The Use of Low-Dose Carvedilol to Improve Hypoglycemia Awareness in Patients with T1DM

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IRB #00108879

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Investigational Agent	Carvedilol
IND Number	[IND number issued by FDA, if applicable]

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LIST OF ABBREVIATIONS

AE	Adverse Event
ANOVA	Analysis of Variance
BMI	Body Mass Index
BP	Blood Pressure
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CGM	Continuous Glucose Monitoring
CIOMS	Council for International Organizations of Medical Science
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DCC	Data Coordinating Center
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ERICA	Electronic Research Integrity and Compliance Administration System
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart Rate
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NIH	National Institutes of Health
PI	Principal Investigator
SAE	Serious Adverse Event
VMH	Ventromedial Hypothalamus

STATEMENT OF COMPLIANCE

I confirm that I have read this protocol, and I will conduct the study as outlined herein and according to the ethical principles stated in the latest version of the Declaration of Helsinki, the U.S. code of federal regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312 and/or 21 CFR Part 812), and the applicable ICH guidelines for Good Clinical Practice (GCP).

I will promptly submit the protocol to the IRB for review and approval. Once the protocol has been approved by the IRB, I understand that any modifications made during the course of the study must first be approved by the IRB prior to implementation except when such modification is made to remove an immediate hazard to the subject.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study treatment, the conduct of the study, and the obligations of confidentiality.

Note: This document is signed electronically through submission and approval by the Principal Investigator in the University of Utah IRB Electronic Research Integrity and Compliance Administration (ERICA) system.

STUDY SUMMARY

Title	The Use of Low-Dose Carvedilol to Improve Hypoglycemia Awareness in Patients with T1DM
Short Title	Carvedilol for Hypoglycemia Unawareness
Protocol Number	IRB #: 00108879
IND	Exemption Approved
Phase	Phase II
Design	Double-blinded, randomized, placebo-controlled study.
Study Duration	From Fall 2018 to Spring 2020
Study Center(s)	University of Utah Health
Objectives	The aim of the study is to investigate whether low-dose carvedilol treatment can improve hypoglycemia awareness and the counterregulatory hormone responses to hypoglycemia in Type 1 diabetic patients with impaired awareness of hypoglycemia.
Number of Subjects	A total of 21 Type 1 diabetic patients with impaired awareness of hypoglycemia will be recruited for the study – 7 subjects will be treated with placebo and 7 subjects will be treated with one of two different doses of carvedilol (2.5 or 3.125mg).
Diagnosis and Main Eligibility Criteria	Patients with Type 1 diabetes and hypoglycemia unawareness will be included in this study. <u>Inclusion criteria:</u> • Type 1 diabetes of >5yrs duration • >18yrs of age • Impaired hypoglycemia awareness/ unawareness assessed by validated questionnaires. • Intensive insulin treatment as defined by multiple daily insulin injections (3 or more) or insulin pump therapy. • Negative pregnancy test. • Signed informed consent. <u>Exclusion Criteria:</u> • Major medical disorders (including liver disease, cardiovascular disease, kidney disease, COPD, asthma, active malignancy and HIV).

	• Overt diabetic complications (neuropathy, nephropathy, retinopathy). • Anemia. • Current or recent use of beta-blocker therapy. • Use of diuretics. • Allergies or contraindications to beta-blockers or heparin. • Pregnant. • Use of benzodiazepines. • Alcohol, drug or medication abuse. • Frequent use of acetaminophen.
Study Product, Dose, Route, Regimen	Hypoglycemia unaware T1DM subjects will be randomly segregated into one of two therapeutic treatment groups: Placebo or Carvedilol. Carvedilol will be administered orally at either 2.5mg or 3.125mg twice daily. Likewise, the placebo drug will also be administered orally twice daily.
Duration of Administration	4 weeks
Reference Therapy	Placebo
Statistical Methodology	Treatment effects will be analyzed using one- or two-way analysis of variance (ANOVA) for independent or repeated measures as appropriate, followed by suitable *post-hoc* analysis. Analyses will be performed using the GraphPad Prism® or SAS® suite of software.

STUDY SCHEMA

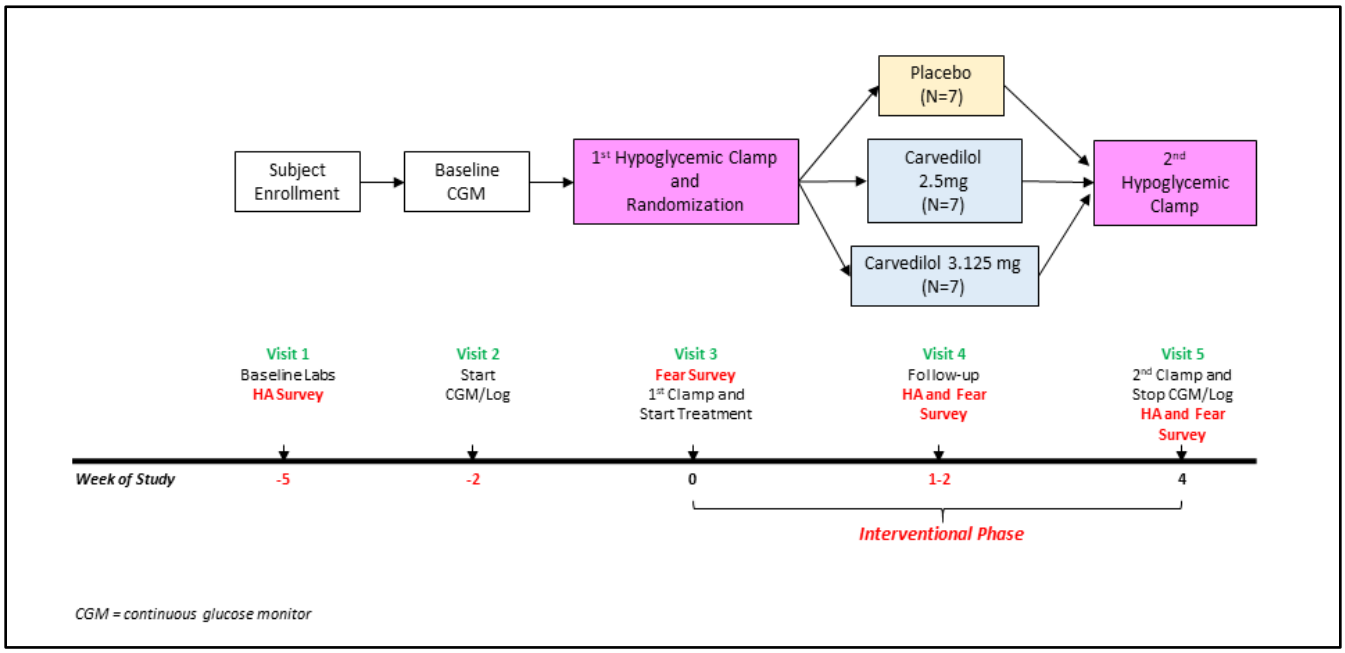


Figure 1: Schematic of the study design.

1. OBJECTIVES

1.1 Primary Objectives and Endpoints

- To investigate whether T1DM patients with impaired awareness of hypoglycemia receiving carvedilol treatment will show marked improvements in hypoglycemia symptomatic awareness scores, as well as improvements in their counterregulatory hormone responses to hypoglycemia.

1.2 Secondary Objectives and Endpoints

- To investigate whether carvedilol treatment reduces the amount of time T1DM patients with hypoglycemia unawareness spend in the hypoglycemic range as assessed by blinded CGM.
- To investigate whether carvedilol reduces the fear of hypoglycemia.

2 BACKGROUND

Hypoglycemia elicits a multifaceted hormonal response that helps restore glycemic levels to normal. As blood glucose levels start to fall, insulin secretion ceases. At the top of this hierarchy of counterregulatory responses are glucagon and epinephrine which are the two principal hormones that act rapidly to increase glucose production and inhibit glucose utilization to raise plasma glucose levels back to normal[1]. In cases of prolonged and/or more severe hypoglycemia, growth hormone and cortisol are mobilized to stimulate the synthesis of gluconeogenic enzymes and inhibit glucose utilization[2]. In non-diabetic individuals, glucagon and epinephrine are usually very effective and the latter responses are rarely required in the acute situation. In contrast, impaired glucose counterregulation presents itself in longstanding diabetes and with antecedent hypoglycemia[3]. Within the first five years after the onset of Type 1 diabetes (T1DM), the primary defense against hypoglycemia, the release of glucagon, either becomes significantly attenuated or is completely absent in diabetic patients and this impairment appears to be specific for the stimulus of hypoglycemia[4,5]. Hence, patients with diabetes primarily depend on the release of epinephrine as their main defense against hypoglycemia. Unfortunately, with longer duration of diabetes and especially with poor glycemic control, epinephrine secretion is also compromised, making these patients even more vulnerable to the threat of hypoglycemia[5-7]. In patients with diabetes, hypoglycemia arises from the interplay of a relative excess of exogenous insulin and defective glucose counterregulation and it remains a limiting factor in attaining proper glycemic management. Both the Diabetes Control and Complications Trial (DCCT) conducted in Type 1 patients[8] and the United Kingdom Prospective Diabetes Study (UKPDS) conducted in Type 2 patients[9], have established the importance of maintaining good glucose control over a lifetime of diabetes to avoid cardiovascular, renal and neurological complications. However, lowering glycemic goals for diabetic patients increases their risk for hypoglycemia exposure. According to the DCCT, T1DM patients put on intensive insulin therapy, though having improved outcomes for diabetic complications, are at a 3-fold higher risk of experiencing severe hypoglycemia compared to those on conventional insulin therapy[10]. Moreover, recent antecedent hypoglycemia reduces autonomic (epinephrine) and symptomatic (which normally

prompts behavioral defenses such as eating) responses to subsequent bouts of hypoglycemia[11-14]. Thus begins the vicious cycle of recurrent hypoglycemia (RH) where hypoglycemia leads to further impairment of counterregulatory responses which in turn, begets more hypoglycemia and so forth. Because of the imperfections of current insulin therapies, those patients attempting to achieve glycemic control suffer an untold number of asymptomatic hypoglycemic episodes. Current estimates of symptomatic hypoglycemic episodes range from 2-3 incidences per week on average and severe, debilitating episodes occur once or twice each year. Therefore, developing therapies to prevent or eliminate hypoglycemia is of great importance.

Sensors that detect changes in blood glucose levels and initiate glucose counterregulatory responses have been identified in both the periphery[15-20] and within the brain[21-29]. These sensors have been localized to the hepatic portal vein[17,18], the carotid body[30] and the brain[21,22,24,31]. In the brain, the dominant sensors are located in the hypothalamus. While peripheral glucose sensors may play a role in mediating the immediate counterregulatory responses to hypoglycemia, it is thought that glucosensors located within the brain may have a redundant regulatory and/or modulatory role in regulating glucose counterregulatory responses. It is well established that brain glucose sensors are crucial for detecting falling blood glucose levels and for initiating counterregulatory responses[22,31]. These sensors are located in the hindbrain, the lateral hypothalamus, the paraventricular nucleus, the dorsal hypothalamus and the ventromedial hypothalamus (VMH)[22,27,32-34]. The neurons within the VMH contain much of the same glucose sensing machinery as the pancreatic β-cells[29]. To date, two main types of glucose sensing neurons have been identified in the brain – those that increase their firing rate in response to increases in glucose levels, the "glucose-excited" or GE neurons and those that decrease their firing rate in response to increasing glucose levels, the "glucose-inhibited" or GI neurons[35-38]. The mechanism by which GE neurons sense changes in blood glucose concentrations is believed to be similar to that used by pancreatic β-cells[39] whereas GI neurons respond to decreases in ambient glucose levels through activation of the metabolic fuel sensor, AMP kinase (AMPK), and closure of chloride channels that result in increased activity of GI neurons[28,40]. Although many of these sensing components have been identified, it is still not entirely clear how glucose sensing neurons regulate counterregulatory hormone release. It has been proposed that alterations in the firing rates of VMH glucose sensing neurons in response to glucose or fuel deficits can inhibit (as is the case for GE neurons) or stimulate (as is the case for GI neurons) the exocytosis of vesicles containing neurotransmitters that can modulate the counterregulatory hormone response[39,41,42].

The inhibitory neurotransmitter, GABA, and the stimulatory neurotransmitters, glutamate and norepinephrine, act within the VMH to suppress or stimulate the counterregulatory responses to hypoglycemia, respectively. In response to an initial bout of hypoglycemia, VMH GABA levels decrease while glutamate and norepinephrine levels increase, allowing for activation of the counterregulatory hormone responses[43-47]. While these studies underscore the importance of VMH neurotransmitter signaling in regulating glucose homeostatic mechanisms, to date, the mechanisms that lead to their dysregulation in models of counterregulatory failure are not entirely clear. Recent evidence suggests that lactate, which serves as an alternate fuel substrate in the brain, plays an important role in precipitating the defects noted above[48,49]. Lactate produced from neighboring astrocytes can supplement higher energy requirements during periods of increased neuronal activity or when glucose supply is limited[50]. If this is

the case, then lactate can be used in place of glucose as a fuel for VMH glucose sensing neurons, preventing them from detecting a fall in glucose levels, causing (inhibitory) GABA tone to be enhanced and (stimulatory) glutamate output to be reduced. Together, these actions ultimately suppress the release of counterregulatory hormones. In recent years, it has been shown that lactate levels are sensed by the brain and more specifically, can act in the hypothalamus to regulate glucose homeostasis, appetite and body weight[51-56]. Lactate prevents the activation of hypothalamic neurons during glucose deprivation[51,57] and more pertinent to this application, attenuates glucose counterregulatory responses to hypoglycemia when locally administered into the VMH[58]. Data from our research group revealed VMH extracellular lactate concentrations are elevated in RH and diabetic animals and in particular, these conditions also increase expression of the lactate transporter, MCT2, in the VMH[48]. When VMH lactate uptake is pharmacologically inhibited using 4CIN, we can improve neurotransmitter and counterregulatory responses in both RH and diabetic animals, suggesting lactate plays an important role in dysregulating neurotransmitter systems in the VMH, which in turn, impairs counterregulatory responses. *Our preliminary data suggests that therapeutic strategies that can reduce brain lactate levels, may help restore hypothalamic glucose sensing mechanisms and the counterregulatory response to hypoglycemia.* Hence, identifying the mechanisms that increase VMH lactate levels may lead to suitable therapeutic strategies to prevent hypoglycemia.

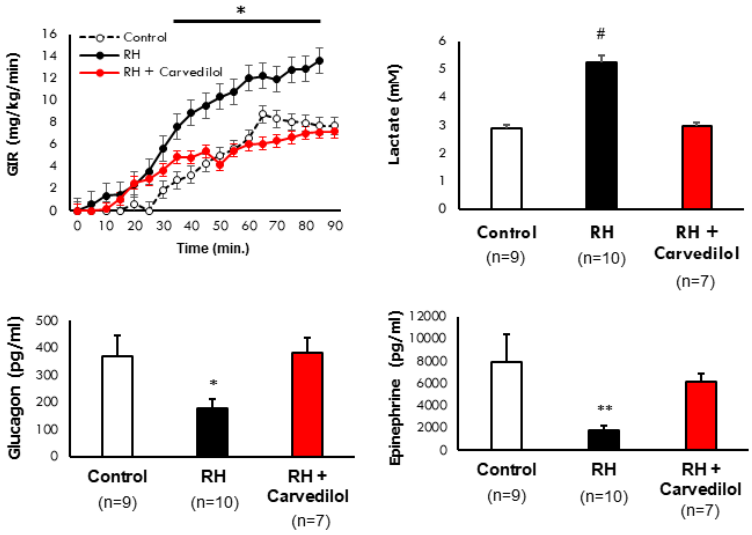
To this end, we investigated one mechanism that can enhance lactate production and/or utilization in the brain - activation of norepinephrine (NE) receptors. Norepinephrine can enhance lactate production from astrocytes and it can also increase the uptake of lactate into neurons through the activation of β2-adrenergic receptors (β2AR), potentially helping to co-ordinate both the supply and uptake of lactate into neurons [59-61]. Normally, in response to an acute bout of hypoglycemia, VMH norepinephrine levels rise and act through β2ARs to enhance the sympathoadrenal response[46,62,63]. Although activation of VMH β2ARs augments the counterregulatory response during *acute* bouts of hypoglycemia, less is known about the effects of RH on this neurotransmitter system. It has been reported that VMH NE levels are not altered by successive bouts of hypoglycemia, suggesting activation of the VMH NE system is not dampened by RH and that its suppressive effects on counterregulatory hormone release may lie downstream of NE release [64]. In support of this finding, *adrenergic blockade during antecedent bouts of hypoglycemia was shown to prevent counterregulatory failure* in healthy human subjects[65]. Therefore, while *acute* activation of VMH adrenergic receptors may be beneficial in its capacity to enhance the counterregulatory response, *repeated* activation of this neurotransmitter system may contribute to counterregulatory failure, but the mechanism(s) by which this occurs have not been fully identified.

To evaluate whether repeated activation of the VMH NE system contributes to counterregulatory failure, we locally microinjected NE into the VMH of non-diabetic, hypoglycemia-naïve rats for 3hrs/d for 3 consecutive days before subjecting the animals to a hypoglycemic glucose clamp on day 4 (Figure 1). Repeated activation of the VMH NE system in the absence of hypoglycemia, increased VMH lactate levels and more importantly, blunted the counterregulatory hormone responses to hypoglycemia. This phenomenon was recapitulated with microinjection of salbutamol, a short-acting β2AR agonist, into the VMH using the same protocol as for NE, suggesting the suppressive effects of NE are mediated through VMH β2ARs. In a subgroup of animals treated with NE, we administered 4CIN into the VMH to block

the uptake of lactate into neurons immediately prior to the hypoglycemic clamp. In this group, we saw the suppressive effects of NE treatment on glucose counterregulation were completely abolished. Hence, our preliminary data suggests that *repeated activation of the VMH NE system plays a role in the development of counterregulatory failure, in part by enhancing central lactate production and therefore, the use of β-adrenergic blockers may be a promising treatment to preserve the responses to hypoglycemia*. As proof-of-concept, our preliminary data shows that RH rats treated with low doses of the non-specific β-blocker, carvedilol (3mg/kg, *intraperitoneally*), during the induction of RH, required less exogenous glucose during the hypoglycemic clamp compared to RH animals treated with vehicle (Figure 2). More importantly, we also saw reductions in VMH lactate levels and significant improvements in the counterregulatory hormone responses to hypoglycemia in the carvedilol-treated RH animals.

It is important to note, however, that treatment of diabetic patients with β-blockers has been somewhat controversial due to its perceived potential to attenuate the responses to hypoglycemia. However, compelling clinical evidence to support this possibility is lacking. In the recent GEMINI study which evaluated the use of two different β-blockers in T2DM patients with hypertension, carvedilol, was shown to effectively reduce overall hypoglycemia burden scores[66]. Likewise, the vast majority of clinical studies show that treatment of non-diabetic and T1DM patients with β-blockers *improved* counterregulatory hormone responses *without* increasing the frequency of hypoglycemia or hypoglycemia unawareness[67-72], although loss of tremulousness and reduced tachycardia were reported in some studies. One study found that treatment of T1DM hypoglycemia aware patients with propanolol reduced glycemic thresholds for adrenergic symptoms, but this deficit was offset by *higher hypoglycemia symptom scores* stemming from *augmented* diaphoresis[73]. Other neuroglycopenic symptoms were *not* affected. Moreover, in a prospective study of 150 diabetic patients, 50 of whom were on β-blockers, no difference in the incidence of hypoglycemic unconsciousness was noted between those patients who were on β-blockers compared to those who were not, suggesting the use of β-blockers in T1DM patients does *not* increase the incidence of severe hypoglycemia [68] and may be a safe therapeutic option that can be used in conjunction with insulin. Therefore, the current application will evaluate the effectiveness of low-dose carvedilol as a treatment for restoring the counterregulatory hormone responses to hypoglycemia and improving hypoglycemia awareness in patients with Type 1 diabetes.

Figure 2: RH (black bars) raised VMH lactate and blunted the counterregulatory responses to hypoglycemia. Treatment with carvedilol reduced VMH lactate and improved the counterregulatory hormone responses (red bars). #P<0.0001 vs Control. *P<0.05 vs Control. **P<0.02 vs. Control.



Carvedilol is a third generation non-selective, vasodilating β-blocker, which is FDA-approved for the treatment of congestive heart failure and hypertension. Carvedilol mainly blocks β2- and β1-adrenergic receptors and some α1-adrenergic receptors. Due to its lipophilic nature, carvedilol readily crosses the blood-brain-barrier. As the brain is our primary target, this beneficial pharmacokinetic property of carvedilol improves CNS bioavailability, allowing lower doses to be used to deliver treatment to the brain. With lower doses, we can reduce the potential for side effects stemming from unnecessary exposure of peripheral tissues to high levels of β-adrenergic blockade Based on data gathered by the Food and Drug Administration (FDA), in a population study of 36,044 patients who reported having side effects while taking carvedilol, only ~1% of these patients experienced hypoglycemia. As carvedilol is currently FDA-approved for clinical use, it can potentially be repurposed for use as a preventive treatment for hypoglycemia. Therefore, we are proposing to evaluate the effectiveness of low-dose carvedilol treatment for 4 weeks as a treatment for restoring the counterregulatory hormone responses to hypoglycemia and improving hypoglycemia awareness in patients with Type 1 diabetes.

3 DRUG INFORMATION

In this study, carvedilol will be administered twice daily to the intervention group. Carvedilol is currently approved by the FDA for indications that are unrelated to the current study. The following is a summary of the FDA label for carvedilol which can be found in the following link: https://www.accessdata.fda.gov/drugsatfda_docs/label/2005/ 020297 s013lbl.pdf.

Carvedilol is a white to off-white powder with a molecular weight of 406.5 and a molecular formula of $C_{24}H_{26}N_2O_4$. Carvedilol is rapidly and extensively absorbed following oral administration, with absolute bioavailability of approximately 25% to 35% due to a significant degree of first-pass metabolism.

Following oral administration, the apparent mean terminal elimination half-life of carvedilol generally ranges from 7 to 10 hours. Plasma concentrations achieved are proportional to the oral dose administered. Carvedilol is more than 98% bound to plasma proteins, primarily with albumin. The plasma-protein binding is independent of concentration over the therapeutic range. Carvedilol is extensively metabolized. Less than 2% of the dose is excreted unchanged in the urine. The oxidative metabolites are further metabolized by conjugation via glucuronidation and sulfation. The metabolites of carvedilol are excreted primarily via the bile into the feces. Plasma concentrations of the active metabolites are about one-tenth of those observed for carvedilol and have pharmacokinetics similar to the parent. The primary P450 enzymes responsible for the metabolism of carvedilol in human liver microsomes are CYP2D6 and CYP2C9 and to a lesser extent CYP3A4, 2C19, 1A2, and 2E1. Since carvedilol undergoes substantial oxidative metabolism, the metabolism and pharmacokinetics of carvedilol may be affected by induction or inhibition of cytochrome P450 enzymes such as Rifampin, Cimetidine and Digoxin.

Indications for use of carvedilol are congestive heart failure, left ventricular dysfunction following myocardial infarction and hypertension. It is contraindicated in patients with bronchial asthma or related

bronchospastic conditions, in patients with hypersensitivity to any component of the product second- or third-degree AV block, sick sinus syndrome or severe bradycardia (unless a permanent pacemaker is in place), or in patients with cardiogenic shock or who have decompensated heart failure requiring the use of intravenous inotropic therapy. It is also not recommended in patients with clinically manifest hepatic impairment.

In terms of pregnancy and teratogenic effects, carvedilol is categorized in Pregnancy Category C. Studies performed in pregnant rats and rabbits given carvedilol revealed increased post-implantation loss in rats at doses of 300 mg/kg/day (50 times the MRHD as mg/m^2) and in rabbits at doses of 75 mg/kg/day (25 times the MRHD as mg/m^2). In rats the no-observed-effect level for developmental toxicity was 60 mg/kg/day (10 times the MRHD as mg/m^2); in rabbits it was 15 mg/kg/day (5 times the MRHD as mg/m^2). There are no adequate and well-controlled studies in pregnant women.

The most common side effects are:

Body as a Whole: allergy, malaise, hypovolemia, fever, leg edema.
Cardiovascular: fluid overload, postural hypotension, aggravated angina pectoris, AV block, palpitation, hypertension.
Central and Peripheral Nervous System: hypesthesia, vertigo, paresthesia.
Gastrointestinal: melena, periodontitis.
Liver and Biliary System: SGPT increased, SGOT increased.
Metabolic and Nutritional: hyperuricemia, hypoglycemia, hyponatremia, increased alkaline phosphatase, glycosuria, hypervolemia, diabetes mellitus, GGT increased, weight loss, hyperkalemia, creatinine increased.
Musculoskeletal: muscle cramps.
Platelet, Bleeding and Clotting: prothrombin decreased, purpura, thrombocytopenia.
Psychiatric: somnolence.
Reproductive, male: impotence.
Special Senses: blurred vision.
Urinary System: renal insufficiency, albuminuria, hematuria.

In clinical trials, carvedilol has been shown to cause bradycardia in about 2-9% of hypertensive patients. In clinical practice, if the pulse rate drops below 55 beats/minute, the dosage is typically reduced.

In the United States, the FDA recommend the starting dose of carvedilol to be 3.125 mg, twice daily for 2 weeks. Patients who tolerate a dose of 3.125 mg twice daily may have their dose increased to 6.25, 12.5, and 25 mg twice daily over successive intervals of at least 2 weeks. In clinical practice, patients should be advised that initiation of treatment and (to a lesser extent) dosage increases may be associated with transient symptoms of dizziness or lightheadedness (and rarely syncope) within the first hour after dosing. Thus, during these periods they should avoid situations such as driving or hazardous tasks, where

symptoms could result in injury. In addition, carvedilol should be taken with food to slow the rate of absorption. In Japan, a lower dose of carvedilol (2.5 mg twice daily) has been used and tested for clinical efficacy[74].

4 STUDY DESIGN

4.1 Description

The current study will be a double-blinded, placebo-controlled study in which Type 1 diabetic patients with hypoglycemia unawareness are randomized to either 1) placebo treatment, 2) 2.5mg carvedilol twice daily or 3) 3.125mg carvedilol twice daily. After enrollment, the participants will be placed on (blinded) CGM monitoring which will be continued until the end of the study. Additionally, participants will be provided with a glucometer to measure their blood glucose and for calibrating the CGMs during the study. One week after CGM placement, the participants will undergo the first hypoglycemic clamp study to obtain baseline measures of hypoglycemia frequency (from downloaded CGM data), hypoglycemia awareness scores and hormone responses. Following the initial clamp procedure, the patients will be randomized to either 4 weeks use of carvedilol or placebo. After the 4 weeks of treatment, the participants will undergo a second hypoglycemic clamp session. Patients will be hospitalized the night before each clamp study to ensure stable glucose levels.

4.2 Dose Limiting Toxicity

Patients allocated to the drug treatment groups will receive a dose of either 2.5mg or 3.125mg of carvedilol twice daily. The goal is to use the lowest efficacious dose for improving hypoglycemia awareness in T1DM patients with little to no side effects. Our preclinical data suggests that at higher doses, β-blockers provide no benefits in terms of improving hypoglycemia awareness.

4.3 Number of Subjects

21 Type 1 diabetic patients with hypoglycemia unawareness will be recruited for the study. The 21 T1DM patients will be randomly allocated to one of the following treatments: 1) placebo (N=7), 2) 2.5mg carvedilol twice daily (N=7) or 3) 3.125mg carvedilol twice daily (N=7). Placebo will be taken twice daily.

4.4 Number of Study Centers

This is a single-center study being conducted at the University of Utah.

4.5 Study Duration

The enrollment of patients will begin in the late fall of 2018. We anticipate that the study procedures will begin in the fall of 2018 and continue until spring 2020. Data analysis and publication of the results are expected to be completed in the late spring or summer of 2020.

5 ELIGIBILITY CRITERIA

This checklist is used to determine subject eligibility; it should be completed for each subject, including review and signature by the enrolling investigator and filed in the subject research chart.

Subject ID: _____ Subject Initials: _____

5.1 Inclusion Criteria

Yes/No (a response of 'No' indicates the subject is **ineligible**)

1. _____ Type 1 diabetes for more than 5 years
2. _____ Age > 18 years
3. _____ Presence of impaired hypoglycemia awareness/unawareness
4. _____ Intensive insulin treatment as defined by multiple daily insulin injections (3 or more) or insulin pump therapy
5. _____ Negative pregnancy test
6. _____ Able to provide informed consent and willing to sign an approved consent form that conforms to federal and institutional guidelines.

5.2 Exclusion Criteria

Yes/No (a response of "Yes" indicates the subject is **ineligible**)

1. _____ Major medical disorders (including liver disease, cardiovascular disease, kidney disease, COPD, asthma, active malignancy or HIV)
2. _____ Overt diabetes complications (neuropathy, nephropathy, retinopathy)
3. _____ Anemia
4. _____ Current or recent use of beta-blocker therapy
5. _____ Use of diuretics
6. _____ Allergies or contraindications to beta-blockers or heparin
7. _____ Use of benzodiazepines
8. _____ Alcohol, drug or medication abuse
9. _____ Frequent use of acetaminophen

I verify that this subject meets all eligibility criteria for enrollment onto this study

_____ _____

Investigator Signature Date

6 TREATMENT PLAN

6.1 Administration Schedule

The treatment group will receive an oral dose of either 2.5mg or 3.125mg carvedilol twice daily for 4 weeks. The placebo group will receive a placebo pill twice daily for 4 weeks.

6.2 Treatment (Carvedilol)

6.2.1.1 How Supplied, Stored, Packaged and Labeled

Carvedilol will be supplied by Investigational Drug Services (IDS) at the University of Utah Hospital. The carvedilol capsules will be over-encapsulated for blinding. Likewise, matching placebo capsules will be produced.

The capsules will be stored at 20° to 25°C (68° to 77°F); excursions permitted to 15° to 30°C (59° to 86°F). They will be protected from light, moisture, freezing and excessive heat. They will be dispensed in a tight, light-resistant container as defined in the USP in a locked cabinet where only staff associated with the study has access to.

6.2.2 Preparation and Administration

Investigation Drug Services (IDS) will prepare the capsules with either carvedilol or placebo in a blinded fashion. Each container will have a number and will match the number the patient receives at randomization. Both the investigational staff and the patients will be blinded to the treatment that the patient is receiving. Randomization will be conducted by the Study Design and Biostatics Center (SDBC) who will provide a randomization code to IDS. The randomization code will be stored in a central database and the file will not be opened until the study is complete or if patient safety requires it.

At randomization, the patients will each receive their own container matching the randomization number. The container will have enough capsules for the 4-week treatment period. On the day of the second hypoglycemic clamp study, the subjects will be asked to return the container including any unused capsules.

6.2.3 Accountability and Compliance

The study investigators will keep a record of the container that each patient receives. This information will be noted in the participant's clinical research file. When the patients return for the second hypoglycemic clamp study, they will bring their container and the remaining pills will be counted and noted in the clinical research file as well. The patient will also be asked to assess how often, if at all, he or she forgot to take the medication.

6.3 Duration of Therapy

The period of treatment is 4 weeks (28 days).

Subjects will be withdrawn or discontinued from the study if any of the following occurs:

- The subject withdraws consent for further participation in the clinical trial. At any time during the trial and without the need to provide a reason or rationale, a subject may choose to decline to participate further in the study. The subject will not suffer any consequences as a result.
- Subject is lost to follow-up despite efforts to contact them.
- Episodes of severe bradycardia and hypotension requiring hospitalization.
- Episodes of severe hypoglycemia develop which involve seizure development or which require hospitalization. The patient population that we are studying are Type 1 diabetic individuals with impaired awareness of hypoglycemia. Thus, these patients are at a higher risk of experiencing hypoglycemia, and it is expected that these patients will have episodes of hypoglycemia (whether treated with investigational drug or placebo). Patients are required to report all episodes of hypoglycemia. Hypoglycemic episodes can range from mild-moderate to severe. Severe hypoglycemia is defined as "hypoglycemic episodes with severe cognitive impairment requiring external assistance for recovery". In the event the study participant experiences an episode of severe hypoglycemia, the circumstances regarding the patient's episode of hypoglycemia will be evaluated by our medical staff who have expertise in treating patients with Type 1 diabetes. Decisions will be made on a case-by-case basis as to whether it is safe for the participant to continue in the study. Obviously the severity of hypoglycemia will impact this clinical decision. Specifically, in the event the participant experiences an unexplained episode of severe hypoglycemia that involves loss of consciousness, seizures or hospitalization, the participant will be immediately removed from the study, the study drug treatment condition will be unblinded, and the IRB notified. For other cases, we will evaluate the circumstances regarding the patient's episode of hypoglycemia individually, and determine whether it is medially safe for the participant to continue in the study. If the participant is withdrawn from the study, the study drug treatment condition will be unblinded, and the IRB notified. As a part of the usual standard of medical care, any participant who experiences severe hypoglycemia will also receive education on how to avoid hypoglycemia.

Subjects may be withdrawn or discontinued from the clinical trial for the following reasons:

- The subject is non-compliant with study treatment requirements and/or protocol procedures, including the use of prohibited concomitant medications.
- The occurrence of an AE, laboratory abnormality or other medical condition or situation such that in the investigator's opinion, continued participation in the study would not be in the best interest of the subject.

7 TOXICITIES AND DOSE MODIFICATION

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) version 4.0 for adverse event and serious adverse event reporting.

7.1 Dose Modification

In this study, participants will receive either a 2.5mg or 3.125mg of carvedilol twice daily. Patients will not have the dose increased. However, if patients experience profound side effects due to the medication such as hypotension and syncope, the PI can reduce the dose by one-half (i.e. one dose per day vs twice daily) or consider withdrawing the participant (see section 6.3). If treatment is delayed, the subject's participation in the study will be discontinued.

7.2 Supportive Care

All supportive measures consistent with optimal subject care will be given throughout the study and all participants will be given a telephone number which they can call if they have any questions or concerns regarding the study and/or their participation in the study.

8 STUDY CALENDAR

Period	Screening	Treatment Period			
			Intervention Phase (4 weeks)		
		Baseline CGM	1st Hypoglycemic Clamp Study	Follow-Up	2nd Hypoglycemic Clamp Study
Visit No.	1	2	3	4	5
Week[1]	-5 to -2	-2	0	1-2	4
Assessments					
Informed consent	X				
Inclusion/Exclusion criteria	X				
Demographics	X				
Vital signs	X		X	X	X
Physical Examination	X				
Pregnancy Test	X	X	X		X
ECG	X		X		X
Hematology/Chemistry/ Urinalysis[2]	X				
AE Assessment		X	X	X	X
Concomitant Medications	X	X	X	X	X
CGM and Glucometer		X[3]	X	X	X[3]
Fear Survey			X	X	X
Hypoglycemia Awareness Survey	X			X	X
Overnight Hospital Admission			X		X
Investigational Drug Treatment			X[4]	X	X[4]
Primary Objective Assessment			X		X
Secondary Objective Assessment			X		X

1. All screening procedures should be completed within 4 weeks of study enrollment (Visit 1), with the exception of laboratory tests which must be completed within 2 weeks, and pregnancy test which must be completed within 48 hours of screening (Visit 1). All visits within the "Treatment Period", except the 2nd hypoglycemic clamp study, should occur within ± 3 days of target date. The 2nd hypoglycemia clamp may be postponed up to 7 days due to hypoglycemia prior to the clamp study.
2. Hematology labs include CGC with differential and platelets; Chemistry labs include c-peptide, HbA1c, Hemoglobin, Albumin, Alkaline Phosphatase, Aspartate Aminotransferase, Alanine Aminotransferase, Total Bilirubin, Creatinine, Glucose, Potassium, TSH, Sodium and Urea Nitrogen; Urinalysis is a dipstick urinalysis including Protein, Hemoglobin and Glucose

3. The CGM is placed after enrollment and until the completion of the second hypoglycemic clamp study. Bayer Contour Next EZ® glucometer will be provided to participant and they will be required to use it to monitor their blood glucose and to calibrate the blinded CGMs.
4. The investigational drug treatment is to be started after the first hypoglycemic clamp study and completed on the day prior to the second hypoglycemic clamp study.

9 STUDY PROCEDURES

Detailed descriptions of subject evaluations required for this protocol are described in this section. These evaluations will be performed during the indicated visits/weeks of the study as described in the Study Calendar and in this section.

All data collected are to be recorded on source documents and entered into the appropriate CRF page.

The PI is responsible for maintaining a record of all subjects pre-screened, screened, and enrolled into the study. All subjects must provide written informed consent before the performance of any study procedures.

9.1 Informed Consent

Prior to conducting any study-related procedures, written informed consent will be obtained from the subject. The nature, scope and possible consequences, including risks and benefits, of the study will be explained to the subject by the PI or designee in accordance with the regulations and guidelines in the Statement of Compliance.

9.2 Study Entrance Criteria

At screening, each subject will be assessed for eligibility against the inclusion/exclusion criteria. Subjects who do not meet the study entrance criteria will not be allowed to enroll and participate in the study. The reason(s) for ineligibility will be documented in the subject research chart.

9.3 Demographics, Medical History and Hypoglycemia Awareness Survey

Subject information including gender, age, date of birth, race, ethnicity, diagnosis of (condition), and other relevant past medical history will be collected during the screening period and recorded in the appropriate CRF. Subjects will also be administered a questionnaire to evaluate hypoglycemia awareness.

9.4 Laboratory Variables

A 30mL blood draw will be conducted to obtain blood samples for routine and safety clinical laboratory tests (hematology, clinical chemistry) as indicated below. In addition, participants will be asked to leave a 100-120mL sample of urine for urinalysis and pregnancy testing as indicated below. Analysis of laboratory samples will be performed by ARUP Laboratories: 500 Chipeta Way Salt Lake City, UT.

9.4.1 Hematology and Clinical Chemistries

Hematology: hematocrit, platelet count, white blood cell count, hemoglobin

Serum Chemistries and Liver Function Tests: potassium, sodium, blood urea nitrogen, albumin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, creatinine,

Additional tests: TSH, glucose levels, C-peptide, HbA1c

9.4.2 Urinalysis

Urinalysis will be performed to evaluate urine microalbumin levels.

9.4.3 Pregnancy Testing

A urinary pregnancy test evaluating human chorionic gonadotropin (hCG) levels will be performed during the initial screening visit, at the time CGM is placed, as well as the night before each of the hypoglycemic clamp studies.

9.4.4 Specimen Collection, Preparation, Storage and Shipping

The collection, preparation, storage and shipping of specimens will be conducted in accordance with procedures outlined by ARUP Laboratories. Blood samples collected for assessment of plasma glucose during the clamping procedure will be collected into heparin coated tubes to prevent coagulation.

9.5 Physical Examination

A complete physical examination including assessment of the respiratory system, cardiovascular system, abdomen as well as specific tests for neuropathy will be conducted at the screening visit.

9.6 Vital Signs

Vital signs, including measurement of systolic and diastolic blood pressure, pulse, heart rate, respiration rate and O_2 saturation will be measured at the screening visit, as well as during the hypoglycemic clamp sessions.

9.7 Electrocardiogram

An ECG will be completed during the screening period to assess for cardiac conduction abnormalities.

9.8 Continuous Glucose Monitor (CGM) and Glucometer

A blinded continuous glucose monitor (CGM) will be placed after enrollment and continue until the end of the second hypoglycemic clamp study. At this time, the participants will also be issued a Bayer Contour Next EZ® glucometer which they will use to monitor their blood glucose for the duration of the study and also to calibrate the CGMs. Instructions on how to use the glucometer will be provided at this time.

9.9 Concurrent Medications

All prescription and non-prescription medications including pharmacologic doses of vitamins, herbal medicines, or other non-traditional medicines, taken from 5 weeks prior to the first dose of carvedilol through the last study visit must be documented and recorded in the CRF.

9.9.1 Prohibited Concurrent Medications

The following medications are prohibited during the study:

- Use of any other beta blocker
- Use of diuretics
- Use of benzodiazepines
- Use of acetaminophen
- Use of SSRIs, naltrexone and other medications that have been proposed to have the potential to improve hypoglycemia awareness or prevent the development of impaired awareness of hypoglycemia.

9.10 The Clamp Procedure

Patients will be asked to reduce the dose of long-acting insulin 24 hours prior to the clamp study if applicable. The night before the clamp study, the subjects will be admitted into the CSC and the CGMs will be checked for hypoglycemia (reading <75-80mg/dl). We are setting this cut-off a little higher than the clinical definition of hypoglycemia (<70mg/dL) to ensure central mechanisms that are normally triggered by mild hypoglycemia are not are not activated[75]. If hypoglycemia (<75-80mg/dL) is noted within 24 hours of beginning the clamp study, then the study will be delayed for one week. Qualifying subjects will be provided with a standardized low glycemic index meal to avoid fluctuations in blood glucose levels during the overnight fast. The CSC nursing staff will introduce an intravenous (antecubital vein) insulin drip in the subject and blood glucose levels will be maintained between 90-120mg/dl during the overnight fasting period. Blood glucose concentrations will be determined from a fingerstick using a point-of-care glucometer in order to titrate the insulin drip.

First Hypoglycemic Clamp*:* The next day, the patient will be asked to void their bladder prior to the start of the clamping procedure. The participant will then remain in bed in a supine position for the procedure. The participant will be asked to refrain from watching television during the procedure. A second catheter will be inserted into the contralateral hand vein for blood sampling and the sampling hand placed into a heated (~55°C) box to arterialize the blood. An ECG will also be connected to the subject to monitor heart electrical activity. Heart rate, blood pressure and ECG will be monitored every 15 minutes throughout the study. Prior to the drawing of each blood sample, the deadspace volume (~5-6ml) will be withdrawn into a syringe. A new syringe will be replaced onto the luer lock and the required blood volume drawn into new syringe. Following blood collection, the collected deadspace volume will be reinfused, followed by a saline infusion through the intravenous line to clear it of residual blood. Baseline blood samples (30mL) will be

collected to measure glucose, HbA1c, hematocrit and hormones (glucagon, catecholamines, insulin, cortisol, growth hormone, pancreatic polypeptide). Blood samples collected for assessment of plasma glucose (1mL) during the clamping procedure will be aliquoted into heparin coated 1ml Eppendorf tubes to prevent coagulation and processed immediately in the YSI and/or Analox Instruments glucose analyzer. In addition, the participants will be asked to fill in questionnaires to assess their perception of hypoglycemic symptoms (Edinburgh Hypoglycemia Symptom Scale) and their fear of hypoglycemia. Subsequently, the subjects will be given KCl tablets (20-40mEq) to help maintain potassium levels during the clamping procedure. Once assessment of baseline parameters has been completed, constant insulin (2mU/kg/min) and variable 20% dextrose infusions will be started at -60 minutes to establish stable glucose levels of ~90mg/dl before gradually lowering plasma glucose levels in three steps from 90-65-55-45 mg/dl by gradually reducing the glucose infusion rate. Plasma glucose levels will be monitored from a 1ml blood sample every 5min using a YSI and/or Analox glucose analyzer. A second Analox Instruments glucose analyzer and glucometer will be kept on hand as a backup. It is important that the glucose levels *NOT* be communicated verbally during the clamping procedure as notification of declining glucose concentrations to the participant might trigger confounding (i.e. artificially higher) awareness scores or stress hormone responses. Each glycemic step will be held for 30 minutes, allowing enough time for blood collection (~30mL at each glycemic plateau) and completion of the Edinburgh questionnaire at each glycemic plateau. At each glycemic step, plasma hormones (glucagon, catecholamines, insulin, cortisol, growth hormone, pancreatic polypeptide) will be evaluated. The frequent assessment of hypoglycemia symptom scores allows us to identify the threshold at which hypoglycemic symptoms are first noticed by the subject. We chose glycemic targets of 55 and 45mg/dl as hypoglycemic symptoms generally first appear between 55-65mg/dl in hypoglycemia aware individuals. In contrast, symptoms may not manifest in hypoglycemia unaware patients until glucose levels reach 45mg/dl. Targets of 55 and 45mg/dl allow us to safely determine whether carvedilol treatment raises the glucose threshold at which symptoms are first detected (i.e. improving hypoglycemia awareness). No more than 500mL of blood will be collected throughout the entire clamping procedure which is equivalent to the volume of a single blood donation. After the clamp, the participant's glucose levels will be recovered to normal by stopping the insulin infusion and raising the glucose infusion rate. In addition, a meal will be provided to aid recovery. This first set of clamps will establish a baseline response in the participant prior to the start of the intervention phase of the study. **Dispersal of Intervention Drug:** At the end of the clamp procedure, the subjects will be supplied with either placebo or one of two different doses of carvedilol, 2.5mg or 3.125mg, along with instructions on how to take the medication. The research staff and participant will be blinded to the treatment being received. The initial dose will be taken on the day following the clamp. In addition, the participant will also receive a hypoglycemia log book along with instructions on how to document hypoglycemia incidences and whom to contact in case of medical concerns. Once the participant's blood glucose levels are stable and he/she is ready to be discharged, we will provide them with glucose tablets and we will cover the cost of transportation home as a precautionary measure.

Follow-Up Visit: About 7-14 days after the start of the intervention phase, the subjects will be asked to come into the clinic for a follow-up visit where vital signs, AE and CGM data will be monitored or evaluated. Subjects will also be asked to complete a hypoglycemia awareness and fear survey at this time.

Second Hypoglycemic Clamp*:* At the end of the four-week intervention phase, the subjects will return to the CSC for the overnight stay and second hypoglycemic clamp procedure. The second hypoglycemic clamp procedure will be identical to the first described above.

9.11 Efficacy Measurements

On all treatment visit days, dosing will occur after all of the safety and efficacy assessments that are scheduled for that particular visit have been completed.

9.11.1 Primary Objective Assessment

The primary objective assessments are 1) improvements in the hypoglycemia symptom scores at each of the (hypoglycemic) glycemic plateaus, 2) shift in the threshold for the detection of hypoglycemia and 3) whether counterregulatory hormone responses improve during the second hypoglycemic clamp in T1DM patients with impaired awareness of hypoglycemia after 4 weeks of carvedilol treatment compared to their baseline and/or compared to the placebo-treated control group.

The symptom scores will be assessed by the Edinburgh Hypoglycemia Symptom Score. Results will be kept in the patients CRF. After the study is complete, the results will be loaded into a secure electronic database and analysis will begin. When reporting the results of the hypoglycemia symptom scores, we will evaluate individual as well as overall or total symptom scores. In addition, symptoms will be grouped into autonomous, neuroglycopenic and other symptoms for evaluation.

The counterregulatory hormone responses (glucagon, epinephrine, norepinephrine, cortisol and growth hormone) will be analyzed from the drawn blood samples. Whole blood samples will be collected and aliquoted into vacutainers containing the appropriate preservatives and anticoagulants, spun down and the plasma portion will be stored in a -80℃ freezer until the samples are analyzed.

9.11.2 Secondary Objective Assessment

The secondary objective assessment will establish whether the frequency of CGM-determined hypoglycemic episodes differs during 4 weeks of carvedilol treatment compared to baseline and compared to the placebo-treated control group.

Patients will wear a blinded CGM that is installed 14 days prior the first clamp study and the device will continue to be worn throughout the entire study. These CGMs will be calibrated using the provided Bayer Contour Next EZ® glucometer. During the first clamp study, the follow-up visit and the second clamp study, data from the CGM will be downloaded and saved onto a secure server and exported into an Excel format for analysis. We will identify the number hypoglycemic excursions during the intervention period and compare this to the number of hypoglycemic episodes encountered during the 7-day period before the first clamp study, the "baseline" period. Comparisons between treatment groups at baseline and following the intervention period, as well as differences from baseline within each treatment group will be made.

Analysis of the CGM data will emphasize the number of hypoglycemic episodes as well as the duration and depth of the hypoglycemic episodes. A single hypoglycemic episode is defined as a glucose reading <70 mg/dL, followed by a reading ≥70mg/dL, lasting at least 15 minutes or at least two readings below 70mg/dL over 15 minutes. If there is less than 30 minutes between two registered episodes of hypoglycemia these are considered as one episode.

We will also evaluate the answers to the fear surveys to determine whether those patients who are treated with carvedilol have a reduction in their fear of hypoglycemia compared to those on placebo.

9.12 Safety Measurements

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) version 4.0 for AE and SAE reporting. An electronic copy of the CTCAE v.4 can be found at: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

9.12.1 Adverse Events

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug treatment, even if the event is not considered to be related to the study drug. For the purposes of this study, the terms toxicity and adverse event are used interchangeably. Medical conditions/diseases present before starting on the study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy

Collection of adverse events will begin following the initial treatment with carvedilol and continue through to the last visit (the second clamp study). Adverse events will be documented in the study source record and recorded in the CRF.

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit or phone contact during the study. Adverse events may also be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test or other assessments. As much as possible, each adverse event should be evaluated to determine:

1. The severity grade based on CTCAE v.4
2. The relationship of the event to the study drug(s): definite, probable, possible, unlikely, not related.
3. The duration: start and end dates, or continuing at final follow-up visit
4. Action taken: (e.g. study drug interrupted or dosage modified, concomitant medication taken, non-drug therapy, hospitalization, or no action taken)
5. Whether the event constitutes a Serious Adverse Event (SAE)

All adverse events will be treated appropriately. Such treatment may include changes in study drug treatment as listed in the dose modification section of this protocol (see section 7 for guidance). Once an adverse event is detected, it should be followed until its resolution and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

9.12.2 Serious Adverse Events

Information about all serious adverse events will be documented in the study source record and recorded in the CRF. A serious adverse event is an undesirable sign, symptom or medical condition which:

- Is fatal or life-threatening
- Results in persistent or significant disability or incapacity
- Requires inpatient hospitalization or prolongation of existing hospitalization (unless the hospitalization is for routine treatment or monitoring of the studied indication, or elective or pre-planned treatment of a pre-existing condition unrelated to the indication under study)
- Causes a congenital anomaly or birth defect
- Is medically significant, i.e., defined as an event that jeopardizes the subject or may require medical or surgical intervention to prevent one of the outcomes listed above

Toxicities which fall within the definitions listed above must be documented as an SAE regardless if they are felt to be treatment related or not.

9.12.3 Serious Adverse Event Reporting Requirements

An event determined to be an SAE must be reported to the IRB and the FDA according to the requirements described below:

IRB Notification:
The PI is responsible to report SAEs that meet the definition of an Unanticipated Problem through the ERICA system *within 10 working days* from the time the investigator learns of the event. An Unanticipated Problem is an event that is:

- **Unexpected** – unforeseen by the investigator in terms of nature, severity, or frequency, given the research procedures and the subject population being studied
- **Related or Probably Related** – determined by the investigator to be related or probably related to participation in the clinical trial
- **Greater Risk** – the severity or scope of the event suggests that the research places subjects or others at greater risk of harm than was previously known or recognized

FDA Notification:
FDA regulations require the PI to report any serious and unexpected adverse event for which there is a reasonable possibility that the drug caused the event. Fatal or life-threatening events that meet these criteria must be reported *within 7 calendar days* from the time the investigator learns of the event. All other reportable events must be reported *within 15 calendar days*. The SAE should be reported on a MedWatch form and submitted as an amendment to the IND. For studies without an IND, the MedWatch report should be submitted to the FDA through the voluntary reporting method.

9.12.4 Reporting of Pregnancy

Although pregnancy is not considered an adverse event, it is the responsibility of the PI or their designee to report any pregnancy or lactation in a subject, including the pregnancy of a male subjects' female partner as an SAE. Pregnancies or lactation that occurs during the course of the trial or within 30 days of completing the trial or starting another new therapy, whichever is earlier, must be reported to the IRB, FDA, and the funding sponsor as applicable. *All subjects and female partners who become pregnant must be followed to the completion/termination of the pregnancy.* Pregnancy outcomes of spontaneous abortion, missed abortion, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events.

9.13 Study Halting Rules

There are no study halting rules for this study.

10 STATISTICAL CONSIDERATIONS

A total of 21 T1DM patients with impaired awareness of hypoglycemia will be sought for this study. For the dose response study, we plan to enroll 21 of these patients who will be randomized to either placebo (N=7) or carvedilol (2.5mg or 3.125mg twice daily; N=7 each).

In estimating the appropriate sample sizes required to yield adequate statistical power, assuming a Type II error rate (β) of no more than 20% for a given test and a Type I error rate of α=5%, we used the following: Power ≥ 1-β = 1-0.2 = 0.8

Treatment effects will be analyzed using one- or two-way analysis of variance (ANOVA) for independent or repeated measures as appropriate, followed by suitable *post-hoc* analysis. Analyses will be performed using the GraphPad Prism® or SAS® suite of software. We will use a significance level of $\alpha<0.05$.

Primary variables:

a) Symptom Scores (autonomous, neuroglycopenic and other symptoms of hypoglycemia)
b) Counterregulatory Hormone Responses (glucagon, epinephrine, norepinephrine, growth hormone and cortisol levels)

Secondary variables:

a) Frequency of hypoglycemia assessed by CGM.

The primary and secondary variables will be analyzed by the statistical approaches mentioned above.

11 DATA HANDLING AND RECORD KEEPING

Data collection is the responsibility of the clinical trial staff under the supervision of the PI. The investigator is ultimately responsible for ensuring the integrity, accuracy, completeness, legibility, and timeliness of the data being reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original source data.

Case Report Forms (CRFs) will be utilized to capture data in the clinical study. Data collection processes and procedures will be reviewed and validated to ensure completeness, accuracy, reliability and consistency.

The PI and study team will conduct periodic reviews of the source data records and CRF database for completeness, consistency and accuracy. Study data will be collected and reported to the DSMB as required by the data and safety monitoring plan.

11.1 Records Retention

FDA investigational drug and device regulations, and GCP guidelines state that clinical trial records and essential documents should be retained for a minimum of 2 years after the last approval of a marketing application, or if no application is to be filed or if the application is not approved, at least 2 years have elapsed since the formal discontinuation or conclusion of the clinical trial. Refer to SOP #CTO-04 for maintenance and archiving of clinical trial records.

11.2 Disclosure and Publication Policy

This study will comply with the FDAAA regulations and NIH policy for registration and results reporting in the ClinicalTrials.gov database, as applicable. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. Registration of the study in the ClinicalTrials.gov database prior to study initiation will comply with this policy.

The results from the study will be summarized in an article, with the intention of publication in a peer-reviewed scientific journal. Positive, negative and inconclusive results will be published. Dr. Chan will decide the order of the authors and the author disclosure will follow the Vancouver declaration.

12 ETHICAL AND REGULATORY CONSIDERATIONS

12.1 Institutional Review

The Institutional Review Board will review the application, protocol and all appropriate documentation in order to safeguard the rights, safety, and well-being of study subjects. The study will only be conducted at study centers where IRB approval has been obtained. The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by the IRB. If the protocol is amended, changes will not be implemented without prior review and approval from the IRB, except where necessary to eliminate an immediate hazard to study subjects.

12.2 Data and Safety Monitoring Plan

The clinical trial may be selected for audit within the scope of the Internal Audit Program. The purpose of an audit is to determine and evaluate adherence to applicable federal regulations, to the study protocol and to GCP principles. The PI is responsible for working with the auditor in providing all needed study records and for developing corrective actions where necessary and ensuring complete and adequate responses to internal audit findings.

If the study is selected for an FDA or other federal agency inspection, the PI will make requested study records and study personnel records available for review (refer to SOP REG-01: FDA Inspections).

12.3 Protocol Deviations

A protocol deviation (or violation) is any departure from the defined procedures and treatment plans as outlined in the protocol version submitted and previously approved by the IRB. Protocol deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study thus jeopardizing the justification for the research. Protocol deviations are unplanned and unintentional events.

Because some protocol deviations pose no plausible threat to participant safety or scientific integrity, reporting is left to the discretion of the PI within the context of the guidelines below. The IRB requires the **prompt reporting** (*within 10 working days*) of protocol deviations which are:

- Exceptions to eligibility criteria
- Intended to eliminate apparent immediate hazard to a research participant
- Harmful – caused harm to participants or others, or placed them at increased risk of harm, including physical, psychological, economic, or social harm
- Possible serious or continued noncompliance

12.4 FDA Annual Reporting

Within 60 days of the anniversary date that the IND went into effect, a brief report of the progress of the investigation will be submitted to the FDA following the requirements set out in 21 CFR Part 312.33.

12.5 Clinical Trials Database

The clinical trial will be registered at initiation and study results will be reported on the ClinicalTrials.gov database as required per 42 CFR Part 11.

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