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Cover Page:

Official Title of the Study:

**A Study to Determine if Caffeine
Accelerates Emergence From Anesthesia**

NCT Number:

NCT02567968

Statistical Calculation:

There was no human data for estimating the number of test subjects to use. We used our own data from rats to provide guidance.

Why expose test subjects to 2 rounds of isoflurane anesthesia, once with caffeine and once without? How many human subjects will be required? There were several reasons why our sample size might be in error. First, rats are not people. They may get rid of isoflurane differently than do humans. In addition, rats are genetically more similar to each other than are humans. That would tend to increase human variance relative to rat. On the other hand, the equipment used to anesthetize rats is less sophisticated than that in humans. In humans, the expired anesthetic concentration is measured, thereby allowing for precise anesthetic concentration, in the lungs. This will tend to lower human variance. In our studies rats were used 4 times (twice with a saline injection twice with an injection of saline containing caffeine). This will increase variance in humans.

Statistical information from experiments already completed (Wang *et al.*, 2014):

We use the following stringent criteria to determine sample size: Power will be set to 95% and $p \leq 0.01$.

Rats waking from isoflurane anesthesia with 5 mg/ kg caffeine:

(Control - saline injection) - Mean = 540 sec, SD = 159.04
(Test - 5 mg/ kg caffeine in saline injection) - Mean = 269.67 sec, SD = 103.69

At this concentration of caffeine we would need $n=12$ measurements for control and $n=12$ measurements for caffeine to meet our stated stringent criteria.

Rats waking from isoflurane anesthesia with 25 mg/ kg caffeine:

(Control - saline injection) - Mean = 477.32 sec, SD = 146.32
(Test - 25 mg/ kg caffeine in saline injection) - Mean = 191.25 sec, SD = 103.69

We would need $n=7$ measurements for control and $n=7$ measurements for caffeine to meet our stated criteria.

The caffeine formulation and concentration used in the current study was 15 mg/ kg caffeine citrate. This is equivalent to 7.5 mg/ kg of caffeine base, which was used in the rat study. So we would require fewer test subjects than the 5 mg/kg data shown above, but more than that for 25 mg/ kg. In addition, the 7.5 mg/ kg was located at the steepest part of the caffeine dose response curve, very close to the EC_{50} value. The 25 mg/ kg was near saturation of the dose response curve. Our best estimate from the data shown above and the dose response curve shown in (Wang *et al.*, 2014) is that we would require $\sim n=10$ test subjects for a concentration of 7.5 mg/ kg caffeine.

But another adjustment needed to be made. The rats were tested at ~ 1.5 MAC of isoflurane, whereas the human volunteers were tested at 1 MAC. The lower isoflurane concentration should be easier for caffeine to antagonize and will require fewer test subjects. Although just an estimate, we hypothesized that $n=8$ test subjects, with each subject tested twice, would be sufficient to answer the question of whether caffeine could accelerate emergence from anesthesia.

Statistical Test:

Data analysis for this crossover trial will use a paired t-test, comparing subjects receiving caffeine to those receiving saline.

Protocol:

Each test subject followed the schedule outlined below.
Schema:

Procedure	Screening Session 1	Study Session 1	Study Session 2
Time	1 week prior to study	Start of Study: week 0	Week 2-4
Explain Trial to subject Obtain Consent	X		
Medical History	X		
Physical exam	X		
Vital signs	X	X	X
EKG	X	X	X
Urine Toxicology screen	X	X	X
Enroll Subject in Trial	X		
Psychomotor Training session	X		
Propofol Induction – Isoflurane maintenance		X	X
**Saline Control injection		X	
**caffeine (15 mg/ kg) injection			X
Psychomotor session 1 + 2		X	X
Adverse Event report		X	X

**Please note that the drugs were given in a double-blind manner and were prepared by a hospital pharmacist.

SUBJECT SELECTION AND WITHDRAWAL

Number of Subjects

For this study 8 subjects were tested. There was a progress report to the IRB after 6 subjects.

Gender, Age, Racial and Ethnic Origin of Subjects

Healthy male, ages 25 – 40, all race and ethnic origin inclusive.

Our animal studies were carried out in adult male rats equivalent to the age range listed above. At present we would like to reproduce our animal studies in humans, introducing as few new variables as possible. The effects of anesthesia and the study drug on fetuses are not entirely known. The tests for early pregnancy are not 100% reliable. In males, pregnancy is not a possibility. Children can have adverse reactions to anesthetics, and they will not be recruited. Therefore, male volunteers are selected in this study. In order to further reduce the variables and minimize the risks for the subjects, exclusion criteria are used to select the subjects who are most suitable for this primary study.

Inclusion Criteria

1. Age 25-40
2. Male
3. Normal healthy subject without systematic diseases or conditions
4. Metabolic Equivalents of Functional Capacity ≥ 5
5. Low risk for Obstructive Sleep Apnea (OSA) based on the screening test (STOP-bang score established by American Society of Sleep Apnea): Yes to > 3 items- high risk of OSA
6. No History of Arrhythmia (Baseline EKG will be obtained during the history and physical session), seizure, liver and kidney diseases
7. BMI < 30 kg/m²
8. No prior difficulty with anesthesia
9. No personal or family history of malignant hyperthermia
10. No history of any mental illness
11. No history of drugs or alcohol abuse (urine drug screens required).
12. Subjects capable of giving consent.
13. Living less than 30 miles away from UC.
14. No history of seizure disorders.
15. No history of head trauma.

Exclusion Criteria

1. Age < 25 or > 40
2. Female
3. ASA physical status > 1 (normal healthy subject without systematic diseases or conditions)
4. Metabolic Equivalents of Functional Capacity (METs) < 5
5. High risks for Obstructive Sleep Apnea (OSA) based on the screening test (STOP-bang score established by American Society of Sleep Apnea): Yes to > 3 items- high risk of OSA
6. History Arrhythmia (Baseline EKG will be obtained during the history and physical session), seizure, liver and kidney diseases
7. BMI > 30 kg/m²
8. Prior difficulty with anesthesia
9. Personal or family history of malignant hyperthermia
10. History of any mental illness

11. History of drugs or alcohol abuse (urine drug screens required)
12. Subjects capable of giving consent
13. Living more than 30 miles away.
14. History of seizure disorders.
15. History of head trauma.

Vulnerable Subjects

No vulnerable subjects were used.

Subject Identification & Recruitment

Brochures, flyers and posters were distributed throughout Chicago.

Location

The University of Chicago Center for Care and Discovery recovery room. This facility is outfitted with all the necessary emergency and equipment required to maximize safety. The isolation recovery room that we use is perfect for our purposes since it is soundproof.

Informed Consent Process

A week (or more) prior to the first anesthesia session, one of the anesthesiologists provided a verbal explanation of the study to the subject and answered all questions regarding this study. Afterwards a written description of the study was provided to the subject. The subject were required to describe the study in their own words showing that they understand the purpose of the study, the risks involved and their own role in the study. If the risks were not well understood, the members of our team explain them again, in detail. The subject and the person who administered the informed consent signed and dated the document. A copy of the informed consent form was given to the subject and the original was kept in the subject's record.

Formulation of the Study Drug

Caffeine citrate (caffeine), is available commercially. It was ordered by the University of Chicago hospital pharmacy. Placebo control was a sterile saline solution.

Treatment Regimen

The study drug (caffeine citrate) or the placebo (a saline solution) was given intravenously 10 min before the end of 1 hour isoflurane anesthesia. Caffeine was administered at a dose of 15 mg/ kg. There will be a single injection of either caffeine or a saline placebo control solution in the anesthetized test subjects. The drug was injected slowly across a 10-minute interval, in order to prevent concentration spikes in the subject. A pump infuser was used to introduce the drug. The subjects will be told not to eat or drink for 8 hours prior to anesthesia (see below for a more detailed description).

An example of the method of administration may be illustrative. The drug, caffeine citrate and comes in a single dosage of 20 mg caffeine per ml of solution. Let's say a volunteer weighs 65 kg. The dose we propose for this trial is 15 mg/ kg. Therefore the volunteer will get a 48.75 ml injection of caffeine citrate. In this case the subject will get 975 mg of caffeine citrate that is equivalent to 487.5 mg of pure caffeine.

Preparation and Administration of Study Drug

All drugs will be stored and/ or prepared by the IDS group and provided to the research team on the day of each session. IDS maintains drug accountability records in compliance with state and federal regulations and the specific requirements of each protocol. At study termination when study drug has been either returned to the sponsor or destroyed, all drug accountability records and study-related documents were transferred to the investigator.

Medications and Therapies

Our goal is to identify a relatively young cohort of healthy volunteers. Thus patients taking drugs for chronic conditions will not be chosen.

Propofol is the preferred agent for the induction phase because it does not cause airway irritation. Isoflurane was used as the main anesthetic agent as it is an extremely safe anesthetic that has been used for decades. During this study, we do not plan to use other anesthetic agents, such as midazolam, fentanyl, and muscle relaxants to minimize the interactions of these drugs to our study drug. However, propofol and muscle relaxants were available at the bedside in case they are needed for the management of unexpected airway situations, such as laryngeal spasm. If an unexpected airway emergency occurs, the ASA difficult airway algorithm was to be applied for airway management. None occurred. The subject was allowed to breathe spontaneously unless the tidal volume is less than 3 ml/kg. We optimized the tidal volume at around 5 ml/kg to make sure each subject receives similar amount of isoflurane for an hour. Pressure support mode to be applied to optimize the tidal volume if necessary. This was done transiently for one subject on one visit. The study drug (caffeine citrate) or the placebo control was given intravenously 10 min before the end of 1-hour isoflurane anesthesia. The anesthesiologists were blinded to the medication injected to the subject since the drug or normal saline is prepared by the pharmacists in the OR pharmacy. When isoflurane was turned off, the subject eliminated isoflurane passively via breathing.

There were two anesthesiologists present during the entire period that anesthesia was administered. Each subject was given a dose of antiemetic medication, zofran (4 mg) 20 minutes before the end of the anesthesia to minimize the incidence of post anesthesia nausea and vomiting. During the period of anesthesia, kept the subject's BP within 20% of its pre-anesthesia value. If the BP fell more than 20% of the baseline, IV fluid was given to replete the fluid deficit due to the restriction of fluid intake prior to the study. If that was not sufficient to raise the BP, we planned on reducing the concentrations of isoflurane by 0.1% at a time until we can maintain the BP close to the normal range. This never occurred. Vasoactive medications were available. Volunteer safety is our first priority.

[Intraoperative hypotension is not well defined (see (Bijker *et al.*, 2007)). In clinical practice, changes of 20 - 30% of the baseline mean MP or SBP are well accepted. Transient change of BP in this range is usually clinically insignificant. Healthy volunteers tolerate these changes better than sick surgical patients. In general, lowering of blood pressure by an anesthetic is also dose dependent. Hemodynamic changes are very fluid during anesthesia. It is important not to overcorrect the minor transient change of BP and heart rate].

Procedures:

Each subject was required to attend 3 sessions to complete the study.

Screening Session 1

In the first screening session, an anesthesiologist explained the purpose of the trial and carefully went over the consent form. If the subject was willing to enroll the consent form was signed. Next, the anesthesiologist took a detailed medical history and performed a physical examination of the subject. A baseline EKG and urine toxicology screen test were obtained. If the subject met the criteria for the study, the subject was enrolled in the trial. At this point a pre-study training session was provided to familiarize the subject with the psychomotor tests.

Study sessions 2 – 3

In the following two sessions, each subject received isoflurane general anesthesia in one session (with a saline injection as a placebo control) and receive isoflurane general anesthesia and caffeine citrate (15 mg/ kg equal to 7.5 mg/ kg of pure caffeine) in the other session in a randomized manner. For each subject, each session involving anesthesia was at least 2 weeks apart.

For each session, subjects were asked not to eat and drink 8 hours prior to the study in order to minimize the potential risk for aspiration during anesthesia. They were asked to refrain from alcohol or drug use for 24 hours prior to the sessions. A toxicology screen was used for each session. Once the subject was checked in, a peripheral intravenous catheter (IV) will be inserted on one of the arms by the anesthesiologist. The IV was used to administer medications during the course of the study. After the IV insertion, American Society of Anesthesiologists (ASA) standard monitoring, including EKG, blood pressure (BP), respiratory rate, end tidal CO₂, pulse oximetry, BIS monitor and temperature, were used to assess the subject (see below for further details). The subject was then be asked to breathe 100% O₂ via a facemask for two minutes. After the lungs were filled with 100% O₂, the subject was given propofol (2mg/kg), as an IV bolus, to induce anesthesia. Within 1-2 minutes, the subject became unconscious. If the subject was still conscious, an extra dose of propofol (0.5 /kg) was given until the subject was in a deeper stage of anesthesia. The extra dose was common as the young healthy volunteers appeared somewhat resistant to the propofol. A Laryngeal Mask Airway (LMA), a rubber airway device, was inserted via the mouth into the larynx. This device helped to optimize the patency of the airway while the subject was completely anesthetized. This allowed the subject to breathe easier without the help of a ventilator. This period is the induction phase of anesthesia. After induction, the subject was given isoflurane (1.0 MAC, as adjusted by age and weigh by the anesthesia machine) for 60 minutes. This period is called the maintenance phase of anesthesia. 60 minutes ensured that each subject reached anesthetic equilibrium. Oxygen flow was kept at 2 L/min during the maintenance phase. Ten minutes before terminating the isoflurane, volunteers were infused with a solution containing saline (control) or with caffeine (test). This was done in a completely blinded fashion. After the termination of isoflurane, the subject were allowed to wake up, without any stimulation. This phase is called the emergence phase of anesthesia. During the emergence phase, Oxygen flow went up to 8 L/min to avoid rebreathing. Our goal was to test whether the caffeine shortens the emergence phase of anesthesia.

[EKG, respiratory rate, end tidal CO₂, pulse oximetry and temperature are monitored continuously, once the volunteer is connected to the monitoring machines. A bispectral index (BIS) monitor, will continuously measure depth of anesthesia. Prior to starting the procedure, blood pressure was measured every 5 minutes; this measurement of blood pressure will provide the baseline for subsequent BP measurements. Blood pressure was

be measured every 2 minutes during the first 10 min of the anesthesia, which includes the induction period. BP was measured every 5 min throughout the course of anesthesia and the first 30 min in the PACU. After that, BP will be measured every 10 min until the subject was discharged. This data was stored automatically for each patient in our hospital's Epic database].

Propofol is the preferred agent for the induction phase because it does not cause airway irritation. Additionally, propofol is normally used for anesthesia induction in human patients. There are several advantages to use isoflurane as the main anesthetic agent. Most importantly, it is an extremely safe anesthetic that has been used for decades. All our preliminary animal data was generated with isoflurane. Because it takes slightly longer to "wake up" from isoflurane anesthesia, compared to other volatile anesthetics like sevoflurane, it allowed us to better differentiate the actions of the caffeine. During this study, we did not use other anesthetic agents, such as midazolam, fentanyl, and muscle relaxants to minimize the interactions of these drugs to our study drug. However, propofol and muscle relaxants were available at the bedside in case they are needed for the management of unexpected airway situations, such as laryngeal spasm. If an unexpected airway emergency occurred, the ASA difficult airway algorithm was to be applied for airway management. This did not happen. The subject was allowed to breathe spontaneously unless the tidal volume was less than 3 ml/kg. This occurred in a single instance. We optimized the tidal volume at around 5 ml/kg to make sure each subject receives similar amount of isoflurane for an hour. Pressure support mode could be applied to optimize the tidal volume if necessary. The study drug (caffeine citrate) or the placebo (saline) were given intravenously 10 min before the end of 1 hour isoflurane anesthesia. The anesthesiologists were blinded to the medication injected to the subject since the pharmacists in the IDS GROUP prepare the drug or the placebo control. Both the caffeine and the saline simply look like water. When isoflurane was turned off, the subject eliminated isoflurane passively via breathing. We measured the time between when the isoflurane is stopped to the awakening of the subject. We recorded the time to recovery of the gag reflex, until the subject's eyes opened, until the subject's mouth opened, and the time until the subject responded to the command to grip the hand of the attending physician or to open eyes and the removal of the laryngeal mask. After the subject was awake and relatively alert, he was asked to perform a series of psychomotor tests to measure his cognitive function. These simple cognitive tests were used to determine if they were still impaired by anesthetic. Both "control" and test subjects will be required to complete the same tests. In total there were 3 different tests. The tests were repeated every 15 minutes in order to obtain a cognitive function recovery time course.

There were at least two anesthesiologists present during the entire period of anesthesia. Each subject will be given a dose of antiemetic medication, zofran (4 mg) 20 minutes before the end of the anesthesia to minimize the incidence of post anesthesia nausea and vomiting. One hour after the isoflurane was stopped, if the subject was awake, oriented and showing stable vital signs, one of the anesthesiologists was allowed to leave the room to prepare for the afternoon session. There was always be at least one anesthesiologist with the test subject at all times during the late part of the recovery phase. Most of the time there were two anesthesiologists.

At the conclusion of the study, we discharged the subject based on the criteria used in our post anesthesia care unit in the hospitals. In general, the subject should be awake and alert with the stable vital signs. He should be able to drink clear liquid, void and ambulate before discharge home. We called a car service, or Uber, for him without cost to the subject. The subject was required to live within 30 miles of The University of Chicago to make sessions easily accessible. Upon discharge, the subject was required to be accompanied by a responsible adult. Each session for any given subject will be at least 2 weeks apart. The total time of each session, including the pre-anesthesia preparation,

testing and recovery from anesthesia, was ~4 hours. We called the subject on the same day and the next day after each session to make sure the subject was fully recovered without any adverse event. The subject received another call a week after the procedure.

Subjects will be tested in this manner a total of two times. In one of the tests they were anesthetized with isoflurane and injected with a saline solution and in one test they will be anesthetized with isoflurane and then injected with caffeine citrate (15 mg/ kg).

References:

- Bijker JB, van Klei WA, Kappen TH, van Wolfswinkel L, Moons KG & Kalkman CJ. (2007). Incidence of intraoperative hypotension as a function of the chosen definition: literature definitions applied to a retrospective cohort using automated data collection. *Anesthesiology* **107**, 213-220.
- Wang Q, Fong R, Mason P, Fox AP & Xie Z. (2014). Caffeine accelerates recovery from general anesthesia. *Journal of Neurophysiology* **111**, 1331-1340.