A randomized double blinded study to examine the use of N-acetyl cysteine for the prevention and treatment of HAAF in patients with type 1 diabetes.

Regulatory Sponsor:	Elizabeth Seaquist MD University of Minnesota 612-626-4833
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List of Abbreviations

List all abbreviations used in protocol

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 Harmonisation
IDS	Investigational Drug Services
IRB	Institutional Review Board
PHI	Protected Health Information
SAE	Serious Adverse Event

Study Summary

Title	*A randomized double blinded study to examine the use of N-acetyl cysteine for the prevention and treatment of HAAF in patients with type 1 diabetes*
Short Title	*NAC for HAAF*
Protocol Number	*NA*
Phase	*I/II*
Methodology	*This is a single center, double blind randomized cross over design trial that will compare the impact of N-acetyl cysteine (200 mg/kg) vs. saline infusion during experimental hypoglycemia on day one on the responses to experimental hypoglycemia on day two. 18 participants will be studied twice, 8 weeks apart. On each occasion they will undergo a 2 hour hypoglycemic clamp (target 50 mg/dl) in the morning and in the afternoon on day one and then again on the morning of day 2. During the morning clamps, samples will be collected for later measurement of serum epinephrine levels, plasma and red blood cell NAC, cysteine, and glutathione concentrations and GSH/GSSG ratios (redox status), and participants will be asked to complete a hypoglycemia symptom questionnaire*
Study Duration	*10-12 weeks per subject*
Study Center(s)	*Single Center*
Objectives	*Primary endpoint will be the within person difference in epinephrine secretion during the morning episodes of hypoglycemia on days one and two under the two treatment conditions. 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 $\leq$ 55 mg/dl) in the clamp studies done in the mornings of days 1, 2, and 3 of both parts 1 and 2.*
Number of Subjects	*36*
Diagnosis and Main Inclusion Criteria	*Healthy adults ages 18-65 years with hemoglobin A1c < 6.0%. Exclusion criteria will include history of diabetes, hyperglycemia, stroke, seizures, arrhythmias, active cardiac disease; pregnancy or plan to become pregnant during the study period; diagnosis of asthma; use of anti-oxidants or drugs that can alter glucose metabolism; and concomitant medical problems that may prevent the subject from successfully completing the protocol.*
Study Product, Dose, Route, Regimen	*N-acetyl cysteine 200 mg/kg (or saline) IV infusion (given as* a 150 mg/kg loading dose over the first hour and then followed by a 50 mg/kg maintenance dose infused over the next 4 hours on day 1 of each of the two parts of the study)
Duration of administration	*5 hours on day one*

Reference therapy	*NAC will be tested against placebo.*
Statistical Methodology	*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 NAC infusion experiment and one from their saline infusion experiment. We will fit a general linear mixed model with fixed effects for treatment (NAC vs. saline), treatment order (NAC first vs. saline 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, gender, and duration of diabetes. Diagnostics will be examined to assess model assumptions. Secondary outcomes include: the within-person difference in peak epinephrine response between the first hypoglycemic clamp (when an infusion is given) and the third hypoglycemic clamp (when no infusion is given) of each infusion experiment; symptom scores during the third* hypoglycemic *clamp of each infusion experiment; and the within-person difference in symptom scores between the first hypoglycemic clamp and the third hypoglycemic clamp of each infusion experiment*

1 Introduction

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 (FDA Title 21 part 312 or 812 and International Conference on Harmonisation guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

The purpose of this project is to evaluate N-acetyl cysteine (NAC) as a therapy for the prevention and treatment of hypoglycemia associated autonomic failure (HAAF) in patients with type 1 diabetes. Impaired awareness of hypoglycemia occurs in approximately 25% of adults with type 1 diabetes and is associated with a six fold higher risk of having severe hypoglycemia that requires the assistance of another to recognize and treat. HAAF is also the primary barrier to achieving the level of glucose control necessary to prevent the microvascular complications of the disease and contributes to the mortality seen in patients with T1DM. To fully evaluate NAC as a therapy, it is imperative that its action in humans be examined.

1.2 Investigational Agent

N-acetyl cysteinev(or saline) IV infusion given as a 150 mg/kg loading dose over the first hour and then follow that with a 50 mg/kg maintenance dose infused over the next 4 hours

1.3 Preclinical Data

In animal models, administration of N-acetyl cysteine (NAC) during episodes of hypoglycemia prevents the development of HAAF, possibly by preventing the hypoglycemia induced rise in hypothalamic reactive oxygen species known to occur during acute episodes of hypoglycemia .

1.4 Clinical Data to Date

The sponsor investigator completed a randomized placebo controlled trial of naltrexone as a therapy of patients with type 1 diabetes hypoglycemia unawareness in which 30 subjects enrolled over 3 years. One third were unable to complete the study: one because of rising liver function tests during the drug treatment and the rest for technical reasons related to the magnetic resonance imaging part of the study. In another study in which the default mode network was evaluated during hypoglycemia, we enrolled 11 people with type 1 diabetes and awareness of hypoglycemia, 9 people with type 1 diabetes and unawareness' of hypoglycemia, and 11 controls over an 18 months period. NAC has not been used in humans as a treatment of hypoglycemia unawareness in type 1 diabetes.

1.5 Dose Rationale and Risk/Benefits

This is a proof of principle experiment in which the maximal dose of NAC approved for human use will be intravenously infused to ensure that high serum concentrations of the drug are achieved. We propose a 200 mg/kg dose in which we will infuse a 150 mg/kg loading dose over the first hour followed by a 50 mg/kg maintenance dose infused over the next 4 hours, as is recommended for acetaminophen overdose,

In the package insert, the manufacturer includes a table that lists the incidence of drug related adverse events following drug administration. Data from 180 individuals are included in the table; 10 experienced mild anaphylactoid reactions (6%), 16 experienced moderate reactions (9%) and 2 experienced severe reactions (1%). All of these reactions occurred within the first 2 hours following the drug administration, following a dosage schedule we plan to use for this protocol. The package insert also includes a table that summarizes the adverse events observed in 76 published articles in which NAC was intravenously infused. The Rocky Mountain Poison and Drug Center prepared this table from information collected on 2040 subjects. 1.67% were noted to have urticara, 1.47% had vasodilatation and rash, 0.78% had hypotension. Our protocol excludes individuals with asthma or with a history of bronchospasm in an effort to reduce the risk of severe anaphylactoid reactions. We believe the small risk of adverse events from NAC exposure are reasonable given the impact of hypoglycemia unanwareness on patients with diabetes. The first sign that these patients have hyogycmeia is confusion or loss of consciousness, both of which can lead to injury from accidents. If untreted, this hypogycmeia could cause seizures and death. The number of patients with type 1 diabetes who experience hypoglycemia unawareness has been estimated to be as high as 25%.

Primary Objective

Primary endpoint will be the within person difference in epinephrine secretion during the morning episodes of hypoglycemia on days one and two under the two treatment conditions. 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 $\leq$ 55 mg/dl) in the clamp studies done in the mornings of days 1 and 2 of both parts 1 and 2.

Secondary Objective

Secondary endpoint will be within person difference in symptom scores collected during the morning episodes of hypoglycemia on days one and two under the two treatment conditions. Symptom scores *during hypoglycemia will be quantified using a previously validated questionnaire at baseline and at the end of the hypoglycemic clamp studies done in the mornings of days 1 and 2 of both parts 1 and 2..*

2 Study Design

2.1 General Design

This is a single center, double blind randomized cross over design trial that will compare the impact of NAC vs. saline infusion during experimental hypoglycemia on day one on the responses to experimental hypoglycemia on day two. Figure 1 shows the protocol in schematic form. We intend to enroll 18 individuals to obtain the complete data sets from 15 participants.

Expected duration of subject participation is 10-12 weeks. This study will consist of a screening visit and two 2-day intervention visits separated by approximately 8 weeks.

2.2 Primary Study Endpoints

Primary endpoint will be the within person difference in epinephrine secretion during the morning episodes of hypoglycemia on days one and two under the two treatment conditions. 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 $\leq$ 55 mg/dl) in the clamp studies done in the mornings of days 1 and 2 of both parts 1 and 2.

2.3 Secondary Study Endpoints

Secondary endpoint will be within person difference in symptom scores collected during the morning episodes of hypoglycemia on days one and two under the two treatment conditions. Symptom scores during hypoglycemia will be quantified using a previously validated questionnaire at baseline and at the end of the hypoglycemic clamp studies done in the mornings of days 1 and 2 of both parts 1 and 2.

2.4 Primary Safety Endpoints

The safety endpoint is ascertainment of symptoms related to allergic reactions (pruritus, swelling/tingling of mouth or tongue, shortness of breath) at baseline and at 30, 60, and 90 minutes after the start of the NAC/saline infusion. Participants' symptoms will be ascertained by the use of a standardized questionnaire.

3 Subject Selection and Withdrawal

3.1 Inclusion Criteria

- Healthy volunteer
- Age 18 – 65 years
- Hemoglobin A1c < 6.0%

3.2 Exclusion Criteria

- History of diabetes, hyperglycemia, stroke, seizures, arrhythmias, active cardiac disease
- Pregnancy or plan to become pregnant during the study period
- Diagnosis of asthma (increases risk of hypersensitivity reactions to NAC)
- Use of anti-oxidants or drugs that can alter glucose metabolism
- Concomitant medical problems that may prevent the subject from successfully completing the protocol
- Unwillingness to avoid exercise during the 7 days before each part of the study

3.3 Subject Recruitment and Screening

Subjects will be recruited from a registry of normal volunteers who are interested in participating in research that is maintained by the investigator. In addition, subjects will be drawn from the community using IRB approved public notices. The only laboratory testing necessary to meet inclusion/exclusion criteria will be a hemoglobin A1c done in the last month.

3.4 Early Withdrawal of Subjects

3.4.1 When and How to Withdraw Subjects

Subjects will be withdrawn at any point during the study if they remove their consent or if they develop any of the exclusion criteria after enrollment. In addition, any subjects who experience the symptoms of an allergic reaction to the NAC severe enough that the infusion is stopped early will be withdrawn from the study at the time the infusion is stopped (before unblinding that participant's treatment assignment). Procedures for withdrawal include collecting the necessary information to provide them with the compensation due them for the time they devoted to the study. At the end of the study, after treatment assignments are unblinded, a letter will be sent to the any subject withdrawn because of symptoms of an allergic reaction to NAC informing them of the treatment they were given. If they had been given NAC, we will instruct them to share the letter with their personal physician so the allergy could be noted in the clinical record.

3.4.2 Data Collection and Follow-up for Withdrawn Subjects

Data will be collected per the protocol up until the point the subject is withdrawn from the study. No additional data will be collected from subjects after their withdrawal unless they were withdrawn due to early stopping of the infusion. For such participants, a follow-up phone call within 1-2 days will ascertain their status and if hospitalized we will request consent to access hospitalization records. NAC is already used in clinical practice for other indications and it does not have an effect on survival.

4 Study Drug

4.1 Description

N-acetyl cysteine (or saline) IV infusion given as a 150 mg/kg loading dose over the first hour and then follow that with a 50 mg/kg maintenance dose infused over the next 4 hours

4.2 Treatment Regimen

A *150 mg/kg loading dose of NAC or saline over the first hour followed by a 50 mg/kg maintenance dose infused over the next 4 hours on* days one *of both parts of the study.*

4.3 Method for Assigning Subjects to Treatment Groups

The investigational pharmacy at the University of Minnesota will be responsible for implementing the randomization order in which participants are given NAC or saline, prepared by the study statistician. This information will be blinded to investigators and participants and they will be unblinded only at the end of the study.

4.4 Subject Compliance Monitoring

Treatment will be witnessed by the investigators, so compliance will be assumed.

4.5 Prior and Concomitant Therapy

No restrictions

4.6 Packaging

- See Section 5.1

4.7 Blinding of Study Drug

The investigational pharmacy at the University of Minnesota Medical Center will prepare and dispense NAC or saline in blinded fashion. The solutions will be prepared to look the same so blinding is maintained. Half normal saline (0.45% NaCl) will be used as the dilutent in the solution. Only the investigational pharmacy will have record of the drug assignment link to participant IDs.

4.8 Receiving, Storage, Dispensing and Return

4.8.1 Receipt of Drug Supplies

Upon receipt of the study treatment supplies, an inventory will be performed and a drug receipt log filled out and signed by the person accepting the shipment. The designated study staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files.

4.8.2 Storage

See section 5.91

4.8.3 Dispensing of Study Drug

The investigational pharmacy at the University of Minnesota Medical Center will dispense the study drug or saline and will follow standard pharmaceutical protocols to reconcile the stock.

4.8.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

5 Study Procedures

This study will consist of a screening visit and two 3-day intervention visits separated by approximately 8 weeks.

At the screening visit (approximately 2-4 days prior to study day one) informed consent will be obtained, the clinical history will be reviewed to be certain a volunteer meets the inclusion critieria and baseline hemoglobin A1c will be obtained.

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 glucose, epinephrine, norepinephrine, cortisol, cysteine and glutathione measurements. Subjects will then be given diphenhydramine 25 mg IV push immediately priot to the start of a 60 minute infusion of NAC (150 mg) or a similar volume of saline between 8 am and 9 am, followed by a four hour infusion of 50 mg of NAC. Thirty minutes after the start of the NAC infusion, a hyperinsulinemic (2.0 mu/kg/min) hypoglycemic (target = 50 mg/dl) clamp protocol will be started. During the morning study, blood samples will be collected every 5 minutes for monitoring of blood glucose levels and every 15 minutes for later measurement of serum epinephrine, norepinephrine, cortisol. Plasma and red blood cells samples will also be collected every 15 minutes for later measurement of NAC, cysteine, glutathione, and GSH/GSSG ratios (redox status). During the final 15 minutes of the morning clamp, subjects will be asked to quantitate their symptoms using a standardized method (13). At the completion of the morning clamp, glucose will be given to return the participant to euglycemia. 2 hours after the end of the morning clamp, a second hyperinsulinemic (2.0 mu/kg/min) hypoglycemic (target = 50 mg/dl) clamp protocol will be followed over two hours. The afternoon clamp study 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 NAC,

cysteine, glutathione, and GSH/GSSG ratios. After the completion of the afternoon clamps, 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. At 7 AM on day 2 they will under a single 2 hour hyperinsulinemic hypoglycemic stepped clamp (75, 65, 55, 45 mg/dl targets) during which blood samples will be collected as on the morning of day 1. Symptom scores will be collected in the final 15 minutes of each step in the clamp. At the end of the study, infusions will be stopped, they will be fed and meal and then be 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 in a blinded fashion. 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
Screening	• Confirm subject qualification • Obtain informed consent • Baseline A1c
Part 1 Day 1	• Randomization • Morning (8 – 10 AM) hyperinsulinemic hypoglycemic clamp study with infusion of NAC or saline depending on randomization assignment, collection of counterregulatory hormones and hypoglycemia symptom scores • Afternoon (1 – 3 PM) hyperinsulinemic hypoglycemic clamp study with infusion of NAC or saline depending on randomization assignment
Part 1 Day 2	• Hyperinsulinemic stepped 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 infusion of NAC or saline depending on randomization assignment, collection of counterregulatory hormones and hypoglycemia symptom scores • Afternoon (1 – 3 PM) hyperinsulinemic hypoglycemic clamp study with infusion of NAC or saline depending on randomization assignment
Part 2 Day 2	Hyperinsulinemic hypoglycemic clamp study with collection of counterregulatory hormones and hypoglycemia symptom scores • Lunch

6 Statistical Plan

6.1 Sample Size Determination

We propose a two-period two-treatment cross-over study, where each participant gets NAC infusion and saline infusion, in randomized order as shown in Figure under 2.1. 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 epinephrine response from their NAC infusion experiment and one epinephrine response from their saline infusion 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 NAC prevents HAAF, then the epinephrine response during the third hypoglycemic clamp after NAC infusion will still be high, while the epinephrine response during the third hypoglycemic camp after saline infusion will be blunted. Therefore ***this within-person difference in epinephrine response will be large and positive***. Assuming a one-sided type I error of 5% and a power of 85%, we can detect an epinephrine response difference between NAC and saline of at least 154 units. This magnitude of difference is comparable to the difference between healthy controls and subjects with T1DM and hypoglycemia unawareness that we found in a previous study. In that study, participants underwent a hyperinsulinemic hypoglycemic clamp and the epinephrine response during hypoglycemia in the subjects with T1DM was 121 ± 168 pg/ml vs. 278 ± 134 pg/ml in controls.

6.2 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 NAC infusion experiment and one from their saline infusion experiment. We will fit a general linear mixed model with fixed effects for treatment (NAC vs. saline), treatment order (NAC first vs. saline 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, gender, and duration of diabetes. Diagnostics will be examined to assess model assumptions. Secondary outcomes include: the within-person difference in peak epinephrine response between the first hypoglycemic clamp (when an infusion is given) and the third hypoglycemic clamp (when no infusion is given) of each infusion experiment; symptom scores during the third hypoglycemic clamp of each infusion experiment; and the within-person difference in symptom scores between the first hypoglycemic clamp and the third hypoglycemic clamp of each infusion experiment.

6.3 Subject Population(s) for Analysis

In this version of the protocol, we intend to only study healthy volunteers. In the original version, we intended to enroll humans with type 1 diabetes of less than 20 years duration, with A1c 6.9-8.2%, who were aware of hypoglycemia but were unable to reach our recruitment goals with this population. More than 45 potential participants were contacted over 14 months and only one subject was able to complete the entire protocol. The reasons potential participants refused enrollment included strong preferences to not experience hypoglycemia and inability to commit the time necessary to complete the study. Previously our group has had no trouble enrolling enough normal volunteers to participate in studies in which they were asked to experience several periods of hypoglycemia. Thus, we believe that we will meet our recruitment goals by changing the population to normal volunteers. In addition, this is a proof of principle study in which the impact of NAC 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 redesign will allow us to determine if NAC works under controlled conditions. If it does, it will provide rationale to use in in patient populations in the future.

Exclusion criteria will include history of diabetes, hyperglycemia, stroke, seizures, arrhythmias, active cardiac disease, pregnancy or plan to become pregnant during the study period; diagnosis of asthma (increases risk of hypersensitivity reactions to NAC); use of anti-oxidants or drugs that can alter glucose metabolism; concomitant medical problems that may prevent the subject from successfully completing the protocol; and an unwillingness to avoid exercise during the 7 days before each part of the study.

7 Safety and Adverse Events

7.1 Definitions

Adverse Event

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 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.

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs a hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event.

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality.
- The abnormality suggests a disease and/or organ toxicity.

- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

7.2 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 will 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 will 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 will 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.

7.3 Reporting of Serious Adverse Events

7.3.1 Study Sponsor Notification by Investigator

A serious adverse event must be reported to the data safety and monitoring board within 24 hours of finding out of the event using a Serious Adverse Event (SAE) form. The investigator will keep a copy of this SAE form on file at the study site.

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 of ongoing serious adverse events should be provided promptly to the data and safety monitoring board.

7.3.2 IRB Notification by Investigator

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.

7.3.3 FDA Notification by Sponsor

The study sponsor shall notify the FDA by telephone or by facsimile 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 data and safety monitoring board'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 investigator 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.

Agency	Criteria for Reporting	Timeframe	Form to Use	Submission address/fax numbers
U of MN IRB	SAE: fatal, life-threatening or serious, unexpected, at least possibly related	10 working days	UMCC SAE	MMC 820
FDA	SAE: fatal, life-threatening,	7 calendar days	FDA prefers MedWatch	Fax: 1 (800) FDA - 0178

	unexpected, at least possible related		3500a Form but alternative formats are acceptable (e.g. summary letter)	
	SAE: serious, unexpected, at least possibly related	15 calendar days		

7.4 Unblinding Procedures

Unblinding of participants and investigators will be done at the end of the study and a letter will be sent to any subject withdrawn because of symptoms of an allergic reaction to NAC informing them of the treatment they were given. If they had been given NAC, we will instruct them to share the letter with their personal physician so the allergy could be noted in the clinical record.

7.5 Stopping Rules

Individuals will be withdrawn from the trial if they experience any symptoms of allergy to NAC severe enough to require early stopping of the infusion, including feeling itchy on their skin, having tingling or swelling in their mouth or tongue, or having trouble breathing.

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 the study statistician Dr. Lynn Eberly, endocrinologist Dr. Lisa Chow (who is independent of the study team), investigational pharmacist Darlette Luke, RPharm, and endocrinologist Dr. Anthony McCall (who is independent of the study team) from the University of Virginia. DSMB members will be unblinded to treatment assignments. They will review (1) incidence of early stopping of an infusion and (2) incidence of any adverse or serious adverse events on at least a monthly basis. After an experiment where the infusion was stopped early and an adverse or serious adverse event was reported, they will review that experiment's data within two weeks.

Participant accrual will be halted if an excessively high rate of participants with allergic symptoms requiring early stopping of the infusion is seen. Statistical sequential boundaries on the cumulative number of participants with allergic symptoms will be used to monitor the rate. If allergic symptoms requiring early stopping of the infusion are noted during an experiment, the participant will be withdrawn from the trial; the withdrawal will be carried out by blinded study staff, thus without knowledge of whether the infusion given was NAC or saline. The DSMB will then review the unblinded participant data within two weeks of the experiment. If the infusion given was saline, the reaction will be denoted in DSMB records as a placebo reaction, and that participant will not be counted towards the cumulative number of participants with allergic symptoms to NAC. If

the infusion given was NAC, and in the DSMB's judgment the reaction was likely related to NAC, then the reaction will be denoted in DSMB records as an allergic reaction to NAC, and that participant will be counted towards the cumulative number of participants with allergic symptoms to NAC. The DSMB will inform the PI that there was an allergic reaction to NAC, and at study's end the project PI will notify the participant that they should inform their personal care provider that an allergic reaction to NAC was observed.

Specifically, the trial will be stopped early if the number of participants with an allergic reaction to NAC is equal to or exceeds the boundary number (bn) out of n participants who have completed at least the Part 1 infusion (Table below). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.05 when the rate of allergic symptoms is equal to the acceptable rate of 10%.

Cumulative early stopping rule for allergic symptoms to NAC with 10% as the maximum allowable rate of allergic symptoms.

Number who have completed at least one Part	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Boundary, bn	-	2	3	3	3	3	4	4	4	4	4	4	5	5	5	5	5	6

7.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

7.6.1 Internal Data and Safety Monitoring Board

We have formed a DSMB that will consists of the study statistician Dr. Lynn Eberly, endocrinologist Dr. Lisa Chow (chair), endocrinologist Dr. Anthony McCall from the University of Virginia, and investigational pharmacist Darlette Luke. They will review the data collected at each experiment on a monthly basis and more frequently if adverse events are reported (as described above in 7.5). In addition, we will file an IND with the FDA for the use of N-acetyl cysteine for the purpose outlined in this application. Past INDs filed by Dr. Lisa Coles for the IV use of N-acetyl cysteine in the study of neurological disease were given an exempt status so we anticipate the FDA will give the IND for this study and exempt status as well, but if not it will be monitored by the staff of

the Clinical and Translational Sciences Institute at the University of Minnesota. They have extensive experience with the monitoring of investigator-initiated trials at the University of Minnesota, including for Dr. Seaquist who recently completed trial of naltrexone as a therapy for hypoglycemia unawareness in type 1 diabetes. Adverse events will be filed with the University of Minnesota IRB according to requirements.

8 Data Handling and Record Keeping

8.1 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.

8.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Data generated by the methods described in the protocol will be recorded in the subjects' medical records and/or study progress notes. Data may be transcribed legibly on CRFs supplied for each subject or directly inputted into an electronic system or any combination thereof.

8.3 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.

8.4 Records Retention

It is the investigator’s responsibility to retain study essential documents for at least 6 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 6 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

9 Study Monitoring, Auditing, and Inspecting

9.1 Study Monitoring Plan

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 the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

9.2 Auditing and Inspecting

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.

10 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 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. The investigator should provide a list of IRB members and their affiliate to the sponsor.

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 is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

11 Study Finances

11.1 Funding Source

This study is funded by the Juvenile Diabetes Research Foundation.

11.2 Conflict 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.

11.3 Subject Stipends or Payments

Subjects will be paid $25 for the screening visit, $150 per day for each day of Part one they complete, and $200 for each day of Part Two they complete to cover travel expenses and time lost from work for each day of study.

12 Publication Plan

The results of the study will be published.

13 References

14 Attachments