Effects of Different Statins on Insulin Storage and Secretion from Pancreatic β -cells: Mechanisms and Clinical Implications

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Introduction: The incidence of cardiovascular disease is far greater in diabetics than non-diabetics¹. Statins are very effective for lipid lowering but new research reports a 10-12% increase in new-onset type-2 diabetes among patients receiving statin therapy². Mechanisms involving decreased β - cell function could be the link between statin therapy and type-2 diabetes³.

Methods:
Insulin secretion: INS-1 cells were cultured in 11 mM glucose to simulate the high levels in diabetes and plated in 96-well plates to perform secretion experiments (~120,000 cells/well). Pitavastatin and Simvastatin were added to cell cultures 72 h prior to experiments at the concentrations in FIGURE 1. Cells were then refreshed by pre-incubation 2 mM glucose for 2 h before adding 12 mM glucose for 2 h at 37°C. Cells were then cooled and sampled for insulin release measured using a FRET-based insulin assay (CisBio, Bedford, MA).
Insulin content: Insulin content was measured to determine the effect of each statin after chronic incubation (72 h). Cells were lysed in cold PBS containing 0.1% Triton X-100 and 25 mM NaOH to extract the insulin content.
Calcium Oscillation: Calcium imaging was measured by Fura-2 fluorescence on an Olympus DSU spinning disk confocal microscope.
Statistical Analysis: All experiments were performed three to five times. Data was analyzed via t-test and ANOVA. * = p<0.05.

Results: At low concentrations (25-100 nM) Simvastatin but not Pitavastatin inhibited GSIS from cultured clonal pancreatic β -cells, an effect that was dependent on insulin content of these cells. Only at higher concentrations (200 nM) did both statins reduce insulin content. Ca^{2+} oscillation frequency was similar; however, Simvastatin treatment increased the amplitude of Ca^{2+} oscillations, whereas Pitavastatin increased average basal Ca^{2+} levels without affecting amplitude of oscillations compared with control.
Conclusions: The differential inhibition of GSIS by Simvastatin compared with Pitavastatin could be due to a depletion of cellular insulin content and reduced insulin secretion. Ca^{2+} oscillations also differed between statins with respect to amplitude and basal intracellular Ca^{2+} content. Our results suggest explanations for clinical differences between statins and demonstrate the need for additional exploration of the mechanisms of action of statins in pancreatic β -cells.
Future Directions: Future studies will have to further examine statin variability and direct effects on β -cell plasma membranes, including the role of intracellular Ca^{2+} and associated differences in Ca^{2+} oscillations and relationships to glucose metabolism and insulin secretion.

Figure 1: GSIS in cells exposed to statins. *Simvastatin caused a decrease in GSIS at all doses. In contrast, Pitavastatin showed no effect on insulin secretion.*

Figure 2: (A) *Simvastatin caused a decrease in insulin content at all doses while Pitavastatin had no such effect until 200 nM. (B)* *Simvastatin and Pitavastatin did not have an effect on secretion as a percent of insulin content up to 50 nM. Pitavastatin did cause an increase in secretion when corrected for content at 200 nM.*

Figure 3: Calcium oscillations measured by fluorescence for Pitavastatin & Simvastatin at 200 nM. Ca^{2+} signaling results from a complex interplay between activation and inactivation of permeable intracellular and extracellular Ca^{2+} channels (Ca^{2+} oscillations). There is a close correlation between glucose dependent insulin release, Ca^{2+} influx and insulin exocytosis. Statin treatment (200 nM, 72 h) did not adversely affect the frequency of intracellular Ca^{2+} oscillations; however, statins differed in their average basal level of intracellular Ca^{2+} and amplitude of Ca^{2+} compared with control.

Summary: Compared to Simvastatin, Pitavastatin had a minimal effect on GSIS. At 50 nM, Pitavastatin preserved insulin content better than Simvastatin. Although the high dose of 200 nM for both statins reduced insulin content, insulin secretion was maintained with Pitavastatin.