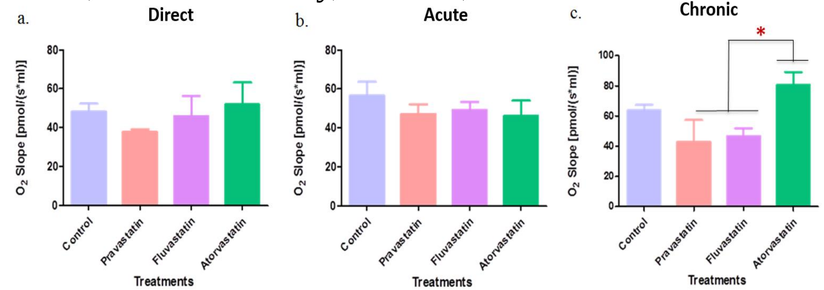


Figure 3: Succinate induced complex II mitochondrial respiration. The effect of a direct (a) (n=3), acute - 2 hour (b) (n=7-9) and chronic - 48 hour (c) (n=5) treatment with 10 µM atorvastatin, fluvastatin and pravastatin on succinate (10 mM) induced mitochondrial respiration of MIN6 clonal cells. Values are as depicted as mean ± SEM, p value < 0.05.

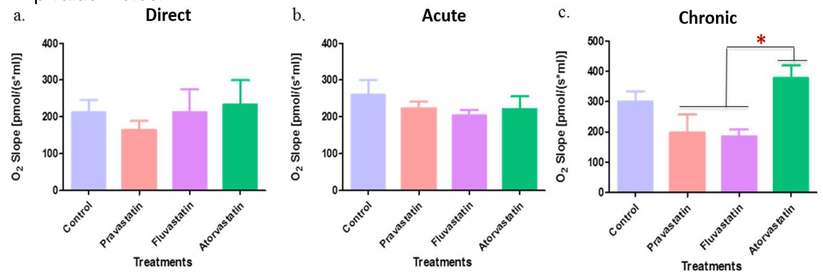


Figure 4: 4mM ADP induced mitochondrial respiration. The effect of a direct (a) (n=3), acute - 2 hour (b) (n=7-9) and chronic - 48 hour (c) (n=5) treatment with 10 µM atorvastatin, fluvastatin and pravastatin on the 4mM ADP induced mitochondrial respiration of MIN6 clonal cells. Values are as depicted as mean ± SEM, p value < 0.05.

Summary of Results

- GSIS and ELISA assays showed a **significant** decrease in statin induced insulin secretion.
- **No significant** decrease in oxygen consumption.
- Atorvastatin **increased** by 42-51% compared to other drugs.
- Flow cytometry is showed **no drug toxicity.**

Conclusion

- Statin induced ↓ insulin secretion but no effect on mitochondrial respiration.
- Atorvastatin induced increase in respiration – activation of **AMPK.**
- Shows the feasibility of testing the **action of candidate drugs** in MIN6 clonal cells.

Acknowledgements

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