Clinical Study Protocol

Intra-nasal naloxone for treatment of impaired awareness of hypoglycemia

Regulatory Sponsor: Amir Moheet
Assistant Professor

Study Product: Naloxone

Protocol Identifiers: **CTSI # 22405**

IND Number: IND exempt/ IND 129763

Trial Registration: **NCT02700048**

Version / Date: Version 05; Jan 31, 2019

Statement of Compliance

This document is a protocol for a human research study. This study will be conducted according to US and International standards of Good Clinical Practice, applicable government regulations and Institutional research policies and procedures.

All individuals responsible for the design and conduct of this study have completed Human Subjects Protection Training and are qualified to be conducting this research prior to the enrollment of any subjects.

As Principal Investigator, I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol. Modifications to the study are acceptable only with an approved protocol amendment. I agree to obtain approval from the IRB and/or regulatory bodies of competent jurisdiction, for the protocol and informed consent before initiating the study, to obtain consent from subjects prior to their enrollment in the study, to collect and record data as required by this protocol and case report forms, to prepare adverse event and study reports as required by this protocol and to maintain study documentation for the period of time required.

_____ _____

Amir Moheet/Assistant Professor Date

As a Sub-Investigator, I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol. Modifications to the study are acceptable only with an approved protocol amendment. I agree to obtain approval from the IRB and/or regulatory bodies of competent jurisdiction, for the protocol and informed consent before initiating the study, to obtain consent from subjects prior to their enrollment in the study, to collect and record data as required by this protocol and case report forms, to prepare adverse event and study reports as required by this protocol and to maintain study documentation for the period of time required.

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Lisa Coles/Research Assistant Professor Date

Version History

Version #	Version Date	Significant Changes from Previous Version
01	02/03/2016	Original Protocol Version
02	04/25/2016	Added additional justification for sample size
03	06/28/2016	Change randomization for the first 3 subjects to naloxone
04	01/05/2017	Add 8mg dose of naloxone; change randomization for the first 8 subjects to naloxone
05	01/31/2019	Update study location

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Study Summary

Title	Intra-nasal Naloxone for treatment of impaired awareness of hypoglycemia
Short Title	Intra-nasal Naloxone for impaired awareness of hypoglycemia
IRB Number	1603M84961
Study Sponsor	Amir Moheet, MD
Phase	1
Principal Investigator	Amir Moheet, MD
Study Design	Single center, single-blind, randomized, cross-over
Study Duration	24 months
Study Center(s)	Clinical Research Unit (CRU)
Objectives	*Primary Objective:* To evaluate intra-nasal Naloxone as a therapy for prevention and treatment of impaired awareness of hypoglycemia. *Secondary Objective:* Characterize naloxone pharmacokinetics following multiple administrations.
Number of Subjects	18
Main Inclusion Criteria	Males and females between the ages of 18-65 years
Study Product, Dose, Route, Regimen	Narcan (naloxone hydrochloride) intranasal spray, 4 mg/nostril repeated 4 times within 8 hours alternating nostrils.
Duration of Administration	4 doses of 4 mg or 2 doses of 8 mg administered over 1 day.
Endpoints	*Primary endpoint* will be the within person difference in peak epinephrine secretion during the morning episodes of hypoglycemia on days one and two; we will compare this difference between the two treatment conditions. *Secondary endpoint* will be naloxone pharmacokinetics including maximum concentration (Cmax), time to maximum concentration (Tmax) and area under the curve (AUC).
Statistical Methods	We will fit a general linear mixed model with fixed effects for treatment (naloxone vs. placebo), treatment order (naloxone first vs. placebo first), and period (Part 1 vs. Part 2) and a random effect for participant. We will also consider adjustment for important baseline characteristics, such as age and sex. Diagnostics will be examined to assess model assumptions.

List of Abbreviations

AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICH	International Conference on Harmonization
IDS	Investigational Drug Services
IRB	Institutional Review Board
PHI	Protected Health Information
SAE	Serious Adverse Event

1.0 Study Contact Information

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2.0 Introduction / Background and Rationale

Iatrogenic hypoglycemia is a common and feared complication of insulin therapy. Recurrent exposure to iatrogenic hypoglycemia in patients with insulin treated diabetes can lead to development of impaired awareness of hypoglycemia (IAH) (1), a condition that is estimated to occur in 20% of patients with type 1 diabetes (T1D) (2). Fear of hypoglycemia can limit the ability of patients with diabetes to achieve the glycemic control shown to prevent complications of diabetes. Strict avoidance of hypoglycemia has been shown to partially restore awareness of hypoglycemia; however it is very difficult to achieve and maintain over the long term (3-5). Consequently, there has been great interest in developing therapies that will prevent and/or reverse IAH in diabetes.

One potential therapy to prevent or reverse IAH in diabetes may be opioid receptor antagonists. Endogenous opiates have been shown to modulate hormonal responses during hypoglycemia and may play a role in the development of IAH (6). Intravenous (IV) administration of naloxone, an opioid receptor antagonist, during hypoglycemia has been shown to augment the counterregulatory response to hypoglycemia in dogs (7) and humans (8). When infused during antecedent hypoglycemia, IV naloxone has been shown to prevent development of defective counterregulatory hormone response to subsequent hypoglycemia in healthy humans (9) and in patients with T1D (10). However, IV dosing of Naloxone is not feasible for chronic outpatient therapy. The usual alternative of oral dosing is hindered by large first pass effects, low oral bioavailability, and high variability. Intra-nasal (IN) route of administration is a novel approach to provide adequate systemic exposure in easy to use delivery route. IN naloxone is currently being used for acute management of opioid overuse.

The long-term goal of this project is to test the hypothesis that IN naloxone therapy in subjects with T1D and IAH will improve counter regulatory hormone responses during hypoglycemia and increase recognition of hypoglycemia symptoms. The purpose of this project is to evaluate IN naloxone as a therapy for the prevention and treatment of IAH. IAH can be reproduced experimentally by inducing recurrent hypoglycemia in healthy subjects (11), providing a robust investigational model for studying mechanism and treatments for IAH. **In this study we will test the hypothesis that IN naloxone therapy will prevent the development of IAH in healthy subjects who are exposed to experimental hypoglycemia**.

Rationale for route of administration, dose, and regimen: Intranasal delivery was selected in order to non-invasively produce similar naloxone concentrations to those attained from the IV dosing used in previous studies which demonstrated the beneficial effects of naloxone (9,10). Two, 4 mg doses, one to each nostril, administered 1 hr apart are expected to produce similar naloxone concentrations as a 0.4 mg/kg/mL dose administered over 90 minutes which was the dose used for the IV study (9). The half-life is reported to be 2.1 hours (Narcan package label) suggesting the drug will remain at elevated levels for ~ 4 hours (2 half-lives) after the second dose. A third 4 mg dose will be administered ~4 hrs after the second to remain levels similar to intravenous dosing. When a single 8 mg dose was administered to healthy volunteers, adverse events included increased blood pressure, musculoskeletal pain, headache, nasal dryness, nasal edema, nasal congestion, and nasal inflammation. These symptoms will be monitored throughout the study.

3.0 Investigational Agent(s)

3.1 Description / Indications

Narcan® (naloxone hydrochloride) intranasal spray (4 mg in 0.1 mL) will be used for this study. Narcan® intransal spray, manufactured by ADAPT Pharma is approved by the FDA for the complete or partial reversal of opioid depression, including respiratory depression, induced by natural and synthetic opioids. The placebo will consist of a saline intranasal spray (0.09% sodium chloride).

3.2 Drug Accountability

3.2.1 Drug Receipt

The Narcan® and placebo intranasal sprays will be shipped from different supplier/ distributer directly to Fairview Investigational Drug Services. For the drug product, the same manufacturer and lot will be used for the entire study.

3.2.2 Packaging

The subjects will receive four 4 mg doses or two 8 mg doses of IN naloxone or placebo which will be dispensed by Fairview Investigational Drug Services, prepared by the Investigators and given to the subject on site. No packaging will be necessary, as no study drug will leave the site.

3.2.3 Storage

Naloxone hydrochloride and placebo intranasal solutions can be stored at room temperature 15C to 25° C.

3.2.4 Return or Destruction of Study Drug

Unused drug will be returned to IDS where it will be destroyed under standard operating procedures.

4.0 Study Objectives

4.1 Primary Objective

Primary endpoint will be the within person difference in peak epinephrine secretion during the morning episodes of hypoglycemia on days one and two; we will compare this difference between under the two treatment conditions (parts 1 and 2). Epinephrine secretion during hypoglycemia is assessed by collecting blood samples for measurement of epinephrine concentrations at baseline and every 15 minutes during the period of

hypoglycemia (starting at point where blood glucose is first < 55 mg/dl) in the clamp studies done in the mornings of days 1 and 2 of both parts 1 and 2.

4.2 Secondary Objective

Characterize naloxone pharmacokinetics following multiple intranasal administrations.

5.0 Study Design

5.1 Overview of Study Design

This is a single center, single-blind randomized cross over design trial that will compare the impact of intra-nasal naloxone vs. intra-nasal placebo administration during experimental hypoglycemia on day one vs. day two. We intend to enroll 18 individuals to obtain the complete data sets from 15 participants. Initially up to 8 of these subjects will receive intra-nasal naloxone first prior to placebo in order to evaluate tolerability and to ensure that the desired drug concentrations are achieved. Double-blinding is not possible because we will not be able to replicate for the placebo (placebo) the same delivery device as is being provided by the manufacturer for the naloxone; the manufacturer is unable to provide placebo-charged devices for us to use as placebo. Our own labeling will be put over both naloxone and placebo devices in order to obscure any device labeling that would break the blind for the participant. Expected duration of subject participation is 10-12 weeks. This study will consist of two 2-day intervention visits separated by approximately 8 weeks.

5.2 Anticipated Duration of the Clinical Investigation

We anticipate the entire duration of the investigation to be 24 months.

Dates	Goal	Responsible party(ies)
Month 1-2	Application for FDA approval , response to stipulations, and approval by University of Minnesota	Moheet, Coles, Seaquist
Month 3-18	Recruitment and screening of subjects	Moheet, Coles, Kumar
Months 3-18	Performing the experiments, laboratory analysis of plasma hormone and naloxone concentrations.	Moheet, Coles, Kumar, Mishra
Month12-24	Preparation of manuscript and abstracts for presentation to national medical societies and for publication in peer reviewed journals	Moheet, Coles, Seaquist

5.3 Evaluation Criteria

5.3.1 Primary Clinical Endpoint

Primary endpoint will be the within person difference in peak epinephrine secretion during the morning episodes of hypoglycemia on days one and two; we will

compare this difference between the two treatment conditions (parts 1 and 2). Epinephrine secretion during hypoglycemia is assessed by collecting blood samples for measurement of epinephrine concentrations at baseline and every 15 minutes during the period of hypoglycemia (starting at point where blood glucose is first < 55 mg/dl) in the clamp studies done in the mornings of days 1 and 2 of both parts 1 and 2.

5.3.2 Secondary Clinical Endpoint

To characterize the pharmacokinetic parameters of intranasal naloxone which will include C_{max} (ng/ml), T_{max} (minutes), half-life (hours), AUC (0-> infinity), clearances (CL), and volume of distributions (V). Please refer to Section 6.4.3 for parameter definitions.

5.4 Study Population

18 subjects 18-65 years of age will be enrolled in the study. The investigators will recruit and screen subjects.

5.4.1 Sample Size

We plan to enroll 18 subjects.

We propose a two-period two-treatment cross-over study, where each participant gets intra-nasal naloxone and intra-nasal placebo, in randomized order. Primary endpoint will be the within person difference in peak epinephrine secretion during the morning episodes of hypoglycemia on days one and two; we will compare this difference between under the two treatment conditions. Thus each participant will have two observations: one epinephrine response from their naloxone experiment and one epinephrine response from their placebo experiment. We expect to recruit 18 individuals in order to complete 15 studies. Additional participants will be recruited if needed to have 15 completed studies. If naloxone prevents IAH, then the epinephrine response during the third hypoglycemic clamp after naloxone infusion will still be high, while the epinephrine response during the third hypoglycemic camp after placebo infusion will be blunted. Therefore this within-person difference in epinephrine response will be large and positive. If naloxone does not prevent IAH, then this within-person difference will be close to zero.

In a previous study between healthy controls and subjects with T1D and impaired awareness of hypoglycemia, participants underwent a hyperinsulinemic hypoglycemic clamp (the same clamp procedure as proposed here) and the epinephrine response (the difference in peak epinephrine between euglycemia and hypoglycemia) was 121 ± 168 pg/ml in the subjects with T1D vs. 278 ± 134 pg/ml

in controls. We have powered this study to achieve a similar magnitude of difference between naloxone and placebo (a paired comparison) as was seen previously between controls and diabetics (a two-sample comparison). Assuming 15 completers, a one-sided type I error of 5%, and a power of 85%, a paired difference between naloxone epinephrine response and placebo epinephrine response of at least 157 pg/ml is statistically detectable if the between-person standard deviation of the paired naloxone vs. placebo difference is ***no more than 216 pg/ml***.

This standard deviation of the paired difference is a function of the standard deviation of the epinephrine response and the within-person correlation between the two epinephrine responses (one after naloxone, one after placebo). As can be seen from the Table below, we are conservatively powered, as our preliminary data suggest that our observed standard deviation will be smaller than 216 pg/ml.

Table. Estimated standard deviation (SD) of the paired difference between naloxone epinephrine response and placebo epinephrine response when the within-person correlation is ρ

	ρ = 0.2	**ρ = 0.4**	**ρ = 0.6**	**ρ = 0.8**
SD in epi response = 135	**171**	**148**	**121**	**85**
SD in epi response = 170	**215**	**186**	**152**	**108**

5.4.2 Subject Recruitment

Healthy participants will be recruited from IRB-approved public notices. Dr. Moheet, Dr. Coles or Anjali Kumar will make initial contact with subjects potentially interested and will discuss the investigation and conduct the informed consent discussion at least 24 hours before study medication is to be given.

5.4.3 Subject Screening

Drs. Moheet or Coles will complete a phone screen with each participant. Subjects will sign the consent form on the study visit, and will have a medical history and current medications recorded. A routine physical, and pregnancy test (if relevant), will be completed on the study visit.

5.4.4 Prior and Concomitant Therapy

Neither prescribed, other than hormonal birth control, nor over-the counter medications will be allowed for 48 hours (two days) prior to, nor for 24 hours after, receiving naloxone.

5.4.5 Inclusion Criteria

Subjects will be eligible to participate in the study if all of the following conditions exist:

1. Males and females between the ages of 18-65 years.
2. Subjects are capable of giving informed consent.
3. Female subjects must be post-menopausal for at least 1 year, or surgically incapable of bearing children, or practicing at least one or more of the following methods of contraception for three months prior to, and during the study: hormonal, intrauterine device (IUD), or barrier method in combination with a spermicide.
4. Subject should be medication free, other than hormonal birth control, for 48 hours before through 24 hours after study drug administration. If the need for medication is identified during this time period, it will be discussed with and approved by the PI.

5.4.6 Exclusion Criteria

Subjects will be excluded from participation in the study if any of the following conditions exist:

1. Women who are pregnant.
2. Women who are breastfeeding.
3. Subject has a known hypersensitivity to naloxone.
4. Subject with hypertension
5. Subject has a significant history of cardiac, neurologic, psychiatric, oncologic, endocrine (including diabetes type I or type II), metabolic, renal or hepatic disease
6. Subject has taken or used any investigational drug or device in the 30 days prior to screening.
7. Subject has taken either prescribed or over the counter medication for 48 hours prior to study drug administration on either of the study days, other than hormonal birth control.
8. History of narcotic or heroin abuse.

5.4.7 Exit/Discontinuation Criteria

Subjects will exit the study if any of the following conditions exist:

1. Subject voluntarily withdraws from the study.
2. Subject death.
3. Subject acquires any of the listed exclusion criteria.
4. Subject completes the protocol.
5. Subject is non-compliant with the protocol (See Section 6.7 for definition of non-compliance)

6. Subject's well-being, in the opinion of the Investigator, would be compromised by study continuation.
7. Subject experiences concurrent illness.
8. Subject experiences a serious adverse event.
9. IRB recommendation

Even if subjects who received study drug exit the study, they will be followed for 24 hours to ascertain if any Adverse Event occurs.

6.0 Study Procedures

6.1 Informed Consent

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject participates in any study procedure. This consent form must be signed by the subject, and the investigator-designated research professional obtaining the consent. A blank copy of the IRB-approved form must be kept on-site and by the sponsor-investigator.

6.2 Randomization Scheme

The investigational pharmacy at the University of Minnesota will be responsible for implementing the randomization order in which participants are given naloxone or placebo, prepared by the study statistician.

6.3 Laboratory Testing Procedures

6.3.1 Frequency of Blood Draws

Three to five milliliter blood samples will be collected for determination of plasma naloxone concentrations prior to dosing, at 5 ± 1, 15 ± 2, 30 ± 3 minutes, and at 1.08 ± 5, 1.5 ± 5, 2, 3, 4, and 6 hours after completion of the first IN dose. The actual blood collection times for hours 2 through 6 may deviate by ± 10 minutes from the specified times. In all cases, the actual time of the blood draw will be recorded. Missed samples or breakage of a sample will be recorded.

Baseline blood samples will be drawn for glucose, glucagon, epinephrine, norepinephrine and cortisol before the dose of Naloxone or placebo and before the start of the insulin clamp. During the morning study on day 1, blood samples will be collected every 5 minutes for monitoring of blood glucose levels and every 15 minutes during the last 90 minutes of the clamp, for later measurement of serum glucagon, epinephrine, norepinephrine and cortisol. Afternoon clamp on day 1 will proceed as in the morning except that no serum or plasma will be collected except

for the monitoring of glucose and collection of samples for subsequent measurement of naloxone. On day 2 blood samples will be collected as on the morning of day 1.

6.3.2 Specimen Preparation, Specimen Handling and Storage

Whole blood will be collected and centrifuged at 2000 rpm for 10 minutes to separate plasma. Plasma will be frozen and stored at -20° C or lower until analysis.

6.3.3 Specimen Shipment

Naloxone samples will be transported in an ice packed Styrofoam container directly from CRU to our research lab (LRB/MTRF) by study personnel. At that point, they will be analyzed. Leftover samples will be stored in the freezer until all analysis is complete, then discarded. Counter regulatory hormones samples including glucagon, epinephrine, norepinephrine, and cortisol would be sent to the Vanderbilt Diabetes Research and Training Center Core Laboratory for analysis.

6.4 Clinical Procedures

6.4.1 Treatment Regimen

This study will consist of two 2-day intervention visits separated by approximately 8 weeks.

Subjects will be asked to report to the research center by 7 am on the first day of the study after fasting overnight. On arrival, intravenous lines will be placed in each arm (one will be used for infusions and the other for blood sampling). A minimum of 30 minutes after the IVs are placed, baseline blood samples will be drawn for later measurement of glucose, glucagon, epinephrine, norepinephrine, and cortisol. Subjects will then be given a 4 mg (0.1 ml) or 8 mg dose (0.2 mL of IN naloxone or a similar volume of IN placebo.. After naloxone or placebo dose, a hyperinsulinemic (2.0 mu/kg/min) hypoglycemic (target = 50 mg/dl) clamp protocol will be started. The clamp will be maintained for 2 hours. Blood samples will be collected every 5 minutes for monitoring of blood glucose levels and every 15 minutes during the last 90 minutes for later measurement of serum glucagon, epinephrine, norepinephrine and cortisol. If the subject received 4mg dose of IN naloxone or placebo, a second dose of 4 mg IN naloxone or placebo will be given at +30 min after the start of insulin clamp. Every 15 minutes during the last 90 minutes minutes of the morning clamp, subjects will be asked to quantitate their hypoglycemia related symptoms using a standardized questionnaire (12). At the completion of the morning clamp, glucose will be given to return the participant to normal glucose. 2 hours after the end of the morning clamp, a second 2 hour hyperinsulinemic (2.0 mu/kg/min) hypoglycemic (target = 50 mg/dl) clamp protocol will be started. At the start of this afternoon clamp study, a dose of 4 mg or 8 mg of IN naloxone or placebo will be given. Afternoon clamp will proceed as in the morning except that no serum or plasma will be collected except for the monitoring of glucose and collection of samples for subsequent measurement of

naloxone. If the subject received 4 mg dose of intra-nasal naloxone or placebo, a second dose of 4 mg intra-nasal naloxone or placebo will be given after 30 minutes. After the completion of the afternoon clamp, subjects will be returned to euglycemia and fed a meal. They will then be allowed to leave the research center with instructions to return the next morning after an overnight fast. At 7 AM on day 2 they will undergo a single 2 hour hyperinsulinemic hypoglycemic clamp (target blood glucose 50 mg/dl) during which blood samples will be collected as on the morning of day 1. Symptom scores will be collected every 15 minutes during the last 90 minutes of the clamp. At the end of the study, infusions will be stopped, and participants will be fed a meal and then sent home after they are scheduled to return for Part 2 in 8 weeks. This timing is selected to ensure that female participants are studied at the same phase of the menstrual cycle; males will return for Part 2 in 8±2 weeks. During Part 2 they will receive the treatment not provided during Part 1. The rest of the study protocol will be the same as in Part 1.

A description of the study visits is below:

Visit description	**Expected activities**
Part 1 Day 1	• Randomization • Morning (8 – 10 AM) hyperinsulinemic hypoglycemic clamp study with two, 4 mg doses of naloxone or placebo depending on randomization assignment, collection of counterregulatory hormones and hypoglycemia symptom scores. • Afternoon (1 – 3 PM) hyperinsulinemic hypoglycemic clamp study with single 4 mg dose of naloxone or placebo depending on randomization assignment • Meal
Part 1 Day 2	• Hyperinsulinemic hypoglycemic clamp study with collection of counterregulatory hormones and hypoglycemia symptom scores • Lunch
8 week wash out period	• Usual activities
Part 2 Day 1	• Morning (8 – 10 AM) hyperinsulinemic hypoglycemic clamp study with two 4 mg doses of naloxone or placebo depending on randomization assignment, collection of counterregulatory hormones and hypoglycemia symptom scores • Afternoon (1 – 3 PM) hyperinsulinemic hypoglycemic clamp study with single 4 mg dose of naloxone or placebo depending on randomization assignment • Meal
Part 2 Day 2	• Hyperinsulinemic hypoglycemic clamp study with collection of counterregulatory hormones and hypoglycemia symptom scores. • Lunch

6.4.2 Clinical Assessments During Study

Blood Pressure & Pulse: A CRU staff member will check and record blood pressure and pulse prior to, and then approximately 30 minutes following, each dose and prior to discharge.

Other Side Effects/Tolerability: Side effects and tolerability of the IN administration will be collected as self-reports by subjects during both part 1 and part 2.

Assessment of Safety: Safety assessments include adverse events during the 6-hour period of observation.

6.4.3 Drug Assay and Pharmacokinetic Analyses

A validated, liquid chromatography-mass spectroscopy assay will be used to measure naloxone concentrations.

Non-compartmental Pharmacokinetics Analysis: T_{max} and C_{max} will be determined from visual inspection of the naloxone concentration-time plots. Pharmacokinetic analysis will be performed with WinNonLin (Certara, Princeton, NJ, USA), a pharmacokinetic data

analysis package, and will include calculation of the area under the concentration-time curve (AUC) as well as other parameters as detailed below. Descriptive statistics will be used to summarize the data from this study. Descriptive statistics will include numbers of subjects (n), mean, median, standard deviation (SD), minimum, and maximum for continuous data and frequencies and percentages for categorical data. For each subject, the following pharmacokinetic parameters will be determined:

- C_{max}: maximum observed plasma concentration
- T_{max}: time to C_{max}
- $AUC_{0\text{-}t}$: area under the plasma concentration-versus-time curve from time zero to the last sampling time at which concentrations were at or above the limit of quantification, calculated by the linear trapezoidal rule
- $AUC_{0\text{-}12hr}$: area under the plasma concentration-versus-time curve from time zero to 12 hours post-dose
- $AUC_{0\text{-}\infty}$; area under the plasma concentration-versus-time curve from time zero to infinity, calculated from $AUC_{0\text{-}t} + (Ct/\lambda z)$, where Ct is the last observed quantifiable concentration
- λz: apparent terminal rate constant λz obtained from the slope of the line, fitted by linear least squares regression, through the terminal points of the natural log of the concentration-versus-time plot of these points
- $t_{1/2}$: apparent terminal half-life, calculated from $(\log_e 2)/\lambda z$
- CL: clearance
- V_d: volume of distribution

Plasma naloxone concentrations below the limit of quantification (BLQ) of the assay will be taken as zero for all calculations. All calculations will be made using raw data.

Compartmental Analysis: Naloxone concentration-time data will also be analyzed using compartmental pharmacokinetic modeling (Phoenix Software, Certara, Princeton, NJ). In addition, a nonlinear mixed-effects model will be employed to determine the population pharmacokinetic parameters of interest using NONMEM, a pharmacokinetic software application. Initially, one- and two-compartment pharmacokinetic models with first-order absorption will be explored. Standard diagnostic plots and goodness of fit parameters will be examined at several key stages to guide the choice of the most appropriate structural and statistical components of the model. The final model will be used to simulate naloxone concentrations using varying doses and dosing regimens.

6.5 Follow-Up Procedures and Therapy Transitions

Subjects may contact study personnel after completion of the study with concerns or questions.

6.6 Study Timetable / Schedule of Events

See above section 6.4.1

6.7 Study Protocol Compliance / Treatment Adherence

Safety and tolerability will be evaluated in all subjects who receive study drug whether or not they complete the study. Subjects are required to stay at CRU research facility for 3 hours after the last dose of study drug administration. If they do not complete the study; a new subject will be recruited to ensure a final sample size of 15 completers.

6.8 Protocol Amendments after Study Initiation

Should changes in the study plan or protocol become necessary in the course of the clinical trial, those specific changes will be discussed and agreed upon by the Investigators, and appropriate IRB approval obtained before the changes are implemented. All changes must be documented as protocol amendments.

6.9 Deviations from the Clinical Protocol

When a deviation from the protocol is necessary for an individual subject, the investigator must complete a description of the deviation from the protocol and justification on the Protocol Deviation Form.

6.10 Subject Compensation

Subjects will be compensated $150 for each day of the study. Total compensation will be $600 if they complete the entire study.

7.0 Data Collection and Analysis

7.1 Subject Population(s) for Analysis

In this study we intend to enroll normal volunteers. Previously our group has had no trouble enrolling volunteers to participate in studies in which they were asked to experience several periods of hypoglycemia. This is a proof of principle study in which the impact of IN naloxone on hypoglycemia induced blunting of the counterregulatory response will be measured. Normal volunteers who participate in this protocol are expected to have the same degree of hypoglycemia induced blunting in their counterregulatory response as patients with type 1 diabetes and normal awareness of hypoglycemia. Therefore, this design will allow us to determine if naloxone works under controlled conditions. If it does, it will provide rationale to use in patient populations in the future.

7.2 Statistical Methods

Our primary outcome is the peak epinephrine response during the third hypoglycemic clamp of each infusion experiment, thus each participant will have two observations: one from their naloxone experiment and one from their placebo experiment. We will fit a general linear mixed model with fixed effects for treatment (Naloxone vs. placebo), treatment order (Naloxone first vs. placebo first), and period (Part 1 vs. Part 2) and a random effect for participant. We are confident that our study design, with a washout period of 8 weeks between Parts 1 and 2, has minimized the possibility of a treatment effect from Part 1 that lingers into Part 2. We will also consider adjustment for important baseline characteristics, such as age and sex. Diagnostics will be examined to assess model assumptions.

8.0 Safety and Adverse Events

8.1 Definitions

Adverse Event (AE)

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries will be regarded as adverse events. Abnormal results of laboratory or diagnostic procedures are considered to be adverse events if the abnormality:

- Results in study withdrawal
- Is associated with a serious adverse event
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the Investigator to be of clinical significance.

Adverse Reaction

An adverse reaction is any adverse event caused by a drug. Adverse reactions are a subset of suspected adverse reactions.

Suspected Adverse Reaction

A suspected adverse reaction is an adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Serious Adverse Event (SAE)

A serious adverse event (SAE) is any adverse event that is:

- Fatal
- Life-threatening
- Requires or prolongs a hospital stay
- Results in persistent or significant disability or incapacity

- Results in a congenital anomaly or birth defect

Important medical events are events that may not be immediately life-threatening, but are clearly of major clinical significance, may be SAEs. They may jeopardize the subject, and may require intervention to prevent one or the other serious outcomes noted above.

Attribution

The relationship of the adverse event and the drug or intervention will be classified as follows:

- **Unrelated:** The AE is clearly NOT related to the intervention
- **Unlikely:** The AE is doubtfully related to the intervention
- **Possible:** The AE may be related to the intervention
- **Probable:** The AE is likely related to the intervention
- **Definite:** The AE is clearly related to the intervention.

Hospitalization

Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse effect; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse effect (e.g., for a preexisting condition not associated with a new adverse effect or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse effect.

Expected Adverse Event

Expected adverse events are those that are known to be associated with or have the potential to arise as a consequence of participation in the study.

Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered unexpected if it is not listed in the Investigator Brochure or Protocol at the specificity or severity that has been observed.

Unanticipated Problems Involving Risk To Subjects or Others (UPIRTSO)

An adverse event that in the opinion of the Principal Investigator is unexpected, related to the drug, and serious.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse

events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events

8.3.1 Study Sponsor Notification by Investigators

A serious adverse event must be reported to the study sponsor by telephone or fax (preferred) within 24 hours of the event. A Serious Adverse Event (SAE) Form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone and fax to:

Amir Moheet (phone) 612-624-3209
(pager) 612-899-2840
(fax) 612- 626-3133

At the time of the initial report the following information should be provided:

- Study Identifier
- Study Center
- Subject Number
- A description of the event
- Date of onset
- Current Status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

Within the following 48 hours, the Investigator must provide further information on the serious adverse event in the form of a written narrative. This should include a

copy of the completed Serious Adverse Event Form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor.

8.3.2 IRB Notification by Investigators

Reports of all serious adverse events (including follow-up information) must be submitted to the IRB within 10 working days if it falls under the UPIRTSO guidelines. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

8.3.3 FDA Notification by Sponsor

The study sponsor shall notify the FDA by telephone or by facsimile (preferred) transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information.

If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the study sponsor will submit the adverse event in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

8.3.4 UPIRTSO Events

Investigators are required to submit a report of UPIRTSO events to the IRB within 10 working days of first learning of the event.

8.4 Safety Monitoring Plan

8.4.1 Anticipated Risks / Risk Mitigation

All CRU Research staff members are trained in BLS. During dosing an MD will be present on site. A complete crash cart and AED are available at CRU, and emergency standing orders are in place. 911 will be called for transport if needed.

Study Procedure	Anticipated Risks	Risk Mitigation
Blood draws, IV drug administration	Infection, pain at injection site	Aseptic technique and use of trained personnel at CRU.

Study Procedure	Anticipated Risks	Risk Mitigation
IN naloxone administration	Increased blood pressure, musculoskeletal pain, headache, nasal dryness, nasal edema, nasal congestion, and nasal inflammation.	The risks of IN naloxone will be minimized by excluding individuals who are sensitive to naloxone hydrochloride and those with high blood pressure. We will also use a standardized form to ask subjects about symptoms of musculoskeletal pain, headache, nasal dryness, nasal edema, nasal congestion, and nasal inflammation. blood pressure will be checked approximately 30 minutes following each dose and prior to discharge If symptoms develop the study may be stopped depending on severity and the doctor will monitor the subject until the symptoms are gone.
IN placebo administration	There are no reported adverse events of IN placebo administration	
Hypoglycemia	Sweating, shakiness, confusion, increased heartbeat and feeling "low". If the blood sugar drops below 20 mg/dl, confusion, seizures, coma or abnormalities in heart rhythm can occur.	The risks of hypogycemia will be minimized by monitoring the blood glucose every 5 minutes and provide glucose as needed to achieve the target level of glycemia. Subjects will also wear a pulse oximeter during the study so we can monitor their heart rate. If we become unable to monitor the glucose during the study or if the develop a serious arrhythmia (as defined by the MD or PA present), the study will be stopped and glucose will be given to normalize the blood sugar. Study MD or PA will be present thoughtout the study to

8.4.2 Medical Monitoring for Participant Safety

A Data Safety Monitoring Board (DSMB) will be assembled at the start of the study to regularly monitor patient safety and study documentation of adverse and serious adverse events. This board will consist of 3 members listed below:

1. Lisa Chow, MD. Associate Professor of Medicine, Department of Medicine, University of Minnesota

2. Tasma Harindhanavudh, MD. Assistant Professor of Medicine, Department of Medicine, University of Minnesota
3. Lynn Eberly, PhD. Associate Professor Division of Biostatistics, School of Public Health, University of Minnesota

Dr. Lisa Chow will chair the committee. Drs. Lisa Chow and Lynn Eberly both have prior experience of serving on other DSMB. Drs. Lisa Chow and Tasma Harindhanavudh are endocrinologist and have clinical expertise in this area of research and are both independent of the study team. Dr. Lynn Eberly is the study the study statistician. DSMB members will be unblinded to treatment assignments. They will review (1) incidence of early stopping of a study and (2) incidence of any adverse or serious adverse events, after 5, 10, and 15 randomizations and again at completion of recruitment. After an experiment where the study was stopped early and an adverse or serious adverse event was reported, they will review that experiment's data within two weeks.
The DSMB will be informed of all adverse events-serious adverse events within 48 hours and others within 7 days. The Principal Investigator will oversee the safety of the study, including careful assessment and appropriate reporting of adverse events as outlined in Section 7.4.
DSMB members will not receive any honorarium for serving on this committee.

8.4.3 Study Stopping Rules

If an SAE occurs in any participant, we will ask the DSMB to determine whether the adverse event was due to study medication. If it is deemed probably due to study medication, no further subjects will be given IN naloxone.

8.4.4 Reporting of Pregnancy

The outcome of any pregnancy (e.g., any premature terminations, elective or therapeutic, and any spontaneous abortions or stillbirths, as well as the health status of the mother and child including date of delivery and infant's gender and weight) will be reported. Pregnancies will be followed until birth or outcome is known pending the subject's permission.

9.0 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study

- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

A Case Report Form will be completed for each subject enrolled into the clinical study. The investigator-sponsor will review, approve and sign/date each completed CRF; the investigator-sponsor's signature serving as attestation of the investigator-sponsor's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

We will record the time and amount of blood draws; the time, amount, and route of drug administration; all vital signs (BP, HR, RR);hypoglycemia symptom scores; any side effects reported from study drugs.

9.4 Clinical Reports

An annual progress report will be submitted to the FDA and the IRB. Investigators will submit a final report of the clinical study to the sponsor and reviewing IRB within 3 months of termination or completion of the clinical study or the Investigator's part of the clinical study.

9.5 Records Retention

The investigator-sponsor will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

10.0 Study Monitoring, Auditing, and Inspecting

This study will be monitored according to FDA/GCP guidelines. The Investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.1 Study Monitoring Plan

Independent monitoring of the clinical study for clinical protocol and IND application compliance will be conducted periodically (i.e., at a minimum of annually) by the Clinical and Translational Science Institute, Clinical Trial Monitoring Service, according to the attached monitoring plan.

10.2 Auditing and Inspection

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11.0 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (21 CFR 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

12.0 Study Finances

12.1 Funding Source

This study is supported by funds from University of Minnesota Foundation and we are also applying for additional funds through other funding sources.

12.2 Conflicts of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must refer to the Regents Policies on Individual Conflict of Interest Policy or Institutional Conflict of Interest Policy. These policies require University Faculty and staff to report external professional activities and business and significant financial interests related to his or her University activities by submitting a REPA (Report of External Professional Activities) at least once per year. Faculty and staff should also file a REPA when substantial changes in business or financial interests occur, when an activity that presents a potential conflict of interest is anticipated, or when submitting an application for research support or technology transfer, submitting research protocols to the IRB, or receiving financial contributions. All University of Minnesota investigators will follow the University conflict of interest policy.

13.0 Publications Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14.0 References

1. Cryer PE. Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes. Diabetes 2005;54:3592-601
2. Geddes J, Schopman JE, Zammitt NN, Frier BM. Prevalence of impaired awareness of hypoglycaemia in adults with Type 1 diabetes. Diabetic Med 2008;25:501-4

3. Fanelli C, Pampanelli S, Epifano L, et al. Long-term recovery from unawareness, deficient counterregulation and lack of cognitive dysfunction during hypoglycemia, following institution of rational, intensive insulin therapy in IDDM. Diabetologia.1994; 37:1265-1276
4. Leelarathna L, Little SA, Walkinshaw E, et al. Restoration of Self-Awareness of Hypoglycemia in Adults with Long-Standing Type 1 Diabetes Hyperinsulinemic-hypoglycemic clamp substudy results from the HypoCOMPaSS trial. Diabetes care 2013;36:4063-70
5. Dagogojack S, Rattarasarn C, Cryer PE. Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. Diabetes.1994; 43:1426-1434
6. McCrimmon RJ. The Response to Hypoglycemia: A Role for the Opioid System? J Clin Endocrinol Metab .2011; 96:3357-3359
7. Eltayeb KMA, Brubaker PL, Lickley HLA, Cook E, Vranic M. Effect of opiate-receptor blockade on normoglycemic and hypoglycemic glucoregulation. Am J Physiol .1986; 250:E236-E242
8. Caprio S, Gerety G, Tamborlane WV, et al. Opiate blockade enhances hypoglycemic counterregulation in normal and insulin-dependent diabetic subjects. Am J Physiol.1991; 260:E852-E858
9. Leu J, Cui MH, Shamoon H, Gabriely I. Hypoglycemia-Associated Autonomic Failure Is Prevented by Opioid Receptor Blockade. J Clin Endocrinol Metab.2009;94:3372-3380
10. Vele S, Milman S, Shamoon H, Gabriely I. Opioid Receptor Blockade Improves Hypoglycemia-Associated Autonomic Failure in Type 1 Diabetes Mellitus. J Clin Endocrinol Metab .2011; 96:3424-3431
11. Moheet A, Kumar A, Eberly LE, Roberts KR, Seaquist ER. Hypoglycemia-Associated Autonomic Failure in Healthy Humans: Comparison of Two vs Three Periods of Hypoglycemia on Hypoglycemia-Induced Counterregulatory and Symptom Response 5 Days Later.
12. Towler D, Havlin C, Craft S, Cryer P. Mechanism of awareness of hypoglycemia—perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. Diabetes. 1993;42:1791–1798.