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Verapamil and beta cell function in adults with recent-onset type 1 diabetes

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INTRODUCTORY PARAGRAPH

Pancreatic beta cell loss is a key factor in the pathogenesis of type 1 diabetes (T1D), but therapies to halt this process are lacking. We previously reported that the approved anti-hypertensive calcium channel blocker verapamil, by decreasing the expression of thioredoxin-interacting protein, promotes the survival of insulin-producing beta cells and reverses diabetes in mouse models[1]. To translate these findings into humans, we have conducted a randomized, double-blind, placebo-controlled Phase 2 clinical trial (NCT02372253) to assess the efficacy and safety of oral verapamil added for 12 months to a standard insulin regimen in adult subjects with recent-onset T1D. Verapamil treatment was well tolerated and associated with improved mixed meal-stimulated C-peptide area under the curve as a measure of endogenous beta cell function at 3 and 12 months compared to placebo (pre-specified primary endpoint) as well as with a lower increase in insulin requirements, fewer hypoglycemic events and on target glycemic control (secondary endpoints). Thus, addition of once daily oral verapamil may provide a safe and effective novel approach, to promote endogenous beta cell function and reduce insulin requirements and hypoglycemic episodes in adult individuals with recent-onset T1D.

T1D is characterized by a gradual destruction and loss of insulin-producing pancreatic beta cells resulting in a lifelong dependence on exogenous insulin to maintain normoglycemia. In

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AUTHOR CONTRIBUTIONS

F.O., T.G. and A.J.P. were responsible for patient care, MMTTs and sample and data collection. G.X., T.B.G. and L.A.T. helped with sample preparation, P.L. provided statistical advice. F.O. and A.S. designed the studies and analyzed the results and A.S. wrote the manuscript. All authors reviewed and approved the manuscript.

COMPETING FINANCIAL INTERESTS STATEMENT

None of the authors have any interests to declare.

A Life Sciences Reporting Summary can be found in the Supplementary Information.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

addition, T1D comes with a risk for secondary complications, including cardiovascular disease, kidney failure, blindness and amputations. While often diagnosed in children and young adults, T1D can occur at any age and, unlike previously thought, many patients retain a small number of functioning beta cells even years after diagnosis[2,3]. Moreover, large studies such as the Diabetes Control and Complications Trial (DCCT) have shown that even a small amount of preserved endogenous insulin production has major beneficial effects in terms of outcome, overall glycemic control and prevention of complication[4]. While major advances have been made in terms of sophisticated insulin preparations as well as insulin delivery and glucose monitoring systems, there is still no effective therapeutic approach available that targets diabetic beta cell loss. Attempts to replace beta cells by pancreas or islet transplantation are unfortunately associated with potentially severe side effects due to the necessary immunosuppression. Also, more recent stem cell-derived approaches are still in their infancy, underlining the urgent need for an effective pharmacological approach to promote the patient's own insulin-producing beta cell mass.

In this regard, we discovered thioredoxin-interacting protein (TXNIP), a cellular redox regulator, as an attractive therapeutic target. We originally identified *TXNIP* as the top glucose-induced gene in a human islet microarray study and found that beta cell expression of *TXNIP* is increased in diabetes[5–7]. Moreover, *TXNIP* overexpression induces beta cell apoptosis whereas *TXNIP* deficiency promotes endogenous beta cell survival and prevents diabetes in different mouse models[6,8,9]. We further discovered that the approved anti-hypertensive drug and calcium channel blocker, verapamil, effectively lowers beta cell *TXNIP* expression in rodent beta cells and islets as well as in human islets[1]. This effect is based on the established mode of action of verapamil, i.e. blockade of L-type calcium channels and the resulting decrease in intracellular free calcium leading to inhibition of *TXNIP* transcription[1]. Tissues with high expression levels of L-type calcium channels such as beta cells and the heart are therefore most likely to benefit from the resulting *TXNIP* inhibition. Indeed, verapamil and *TXNIP* downregulation have been shown to have beneficial effects in the diabetic heart[10–12], making adverse effects in this regard unlikely.

Moreover, in mouse models of diabetes, oral administration of verapamil promoted functional beta cell mass and prevented and even reversed overt diabetes[1]. In addition, we found that downregulating *TXNIP* also improves beta cell function and insulin production[13] and secretion[14], which may help increase the amount of insulin synthesized and secreted per beta cell especially in the context of dramatically reduced beta cell mass as in T1D. Finally, TXNIP has also been shown to be involved in inflammasome activation[15], suggesting that its downregulation might also have some anti-inflammatory effects.

Verapamil has been approved by the US Federal Drug Administration and has been widely used as an anti-hypertensive for over 30 years. While few retrospective studies have now emerged suggesting that verapamil use might be associated with reduced risk of developing type 2 diabetes[16–18], no prospective interventional trial had been performed to determine whether our preclinical findings might be translatable to humans and whether verapamil might be able to enhance functional beta cell mass and have beneficial effects in patients with T1D. We therefore conducted a randomized, double-blind, placebo-controlled, pilot-

and-feasibility trial to assess the efficacy and safety of oral verapamil added for 12 months to a standard insulin therapy in adult subjects with recent-onset T1D.

RESULTS

A total of 32 subjects, 18–44 years of age, diagnosed with T1D within the last 3 months were screened with mixed-meal tolerance tests (MMTT) for the presence of a minimal stimulated C-peptide value of ≥ 0.2 nmol/L and at least one positive T1D associated auto-antibody (Fig. 1). Out of these, 26 subjects qualified and underwent randomization to receive oral verapamil or placebo in addition to their insulin therapy. Two subjects (8%) that had randomized to the verapamil group had to be excluded prior to the collection of any outcome information (one relapsed into drug abuse and one refused to take scheduled insulin) resulting in a verapamil group of 11 subjects and a placebo group of 13 subjects that completed the one year trial. Baseline characteristics were well balanced between the two treatment groups and none showed any significant difference (Supplemental Table 1). Also, the average %HbA1c was very similar in the verapamil and the placebo group with 6.6 and 6.8, respectively.

To assess endogenous beta cell function, our primary endpoint, we measured the MMTT stimulated C-peptide area under the curve (AUC) at baseline, 3 months and at 12 months. Indeed, repeated measures ANOVA suggested a significant difference among group/time means. While the 2-sided Student's *t*-test showed that there was no significant difference at the 0-month baseline ($P = 0.300$), the stimulated C-peptide AUC was significantly larger in the verapamil group compared to placebo at both 3 months ($P = 0.0270$) and 12 months ($P = 0.0186$) (Fig. 2a). We also performed ANCOVA model adjusting for baseline to correct for any pre-existing difference at 0 months and the results still revealed that the stimulated C-peptide AUC was significantly larger in the verapamil group compared to placebo at both 3 and 12 months ($P = 0.0334$ and $P = 0.0377$, respectively). In addition, we assessed the change from baseline at 3 and 12 months in each individual and the results revealed again that subjects on verapamil maintained a significantly higher percentage of their stimulated C-peptide AUC as compared to those on placebo ($P = 0.0491$ and $P = 0.0451$, respectively) (Fig. 2b). Moreover, we also imputed the missing values for the two subjects that had to be excluded using a multiple imputation approach, re-analyzed the outcomes using the imputed data and conducted a sensitivity analysis. Of note, the results from the sensitivity analysis were consistent with the results presented above (Supplemental Table 2).

To assess changes in exogenous insulin requirements, one of the secondary endpoints, we also analyzed the total daily dose of insulin (TDDI) required to maintain glycemic control. At baseline, insulin requirements were 0.26 units/kg/day for both the verapamil and the placebo group. However, while by 12 months the TDDI increased 70% in the placebo group consistent with disease progression, the increase was only 27% in the verapamil group resulting in a significant treatment difference of −43%, 95% CI −84 to −1 ($P = 0.0312$) (Fig 3a). Of note, both groups maintained excellent glycemic control throughout the trial as demonstrated by average %HbA1c measurements between 6–7 (Fig. 3b). At 6 months there was a non-significant trend towards a lower %HbA1c in the verapamil group ($P = 0.083$). Moreover, the average rate of hypoglycemic events defined as blood glucose episodes of ≤

2.2mmol/L per month, was 0.5 events/month in the verapamil group as compared to 2.7 events/month with placebo (treatment difference −2.2 events/month, 95% CI −4.2 to −0.1, P = 0.0387) (Fig. 3c). Also, although not significant, verapamil-treated subjects spent more time within the target range of 3.9–10 mmol/L blood glucose (82% vs 72%, P = 0.130) and less time in the hyperglycemic (>10 mmol/L) or hypoglycemic (<3.9 mmol/L) range as compared to the placebo group and assessed using continuous glucose monitoring system data (Fig. 3d). Of note, verapamil treatment did not affect fasting serum glucagon levels (20.5 ng/L in the verapamil group vs 20.9 ng/L in the placebo group, P = 0.938). Together, these findings indicate that less exogenous insulin was required in the verapamil group to achieve equally good or better glycemic control as compared to the placebo group and suggest that oral verapamil can promote and preserve endogenous beta cell function in adults with recent onset T1D.

We also found that overall adverse events were very mild and none required treatment discontinuation or dose interruption or reduction (Supplemental Table 3). The only adverse event that occurred in a higher incidence in the verapamil group was constipation, which is consistent with it being known as the most common side effect of verapamil. However, reported symptoms were mild and did not require any medical intervention. Also, there were no severe hypoglycemic episodes requiring assistance of another person as defined by the American Diabetes Association in either treatment group. Importantly, verapamil did not cause any hypotension even in these normotensive subjects and monitoring of the participants' systolic and diastolic blood pressure throughout the study did not reveal any trend towards lower levels in the verapamil group (Fig. 4a,**b**). Moreover, all subjects maintained a normal heart rate (Fig. 4c) and electrocardiogram (EKG) analysis revealed that verapamil treatment did not cause any alteration in the QT or PR interval (Fig. 4d,**e**).

DISCUSSION

Taken together, this randomized, double-blind, placebo-controlled Phase 2 trial represents the first prospective study demonstrating that oral verapamil added to standard insulin therapy promotes endogenous beta cell function and lowers exogenous insulin requirements and hypoglycemic episodes in recent-onset adult T1D patients. Overall, verapamil was very well tolerated and aside from mild constipation no clinically significant adverse events were reported consistent with its proven safety profile. Of note, no hypotension and no EKG changes were observed either, demonstrating that verapamil can also be used safely in younger, normotensive subjects with T1D.

The fact that verapamil has now been found to be effective in promoting endogenous beta cell function as assessed by the validated method of MMTT stimulated C-peptide AUC in these individuals with T1D is consistent with our preclinical studies in isolated human islets and mouse models of diabetes, where verapamil has been shown to reduce the detrimental expression of *TXNIP*, prevent beta cell apoptosis and promote beta cell mass and improve glucose homeostasis[1]. However, we cannot rule out the possibility that verapamil also improved insulin sensitivity. In fact, improved insulin sensitivity could lead to overall better blood glucose control and account for the other associated secondary outcomes including reduction in exogenous insulin requirements, which in turn could reduce the number of

hypoglycemic episodes. In type 2 diabetes verapamil has also been suggested to possibly inhibit gluconeogenesis[19]. While there is no indication that it did so in our study participants and glucagon levels remained unchanged, we cannot exclude that such an effect contributed to the observed beneficial effects of verapamil on blood glucose.

Our results and the notion of verapamil having beneficial effects in the context of diabetes are further supported by retrospective studies focusing on type 2 diabetes. These include a recent study using Taiwan's National Health Insurance Research Database, where verapamil use was found to be associated with reduced incidence of newly diagnosed type 2 diabetes[16] as well as previous International Verapamil SR/Trandolapril (INVEST) spin-off studies that also suggested that participants in the verapamil arm had a lower risk of developing diabetes[17,18]. In addition, in an association study using the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort, we found that diabetic verapamil users had fasting blood glucose levels that were up to 2.1 mmol/L lower as compared to subjects with diabetes not on calcium channel blockers[20].

Interventional trials in T1D have primarily focused on suppressing or modulating the immune response[21–25]; however, it has now become apparent that to achieve sustained improvement, effective approaches to promote functional beta cell mass will have to be applied simultaneously[24,26]. Our findings now suggest that verapamil may represent such a novel and rational approach to enhance endogenous beta cell function. In addition, verapamil treatment was well tolerated, was not associated with any clinically significant adverse events and seemed to be effective even when just added to a standard insulin regimen. In future studies, though, it will also be interesting to test verapamil in combination with immune modulatory interventions.

The main limitation of this pilot-and-feasibility trial is the relatively small number of subjects included and it will have to be confirmed in future larger trials with longer follow-up. Also, since no *in vivo* measures are available to assess islet TXNIP levels in humans, it remains unclear whether verapamil was working in the study participants as it did in the prior rodent studies. On the other hand, our study sample seems to be representative of the T1D population, as the absolute numbers of C-peptide AUC at baseline and over time were very comparable to those observed in large T1D trials[27,28]. In addition, our trial was randomized, double-blind and placebo controlled and the study groups ended up being well-balanced in terms of their baseline characteristics, glycemic control and insulin requirements. Also, while we monitored participants for a full year rather than focusing just on the first few months, future longer-term studies wil1 have to determine whether beneficial effects can be sustained with (or without) continuous verapamil treatment. It will also be interesting to mine the electronic health record data for any epidemiological evidence that verapamil might improve diabetes control in subjects with T1D. Finally, based on these promising results in adults with T1D, the safety and efficacy of oral verapamil will also have to be tested in a pediatric T1D population. Since the natural course of T1D can be quite different and is often more aggressive in children, the results of the current study may not automatically translate into pediatric individuals and it is particularly important to first confirm that verapamil also has beneficial effects in this unique population.

In conclusion, once daily oral verapamil added to a standard insulin regimen may provide a safe and effective novel approach, to promote and preserve the person's own beta cell function, delay beta cell loss and disease progression for at least a year, and reduce insulin requirements and hypoglycemic episodes in recent-onset adult subjects with T1D.

ONLINE METHODS

Study design and participants:

The trial protocol was approved by the Institutional Review Board of the University of Alabama at Birmingham (UAB), in compliance with all ethical regulations and all patients provided written informed consent. The trial was also officially registered with ClinicalTrials.gov (NCT02372253). The trial was designed as a double-blind, placebo-controlled study with participants being randomized in a 1:1 ratio, with a simple randomization approach using computer-generated random numbers, to receive oral verapamil or matching placebo, in addition to standard care with continuous subcutaneous insulin infusion via an insulin pump. No other diabetes medications were permitted during the study. The pre-specified primary endpoint was endogenous beta cell function as determined by stimulated C-peptide during a 2h mixed meal tolerance test (MMTT), as previously validated[27,29,30]. Secondary endpoints included changes in exogenous insulin requirements as assessed by total daily dose of insulin (TDDI) and overall glycemic control as assessed by HbA1c, hypoglycemic events and use of a continuous glucose monitoring system (CGMS). This pilot study was not powered to detect differences in these secondary endpoints. Also, since improved blood glucose control could affect all of them, these secondary outcomes are likely to be linked and may not represent separate issues.

Eligible participants were 18–45 years of age and had been diagnosed with T1D within the last 3 months. The individual diagnostic factors are listed in Supplemental Table 4. In addition, they had to have a body mass index (BMI) <30, show positive auto-antibodies to at least one antigen (i.e. GAD65, IA-2, ICA, MIAA, ZnT8) and reach a minimal stimulated C-peptide value of ≥0.2nmol/L during the screening MMTT. Furthermore, participants had to agree to an intensive management of their diabetes with an HbA1c goal of <7.0% and to be willing to wear an insulin pump and CGMS. Individuals with concomitant use of glucagon-like peptide-1 receptor agonists or oral anti-diabetic agents or with any other serious disease were excluded. Other, non-diabetes related medications included one subject on levothyroxine, one on losartan, one on sertraline and one on atorvastatin, doxepin and topiramate,

Participants were randomly assigned to receive a once daily oral dose of sustained-release verapamil (titrated over the first 3 month from 120mg to 360mg) or placebo for a total of 12 months in addition to their insulin therapy. (This dose was chosen based on its proven tolerability and effectiveness in terms of calcium channel blockade and considering that the maximal recommended daily dose for verapamil is 480mg.) Participants were seen monthly during the first three months and then every 3 months until the end of the study and were carefully monitored for any occurrence of hypotension, bradycardia, or EKG changes (PR or QT interval prolongation).

Randomization and masking:

For masking, verapamil (120 mg sustained-release) and placebo were over-encapsulated resulting in identically looking capsules and packaging and were assigned different “lot numbers”. The trial team and investigators had no access to the lot number code throughout the study. Participants were assigned to lot numbers on a continuous basis via web-based computer generated random number sequence. Titration of treatment was achieved by increasing the number of capsules. Participants, care providers and investigators assessing the outcomes remained blinded until completion of the data analysis.

Procedures:

Endogenous beta cell function was assessed at baseline, 3 months and 12 months using stimulated C-peptide AUC during a MMTT as described previously[27,29]. The MMTT was only performed when fasting blood glucose levels were within the range of 3.9–11.1 mmol/L and otherwise the test was rescheduled. Blood samples were collected at −10, 0, 15, 30, 60, 90 and 120 minutes for serum C-peptide. The C-peptide AUC (0–120min) was calculated using the trapezoidal rule and divided by the time of the test to obtain the mean AUC (in nmol/L)[29]. Furthermore, to assess effects on exogenous insulin requirements the change in TDDI from baseline was calculated by analyzing the subject’s mean daily insulin use during the 2 weeks preceding the 3, 6, 9 and 12 months clinic visits. Glycemic control was monitored by measurements of %HbA1c every 3 months as well as the number of hypoglycemic episodes and the percent time spent within the target range of 3.9–10 mmol/L, or above or below it, as calculated based on CGMS data. The main safety parameters included measurements of blood pressure, heart rate, PR and QT intervals by EKG, and blood chemistry including liver function tests.

Statistical Analysis:

Participants’ demographic characteristics and outcomes were summarized as mean and standard errors (SE) for continuous variables; and frequency and proportion for categorical variables. The group comparison of baseline measures was conducted using Chi-square test, Fisher’s exact test, or Student’s *t*-test where appropriate. The normal distribution assumption was checked using Q-Q plots. All tests were two-sided. For the primary and secondary outcomes, a repeated measures two-way ANOVA was conducted first; and then where appropriate the ANCOVA model controlling for the baseline was conducted to compare group means at the 3 or 12 month time points. Sample size estimates were based on the primary outcome of endogenous beta cell function as measured by stimulated C-peptide AUC and on previously reported sample size considerations for studying treatment effects on beta cell function at 12 months in newly diagnosed patients with T1D over the age of 18 years in the T1D Trial Network[31] as well as on recommendations for planning pilot studies in clinical and translational research[32]. Statistical analyses were performed with the use of SAS 9.4 (Cary, NC). Sensitivity analysis was conducted using a multiple imputation approach as described previously[33].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Xu G , Chen J , Jing G & Shalev A Preventing beta-Cell Loss and Diabetes With Calcium Channel Blockers. Diabetes 61, 848–856 (2012). [PubMed: 22442301]
2. Davis AK , et al. Prevalence of detectable C-Peptide according to age at diagnosis and duration of type 1 diabetes. Diabetes Care 38, 476–481 (2015). [PubMed: 25519448]
3. Liu EH , et al. Pancreatic beta cell function persists in many patients with chronic type 1 diabetes, but is not dramatically improved by prolonged immunosuppression and euglycaemia from a beta cell allograft. Diabetologia 52, 1369–1380 (2009). [PubMed: 19418039]
4. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trial. A randomized, controlled trial. The Diabetes Control and Complications Trial Research Group. Ann Intern Med 128, 517–523 (1998). [PubMed: 9518395]
5. Shalev A , et al. Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway. Endocrinology 143, 3695–3698 (2002). [PubMed: 12193586]
6. Chen J , et al. Thioredoxin-Interacting Protein Deficiency Induces Akt/Bcl-xL Signaling and Pancreatic Beta Cell Mass and Protects Against Diabetes. FASEB J 22, 3581–3594 (2008). [PubMed: 18552236]
7. Chen J , Saxena G , Mungrue IN , Lusis AJ & Shalev A Thioredoxin-Interacting Protein: A Critical Link between Glucose Toxicity and Beta Cell Apoptosis. Diabetes 57, 938–944 (2008). [PubMed: 18171713]
8. Minn AH , Hafele C & Shalev A Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. Endocrinology 146, 2397–2405 (2005). [PubMed: 15705778]
9. Minn AH , et al. Gene expression profiling in INS-1 cells overexpressing thioredoxin-interacting protein. Biochem Biophys Res Commun 336, 770–778 (2005). [PubMed: 16143294]
10. Chen J , Cha-Molstad H , Szabo A & Shalev A Diabetes Induces and Calcium Channel Blockers Prevent Cardiac Expression of Pro-Apoptotic Thioredoxin-Interacting Protein. Am J Physiol Endocrinol Metab 296, 1133–1139 (2009).
11. Afzal N , et al. Beneficial effects of verapamil in diabetic cardiomyopathy. Diabetes 37, 936–942 (1988). [PubMed: 3384188]
12. Cohn RD , et al. Prevention of cardiomyopathy in mouse models lacking the smooth muscle sarcoglycan-sarcospan complex. J Clin Invest 107, R1–7 (2001). [PubMed: 11160141]
13. Xu G , Chen J , Jing G & Shalev A Thioredoxin-interacting protein regulates insulin transcription through microRNA-204. Nat Med (2013).
14. Jo S , et al. miR-204 Controls Glucagon-Like Peptide 1 Receptor Expression and Agonist Function. Diabetes 67, 256–264 (2018). [PubMed: 29101219]
15. Zhou R , Tardivel A , Thorens B , Choi I & Tschopp J Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 11, 136–140 (2010). [PubMed: 20023662]
16. Yin T , Kuo SC , Chang YY , Chen YT & Wang KK Verapamil Use Is Associated With Reduction of Newly Diagnosed Diabetes Mellitus. J Clin Endocrinol Metab 102, 2604–2610 (2017). [PubMed: 28368479]
17. Cooper-Dehoff R , et al. Predictors of development of diabetes mellitus in patients with coronary artery disease taking antihypertensive medications (findings from the INternational VErapamil SR-Trandolapril STudy [INVEST]). Am J Cardiol 98, 890–894 (2006). [PubMed: 16996868]

18. Cooper-DeHoff RM , et al. Blood pressure control and cardiovascular outcomes in high-risk Hispanic patients--findings from the International Verapamil SR/Trandolapril Study (INVEST). Am Heart J 151, 1072–1079 (2006). [PubMed: 16644338]
19. Busch Sorensen M , et al. Influence of short term verapamil treatment on glucose metabolism in patients with non-insulin dependent diabetes mellitus. European journal of clinical pharmacology 41, 401–404 (1991). [PubMed: 1761065]
20. Khodneva Y , Shalev A , Frank SJ , Carson AP & Safford MM Calcium channel blocker use is associated with lower fasting serum glucose among adults with diabetes from the REGARDS study. Diabetes Res Clin Pract 115, 115–121 (2016). [PubMed: 26818894]
21. Nambam B , Bratina N & Schatz D Immune Interventions for Type 1 Diabetes Mellitus. Diabetes technology & therapeutics 19, S74–S81 (2017). [PubMed: 28192018]
22. Alhadj Ali M , et al. Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes. Science translational medicine 9(2017).
23. Herold KC , et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. The New England journal of medicine 346, 1692–1698 (2002). [PubMed: 12037148]
24. Aronson R , et al. Low-dose otelixizumab anti-CD3 monoclonal antibody DEFEND-1 study: results of the randomized phase III study in recent-onset human type 1 diabetes. Diabetes Care 37, 2746–2754 (2014). [PubMed: 25011949]
25. Pescovitz MD , et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. The New England journal of medicine 361, 2143–2152 (2009). [PubMed: 19940299]
26. Pozzilli P , Maddaloni E & Buzzetti R Combination immunotherapies for type 1 diabetes mellitus. Nature reviews. Endocrinology 11, 289–297 (2015).
27. Greenbaum CJ , et al. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. Diabetes 61, 2066–2073 (2012). [PubMed: 22688329]
28. Hao W , et al. Fall in C-Peptide During First 4 Years From Diagnosis of Type 1 Diabetes: Variable Relation to Age, HbA1c, and Insulin Dose. Diabetes Care 39, 1664–1670 (2016). [PubMed: 27422577]

METHODS-ONLY REFERENCES

29. Greenbaum CJ , et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. Diabetes Care 31, 1966–1971 (2008). [PubMed: 18628574]
30. Palmer JP , et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21–22 October 2001. Diabetes 53, 250–264 (2004). [PubMed: 14693724]
31. Lachin JM , et al. Sample size requirements for studies of treatment effects on beta-cell function in newly diagnosed type 1 diabetes. PLoS One 6, e26471 (2011). [PubMed: 22102862]
32. Moore CG , Carter RE , Nietert PJ & Stewart PW Recommendations for planning pilot studies in clinical and translational research. Clinical and translational science 4, 332–337 (2011). [PubMed: 22029804]
33. Li P , Stuart EA & Allison DB Multiple Imputation: A Flexible Tool for Handling Missing Data. JAMA 314, 1966–1967 (2015). [PubMed: 26547468]

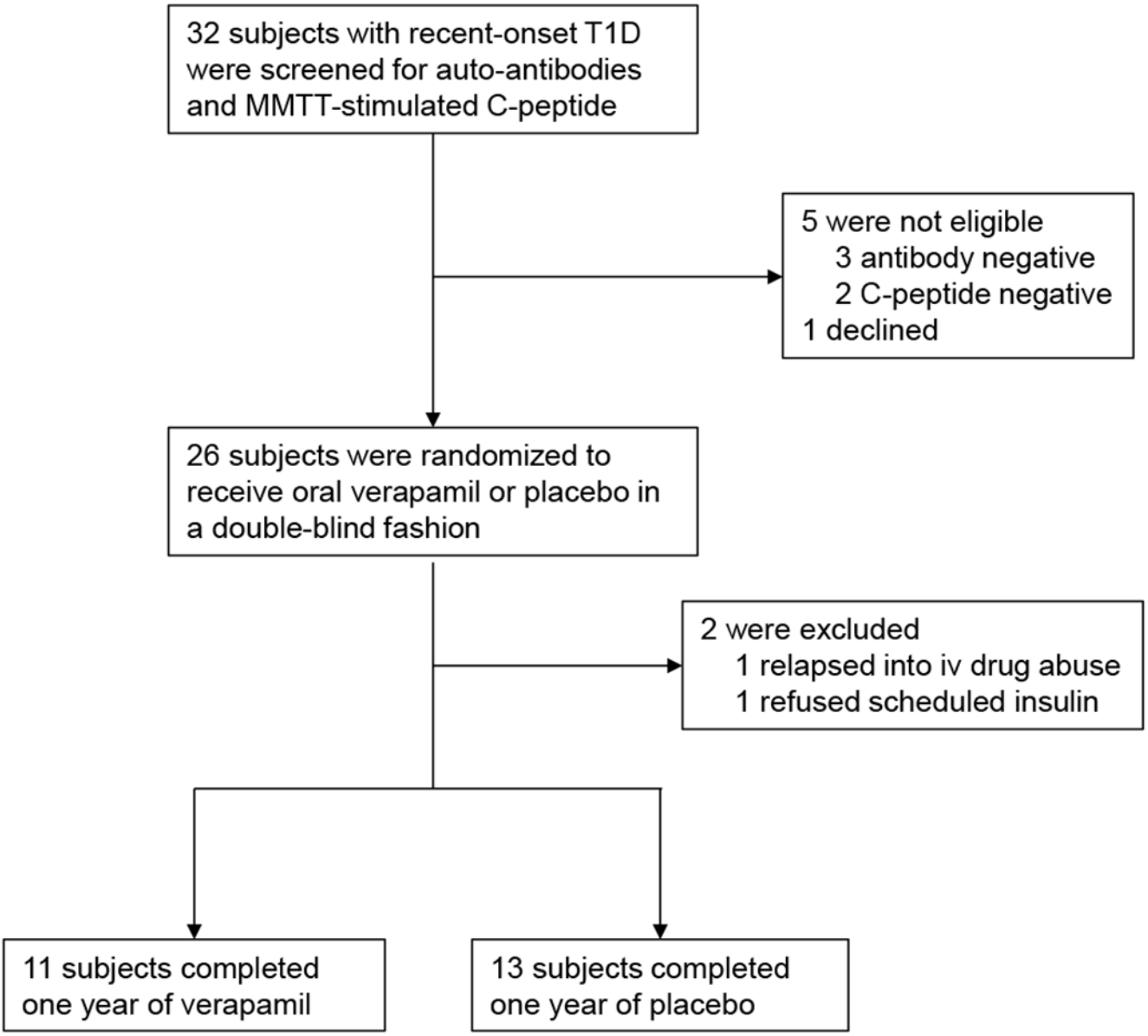

Figure 1.

Screening, Randomization and Treatment. A schematic diagram illustrating the selection procedure for the enrolled individuals in the study. All participants had been diagnosed with T1D within the last 3 months and continued their standard insulin infusion therapy throughout the trial.

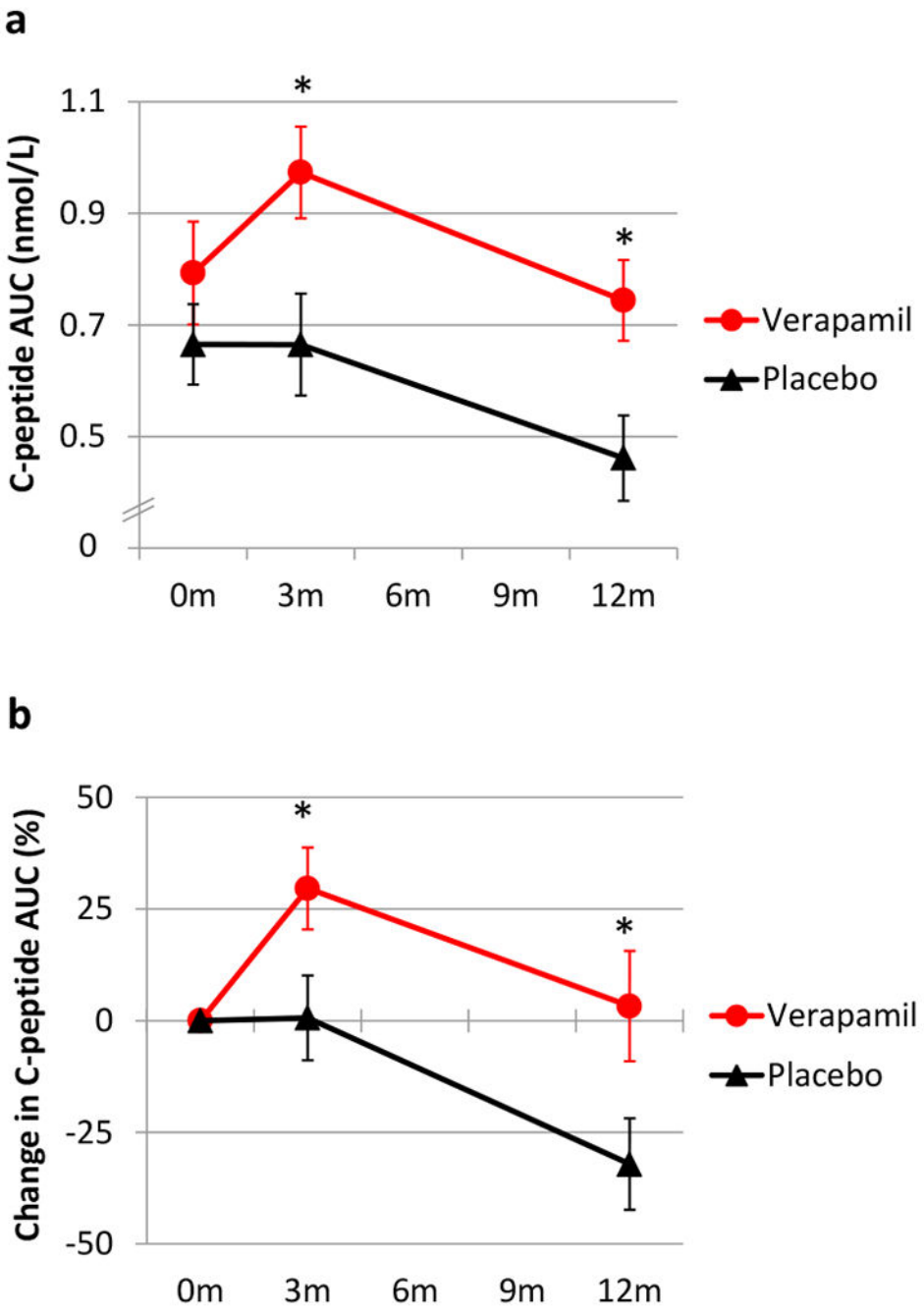

Figure 2.

Verapamil Effects on Endogenous Beta cell Function. (**a**,**b**) Absolute values (**a**) and changes from individual baseline values (**b**) of the mixed meal-stimulated C-peptide area under the curve (AUC) at 0, 3 and 12 months of the trial in all subjects of the verapamil ($n = 11$) and placebo ($n = 13$) groups. Means and SE error bars of are shown. For **a** repeated measures ANOVA: $F_{1,48}$=4.92, $P = 0.0313$; 3 months: two-sided Student's t-test: $t_{22} = -2.37$, $^*P = 0.0270$; (ANCOVA $F_{1,23}$=5.19, $P = 0.0334$); 12 months: treatment difference 0.28 nmol/L, 95% CI 0.05 to 0.51, two-sided Student's t-test: $t_{22} = -2.54$, $^*P = 0.0186$; (ANCOVA

$F_{1,23}$=4.92, P = 0.0377). For **b** repeated measures ANOVA: $F_{1,48}$=4.86, P = 0.0323; 3 months: two-sided Student's t-test: t_{22}=−2.08, *P = 0.0491; 12 months: treatment difference 35.4%, 95% CI 0.8 to 69.9, two-sided Student's t-test: t_{22}=−2.12, *P = 0.0451.

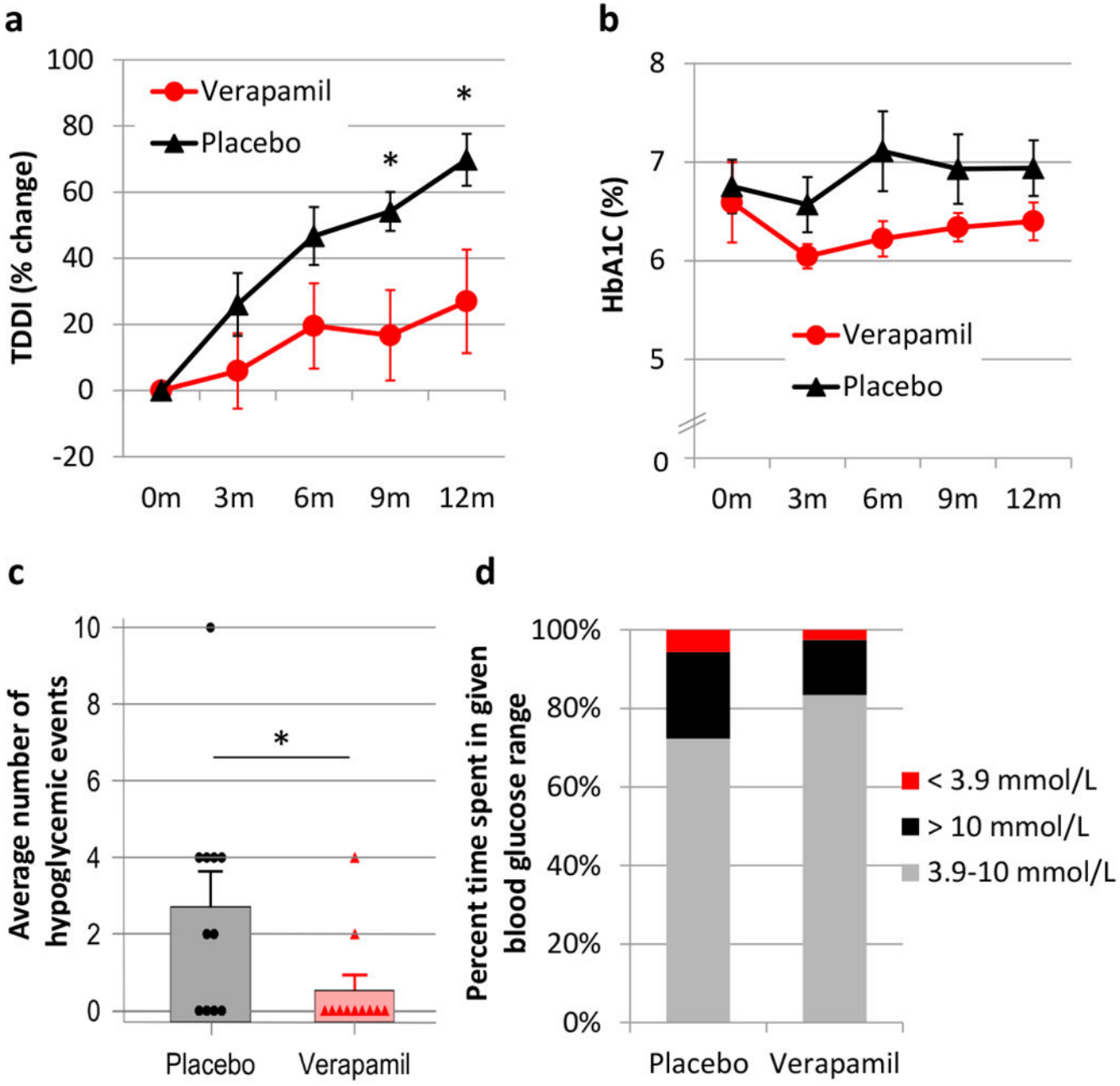

Figure 3.

Verapamil Effects on Glycemic Control and Insulin Requirements. **(a)** Mean percent change in total daily dose of insulin (TDDI) during the trial in the verapamil (n = 10) and placebo (n = 13) groups. Error bars show SE. Repeated measures ANOVA: $F_{1,89}$=4.37, P = 0.0395; 9 months: two-sided Student's t-test: t_{16}=2.41, *P = 0.0281; 12 months: treatment difference −43%, 95% CI −84 to −1, two-sided Student's t -test: t_{17}=2.34, *P = 0.0312. **(b)** Mean values for %HbA1c as measured at 0, 3, 6, 9, and 12 months in the verapamil (n = 11) and placebo (n = 13) groups. **(c)** Average number of hypoglycemic episodes of blood glucose ≤ 2.2 mmol/L per month in the verapamil (n = 11) as compared to the placebo (n = 11) group.

Bars represent means, error bars show SE, dots indicate individual data points. Treatment difference −2.2 events/month, 95% CI −4.2 to −0.1, two-sided Student's *t*-test: t_{20}=−2.21, **P* = 0.0387. **(d)** Percent time spent at the target blood glucose range of 3.9–10 mmol/L (grey), above 10 mmol/L (black) or below 3.9 mmol/L (red) as assessed by continuous glucose monitoring in the verapamil (*n* = 10) and the placebo (*n* = 11) groups.

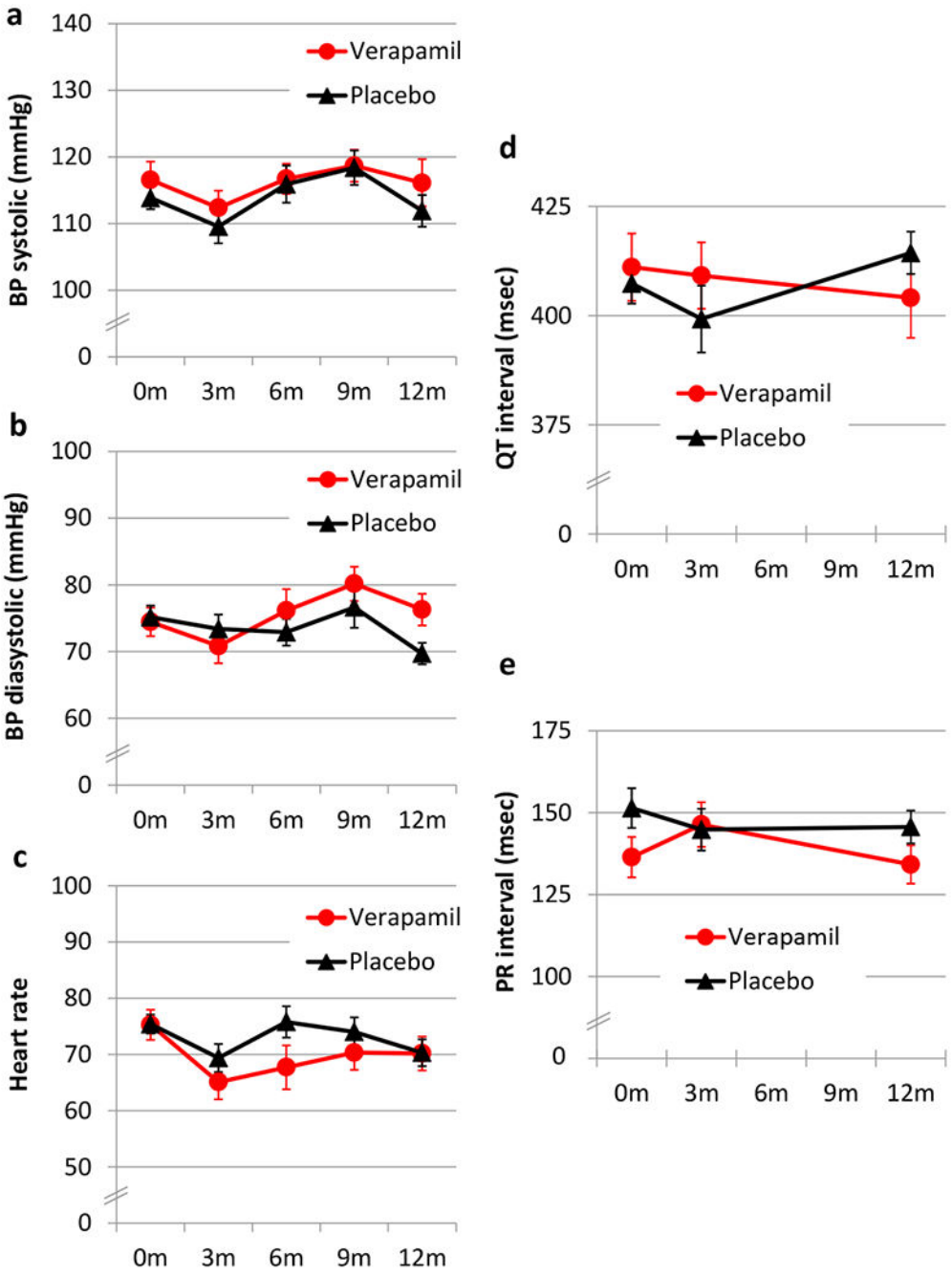

Figure 4.

Blood Pressure and Heart Rate throughout the Trial. **(a-e)** Mean values for systolic (**a**) and diastolic (**b**) blood pressure (BP), heart rate **(c)** and EKG-measured QT (**d**) and PR **(e)** intervals observed in the verapamil ($n = 11$) and placebo ($n = 13$) groups during the 12 month trial. Error bars represent SE.