A Randomized Controlled Trial of R-Form Verapamil Added to Ongoing Metformin Therapy in Patients with Type 2 Diabetes

Chih-Yuan Wang,1,* Kuo-Chin Huang,2,* Chia-Wen Lu,2 Chih-Hsun Chu,3 Chien-Ning Huang,4 Harn-Shen Chen,5 I-Te Lee,6 Jung-Fu Chen,7 Ching-Chu Chen,8 Chung-Sen Chen,9 Chang-Hsun Hsieh,10 Kai-Jen Tien,11 Hung-Yu Chien,12 Yu-Yao Huang,13 Jui-Pao Hsu,14 Guang-Tzuu Shane,14 Ai-Ching Chang,15 Yen-Chieh Wu,15,16 and Wayne Huey-Herng Sheu5,16,*

1Division of Endocrinology and Metabolism, Department of Internal Medicine, National Taiwan University Hospital, Taipei 100229, Taiwan 2Department of Family Medicine, National Taiwan University Hospital, Taipei 100229, Taiwan 3Division of Endocrinology and Metabolism, Department of Internal Medicine, Kaohsiung Veterans General Hospital, Kaohsiung 813414, Taiwan 4Institute of Medicine, Chung Shan Medical University & Hospital, Taichung 402306, Taiwan 5Division of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital, Taipei 112201, Taiwan 6Division of Endocrinology and Metabolism, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung 407219, Taiwan 7Division of Metabolism, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 833401, Taiwan 8Division of Endocrinology and Metabolism, Department of Medicine, China Medical University Hospital, Taichung 404332, Taiwan 9Division of Endocrinology and Metabolism, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung 407219, Taiwan 10Division of Endocrinology and Metabolism, Department of Internal Medicine, Tri-Service General Hospital, Taipei 114202, Taiwan 11Division of Endocrinology and Metabolism, Department of Internal Medicine, Chi Mei Medical Center, Tainan 710402, Taiwan 12Department of Endocrinology and Metabolism, Taipei City Hospital Renai Branch, Taipei 106243, Taiwan 13Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospital, Taoyuan 333423, Taiwan 14Center Laboratories Inc., Taipei 115603, Taiwan 15Lumosa Therapeutics Co., Ltd., Taipei 115603, Taiwan 16Faculty of Medicine, National Yang Ming Chiao Tung University, Taipei 112304, Taiwan

*These authors contributed equally to this work and thus share the first authorship.

Correspondence: Wayne Huey-Herng Sheu, MD, PhD, Division of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; Faculty of Medicine, National Yang Ming Chiao Tung University, No. 201, Sec. 2, Shipai Road, Beitou District, Taipei City 112201, Taiwan. Email: whhsheu@vghtpe.gov.tw.

Abstract

Context: There is a medical need for effective insulin-independent antidiabetic drugs that can promote pancreatic β-cell function and have a low risk of hypoglycemia in type 2 diabetes mellitus (T2DM) patients. R-form verapamil (R-Vera), which is able to enhance the survival of β-cells and has higher cardiovascular safety margin compared with racemic verapamil, was developed as a novel approach for T2DM treatment.

Objective: This randomized, double-blind, placebo-controlled clinical trial was designed to evaluate the efficacy and safety of 3 dosages of R-Vera added to ongoing metformin therapy in T2DM patients who had inadequate glycemic control on metformin alone.

Methods: Participants were randomly assigned in an equal ratio to receive R-Vera 450, 300, or 150 mg per day, or matching placebo, in combination with metformin. The primary endpoint was change in hemoglobin A1c (HbA1c) after 12 weeks of treatment.

Results: A total of 184 eligible participants were randomized to receive either R-Vera or placebo plus metformin. At week 12, significant reductions in HbA1c were observed for R-Vera 300 mg/day (−0.36, P = 0.0098) and 450 mg/day (−0.45, P = 0.0073) compared with placebo. The reduction in HbA1c correlated with decreasing fasting plasma glucose levels and improved HOMA2-β score. Treatment with R-Vera was well tolerated with no hypoglycemic episodes occurring during the trial.

Conclusion: Addition of R-Vera twice daily to ongoing metformin therapy significantly improved glycemic control in T2DM patients. The favorable efficacy and safety profile of R-Vera 300 mg/day can be considered as the appropriate dose for clinical practice.

Key Words: R-form verapamil, metformin, type 2 diabetes, HbA1c, anti-diabetic drug

Abbreviations: AE, adverse event; BMI, body mass index; DM, diabetes mellitus; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A1c; HDL, high-density lipoprotein; HOMA2-β, homeostasis model 2 assessment β-cell; HOMA2-IR, homeostasis model 2 assessment of insulin resistance; LDL, low-density lipoprotein; LS, least squares; R-Vera, R-form verapamil; SBP, systolic blood pressure; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TEAE, treatment-emergent adverse event; TXNIP, thioredoxin-interacting protein.
Diabetes mellitus (DM) is a metabolic disorder that, as a major global public health burden, is projected to affect 700 million people by 2045 (1). It is a major cause of blindness, kidney failure, heart attack, stroke, and lower limb amputation, and the rate of DM-related mortality is about 11.3% among 20- to 79-year-olds globally (2). The increasing prevalence and incidence of DM is primarily due to the increase in the prevalence of type 2 diabetes mellitus (T2DM), which accounts for 90% to 95% of all diabetes cases. The marked decrease in insulin secretion in response to glucose stimulation is largely attributed to a reduction in beta cell mass, ranging from 40% to 63% (3).

Various oral antidiabetic drugs are applicable to T2DM treatment; however, the risk of hypoglycemia, weight gain, and insufficient efficacy may limit their use in some patients. The most important limitation is the insufficient efficacy of these therapies due to the decrease in beta cell mass. While new islet formation and beta cell replication occur normally in patients with T2DM, increased apoptosis is the major cause of decreased beta cell mass. Therapeutic approaches designed to arrest apoptosis could be a significant new development in the management of T2DM, which might shed new light on reversing the disease to a degree, rather than just glycemic control (4).

Incretin hormone mimetics have been shown to ameliorate beta cell apoptosis and increase beta cell mass in rodent models of diabetes; however, these effects have not yet been replicated in human clinical trials, possibly due to intrinsic differences between species, beta cell physiology, and the use of different treatment protocols (5). Verapamil is a phenylalkylamine calcium channel antagonist indicated for management of hypertension, arrhythmia, and angina. In addition to the cardiovascular effects, verapamil has been shown to play a role in preventing insulin-producing beta cell death in vivo and in vitro studies (6, 7). The mechanism of action involves inhibition of extracellular calcium penetration through cell membrane, reduction of intracellular calcium level, and inhibiting the expression of proapoptotic thioredoxin-interacting protein (TXNIP) which would otherwise accumulate in the pancreatic beta cells and increase oxidative toxicity (6, 7). Therefore, verapamil provides potential therapeutic application in the treatment of DM. The safety and efficacy of verapamil for beta cell survival is also currently being tested under an investigational protocol for the treatment of type 1 DM (T1DM). Using insulin production as an indirect measure of beta cell mass, a greater improvement in the C-peptide area under the curve (AUC) was reported in the preliminary results (ClinicalTrials.gov no. NCT02372253). In a systematic review of the effects of verapamil-based treatment on beta cells in 11 controlled clinical trials of patients with T2DM, verapamil treatment was associated with a significant decrease in plasma glucose levels; however, glycated hemoglobin (HbA1c) levels were not significantly improved by this drug (8). An improvement in glycometabolic control was noted in 3 trials involving patients with predicted or known late-stage T2DM, which is a condition characterized by beta cell apoptosis.

Although verapamil is a drug of choice for the treatment of the cardiovascular diseases, adverse effects such as bradycardia and hypotension may occur under high dose. Several side effects were reported in the clinical trials of orally administered verapamil. The most serious adverse reactions reported with verapamil were heart failure, atrioventricular block, hypotension, and rapid ventricular response. Literature reports indicated that the R and S enantiomers had differing levels of pharmacologic activity. In contrast to the R-form, the S-form of verapamil demonstrated 8- to 20-fold higher potency to slow atrioventricular conduction and 15- to 50-fold higher potency to reduce myocardial contractility in animals and humans (9).

Based on the above-mentioned evidence, R-form verapamil is likely to reverse beta cell apoptosis and T2DM progression, with higher cardiovascular safety margin compared with S-form verapamil and racemic products. An oral formulation of R-form verapamil (R-Vera) is under development by Center Laboratories, Inc. as a potential treatment for patients with T2DM. The proposed investigational clinical trial is a dose-finding, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of R-Vera in combination with metformin in patients with T2DM who have inadequate glycemic control on metformin alone.

**Methods**

This multicenter study was conducted in 15 healthcare institutions located in Taiwan and the United States from November 2017 to April 2020 in accordance with the local laws and regulations, the Declaration of Helsinki, good clinical practice guidelines, and the study protocol. The study protocol was approved by the institutional review boards of all participating institutions. All participants provided written informed consent before receiving any study-specific procedures. The study was registered with ClinicalTrials.gov and first posted on October 23, 2017, as NCT03317028.

**Participants**

Individuals who were aged 18-75 years and met all of the following criteria were eligible for this study: 1) were diagnosed with T2DM for at least 12 weeks and had a stable diet and exercise program for at least 8 weeks before screening; 2) had HbA1c of 7% or greater and 10% or less at screening; 3) had fasting plasma glucose (FPG) less than 260 mg/dL; 3) had body mass index (BMI) between 20.0 and 45.0 kg/m²; 4) had adequate liver function and estimated glomerular filtration rate (eGFR) values of 45 mL/min/1.73 m² or greater; 5) had a stable dose of metformin monotherapy of 1500 mg/day or greater (or on maximally tolerated dose for patients who could not titrate up to 1500 mg/day due to safety concerns or side effects of metformin) for at least 12 weeks before enrollment. Female participants of childbearing potential had a negative pregnancy test at screening and agreed to use a highly effective contraceptive method during the study period.

Potential participants who met any of the following conditions were excluded from the study: 1) had used insulin within 12 weeks prior to screening (insulin for emergency use for 7 consecutive days or less was allowed); 2) were likely to use any antihypertensive agents of α-blockers, β-blockers, or non-dihydropyridine calcium channel blockers throughout the study or who were using more than 4 types of antihypertensive agents; 3) had hypotension (resting average systolic blood pressure lower than 90 mmHg) at screening; 4) were on a weight loss program and not in the maintenance phase. Other exclusion criteria included diabetes mellitus other than T2DM and diabetic complications; clinically significant
cardiovascular, gastrointestinal, or renal disease; with severe infection, serious trauma, or perioperative period; or having received any investigational therapy from another clinical trial within 12 weeks prior to screening.

**Study Medication and Intervention**

R-Vera or matching placebo were manufactured by Center Laboratories, Inc. in white or almost white, round shape tablet form for oral administration. Every R-Vera tablet contains 75 mg of R-form verapamil.

In the single-blind placebo run-in period, all participants took 3 placebo tablets orally twice a day after the morning and evening meals for 3 to 4 weeks. During the double-blind treatment period, participants took 3 tablets of R-Vera and/or matching placebo orally twice a day after the morning and evening meals for 12 weeks as follows: 1) Daily dose of 450 mg: 3 tablets of R-Vera each dosing; 2) Daily dose of 300 mg: 2 tablets of R-Vera plus 1 tablet of placebo each dosing; 3) Daily dose of 150 mg: 1 tablet of R-Vera plus 2 tablets of placebo each dosing; and 4) Placebo group: 3 tablets of placebo each dosing.

**Study Design**

This randomized, double-blind, placebo-controlled, parallel-group clinical trial was designed to evaluate the efficacy and safety of 3 doses of R-Vera in combination with metformin in T2DM patients who had been on a stable, maximally tolerated dose of metformin monotherapy yet still had inadequate glycemic control. This study started with a single-blind, placebo run-in period for 3 to 4 weeks, followed by a double-blind treatment period for 12 weeks. Eligible participants were randomly assigned, in a 1:1:1:1 ratio following a computer-generated randomization schedule to receive R-Vera at a daily dose of 450 mg, 300 mg, 150 mg, or matching placebo. The randomization schedule linked sequential numbers to treatment codes allocated at random and was prepared in a 1:1:1:1 randomization ratio using block technology and stratified by region. This randomization schedule was generated and kept secure by the delegated contract research organization (A2 Healthcare Taiwan Corporation) and was not disclosed to the participants, investigators, or any personnel involved in the conduct of this study before the database was locked, except in case of an emergency.

Participants were enrolled in the study for a maximum of 15 to 16 weeks and scheduled to visit the investigational site at weeks 1, 3, 5, 9, and 13 (**Fig. 1**). At every visit, the participant’s weight was measured, and vital signs and physical examinations were conducted. Safety was assessed by recording adverse events, concomitant medications, hematology, serum chemistry, and urinalysis from a clinical laboratory at every visit and 12-lead electrocardiograms at weeks 1, 5, and 13. Efficacy was evaluated by measuring the blood levels of HbA1c, FPG, insulin, and C-peptide, as well as lipid parameters (including total cholesterol, triglycerides, low-density lipoprotein [LDL], and high-density lipoprotein [HDL]) at weeks 1, 5, and 13. The blood samples for the efficacy measurements were collected after fasting (≥ 10 hours) prior to administration of the study drug on that visit day, after the participant had rested for at least 5 minutes in a seated position. All laboratory assays were performed by the certified or accredited clinical laboratory affiliated to each institution that participated in this study. HbA1c was determined by high performance liquid chromatography, FPG by a hexokinase method, insulin by solid-phase radioimmunoassay, and C-peptide by a chemiluminescent immunoassay sandwich method. Enzymatic colorimetric assays were used for total cholesterol, triglyceride, LDL, and HDL.

The drug exposure-response assessment was an optional test and participants who agreed to participate in the assessment provided a separate informed consent. Blood samples were collected before study drug administration (baseline) and at end of treatment (C<sub>trough</sub>) at 12 ± 4 hours from the last

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**Figure 1.** The study design and participant disposition in the 4 treatment groups. Abbreviations: ICF, informed consent form; R-Vera, R-form verapamil.
Efficacy and Safety Assessments
The primary study endpoint was the change in HbA1c from baseline to end of treatment. The secondary endpoints included 1) percentage of participants achieving the target HbA1c of < 7.0% at week 12; 2) changes in FPG and insulin levels; 3) changes in homeostasis model 2 assessment of β-cell function (HOMA2-β) and homeostasis model 2 assessment of insulin resistance (HOMA2-IR); 4) changes in the related cardiovascular risk factors including total cholesterol, triglycerides, LDL, HDL, blood pressure and body weight; 5) the proportion of participants requiring rescue medication; and 6) the drug exposure-response (pharmacokinetics-pharmacodynamics) at steady state.

Safety assessments were conducted throughout the study including adverse events; abnormal changes in vital signs, clinical laboratory results, or 12-lead electrocardiograms; and occurrence of hypotension and hypoglycemic events.

Statistical Analysis
Assuming a standard deviation of 0.87% for measurements of HbA1c and that significance is evaluated at $\alpha = 0.05$ using a two-tailed test, approximately 42 participants per treatment group would provide 83% power to detect a true difference between R-Vera and placebo groups of 0.56% in the mean change in HbA1c after 12 weeks of treatment. Assumptions also included an approximate 5% dropout rate and 40% placebo run-in failure rate, therefore, a total of 295 participants were planned to be enrolled in the single-blind placebo run-in period and 177 participants were planned to be randomized.

Data analyses were based on the intention-to-treat (ITT) population, which consisted of all participants who received at least one dose of the study drug during the double-blind treatment period. To assess the change in HbA1c from baseline, the R-Vera and placebo groups were compared using the analysis of covariance (ANCOVA) model with baseline HbA1c level as covariate, and treatment groups and regions as fixed effects. Continuous variables were summarized as number of observations, mean, median, SD, minimum and maximum, and were analyzed by ANCOVA. Pairwise comparison was performed using Student’s t method with overall alpha 0.05 controlled. Categorical variables were summarized as the number of observations, frequency, and percentages as appropriate, and were analyzed by using Chi-square test or Cochran-Mantel-Haenszel (CMH) test stratified by region. All statistical tests are two-sided and P values < 0.05 are regarded as significant.

Incidence of all treatment-emergent adverse events (TEAEs) and the study medication-related adverse events were presented by treatment, body system classification and preferred term according to Medical Dictionary for Regulatory Activities (MedDRA), System Organ Class (SOC) codes.

The last-observation-carried-forward (LOCF) procedure was used to estimate the missing data for efficacy variables. No imputation was performed to estimate missing values for safety variables. Subgroup analysis based on BMI, age, baseline HbA1c, HOMA2-β, HOMA2-IR, duration of diabetes, baseline systolic blood pressure and baseline diastolic blood pressure were performed as part of the ad hoc analysis.

Results
Participant Disposition and Characteristics
A total of 253 participants were screened for the study and 184 participants were eligible and randomized to the 4 treatment groups: 46 in the placebo group, 46 in the 150 mg/day group, 47 in the 300 mg/day group, and 45 in the 450 mg/day group. There were 164 participants in total who completed the study treatment: 39 in the placebo group, 43 in the 150 mg/day group, 42 in the 300 mg/day group, and 40 in the 450 mg/day group. Twenty participants terminated early from the study: 7 in the placebo group, 3 in the 150 mg/day group, 5 in the 300 mg/day group, and 5 in the 450 mg/day group (Fig. 1). The major reasons for early termination from the study were adverse events (placebo group, 0%; R-Vera group, 3.6%) and impossible to continue (placebo group, 10.9%; R-Vera group, 3.6%) including informed consent withdrawals, lost to follow-up, or poor compliance with the study drug administration or protocol procedures.

In total, 32 major protocol deviations and 116 minor protocol deviations were reported. The most common major protocol deviations were a total cumulative dose less than 75% of expected dose (17 participants, 9.2%), FPG re-test not performed (5 participants, 2.7%) and missing posttreatment HbA1c test (5 participants, 2.7%). The most common minor protocol deviations included clinical operation errors (42 participants, 22.8%), placebo run-in period less than 21 days or over 28 days (39 participants, 21.2%), and visit window deviation (24 participants, 13.0%).

The baseline demographic and disease characteristics were summarized in Table 1. The mean age for the total study population was 56.8 years old, 54.3% participants were male, and the mean BMI was 28.81 kg/m². The diabetes mellitus history was also analyzed: the mean disease duration was 6.74 years and mean onset age of disease was 49.52 years old. A total of 180 (97.8%) participants had at least one concurrent condition besides T2DM before entering the study. The most prevalent concurrent conditions were involving the endocrine/metabolic system (134 participants, 72.8%), hypertension (104 participants, 56.5%), and gastrointestinal system (62 participants, 33.7%). Overall, there was no statistically significant difference among the treatment groups in terms of age, gender, body weight, BMI, race, and disease history.

Efficacy
The primary efficacy endpoint was the change from baseline in HbA1c after 12 weeks of treatment. At week 12, participants treated with placebo showed a slight increase in HbA1c with least squares (LS)-mean change of 0.07%, while participants treated with R-Vera showed a reduction in HbA1c and the LS-mean changes from baseline were $-0.18\%$, $-0.28\%$, and $-0.37\%$ for the 150 mg/day, 300 mg/day, and 450 mg/day groups, respectively. Statistically significant reductions in
HbA1c were observed at the dose of 300 mg/day ($P = 0.0373$) and 450 mg/day ($P = 0.0098$) when compared with placebo.

At week 12, 20.0%, 29.5%, and 27.3% of participants in the R-Vera 150 mg/day, 300 mg/day, and 450 mg/day groups, respectively, achieved the HbA1c goal of < 7.0%. In the placebo group, this goal was reached by 6.5% of participants. Significant difference was found between the placebo and R-Vera pooled treatment groups ($P = 0.0222$).

Treatment effects on HbA1c were also analyzed across subgroups defined by age, baseline BMI, HbA1c, HOMA2-β, HOMA2-IR, blood glucose, blood pressure, and duration of diabetes. The subgroup analysis results suggested that participants with higher baseline HbA1c (HbA1c ≥ 8.0%), worse β-cell function (HOMA2-β < median), and higher FPG level (> 130 mg/dL) had greater HbA1c reductions from baseline when treated with R-Vera compared with placebo (Fig. 2).

Treatment with medium to high doses of R-Vera resulted in clear reductions from baseline in FPG compared with placebo at week 12 (Table 2), although the difference among treatment groups was not statistically significant. The time course of FPG reduction was generally stable from 4 weeks through 12 weeks of the treatment period. Similar to changes in HbA1c observed after 12 weeks of treatment, the subgroup analysis results suggested that participants with higher baseline HbA1c (HbA1c ≥ 8.0%) and worse β-cell function (HOMA2-β < median) had better response to R-Vera compared with placebo as an add-on to metformin therapy in terms of FPG reduction (data not shown).

During the study treatment period, no participant required rescue medication, and only one (2.2%) participant from the placebo group met the discontinuation criteria, due to a FPG level of 260 mg/dL or greater for 2 consecutive measurements.

At baseline, participants in the R-Vera treatment groups had better β-cell function with higher HOMA2-β score relative to the placebo group and the difference was statistically significant (R-Vera pooled vs placebo: 59.00 vs 44.46, $P = 0.0243$). After 12 weeks of treatment, participants in the R-Vera treatment groups still showed greater increase in HOMA2-β from baseline compared with participants in the placebo group, although the difference was not significant (Table 2). In contrast, HOMA score for insulin resistance (HOMA2-IR) did not show any indicative changes in either the placebo group or the R-Vera groups (Table 2).

Cardiovascular risk factors including total cholesterol, triglycerides, LDL, HDL, and body weight were explored in this study (Table 2). The lipoprotein panel and body weight results showed that inter-group comparison of the changes from baseline to week 12 did not exhibit any statistically significant difference. The results indicated that R-Vera treatment did not result in any gain of body weight.

A total of 151 participants consented to participate in the drug exposure-response assessment: 35 in the placebo group, 38 in the 150 mg/day group, 43 in the 300 mg/day group, and 35 in the 450 mg/day group. The pharmacokinetic-pharmacodynamic evaluation of R-Vera revealed that after 12 weeks of treatment, the plasma concentration of R-Vera increased dose proportionally in participants who took R-Vera, and HbA1c reductions were also observed in the R-Vera treatment groups with the highest reduction occurring in the 300 mg/day group. At the end of treatment, the baseline-adjusted $C_{\text{trough}}$ of R-Vera for 150 mg/day, 300 mg/day, and

| Table 1. Baseline demographic and disease characteristics$^a$ |
|-----------------------------------------------------------|
| Characteristics                                        | Placebo (n = 46) | R-Vera 150 mg (n = 46) | R-Vera 300 mg (n = 47) | R-Vera 450 mg (n = 45) |
|-----------------------------------------------------------|
| Age, y                                                   | 54.7 (10.26)     | 55.2 (8.91)             | 58.6 (8.50)             | 58.8 (9.51)             |
| Sex, n (%)                                               | 24 (52.2%)       | 22 (47.8%)              | 27 (57.4%)              | 27 (60.0%)              |
| Male                                                     | 22 (47.8%)       | 24 (52.2%)              | 20 (42.6%)              | 18 (40.0%)              |
| Female                                                   | 0 (0.0%)         | 3 (6.5%)                | 3 (6.4%)                | 3 (6.7%)                |
| Race, n (%)                                              | 25 (54.3%)       | 26 (56.5%)              | 26 (55.3%)              | 25 (55.6%)              |
| African American                                         | 0 (0.0%)         | 3 (6.5%)                | 3 (6.4%)                | 3 (6.7%)                |
| Asian                                                    | 20 (43.5%)       | 16 (34.8%)              | 18 (38.3%)              | 17 (37.8%)              |
| Caucasians                                               | 1 (2.2%)         | 1 (2.2%)                | 0 (0.0%)                | 0 (0.0%)                |
| Region, n (%)                                            | 21 (45.7%)       | 20 (43.5%)              | 21 (44.7%)              | 20 (44.4%)              |
| United States                                            | 25 (54.3%)       | 26 (56.5%)              | 26 (55.3%)              | 25 (55.6%)              |
| Body weight, kg                                          | 80.8 (19.78)     | 80.3 (17.69)            | 73.1 (17.07)            | 80.1 (20.03)            |
| BMI, kg/m$^2$                                            | 29.5 (6.04)      | 29.0 (4.58)             | 27.0 (4.68)             | 29.6 (6.29)             |
| Duration of diabetes, yr                                 | 6.4 (4.98)       | 5.9 (6.48)              | 8.3 (6.95)              | 6.4 (4.83)              |
| HbA1c, n (%)                                             | < 8.0%           | 29 (63%)                | 26 (57%)                | 27 (57%)                | 26 (58%)                |
| ≥ 8.0%                                                   | 17 (37%)         | 20 (43%)                | 20 (43%)                | 19 (42%)                |
| HOMA2-β, n (%)                                           | < Median$^b$     | 24 (57%)                | 17 (40%)                | 21 (48%)                | 23 (55%)                |
| ≥ Median$^b$                                             | 18 (43%)         | 25 (60%)                | 23 (52%)                | 19 (45%)                |

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; HOMA2-β, homeostasis model 2 assessment β-cell; R-Vera, R-form verapamil.

$^a$Data are presented in mean ± SD unless otherwise indicated.

$^b$Baseline HOMA2-β median = 45.75%.
The changes in HbA1c from baseline were −0.23%, −0.40%, and −0.34%.

Safety and Tolerability

In total, 44 (23.9%) participants experienced at least one treatment-emergent adverse event (TEAE), as summarized in Table 3, among which 32 (23.2%) participants treated with R-Vera, 12 (26.1%) participants treated with placebo, 1 (0.5%) participant had Grade 3 TEAE, 9 (4.9%) participants had treatment-related AEs, and no participants had serious adverse events (SAEs). There was only one participant from the placebo group who experienced Grade 3 TEAE. This TEAE was increased blood triglycerides was considered unlikely related to the study drug. The participant recovered and continued the study treatment without dose change. The number of treatment-related AEs increased as R-Vera dose levels increased, and 3 out of 6 events in the 450 mg/day group were cardiac disorders, including palpitations, ventricular extrasystoles, and prolonged QT. No participants experienced TEAEs of hypotension or hypoglycemia during the study period. There were no suspected unexpected serious adverse reactions (SUSARs) or deaths reported in this study.

It was also noticed that the reported numbers of urinary tract infection (UTI) were 6 in the placebo group, and 3, 2, 1 in participants treated with R-Vera 150 mg/day, 300 mg/day, and 450 mg/day, respectively. The result showed much lower incidence of urinary tract infection in the R-Vera treatment groups compared with the placebo group (R-Vera pooled vs placebo: 4.3% vs 13.0%, P = 0.0388).

Discussion

There has been one systematic review of the beta cell effects of verapamil-based treatment administered either orally or via injection in patients with T2DM. The dosage of oral verapamil alone administration ranged from 80 mg/TID to 270 mg/day in 7 studies, and the treatments in these studies significantly lowered plasma glucose but failed to significantly affect HbA1c values (8). The present phase II clinical trial was the first randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of the combination therapy of R-Vera (150, 300, and 450 mg/day) plus metformin in patients with T2DM who had inadequate glycemic control on metformin alone. Effectively reduced HbA1c level, a greater reduction in FPG, as well as a larger proportion of participants achieving the HbA1c goal of < 7.0% when compared with the placebo group were revealed after 12 weeks of R-Vera in combination with metformin. Further evaluation of efficacy with subgroup analysis demonstrated that participants treated with R-Vera 300 mg/day had the greatest reduction in HbA1c as compared with placebo-treated participants. In terms of safety profile, the number of treatment-related AEs increased as R-Vera dose levels increased. Taken together, the present phase II trial revealed R-Vera plus metformin led to better reduction of HbA1c than previous verapamil-based treatments, and R-Vera 300 mg/day demonstrated a favorable safety profile and substantial clinical activity in participants with T2DM and can be considered as an appropriate dose for further efficacy confirmatory studies.

In this study, the effect of R-Vera on pancreatic beta cell preservation was evaluated by change in HOMA2-β from baseline. After 12 weeks of treatment, participants in the R-Vera treatment groups showed a trend of increase in HOMA2-β from baseline compared with participants in the placebo group, despite the fact that participants in the

450 mg/day were 29.54 ng/mL, 53.28 ng/mL, and 60.28, respectively, and the changes in HbA1c from baseline were −0.23%, −0.40%, and −0.34%.

Safety and Tolerability

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R-Vera treatment groups already had higher HOMA2-β score relative to participants in the placebo group at baseline (Table 2). Apoptosis is the primary mechanism underlying beta cell death in both T1DM and T2DM (10), and beta cell death may be induced by thioredoxin-interacting protein (TXNIP). TXNIP was first identified as the most upregulated

| Parameters                  | Placebo | R-Vera 150 mg | R-Vera 300 mg | R-Vera 450 mg |
|-----------------------------|---------|---------------|---------------|---------------|
| HbA1c%                      | (n = 46) | (n = 45)      | (n = 44)      | (n = 44)      |
| Baseline                    | 7.82 (0.8) | 7.91 (0.9)    | 7.94 (0.8)    | 7.72 (0.8)    |
| Change from baseline        | 0.07 (0.120) | -0.18 (0.123) | -0.28 (0.125) | -0.37 (0.123) |
| Difference vs placebo       | -0.25    | -0.36^        | -0.45^        |               |
| FPG, mg/dL                  | (n = 46) | (n = 45)      | (n = 44)      | (n = 45)      |
| Baseline                    | 159.7 (37.3) | 151.8 (36.4)  | 168.9 (64.0)  | 152.1 (35.3)  |
| Change from baseline        | -2.9 (5.4) | 0.6 (5.5)     | -13.4 (5.7)   | -13.2 (5.5)   |
| Difference vs placebo       | 3.6      | -10.5         | -10.3         |               |
| HOMA2-β, %                  | (n = 41) | (n = 41)      | (n = 40)      | (n = 41)      |
| Baseline                    | 44.5 (21.7) | 62.9 (40.0)   | 57.2 (33.5)   | 57.0 (36.3)   |
| Change from baseline        | 4.6 (5.8) | 12.0 (5.7)    | 9.0 (5.8)     | 18.0 (5.8)    |
| Difference vs placebo       | 7.5      | 4.4           | 13.5          |               |
| HOMA2-IR, %                 | (n = 41) | (n = 41)      | (n = 40)      | (n = 41)      |
| Baseline                    | 1.8 (1.1) | 2.4 (1.7)     | 2.7 (2.6)     | 2.2 (1.4)     |
| Change from baseline        | -0.0 (0.2) | 0.2 (0.2)    | -0.3 (0.2)    | 0.1 (0.2)     |
| Difference vs placebo       | 0.3      | -0.3          | 0.1           |               |
| Total cholesterol, mg/dL    | (n = 46) | (n = 45)      | (n = 44)      | (n = 45)      |
| Baseline                    | 182.2 (39.5) | 168.8 (32.5) | 168.4 (37.2) | 172.4 (40.2) |
| Change from baseline        | -3.6 (3.7) | 5.7 (3.6)     | 0.1 (3.7)     | 1.3 (3.6)     |
| Difference vs placebo       | 9.4      | 3.7           | 4.9           |               |
| Triglycerides, mg/dL        | (n = 46) | (n = 45)      | (n = 44)      | (n = 45)      |
| Baseline                    | 179.7 (101.9) | 174.9 (98.9) | 155.9 (87.3) | 183.4 (115.7) |
| Change from baseline        | -11.9 (9.7) | 10.1 (9.9)    | -20.2 (10.1) | -10.3 (9.9)   |
| Difference vs placebo       | 22       | -8.3          | 1.6           |               |
| LDL, mg/dL                  | (n = 46) | (n = 46)      | (n = 46)      | (n = 44)      |
| Baseline                    | 106.5 (30.7) | 95.5 (30.3)   | 95.6 (30.7)   | 96.6 (32.3)   |
| Change from baseline        | -4.3 (3.2) | 1.6 (3.2)     | -0.3 (3.2)    | 0.3 (3.2)     |
| Difference vs placebo       | 5.92     | 4.0           | 4.6           |               |
| HDL, mg/dL                  | (n = 46) | (n = 45)      | (n = 44)      | (n = 45)      |
| Baseline                    | 47.5 (11.3) | 44.0 (9.4)    | 45.8 (8.8)    | 44.4 (9.1)    |
| Change from baseline        | 0.7 (1.1) | 0.6 (1.1)     | 0.7 (1.1)     | 1.5 (1.1)     |
| Difference vs placebo       | -0.1     | 0.0           | 0.8           |               |
| Body weight, kg             | (n = 46) | (n = 45)      | (n = 44)      | (n = 45)      |
| Baseline                    | 80.8 (19.8) | 80.3 (17.7)   | 73.1 (17.1)   | 80.1 (20.0)   |
| Change from baseline        | -0.4 (0.4) | -0.1 (0.4)    | -0.4 (0.4)    | 0.3 (0.4)     |
| Difference vs placebo       | 0.3      | 0.0           | 0.7           |               |
| SBP, mmHg                   | (n = 39) | (n = 43)      | (n = 42)      | (n = 41)      |
| Baseline                    | 132.9 (13.2) | 133.6 (11.7)  | 131.9 (10.2)  | 133.6 (13.5)  |
| Change from baseline        | -0.3 (1.5) | -4.6 (1.5)    | -2.0 (1.5)    | -3.1 (1.5)    |
| Difference vs placebo       | -4.3^    | -1.7          | -2.7          |               |
| DBP, mmHg                   | (n = 39) | (n = 43)      | (n = 42)      | (n = 41)      |
| Baseline                    | 79.0 (9.1) | 80.7 (8.4)    | 77.6 (8.2)    | 77.6 (9.4)    |
| Change from baseline        | 0.7 (1.1) | -3.6 (1.1)    | -0.5 (1.1)    | -3.6 (1.1)    |
| Difference vs placebo       | -4.2^    | -1.2          | -4.3          |               |

Abbreviations: DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; HOMA2-β, homeostasis model 2 assessment β-cell; HOMA2-IR, homeostasis model 2 assessment of insulin resistance; LDL, low-density lipoprotein; R-Vera, R-form verapamil; SBP, systolic blood pressure.

*Baseline data are mean ± SD unless otherwise indicated.

*Changes from baseline data are LS-mean ± standard error unless otherwise indicated.

*P < 0.05 compared with placebo.
gene in an oligonucleotide microarray study investigating the effects of glucose on isolated human pancreatic islets (11). As a negative regulator of thioredoxin, TXNIP exerts its proapoptotic effects on beta cells by inhibiting thioredoxin and inducing oxidative stress, which is a key element in beta cell glucotoxicity and apoptosis. Importantly, TXNIP is highly regulated by alterations in blood glucose levels (12-14). Calcium channels on beta cells participate in insulin secretion, beta cell physiology, and pathophysiology. As a calcium channel blocker, R-Vera was found to inhibit TXNIP expression and may have mediated a TXNIP-lowering effect on beta cells in our preclinical study (15). In a streptozotocin-induced DM mouse model, TXNIP upregulation and beta cell apoptosis were significantly increased in the islet tissues as compared with the normal mice, and the effect of streptozotocin could be reversed by the administration of 100 mg/kg/day R-Vera for 10 days, which demonstrated significantly decreased TXNIP expression and reduced beta cell apoptosis in the islet in the R-Vera treatment group as compared with the vehicle group (15). The result of improvement in HOMA2-β score in the participants treated with R-Vera in the current study is also consistent with the report by A. Shalev et al (16) that oral verapamil added to a standard insulin regimen for 12 months in adult participants with recent-onset type 1 diabetes showed less increase in insulin requirement, fewer hypoglycemic events, and promotion of endogenous beta cell function, when compared with placebo.

A previous study of the effects of verapamil on cardiovascular responses in normotensive volunteers did not find significant decrease of systolic and diastolic blood pressure at rest during the use of verapamil (3 × 40 mg/day, 3 × 80 mg/day, 3 × 120 mg/day) (17). In addition, verapamil did not induce a change in blood pressure during exercise. Similarly, in a study of the effect of sustained-released verapamil (240 mg/day) on graded endurance exercise in 12 healthy male volunteers, systolic blood pressure (SBP) was not lowered by verapamil, both at rest and during graded exercise (18). In the present study, when stratified by the cutoff point of baseline SBP at 140 mmHg, R-Vera treatment groups showed greater reduction in SBP in week 12 compared with the placebo group for participants with baseline SBP ≥ 140 mmHg and the LS-mean changes from baseline were −5.9, −14.4, −10.5, and −13.4 mmHg for the placebo, 150 mg/day, 300 mg/day, and 450 mg/day treated groups, respectively. This reduction in SBP was not observed in the participants with normal blood pressure whose baseline SBP was less than 140 mmHg. The LS-mean changes were 2.0, −1.0, 1.4, and 0.3 mmHg for placebo, 150 mg/day, 300 mg/day, and 450 mg/day treated groups respectively. Also, no participants experienced hypotension events during the study period. According to the USA Food and Drug Administration guidance for hypertension indication drug labeling, lowering blood pressure reduces the risk of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions. The R-Vera effect on lowering high SBP may provide additional treatment benefits to T2DM patients with concurrent cardiovascular disease.

The primary limitation to generalization of the present result is the relatively small number of enrolled participants and restricted treatment duration (i.e., 12 weeks). A larger number of participants and longer treatment period (for example, 26 weeks or longer) should be considered in future trials to determine whether the beneficial effects can be sustained with (or without) continuous R-Vera treatment. Another limitation of this study was that the study groups were not well balanced in terms of their baseline beta cell function.

### Table 3. Summary of overall safety and selected adverse events over 12 weeks

| Participants, n (%) | Placebo (n = 46) | R-Vera 150 mg (n = 46) | R-Vera 300 mg (n = 47) | R-Vera 450 mg (n = 45) |
|--------------------|-----------------|-----------------------|-----------------------|-----------------------|
| Any AEs            | 12 (26.1)       | 10 (21.7)             | 11 (23.4)             | 11 (24.4)             |
| AEs leading to discontinuation | 0               | 0                     | 1 (2.1)               | 3 (6.7)               |
| AEs related to study drug* | 0               | 1 (2.2)               | 2 (4.3)               | 6 (13.3)              |
| Serious AEs        | 0               | 0                     | 0                     | 0                     |
| Selected AEs       | 0               | 0                     | 0                     | 0                     |
| Hypotension        | 0               | 0                     | 0                     | 0                     |
| Hypoglycemia       | 0               | 0                     | 0                     | 0                     |
| Gastrointestinal disorders |                  |                       |                       |                       |
| Abdominal pain upper | 1 (2.2)          | 0                     | 0                     | 0                     |
| Constipation       | 0               | 1 (2.2)               | 1 (2.2)               | 0                     |
| Abnormal change in 12-lead ECG |                |                       |                       |                       |
| Ventricular extrasystoles | 0              | 0                     | 0                     | 1 (2.1)               |
| Prolonged QT       | 0               | 0                     | 0                     | 1 (2.1)               |
| AEs with frequency ≥ 5% in any group |            |                       |                       |                       |
| Urinary tract infection | 6 (13.0)         | 3 (6.5)               | 2 (4.3)               | 1 (2.2)               |
| Dizziness          | 0               | 0                     | 0                     | 3 (6.7)               |

**Abbreviations:** AE, adverse events; R-Vera, R-form verapamil.

*Possibly, probably, or very likely related to study drug, as assessed by investigators.
Participants in the R-Vera treatment groups appeared to show higher HOMA2-β score relative to the placebo group (R-Vera pooled vs placebo: 59.00 vs 44.46, \(P = 0.0243\)) and the difference was statistically significant. This may in turn result in nonsignificant efficacy of R-Vera on the beta cell function improvement. A well-planned randomization and stratification strategy should be considered in future research.

In conclusion, oral administration of R-Vera twice daily in combination with metformin improved glycemic control in T2DM patients by reducing HbA1c and FPG levels, as well as helped greater proportion of patients achieve the HbA1c goal of < 7.0%. In addition, treatment with R-Vera was generally well tolerated. The favorable efficacy and safety profile of R-Vera 300 mg/day, combined with the potential for beta cell preservation and reduction in high blood pressure, make R-Vera a promising new antidiabetic medication.

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J.P.H. and G.T.S. are employed by Center Laboratories Inc. A.C.C. and Y.C.W. are former employees of Center Laboratories Inc. and are currently employed by Lumosa Therapeutics Co., which is an affiliated company to Center Laboratories Inc. All the other authors declare that they have no conflicts of interest.

Clinical Trial Information

Clinical Trial Registration Number: NCT03317028

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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