Tacrolimus, but not voclosporin, significantly inhibits insulin exocytosis from human islets at clinically relevant trough concentrations

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INTRODUCTION

- Diabetes after organ transplantation is a serious problem caused by exposure to steroids and/or calcineurin inhibitors, particularly tacrolimus (TAC) (Webster et al 2005 *BMJ*).
- Calcineurin is thought to play important roles in pancreatic beta-cells, but the effects of calcineurin inhibitors on human beta-cells remain understudied, despite its clinical importance.
- We and others have shown that TAC and other immunosuppressants have direct deleterious effects on human islets (Johnson et al 2006 *Cell Transplantation*; Dai et al 2020 *JCI Insight*).
- Voclosporin (VCS) is a next generation calcineurin inhibitor that has recently succeeded in Ph3 clinical trials for lupus nephritis, and is in Ph2 trials for other conditions.
- VCS has demonstrated less new-onset diabetes in clinical trials, but the direct effects of this drug on human beta-cell function are unknown.

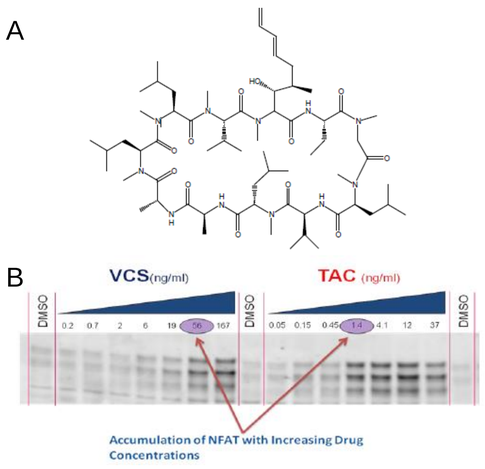

Figure 1| Structure and activity of VCS relative to TAC.
(A) Structure of voclosporin (VCS). **(B)** NFAT accumulation in response to various doses of VCS and TAC.

PROJECT OUTLINE

- We studied 2 clinically relevant doses of TAC (10 ng/ml trough, 30 ng/ml peak) and VCS (20 ng/ml trough, 60 ng/ml peak), meant to approximate the trough and peak concentrations of each drug.
- We compared the effects of TAC and VCS on the dynamics of insulin secretory function using perifusion analysis on non-diabetic cadaveric human islets.
- We compared the effects of TAC and VCS on the programmed cell death rate using the incorporation of propidium iodide under long-term imaging conditions using Molecular Devices ImageXpress^{MICRO} XLS high-content imaging systems.
- We compared the transcriptomic profile of isolated human islets treated with TAC and VCS using RNA sequencing.

Table 1| IDs and characteristics of human islets used in this study.

Donor ID	Age (years)	Sex	HbA1c (%)	BMI
R297	69	Male	No data	27.2
R300	30	Female	No data	25.3
R301	18	Male	5.0	19
R303	56	Female	No data	24.1
R305	60	Male	5.6	21
R306	22	Female	5.3	21.1
R308	20	Male	5.5	19.8
R309	47	Female	5.5	27.4
R310	25	Male	5.4	26.4
R314	31	Female	5.0	30.3
R316	52	Male	5.7	26
R317	54	Male	5.1	26.4
R318	54	Male	5.0	20.5
R319	68	Male	5.0	27.8
R322	44	Female	4.9	23.2
R325	50	Male	No data	30.3
R326	26	Male	5.5	27
R327	57	Male	5.8	33.9
R328	48	Male	5.2	22.7

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- J.D.J. designed and supervised the studies, and is the ultimate guarantor of the work.

RESULTS

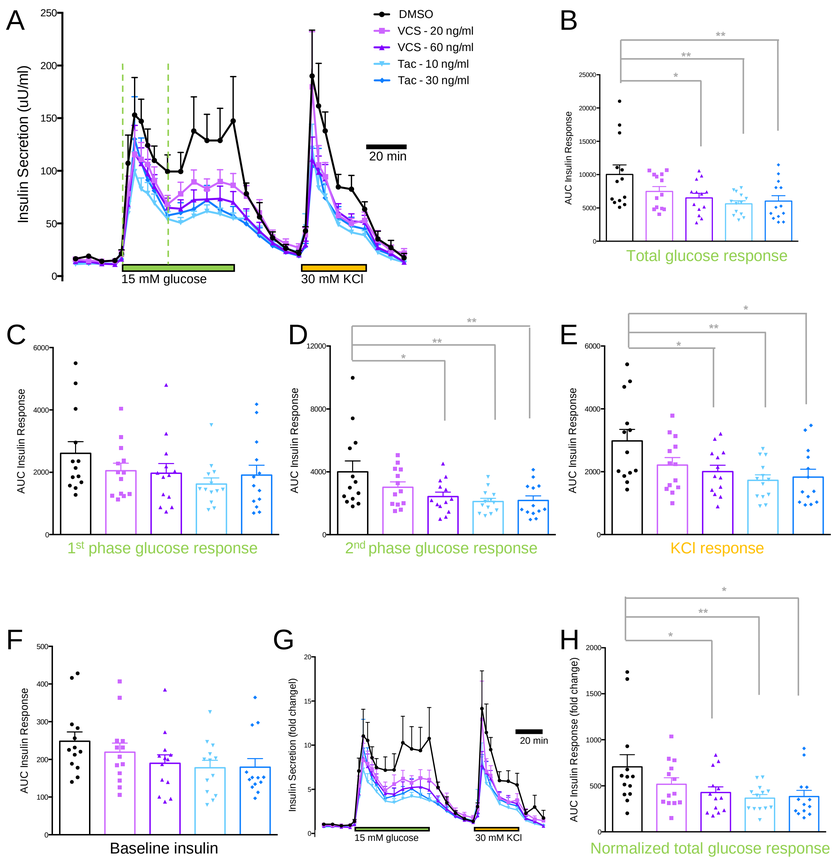

Figure 2| Basal, glucose-stimulated, and KCl-stimulated insulin secretion from human islets treated with peak and trough concentrations of VCS and TAC. (A) Averaged traces of dynamic insulin secretion measurements in the context of 3 mM glucose, or 15 mM glucose or 30 mM KCl (as indicated). **(B)** Total area under the curve (AUC) of the 15 mM glucose response. **(C, D, E)** AUCs of 1st phase and 2nd phase high glucose responses, as well as the KCl response. **(F)** Baseline insulin secretion. **(G,H)** Insulin secretion normalized to baseline, including AUC. n=13

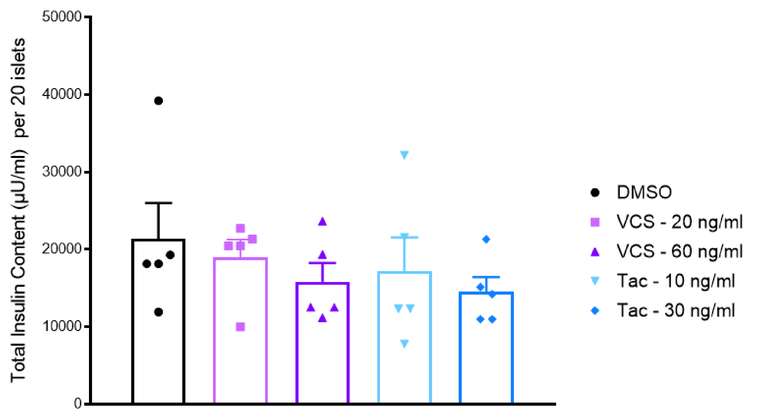

Figure 3| Insulin content from isolated human islets treated with VCS and TAC.
Insulin contents were extracted from 20 islets using the standard acid-ethanol protocol.

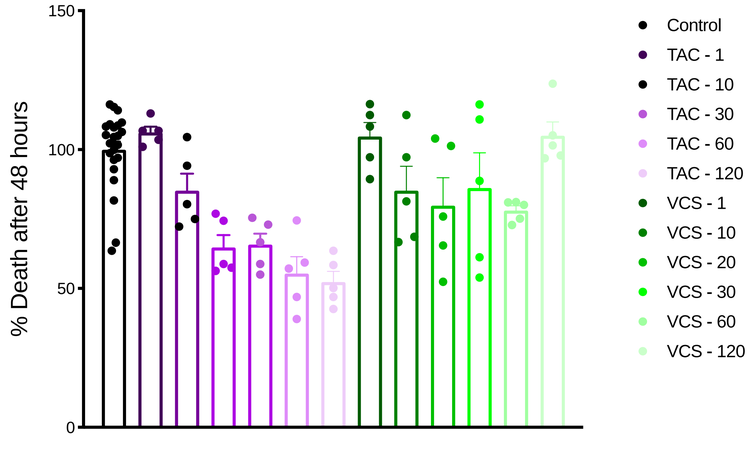

Figure 4| Long-term imaging of islet cell survival. Cell death was assessed every 30 minutes over 48 hours using propidium iodide and Hoechst staining. Results shown are quantified from 5 cultures per drug condition, over two separate runs.

Figure 5| RNA sequencing analysis of human islets treated with TAC and VCS at peak doses. (A) Top differentially expressed mRNAs are shown in blocks. Raw CPM values are shown in the table. **(B)** String analysis of the relationship between differentially expressed mRNAs. **(C)** Significantly enriched gene categories according to Gene Ontology. Protein-protein interaction networks highlight connections between calcineurin/NFAT pathway and deferentially expression genes in **(D)** VCS or **(E)** TAC treatment. Up or down regulated genes are shown in red or green, respectively.

SUMMARY

- TAC, but not VCS, caused a significant impairment of both 15 mM glucose-stimulated insulin secretion and 30 mM KCl-stimulated insulin secretion, pointing to possible a molecular defect in the distal stages of exocytosis after Ca^{2+} entry via voltage-gated Ca^{2+} channels. Both TAC and VCS inhibited insulin secretion at high concentrations. No effects on insulin contents were identified.
- No significant deleterious effects on cell survival were observed with either drug in this model.
- RNA sequencing showed that TAC, and to a lesser extent VCS, decreased the expression of genes that regulate exocytosis, including synaptotagmins (SYT16, SYT5), ER-to-Golgi traffic (PITPNM1), and hormone processing (PAM). Pathway analysis showed a significant enrichment of Gene Ontology terms including ‘syntaxin binding’.
- These data support prior clinical evidence demonstrating the favourable glucose homeostasis profile of VCS versus TAC.