Statistical Analysis Plan

Protocol Title: A randomized, double-blinded and placebo-controlled study on super-oxygenated water

Protocol Number: Inhale-001

ClinicalTrials.gov ID: NCT07016191

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INTRODUCTION

The aim of this project is to test in a single-center randomized clinical trial, if ingestion of INHALE super-oxygenated water can augment blood oxygen saturation (SpO2) and reduce heart rate (primary outcome measures) compared to their pre-ingestion levels as well as boost energy level and brain clarity (secondary outcome measures).

This statistical analysis plan (SAP) will give more detailed descriptions of the endpoints in the study and the corresponding analyses.

STUDY DESIGN

A total of 60 consenting subjects (research participants) will be recruited in the study. The study is a parallel-group clinical trial between two study arms to receive either INHALE super-oxygenated water (test article) or a source and taste-matched normal water (placebo control) randomized at 1:1 ratio with each arm enrolling 30 subjects. This is a double-blind study where neither the subject nor the investigator will know the assigned treatment. An unblinded research staff will perform the randomizations and dispense the blinded waters for use by blinded investigator.

The subjects will ingest 12 oz of water (about 10% of daily fluid intake which is similar to an average beverage size) in one administration. This amount is based upon available literature as a reasonable amount of fluid to drink and is aimed at not causing any dilution effect on blood oxygen saturation. The test article, INHALE water, contains dissolved oxygen at ~56 mg/L without any added sodium, sugar, protein or fat. The source and taste-matched normal water is packaged in the same way as the test article and contains dissolved oxygen at 0 mg/L without any added sodium, sugar, protein or fat. Only one dose (12 oz) of water will be ingested. Subjects will not be allowed to crossover from one arm to the other.

Pre-ingestion or baseline measurements of SpO2 and heart rate will be measured. At one-minute post ingestion, SpO2 and heart rate will be recorded. Subjects will complete clinical outcome assessments as patient reported outcome (PRO) for Energy level and Brain clarity assessment at pre- and post-ingestion time points.

Only one clinic visit will be necessary for a subject to complete the study. The overall study design is presented in Figure 1.

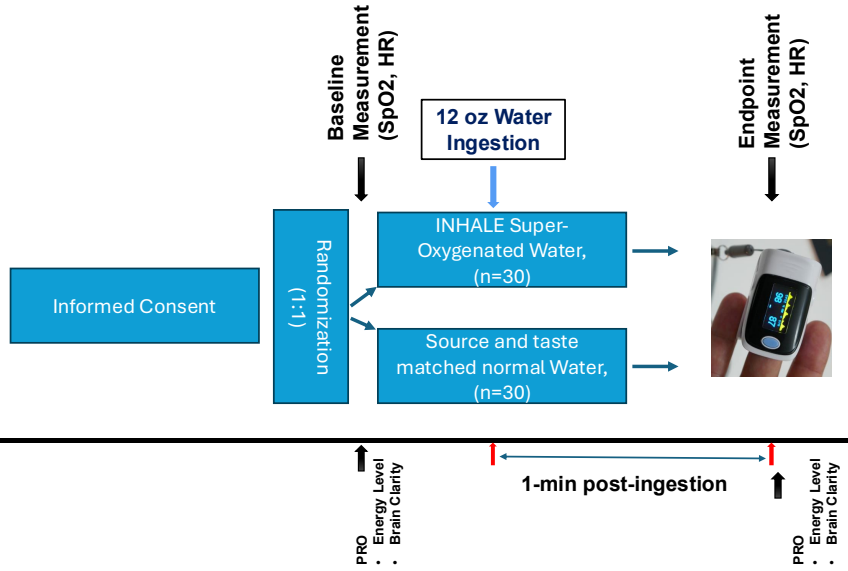

Fig 1: Study design schema

Sample Size Calculation

The primary outcome measure for the power calculation is the difference between INHALE super-oxygenated water and source and taste-matched placebo control water in the change of SpO2 and heart rate. There are no prior studies known to the sponsor that measured similar effects following an oral ingestion to better advise sample size calculation. The sample size of 30 is selected to support analysis for an expected >1% increase in SpO2 and an anticipated >1% decrease in heart rate as continuous measures in the experimental group with an alpha level of 0.05 powered at 80%.

AIMS AND OBJECTIVES

To demonstrate an improvement in blood oxygen saturation and reduction in heart rate following ingestion of super-oxygenated water. Additionally, the study will also measure whether the super-oxygenated water will enhance energy level and brain clarity.

OUTCOMES

Primary and secondary outcome measures will be assessed to answer the study aims and objectives. The analyses of the data are described below.

Primary Outcome Measures

1. Blood oxygen saturation (SpO2). It will be measured at pre-ingestion and at one-minute post-ingestion of water by a pulse oximeter.
2. Heart rate. It will be measured at pre-ingestion and at one-minute post-ingestion of water by a pulse oximeter.

Secondary Outcome Measures

1. Energy level. It will be assessed at pre-ingestion and at one-minute post-ingestion of water through patient reported outcome measure (PRO) with a questionnaire (scale 1-10).
2. Brain clarity. It will be assessed at pre-ingestion and at one-minute post-ingestion of water through a PRO questionnaire (scale 1-10).

Safety Outcomes

Consumption of water is generally considered safe for human consumption. The sponsor is not aware of any health events related to drinking of super-oxygenated water. The production cycle of INHALE superoxygenated water encompasses a closed and aseptic cycle that ensures consistency and sterility of the product.

All adverse events will be collected, graded and reported.

STUDY POPULATION

Study Eligibility- Inclusionary Criteria

1. Legally adult (18 years or older) and either gender (male or female) will qualify
2. Subject must be willing to review and provide an informed consent for participation
3. Subject must agree to the study procedures including water ingestion, and SpO2 and heart rate measurements

Study Eligibility- Exclusionary Criteria

1. Any active and life-threatening medical condition involving hepatic, renal, cardiac, respiratory, endocrinal, or gastrointestinal systems, or any blood disorder that may confound the study
2. Female subjects those are pregnant, nursing or planning to become pregnant
3. Subjects receiving any experimental medications or have undergone a major surgical procedure in last 30 days
4. Subjects previously utilized the test article (INHALE super-oxygenated water) within the last 24 hours, have undergone bariatric surgery or have received weight-loss medication
5. Subjects with known anemia, hemoglobinopathies or other comorbidities necessitating fluid restriction

Intent-to-treat (ITT)

All randomized study subjects will constitute ITT population. Collected data will be processed without any bias or selection and no subject data will be excluded from the analyses.

Per Protocol (PP)

All randomized study subjects completing the whole study period (completing all study assessments). The PP analyses will be performed in case of subjects not completing the entire study. Analyses of this population is seen as a sensitivity analysis to investigate whether the study conclusions are sensitive to assumptions regarding the pattern of missing data.

ANALYSES

The outcome measures will be presented using descriptive statistics; to summarize and describe the main features of the dataset using simple numbers (by the percent, mean, standard deviation and/or standard error) and graphs (scatter plot, histogram), helping to make large amounts of data understandable.

These analyses will provide a concise overview of characteristics like proportion of responders in a study group.

SpO2

The blood oxygen saturation will be measured twice (before and after water ingestion). The post-ingestion SpO2 will be compared with the pre-ingestion SpO2 from the same subject. An increased post-ingestion SpO2 over the pre-ingestion level will constitute treatment response and the SpO2 response rate (n/N) will be measured for both study arms. Pearson Chi-Square test will be applied to analyze categorical data (SpO2 response rate) between the arms for asymptotic significance (2-sided) at 5% level.

Heart Rate

The heart rate will be measured twice (before and after water ingestion). The post-ingestion heart rate will be compared with the pre-ingestion heart rate from the same subject. A reduction in post-ingestion heart rate over the pre-ingestion level will constitute treatment response and the heart rate response rate (n/N) will be measured for both study arms. Pearson Chi-Square test will be applied to analyze categorical data (heart rate response rate) between the arms for asymptotic significance (2-sided) at 5% level.

Energy Level

Energy level as reported by PRO assessment will be measured twice (before and after water ingestion). The post-ingestion energy level will be compared with the pre-ingestion energy level from the same subject. An increased post-ingestion energy level over the pre-ingestion level will constitute treatment response and the energy level response rate (n/N) will be measured for both study arms. Pearson Chi-Square test will be applied to analyze categorical

data (energy level response rate) between the arms for asymptotic significance (2-sided) at 5% level.

Brain Clarity

Brain clarity as reported by PRO assessment will be measured twice (before and after water ingestion). The post-ingestion brain clarity will be compared with the pre-ingestion brain clarity from the same subject. An increased post-ingestion brain clarity over the pre-ingestion level will constitute treatment response and the brain clarity response rate (n/N) will be measured for both study arms. Pearson Chi-Square test will be applied to analyze categorical data (energy level response rate) between the arms for asymptotic significance (2-sided) at 5% level.

MISSING DATA

As the study is designed to collect pairwise data at two time points, missing data (lost or uncollected data) at any time point will make the dataset unusable. When analyzing using the ITT population, simple imputation (last observation carried forward) will be used for both primary and secondary outcomes. A missing numerical data for a time point will be replaced with numerical data collected at the other time point for the same subject.