Inhibition of Polyamine Biosynthesis Preserves Islet β-Cell Function in Type 1 Diabetes

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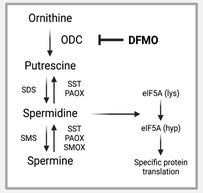
Introduction

- Type 1 diabetes (T1D) is an autoimmune disorder that results in destruction of insulin-producing pancreatic beta cells. Activation of stress-responsive pathways within the beta cell contributes to autoimmune-associated beta cell dysfunction and death in type 1 diabetes.
- Based on preclinical data suggesting a role for polyamines in autoimmunity and cytokine-induced inflammation, and an effect of difluoromethylornithine (DFMO, an irreversible inhibitor of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC)) in vivo to delay diabetes in nonobese diabetic mice, we sought to clarify a direct role for ODC in beta cell stress and to examine if oral DFMO treatment would safely improve beta cell health in human type 1 diabetes.



Results

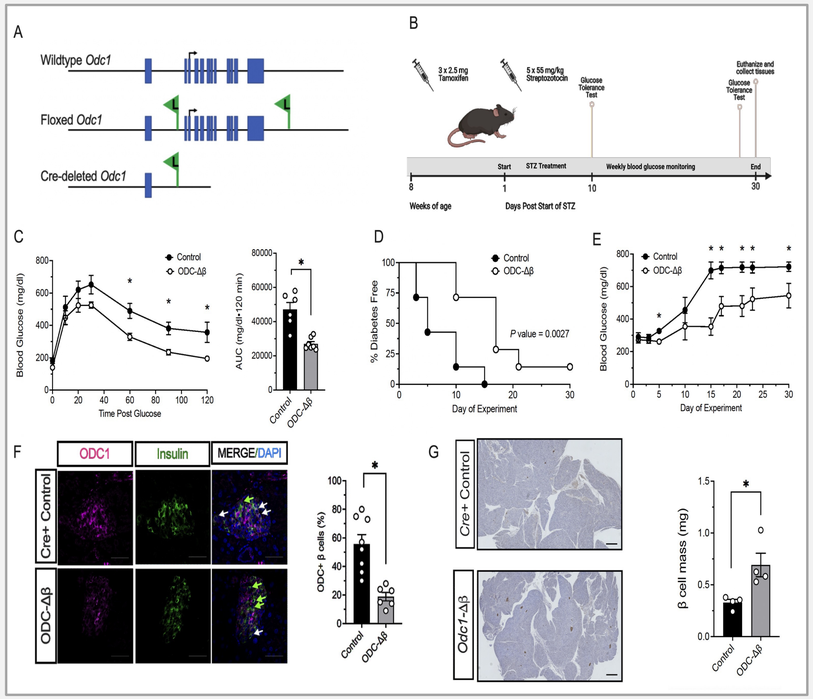


Figure 1: *Odc1*$^{\Delta beta}$ mice are protected from STZ-induced diabetes.
We generated mice with inducible, tissue-specific deletion of the *Odc1* gene encoding ornithine decarboxylase in mice. Administration of tamoxifen to *Odc1*$^{loxP/loxP}$;*MIP1-CreERT* mice at 8 weeks of age resulted in the generation of beta cell–specific knockout mice (*Odc1*$^{\Delta beta}$). (A) We next subjected *Odc1*$^{\Delta beta}$ mice and littermate controls to multiple low-dose streptozotocin (STZ) injections injections mimic the inflammatory milieu of T1D. (B)
(C) GTT at day 4 post STZ-treatment and Area under the curve analysis of GTT. (D) Diabetes incidence; (E) Random-fed blood glucose values. There was a ~65% reduction in the number of β cells exhibiting ODC production in Odc1Δβ mice compared to controls. (F) β cell mass was significantly 2-fold higher in Odc1Δβ mice that received STZ injections (G).

Results

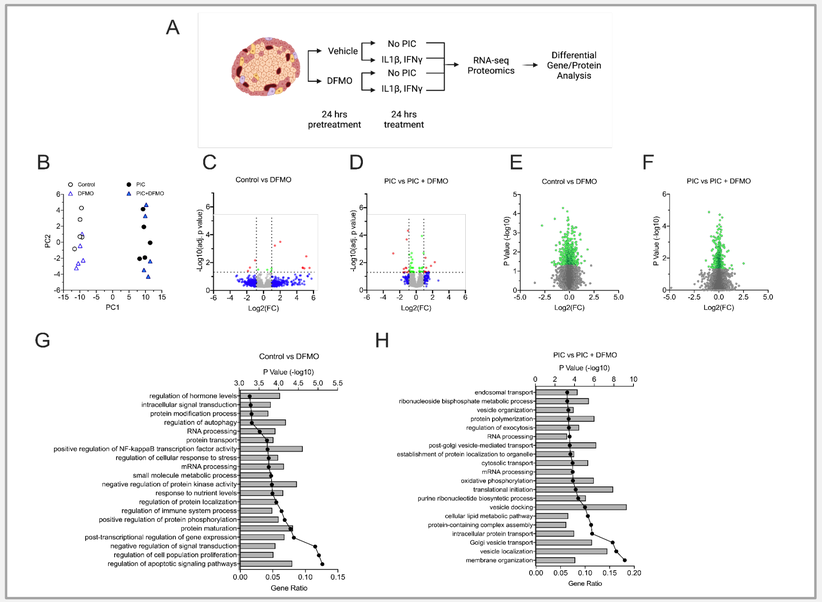


Figure 2: RNA-sequencing and proteomics of human islets treated with DFMO revealed pathways involved in post-transcriptional regulation.
We next performed unbiased RNA sequencing analysis of human islets from 5 donors and data independent analysis mass spectrometry from 6 donors. (A) Schematic of experimental design; (B) PCA analysis of RNA-sequencing outputs; (C-D) Volcano plot of genes identified between different conditions; (E-F) Volcano plot of proteins identified between different conditions; (G-H) Pathways relieved through GO:Biological Process of differentially expressed proteins identified between different conditions.

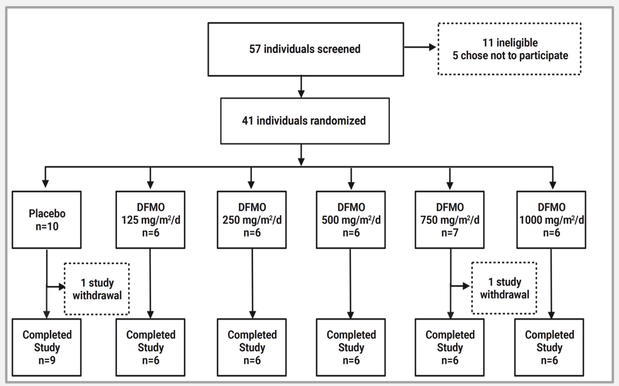


Figure 3: Schematic of the multicenter, double-blind, placebo-controlled, phase I/II dose-ranging clinic trial (ClinicalTrials.gov NCT02384889).
The primary study outcome was assessment of the safety of varying doses of DFMO. Persons with recent onset type 1 diabetes (within 240 days of diagnosis) were enrolled at 3 clinical diabetes centers within the United States. 41 individuals were randomized to 3-month treatment with either placebo or a fixed oral dose DFMO.

Table 1: Baseline characteristics of study participants.

Variable	Placebo (n= 10)	125 mg/m^2 (n = 6)	250 mg/m^2 (n = 6)	500 mg/m^2 (n = 6)	750 mg/m^2 (n = 7)	1000 mg/m^2 (n = 6)
Age (in years)	16.5 (6.5)	17.0 (6.3)	15.0 (2.7)	15.5 (2.6)	16.2 (5.3)	15.7 (2.3)
Race (%)						
Black	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Multiple	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
White	9 (90.0%)	6 (100%)	6 (100%)	6 (100%)	5 (71.4%)	6 (100%)
Number female (%)	5 (50.0%)	1 (16.7%)	3 (50.0%)	1 (16.7%)	4 (57.1%)	3 (50.0%)
BMI (kg/m2)	22.8 (2.9)	21.4 (2.8)	22.1 (4.6)	27.0 (7.2)	23.9 (4.4)	21.1 (3.2)
HbA1c % [mmol/mol]	7.9 (1.4) [63]	7.4 (1.5) [57]	6.7 (1.3) [50]	6.0 (0.3) [42]	6.2 (0.7) [44]	7.2 (1.8) [55]
Days since T1D diagnosis	156.3 (63.5)	166.7 (71.4)	125.7 (58.0)	148.0 (68.4)	128.6 (50.0)	86.8 (29.4)

Inclusion criteria included:
- age 12-40 years
- T1D within 2-8 months
- C-peptide of >0.2pmol/mL
- + titer for islet autoantibody
- No immunomodulatory therapy

No dose-limiting toxicities or serious (>Grade 2) adverse events were observed

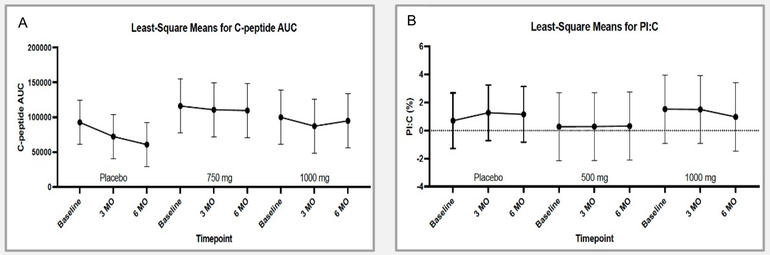


Figure 4: C-peptide and proinsulin results over the course of treatment.

- MMTT-derived adjusted means for C-peptide AUC were significantly higher at the 6-month timepoint in the 125 mg/m^2 (p=0.02), 750 mg/m^2 (p=0.03) and 1000 mg/m^2 DFMO dosing groups (p=0.02; **Figure 4A**).
- MMTT glucose AUC values were only significantly lower in the 125 mg/m^2 (p=0.03) and 750 mg/m^2 (p=0.02) treatment groups compared to placebo at the 6-month timepoint.
- At 6-months only the 1000 mg/m^2 group exhibited a decrease in the PI:C compared to placebo (p=0.04;**Figure 4B**).
- No significant treatment mediated changes in immune cell subsets or phenotypes, including Tregs, Th17, or other CD4+ cells or CD8+ T cells were noted.

Conclusion

- Beta cell-specific deletion of the gene encoding ornithine decarboxylase in mice protected animals against toxin-induced beta cell loss and hyperglycemia, implicating a direct role for ornithine decarboxylase in beta cell stress.
- Consistent with this observation, global transcriptomic and proteomics show that stressed human islets treated with DFMO exhibit alterations in pathways related to endoplasmic reticulum-related protein processing, antigen presentation, and reactive oxygen species.
- This multicenter randomized placebo-controlled trial showed that oral administration of DFMO over a 3-month period is safe and well tolerated in individuals with recent-onset type 1 diabetes. Participants on higher DFMO dose regimens exhibited higher C-peptide AUC 6 months after treatment compared to placebo-treated participants suggesting that DFMO may provide metabolic benefit to preserve beta cell function in type 1 diabetes.

Acknowledgements

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