NAVAL MEDICAL RESEARCH UNIT DAYTON

THE PHARMACOKINETICS AND EFFICACY OF A LOW-DOSE, AQUEOUS, INTRANASAL SCOPOLAMINE SPRAY

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Abstract

The study examined both the pharmacokinetic properties and efficacy of a low-dose, aqueous, intranasal scopolamine spray (INSCOP) as an anti-motion sickness prophylaxis. The study was divided into two phases: a pharmacokinetics (PK) phase, which established pharmacokinetic parameters, and an Efficacy phase, which was a within-subjects, cross-over, double blind, placebo controlled trial testing the efficacy of INSCOP during mechanical rotation. Cognitive testing, blood samples, subjective alertness, and adverse events were collected in both parts. INSCOP is rapidly absorbed, having detectable plasma levels in 10 to 15 minutes for most subjects, and reaching maximum plasma concentrations typically within 60 minutes. Measured by the number of head tilts tolerated during mechanical rotation, subjects receiving INSCOP tolerate an average of 31 head tilts more when using INSCOP compared to a placebo condition. Cognitive assessments and subjective fatigue measurements were not significantly different between conditions. INSCOP's positive efficacy and safety profile having been identified, future studies can address multi-dose schedules and operational field trials.

Introduction

Motion sickness (MS) is a problem for the modern military across the Services, regardless of the mode of transportation. Multiple studies have identified the anticholinergic scopolamine as the most effective medication against MS (25, 27); however, the two most common methods of administration, oral and transdermal, have drawbacks that often compromise its utility, whereas intravenous, intramuscular, and subcutaneous administrative routes are impractical in operational settings. The oral dosage's shortcomings are significant enough that it is not commercially available in the United States: first-pass metabolism decreases bioavailability to between 11 and 48%, and the reduced gastric motility seen in MS can prevent timely absorption (18, 20). Transdermal scopolamine (TDS), while bypassing first-pass metabolism, requires 6 to 8 hours after placement to reach therapeutic plasma levels and continues releasing active medication for up to 72 hours (15, 17). Both methods can produce significant side effects, primarily sedation, and the extended duration of TDS (up to 96 hours) can produce additional detrimental physiological and cognitive side effects (17, 20).

To bypass these handicaps, an intranasal gel formulation of scopolamine (INSCOP) was developed. Intranasal scopolamine was first explored in 1950s military studies, but was abandoned due to the limitations of medication delivery devices at the time and an inability to deliver a properly metered dose (5, 22). With improved delivery devices, a 0.4 mg dose of INSCOP gel was tested in collaborative studies between the Navy and the National Aeronautics and Space Administration (NASA), and proved efficacious in the treatment of laboratory-induced MS, without the sedation or cognitive side effects seen in other administrative routes (21, 22). However, as the gel formulation was originally designed to be used by astronauts in micro-gravity environments, its viscosity and dense particulate size made it less than optimal for military operations in the terrestrial setting.

In response to the limitations of the gel formulation, the Pharmacotherapeutics Laboratory of the SK3 Human Adaptation and Countermeasures Office at NASA under Dr. Lakshmi Putcha developed a finer particulate, moderate pH, INSCOP aqueous spray designed to allow for a more even distribution, improved absorption, and reduced time to maximum plasma concentration (C_{max}).

A pilot Phase II clinical trial of the aqueous spray was conducted at the Naval Medical Research Laboratory (NAMRL) in Pensacola in 2011 with the goal of determining bioavailability, C_{max}, and time to C_{max} (T_{max}). Six subjects (all male) with an average age of 21.67 years (SD = 3.61) self-administered 0.2 mg (0.1 mg/nostril) of aqueous INSCOP. Delivery devices were weighed immediately before and after administration. Blood was collected prior to dosing and 10 times post-dose. Vital signs and subject-reported side effects were assessed concurrently with blood draws. INSCOP concentrations in blood were detected using Liquid Chromatographic-Mass Spectrometric (LC-MS) analysis. All 6 subjects had detectable plasma concentrations within 15 minutes, with a mean T_{max} of 57.6 minutes. C_{max} ranged between 96.4 and 230.5 pg/mL with a mean of 165.6 ± 55.7 pg/mL. The mean area under the curve (AUC_{0-8}), an estimate of bioavailability, was 491.5 pg/ml * $hour^{-1}$. Delivery device weight change was identical across subjects. Cognitive performance, measured via the Automated Neuropsychological Assessment Metrics® (ANAM) batteries, and subjective alertness, measured via the Karolinska Sleepiness Scale (KSS), did not change significantly from pre-dose values. No side effects or adverse events were reported. These results suggested that, similar to the gel formulation, the aqueous INSCOP spray offers fast onset of action with consistent drug delivery at a lower than normal dose and without significant side effects.

Due to Base Realignment and Closure (BRAC), the NAMRL laboratory was closed immediately following the completion of the pilot study, and staff and resources moved to the Naval Medical Research Unit Dayton (NAMRU-D) in 2011. In 2012, Epiomed Therapeutics, Inc. (Epiomed; Irvine, CA)

acquired INSCOP's Investigational New Drug (IND) 033983 and New Drug Application (NDA) 21-095 from NASA through the Space Act Agreement, and in 2013 signed a cooperative research and development agreement (CRADA) with the U.S. Navy for the continuation of development of INSCOP in gel and spray formulation. In 2014, Repurposed Therapeutics, Inc. (Repurposed; Tampa, FL) acquired Epiomed, retaining the partnership with the US Navy. The current study was conducted at NAMRU-D initially with Epiomed and later Repurposed serving as the FDA sponsor and supplier of clinical trial material (CTM).

The objectives of the present study were to determine the pharmacokinetics and efficacy of a 0.2 mg (0.1 mg/nostril) dose of an aqueous intranasal scopolamine spray. The study was a Part IIb clinical trial divided into two parts: Pharmacokinetics (PK), which repeated the pilot study with an expanded sample size, and Efficacy, a randomized, double-blind, placebo-controlled, within-subjects design aimed at testing the efficacy of INSCOP via laboratory-induced MS. Both parts evaluated vital signs, cognitive performance, subjective alertness, and medication side-effect profiles.

Methods

Subjects

All subjects were active-duty military between the ages of 18 years and 59 years. The study protocol was approved by NAMRU-D's Institutional Review Board (IRB). Written informed consent was obtained from each subject prior to participation. Subjects were permitted to participate in both parts of the study, though each part required signing separate consent forms. All consent sessions occurred at NAMRU-D. Financial compensation was addressed prior to subjects signing consent, with subjects receiving $13.64 per blood draw, for a total of $150.04 in PK and $218.24 in Efficacy. No other form of compensation was offered. To determine at least minimal susceptibility to MS, subjects in the Efficacy part completed the Motion Sickness Susceptibility Questionnaire (MSSQ) [Appendix A], requiring a minimum score of 3.0 to participate; subjects with scores below 3.0 were dismissed. All subjects in both parts were screened for any exclusionary health-related habits. All female subjects were given a urine pregnancy test repeated upon every return visit to NAMRU-D; positive results led to immediate exclusion.

PK. Twenty-one subjects (15 male, 6 female) with a mean age of 30.86 years (SD = 12.59) volunteered to participate in PK, of which 13 (10 male, 3 female) with a mean age of 30.31 years (SD = 13.42) completed the study. Descriptive statistics are shown in Table 1.

Efficacy. Forty-two subjects (29 male, 13 female) with a mean age of 30.45 years (SD = 10.46) volunteered to participate in Efficacy, of which 22 (17 male, 5 female) with a mean age of 31 years (SD = 10.8) completed the study. A 23rd subject completed Efficacy, but was excluded from final results due to protocol violations during experimental days. Descriptive statistics are shown in Table 1.

Table 1. Demographics for PK and Efficacy.

	PK		Efficacy	
Gender	**N**	**%**	**N**	**%**
Male	10	77	17	77
Female	3	23	5	23
Age (years)	**Mean**	**SD**	**Mean**	**SD**
Total	30.3	12.1	31.4	10.9
Male	31.9	13.4	33.8	11.9
Female	25.0	3.6	24.4	3.5
Height (inches)				
Total	70.1	3.9	69.6	3.3
Male	71.6	2.6	70.6	2.7
Female	65.2	3.4	65.9	2.9
Weight (pounds)				
Total	178.4	29.3	174.6	27.9
Male	188.2	22.7	181.1	27.0
Female	145.6	27.0	150.0	18.9
MSSQ Score				
Total			13.1	10.2
Male			12.9	11.0
Female			13.7	8.0
Race	**N**	**%**	**N**	**%**
White	9	69	16	73
Black	0	0	0	0
Asian/Pacific Islander	1	8	1	5
Hispanic	2	15	4	18
Other	1	8	1	5

Drug Preparation

Scopolamine hydrobromide, USP, 0.1 mg/ 0.1 mL intranasal spray, delivered via the Aptar Bidose Liquid Spray System was provided by Repurposed. For PK, each medication vial was enclosed in a numbered bag or plastic container and labeled as intranasal scopolamine 0.2 mg / 0.1 g. For Efficacy, active medication and placebo were blinded and numbered, one of each in subsequently numbered bags. The included placebo was a saline spray, also packaged in the Aptar devices. Both INSCOP and placebo were delivered by two pumps of the Aptar actuator, one to each nostril.

Active medication and placebo were formulated and shipped in two separate lots: UMB201107-09 and UMB201411-07, hereafter referenced as lots 2011 and 2014 respectively, per the year each lot was created. Both lots were formulated and packaged at the University of Maryland, with approximately three years separating the two batches. Lot number identification was listed on all CTM and subject data. Lot 2011 was the same lot used in the 2011 pilot study at NAMRL. To ensure the original samples of lot 2011 remained functionally potent due to the three years separating the pilot study at NAMRL and the current study, the Pharmaceutical Department at the University of Maryland conducted an assay of the remaining samples and extended the shelf life until 31 April 2015. On 8 March 2014, NAMRU-D received 15 labeled samples of lot 2011 for use in the PK part. On 19 June 2014, NAMRU-D received 30 sealed bags, each containing one active medication vial and one

placebo vial of lot 2011. On 16 December 2014, NAMRU-D received 20 labeled samples and 30 sealed bags of lot 2014 for continuation of both PK and Efficacy parts.

Motion Stimulus

PK. There was no motion stimulus in the PK part.

Efficacy. Motion stimulus was provided by Neuro Kinetics, Inc.'s Neuro-Otologic Test Center (NOTC), a multi-axis rotating chair contained within a cylindrical sound- and light-proof chamber (Figure 1). The method employed to evoke MS consisted of seated yaw axis rotation at a constant velocity, accompanied by paced, off-axis head movements. This repeatedly-proven provocative effect of Coriolis cross-coupling during off-axis head movements elicits at least minimal stomach symptoms in ~90% of participants within 20 minutes (14). Subjects were secured into the NOTC via a 4-point chest harness and foot straps. Researchers maintained visual contact with subjects via an infrared camera mounted on the arm of the NOTC and audio communications via two-way headsets. A head-mounted gyroscope was placed on each subject allowing tracking of angular head tilt. Prior to beginning rotation, padded head stops attached to the NOTC were adjusted laterally on either side of each subject's head to ensure head tilts were 30˚ in each direction, as measured by the head-mounted gyroscope. Vertical axis rotation began at 1 rpm with an increase of an additional 1 rpm every minute to a maximum of 40 rpm. While rotating, a pre-recorded computerized voice informed subjects to make paced head tilts of 30˚ to the right and left at a rate of 0.125 Hz (right, center, left, and back to center over 16 seconds). The sequence of events that began each minute during motion stimulus was: 1.5 seconds of acceleration ($4^{\circ}/s^2$) followed by 58.5 seconds of constant rotational velocity. At 48 seconds into each minute of rotation, subjects returned their heads to central position and were asked to indicate "yes" or "no" to "stomach awareness" and, if "no", then queried to rate a list of symptoms on a three-point scale , one being none and three being maximum. Symptoms included: nausea, dizziness, sweating, salivation, warmth, drowsiness and headache. If subjects answered "yes" to "stomach awareness", the NOTC would continue rotating for a full minute without advancing stepwise in speed and without head tilt cues. At the end of a full minute, subjects would again be asked to answer "yes" or "no" to "stomach awareness". If no, the NOTC would advance 1 rpm and head tilts would resume. If "yes", researchers would stop the program and remove the subject from the NOTC. Rotation end point was either a full minute of rotation at 40 rpm or a self-declared "moderate stomach awareness" continuing unabated for a full minute.

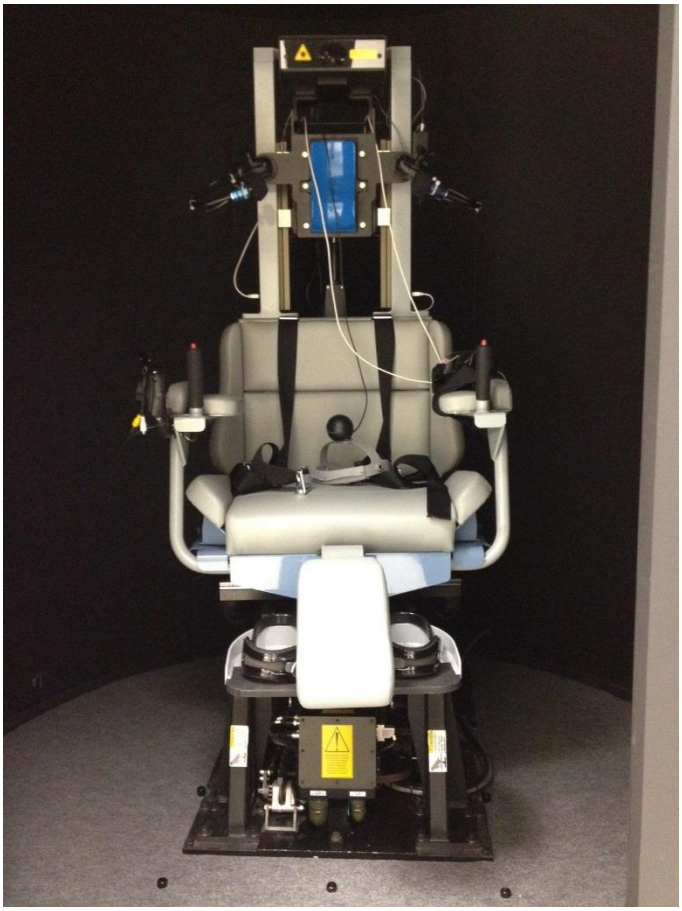

Figure 1. Neuro Kinetics' NOTC.

Measures

Motion Sickness Questionnaires

Modified Motion Sickness Susceptibility Questionnaire – Short Form (MSSQ). This questionnaire determines individual susceptibility to motion sickness and the types of motion most likely to cause sickness during both childhood and the past 10 years. All subjects participating in Efficacy completed the MSSQ. A minimum score of 3.0 was required for participation. The cut-off score was derived from previous research to identify the most appropriate subject population (7, 8).

Motion Sickness Symptom Assessment. The symptoms collected during rotation in Efficacy were derived from symptoms listed in the Pensacola Motion Sickness Questionnaire (MSQ) [9]. Symptoms included: nausea, dizziness, sweating, salivation, warmth, drowsiness and headache for each minute of motion exposure. Subjects were asked to rate each symptom on a scale of one to three, with one representing "none/minimal", two "moderate", and three "maximum". Stomach awareness was reported in a "yes/no" dichotomy. Symptoms were collected at the end of each minute, prior to advancement in rpm. A baseline symptom assessment was conducted before rotation began but after the subjects had been secured in the NOTC. A post-rotation symptom assessment was conducted immediately after rotation had ceased and prior to removing the subject from the NOTC.

Biological Data Collection

Upon arrival on experimental day(s), subjects received an indwelling 20 or 22 gauge catheter with an extension set in the antecubital vein of their non-dominant arm. Blood samples were collected using two 6 mL tubes with Lithium Heparin 95 USP units, the line flushed with 0.9% saline before and after each draw. Prior to taking each sample, a 3 mL additive-free tube of blood was taken and discarded to clear the line of saline. If the indwelling catheter became compromised, the unit was either replaced or a direct stick via 21 gauge collection set was substituted. The maximum amount of blood that could be drawn per subject was 165 mL over 8.5 hours for PK, and 120 mL over 5.5 hours per experimental day for Efficacy. For PK, blood was drawn 11 times: at baseline, and then at 5, 15, 30, 45, 60, 120, 180, 240, 360, and 480 minutes post-dose. For Efficacy, blood was drawn eight times per experimental day: at baseline, and then at 5, 15, 25, 80, 100, 120, and 180 minutes post-dose.

Blood samples in both parts were centrifuged at 3000 rpm at 4° C for 10 minutes for plasma separation. Plasma for each time point was divided equally into three cryovials via sterile pipettes to create three separate sets of plasma samples for each subject. Each cryovial received between 1.5 and 2.0 mg of plasma and was stored in a -80 ° C freezer until transferred for analysis. Plasma samples were assayed for scopolamine quantification using a fully validated LC/MS method by NASA's Pharmacotherapeutics Laboratory.

Physiological Monitoring

Vitals collected included heart rate, systolic and diastolic blood pressure, and oral temperature. Vitals were collected for safety and to provide additional information pertaining to potential medication effects. Vitals were taken concurrently with blood draws in both PK and Efficacy parts.

Cognitive Assessments

For both parts, cognitive performance was measured using six tests from the ANAM® program, including: Code Substitution – Learning (CDS), Code Substitution – Delayed Memory (CDD), Running Memory – Continuous Performance (CPT), Logical Relations (LRS), Matching to Sample (M2S), and Simple Reaction Time (SRT). These tests were chosen for their known sensitivity to medication-induced performance effects (11, 13). All tests were conducted on a laptop, using the mouse only.

CDS emphasized scanning and paired associative learning of symbol-number pairs, with subjects required to identify whether a displayed symbol-number pair is correct compared to a defined key of symbol-number pairs at the top of the computer screen. CDD tested delayed recall on the symbol-number pairs, by removing the key and requiring subjects to recall whether displayed symbol-number pairs were correct corresponding to the key in CDS. CPT assessed sustained attention, concentration and working memory by rapidly displaying a series of numbers whereupon subjects would identify whether the displayed number matched the preceding number or not. LRS assessed abstract reasoning by asking subjects to evaluate whether a statement was true or false in describing the order of two displayed symbols. M2S assessed spatial processing and working memory by displaying a shaded 4x4 sample grid and then replacing it with two comparison grids, one identical to the sample grid and the other differing; subjects would identify the grid identical to the sample grid. SRT measured reaction time by requiring subjects to click the mouse button as rapidly as possible each time a symbol appeared.

The cognitive testing was organized into blocks identical for all subjects. Blocks were identical for both parts: six blocks for PK subjects, five for Efficacy. Three practice sessions occurred prior to the experimental day(s) to ensure subjects reached performance asymptote prior to medication administration; the first two practice sessions consisted of CDS and CDD only, whereas the third practice session consisted of three blocks of all ANAM® tests. A final practice session occurred at the beginning of the experimental day(s). Each experimental day(s) block consisted of (in order): CDS, M2S, SRT, CPT, LRS, and CDD. CDS and CDD were separated by a minimum of 30 minutes, which began at the completion of CDS, to decrease chances of proactive interference. For PK, the ANAM® test batteries were applied prior to dosage, and then at 20, 65, 125, 185, and 365 minutes post-dose. For Efficacy, the ANAM® test batteries were applied prior to dosage, and then at 20, 85, 125, and 185 minutes post-dose. Due to the necessity of acquiring blood samples and the timing of rotation, the ANAM® test battery at 20 minutes post-dose excluded CDS and CDD in both PK and Efficacy.

Subjective Assessments

Karolinska Sleepiness Scale (KSS)

As a primary side effect of higher doses of scopolamine is fatigue, KSS scores were used to identify any potential impact on alertness. The KSS measures subjective alertness using an ascending 9-point scale ranging from “extremely alert” (one) to “extremely sleepy, fighting sleep” (nine). Subjects were instructed to complete a KSS form immediately after completion of LRS in every ANAM® block in both parts; LRS was selected due to the 30 minute delay for CDD and to ensure a KSS was completed for each ANAM® block, as CDD was not included in the 20 minute post-dose block. Previous research linked KSS with objective measures of encephalographic and oculographic signs of sleep onset (1, 10).

Adverse Events (AEs)

In both parts, immediately after vitals and blood had been collected, subjects were asked “How do you feel right now?” and “Is this normal for you at this time of day?”. All answers were recorded and any abnormal response was listed as an adverse event. Any adverse event noted outside of these time points by research staff or reported by subjects was likewise recorded.

Experimental Procedures

Practice and Physical Examination Days

Subjects reported to the lab prior to experimental day(s) on three occasions, each separated by a minimum of 24 hours. For both parts, unless otherwise noted, pre-experimental day(s) sessions were identical. The first visit included the signing of informed consent and completion of the MSSQ (Efficacy only). After consent, subjects were read a list of known side effects and symptoms of allergic reactions to scopolamine as well as necessary corresponding actions should any side effects/reactions occur. Subjects were then screened for exclusionary health-related habits or behaviors via the Confidential Medical Questionnaire (CMQ) [Appendix A] and the Confidential Exclusionary Behavior Questionnaire (CEB) [Appendix A]. The CEB was repeated upon every return visit to the lab, including experimental day(s), to ensure subject compliance. Subjects were excluded from participation for regular tobacco and/or alcohol consumption, vestibular disorders, asthma, sleep apnea, seizure disorders, liver/kidney problems, heart/circulatory disease, high blood pressure, narrow-angle glaucoma, emphysema, peptic ulcers, obstructions of the abdomen or bladder, and enlarged prostate. Subjects were also excluded if having donated blood within 30 days. If subjects were taking any

prescription medication, they were required to receive Medical Monitor clearance prior to study participation. After completion of the forms, subjects practiced a block of ANAM® tests (CDS and CDD). Between the first and second visits, subjects had a fasting blood draw at the Wright-Patterson Medical Center's laboratory. The second visit included completion of a CEB, a second practice block of CDS and CDD, and a brief medical exam by the Medical Monitor. During the medical exam, the Medical Monitor would inform subjects of the lab results and, if not meeting exclusionary criteria, clear subjects for continued study participation. The third visit included completion of a CEB and three blocks of ANAM® tests, including all six tests. Between ANAM® blocks, subjects were briefed on the timeline and protocol of the experimental day(s). If applicable, pregnancy tests were given at each visit.

Experimental Days

PK. Subjects reported to the lab, completing a CEB and a compliance check. Baseline vitals were collected and urine pregnancy tests were completed as applicable. An indwelling catheter was inserted and baseline blood samples drawn. Subjects then completed baseline cognitive testing and KSS. Research assistants demonstrated proper self-administration of medication using placebo spray. Approximately 30 minutes post-arrival, 0.2 mg (0.1 mg/nostril) of INSCOP was self-administered, followed by blood draws, vitals collection, cognitive testing, and the KSS for approximately eight hours post-dose. Caffeine-free beverages and food were provided to subjects throughout the experimental day. When not undergoing testing or biological sample collection, subjects would remain in NAMRU-D's subject lounge. Timeline details are listed in Table 2.

Efficacy. Experimental days were separated by a minimum of one week. Each day's timeline was identical except for the contents of the intranasal spray (active medication or placebo). Subjects reported to the lab and completed a CEB and a compliance check. Baseline vitals were collected and urine pregnancy tests completed if applicable. An indwelling catheter was inserted and baseline blood samples drawn. Subjects then completed baseline cognitive testing and KSS. Research assistants demonstrated proper self-administration of medication using placebo spray. Approximately 30 minutes post-arrival, 0.2 mg (0.1 mg/nostril) of either INSCOP or placebo was self-administered, followed by blood draws, vitals collection, cognitive testing and the KSS. Rotation began approximately 40 minutes post-dose, and was discontinued when subjects either reported "moderate stomach awareness" for a full minute unabated, or the maximum rotation speed of 40 rpm was obtained. Post-rotation, subjects experienced additional blood draws, vitals collection, cognitive testing, and the KSS for approximately two- to three-hours (actual time dependent on rotation length). Total time at the lab during an experimental day approximated 5.5 hours. Caffeine-free beverages and food were provided to subjects throughout the experimental day. When not undergoing testing or biological sample collection, subjects would remain in NAMRU-D's subject lounge. Timeline details are listed in Table 3.

Table 2. PK Experimental Day Timeline.

Time	Post-Dose (in minutes)	Event(s)
0700		Subject arrival, reaffirm consent, compliancy check, pregnancy test if applicable.
0705		Baseline vitals and AE #1, IV insertion
0715		ANAM warm up session
0720		Baseline ANAM #1, Code substitution: Learning #1
0735		Baseline KSS #1
0750		Code Substitution: Delayed Memory #1
0800		Baseline Blood Draw #1
0830	0	Medication Administration
0835	5	Blood Draw #2; Vitals and AE #2
0845	15	Blood Draw #3; Vitals and AE #3
0850	20	ANAM #2, no Code Substitution; KSS #2
0900	30	Blood Draw #4; Vitals and AE #4
0915	45	Blood Draw #5; Vitals and AE #5
0930	60	Blood Draw #6; Vitals and AE #6
0950	80	ANAM #3, Code Substitution: Learning #2; KSS #3
1025	115	Code Substitution: Delayed Memory #2
1030	120	Blood Draw #7; Vitals and AE #7
1035	125	ANAM #4, Code Substitution: Learning #3; KSS #4
1105	155	Code Substitution: Delayed Memory #3
1130	180	Blood Draw #8; Vitals and AE #8
1135	185	ANAM #5, Code Substitution: Learning #4; KSS #5
1205	215	Code Substitution: Delayed Memory #4
1230	240	Blood Draw #9; Vitals and AE #9
1430	360	Blood Draw #10; Vitals and AE #10
1435	365	ANAM #6, Code Substitution: Learning #5; KSS #6
1505	395	Code Substitution: Delayed Memory #5
1630	480	Blood Draw #11; Vitals and AE #11
1635	485	Subject discharged

Table 3. Efficacy Experimental Days Timeline.

Time	Post-Dose (in minutes)	Event(s)
0700		Subject arrival, reaffirm consent, compliancy check, pregnancy test if applicable.
0705		Baseline vitals and AE #1, IV insertion
0715		ANAM warm up session
0720		Baseline ANAM #1, Code substitution: Learning #1
0735		Baseline KSS #1
0750		Code Substitution: Delayed Memory #1
0800		Baseline Blood Draw #1
0830	0	Medication/Placebo Administration
0835	5	Blood Draw #2; Vitals and AE #2
0845	15	Blood Draw #3; Vitals and AE #3
0850	20	ANAM #2, no Code Substitution; KSS #2
0855	25	Blood Draw #4; Vitals and AE #4
0900	30	Rotation in NOTC
0950	80	Blood Draw #5; Vitals and AE #5
0955	85	ANAM #3, Code Substitution: Learning #2; KSS #3
1010	100	Blood Draw #6; Vitals and AE #6
1025	115	Code Substitution: Delayed Memory #2
1030	120	Blood Draw #7; Vitals and AE #7
1035	125	ANAM #4, Code Substitution: Learning #3, KSS #4
1105	155	Code Substitution: Delayed Memory #3
1130	180	Blood Draw #8; Vitals and AE #8
1135	185	ANAM #5, Code Substitution: Learning #4, KSS #5
1205	215	Code Substitution: Delayed Memory #4
1220	230	Subject discharged

Pharmacotherapeutics and Efficacy

The plasma concentrations of INSCOP were determined by a LC-MS method conducted by the Pharmacotherapeutics Laboratory, Johnson Space Center, Houston, TX. Efficacy was determined by the average number of head tilts tolerated per condition. Each minute of rotation was equal to 12 head tilts (not including the rotation minutes after subjects affirmed “moderate stomach awareness”). The stimulus profile was controlled by Labview® software, as was the collection of the total number of head tilts and rotation duration. A research assistant also ensured subjects complied with pre-recorded instructions during rotation via the NOTC-mounted camera.

Statistical Analyses

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL) and Excel 2013 version 15.0 (Microsoft, Inc.), both for Windows® (Microsoft, Inc.).

In Efficacy, paired samples t-tests were conducted to determine whether INSCOP resulted in a significant increase in the total number of head tilts tolerated and rotation time compared to placebo. Paired samples t-tests and log-rank tests were conducted to identify any significant differences in the

number and duration of symptoms experienced during rotation between the INSCOP and placebo conditions.

For both PK and Efficacy, plasma concentration vs. time profiles of INSCOP were analyzed for absorption and bioavailability using moment analysis (5). C_{max} and T_{max} were used to estimate absorption. AUC was used to estimate bioavailability and calculated via the linear trapezoidal method. A series of repeated measures ANOVAs were used to compare bioavailability and plasma concentrations between lots and parts. Due to small sample sizes in PK and differing sample sizes in Efficacy, Mann-Whitney tests were conducted to determine any differences in tested variables between lots.

For PK, a series of repeated measure ANOVAs was conducted to compare changes across time in heart rate, systolic and diastolic blood pressure, temperature, subjective alertness via the KSS, and cognitive performance. For the Efficacy part, a series of repeated measure ANOVAs were conducted on the same variables to compare changes between treatment conditions across time. For both parts, Greenhouse-Geisser corrections were used where Mauchley's test indicated the assumption of sphericity had been violated. Significant effects were explored using post hoc tests with the Sidak correction to control familywise error rate. For both parts, cognitive tests were analyzed via throughput, calculated as the number of correct responses per unit of available response time. As throughput is comprised of both speed and accuracy, it holds higher validity and less variability than measuring either speed or accuracy alone (24, 26).

Results

Results

Rotation

There was a significant difference in the number of head tilts tolerated between INSCOP (222.5 ± 105) and placebo condition (191.7 ± 95.1), $t_{(21)} = 2.48$, $p = 0.02$, with a mean difference between conditions of 30.7 ± 58.2. Of the 14 (68.2%) subjects who tolerated a greater number of head tilts in the INSCOP condition, the mean increase was 62.9 ± 43. In contrast, 5 (22.7%) subjects had a mean of 41 ± 32.1 more head tilts in placebo condition compared to INSCOP, and 3 (13.6%) subjects had an equal number of head tilts in each condition. There were no significant differences in the number or duration of motion sickness symptoms between conditions or lots.

Pharmacokinetics

PK. The pharmacokinetic analysis was conducted using a non-compartmental model. The mean C_{max}, T_{max}, $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}INF}$ are displayed in Table 4. Due to difficulties in retaining venous access, researchers were unable to obtain the desired number of blood samples from three subjects: two subjects were excluded due to multiple missing values. One subject's single missing value at the 30-minute post-dose mark was replaced with an average of the immediate preceding and following values (21.2 pg/mL). There was some variance in dosage delivered, with subjects receiving a mean of 0.19 ± 0.2 mg of INSCOP. A Pearson's Correlation found no relation between dosage and C_{max} or AUC.

Table 4. PK Bioavailability Parameters.

	Total (n=11)		Lot 2011 (n=5)		Lot 2014 (n=6)	
Minutes Post-Dose	Mean	SD	Mean	SD	Mean	SD
Baseline	0	0	0	0	0	0
5	2.6	5.1	4.8	7.0	0.9	2.1
15	31.9	26.0	30.0	15.0	33.4	34.2
30	59.0	30.5	66.5	24.2	59.1	35.9
45	88.8	42.6	109.6	33.0	71.4	44.3
60	110.4	53.0	140.6	51.4	85.2	42.8
120	75.5	40.9	99.7	31.2	55.3	38.7
180	56.1	49.6	88.9	59.0	28.7	12.7
240	37.3	31.4	63.3	27.4	15.6	11.3
360	8.2	10.8	13.7	13.9	3.6	4.9
480	3.7	8.5	5.6	12.6	2.2	3.4
Mean C_{max} (pg/mL)	117.5	56.6	156.3	47.5	85.2	44.2
Mean T_{max} (Minutes)	70.9	36	84	54	60	0
$AUC_{0\text{-}8}$ (pg/ml * h^{-1})	320.5	178.3	455.4	159.6	208.1	99.2
$AUC_{0\text{-}INF}$ (pg/ml * h^{-1})	329.1	190.7	471.0	179.9	210.9	99.7

Figure 3 displays mean plasma concentration levels by total PK subjects and by lot. Absorption was rapid, with 10 of 11 subjects reaching detectable plasma concentrations of INSCOP within 15 minutes post-dose ($\overline{X}$ = 35.1 ± 25.1 pg/mL). By 30 minutes post-dose, 10 of 11 subjects had detectable plasma concentrations ($\overline{X}$ = 62.8 ± 29.3 pg/mL), with final subject lacking due to researchers' inability to obtain blood at that time point. All subjects had detectable concentrations by the 45 minutes post-mark. C_{max} ranged between 62.4 and 217.6 pg/mL with a mean of 117.5 ± 56.6 pg/mL. Ten of 11 subjects reached C_{max} at 60 minutes post-dose, with the final subject peaking at the 180 minute post-dose mark; the latter is notable for having self-identified significant nasal congestion prior to medication administration. T_{max} was 70.9 ± 36 minutes. By the final time point, 8 of 11 subjects had returned to non-detectable levels of plasma concentration, with the remainder (n=3) having a final mean plasma concentration of 13.7 ± 12.5 pg/mL. The mean $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}INF}$ was 320.5 ± 178.3 and 329.1 ± 190.7 pg/ml x h^{-1}, respectively.

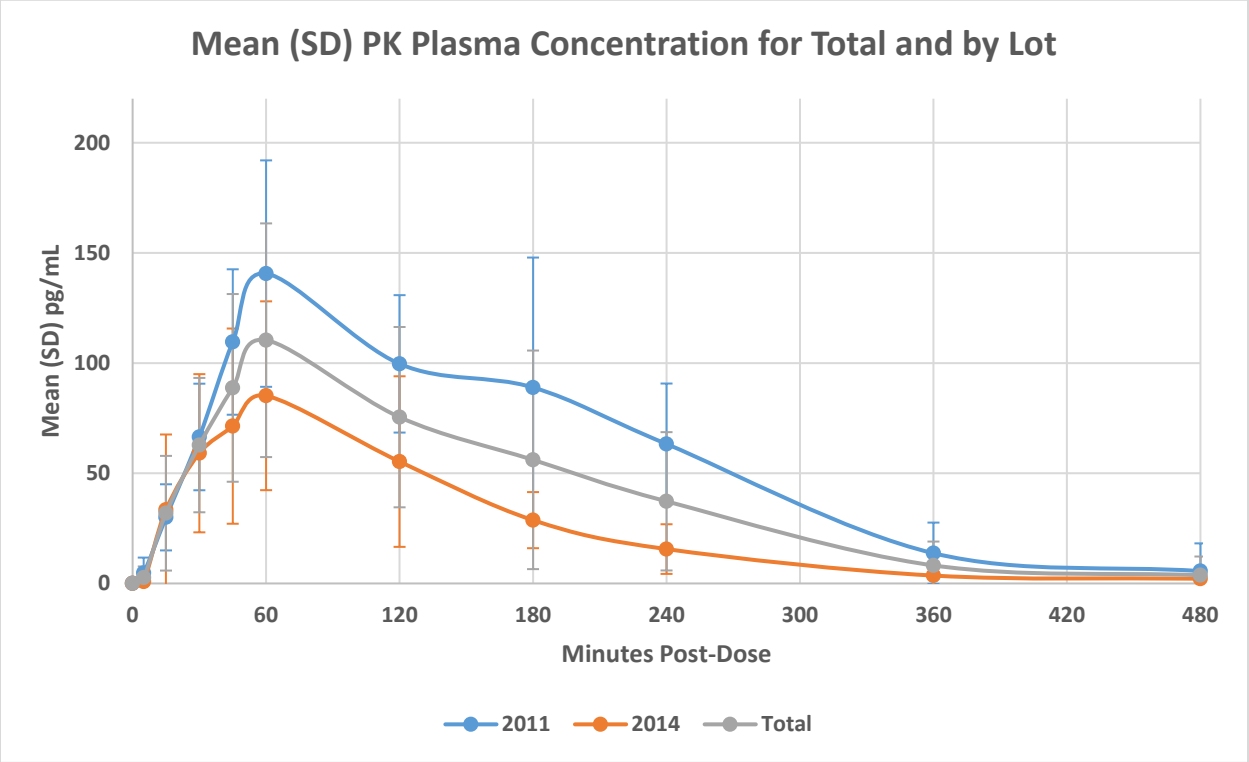

Figure 3. Mean PK Plasma Concentration Total and by Lot.

The analysis of the plasma concentration by lot (Figure 3) found a significant main effect of lot, F (1,9) = 5.96, p = .037, and a significant interaction between lot and concentration, F (10,90) = 2.75, p = .005. The interaction was driven by the 2014 lot's lower plasma concentration levels beginning at one hour post-dose and continuing until the final time-point. Due to unequal variances between lots per Levene's test, a Mann-Whitney test was used to identify the main effect between lots as driven by the time points at two (p = .045), three (p = .018), and four hours post-dose (p = .011). The difference between lots at one hour post-dose was approaching significance, p = .068.

Efficacy. As with PK, the pharmacokinetic analysis for Efficacy was conducted using a non-compartmental model. The mean C_{max}, T_{max}, $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}INF}$ are displayed in Table 5. Figure 4 displays mean plasma concentration levels by total subjects and by lot. Two subjects were excluded due to missing plasma samples. As with the PK part, there was some variance in the weight of dosages delivered. The overall mean dosage delivered was 0.19 ± .01 mg, of which lot 2011's mean dosage (n=15) was 0.19 ± .01 mg compared to a 0.18 ± .01 mg for lot 2014 (n=5). A Pearson's Correlation found no relation between dosage and C_{max} or AUC. Absorption was rapid, with 18 of 20 subjects reaching detectable plasma within 15 minutes post-dose ($\bar{X}$ = 57.0 ± 8.2 pg/mL). By 25 minutes post-dose, 19 subjects had detectable plasma concentrations ($\bar{X}$ = 101.9 ± 14.1 pg/mL). All subjects had detectable concentrations by 45 minutes post-dose. C_{max} ranged between 63.8 and 308.1 pg/mL with a mean of 175.3 ± 62.7 pg/mL. T_{max} was 87 ± 40.2 minutes. The mean $AUC_{0\text{-}3h}$ was 340.2 ± 144.3 pg/ml x h^{-1}. Due to the wide variance in individual plasma levels and available curve data limited to 3 hours post-dose, $AUC_{0\text{-}INF}$ was not calculated.

Table 5. Efficacy Plasma Parameters.

Minutes Post-Dose	Total (n=20) Mean	Total (n=20) SD	Lot 2011 (n=15) Mean	Lot 2011 (n=15) SD	Lot 2014 (n=5) Mean	Lot 2014 (n=5) SD
Baseline	0.0	0.0	0.0	0.0	0.0	0.0
5	6.0	16.6	2.1	4.3	17.9	31.8
15	51.3	38.9	49.1	36.0	57.9	50.9
25	96.8	65.3	90.5	61.5	116.0	80.2
80	150.6	69.9	135.6	71.3	195.3	45.1
100	122.6	53.1	113.0	54.4	151.4	40.3
120	142.4	70.0	122.6	61.6	201.7	64.2
180	97.2	55.7	94.7	62.4	105.0	31.2
Mean C_{max} (pg/mL)	175.3	62.7	158.5	56.6	225.4	56.9
Mean T_{max} (Minutes)	87.0	39.9	82.7	44.3	100.0	20.1
AUC_{0-8} (pg/ml * h^{-1})	340.2	144.3	308.9	139.9	434.2	125.2
AUC_{0-INF} (pg/ml * h^{-1})	NA	NA	NA	NA	NA	NA

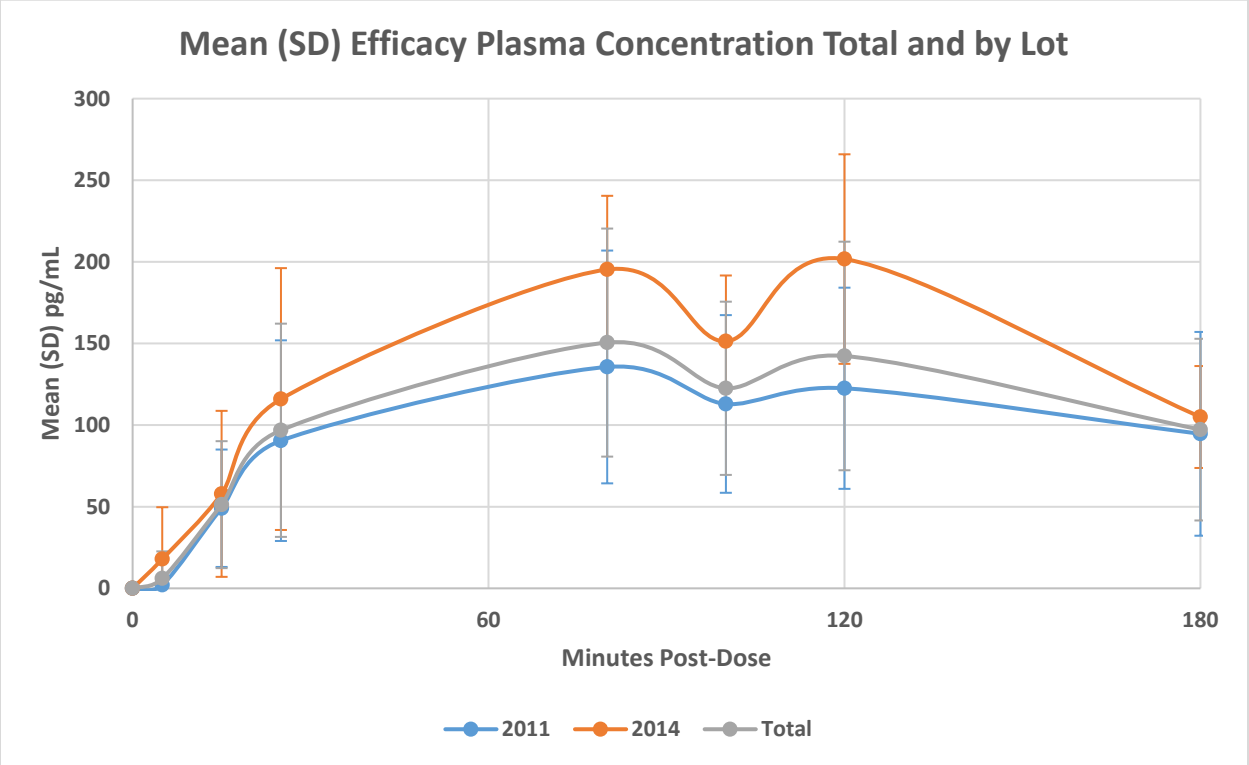

Figure 4. Mean Efficacy Plasma Concentration Total and by Lot.

As with PK, there were differences between lots, though the difference in sample sizes (n=15 for 2011 lot, n=5 for 2014 lot) precluded accurate analysis. The mean C_{max} for the 2011 and 2014 lots were 158.5 ± 56.6 pg/mL and 225.4 ± 56.9 pg/mL, and for AUC_{0-3h} 308.9 ± 139.9 pg/ml x h^{-1} and 434.2 ± 125.2 pg/ml x h^{-1}, respectively. A Mann-Whitney test identified the only significant difference in time

points between lots occurring at the two-hour post-dose mark (p = 0.32), with 122.6 ± 61.6 pg/mL vs 201.1 ± 66.9 pg/mL . The difference between lots in C_{max} approached significance (p = 0.061).

Vitals Results

PK. All 13 subjects were included in the vitals analysis. In the repeated measures ANOVAs conducted for vitals, Mauchley's test indicated that the assumption of sphericity had been violated for overall effect for heart rate, *χ2(54) = 80.86, p = 0.027,* and for KSS, *χ2(14) = 31.91, p = 0.005*; therefore the Greenhouse-Geisser correction for degrees of freedom was used. The analysis conducted on heart rate showed a significant main effect, F (4.65, 55.83) = 11.19, p < .000, driven by the difference between the peak heart rate at baseline ($\bar{X}$ = 62.7 ± 12.9 beats per minute [bpm]) and the nadir at 120 minutes post-dose ($\bar{X}$ = 47.8 ± 6.0 bpm). The analyses conducted on systolic and diastolic blood pressure found no significant main effect, though as with heart rate, the lowest scores occurred at 120-minutes post-dose. Analysis of temperature showed a significant main effect, F (10, 120) = 2.09, p = .030, driven by the temperature nadir at 120 minutes post-dose ($\bar{X}$ = 97.1 ± 0.7 °F) compared to the mean of the remaining 10 time points ($\bar{X}$ = 97.5 ± 0.2 °F).

Efficacy. All 22 subjects were included in the vitals analysis. Analysis of heart rate in the efficacy part found a significant difference between conditions, F (1, 21) = 24.45, p < .000, due to significantly lower mean heart rates in the INSCOP condition from 25 minutes post-dose onwards ($\bar{x}$ = 54.7 ± 8.7 bpm) compared to placebo ($\bar{x}$ = 60.7 ± 9.7 bpm) [Figure 5]. The analysis conducted on blood pressure found a significant difference between treatments for systolic, F (1, 21) = 4.32, p = .050, and approaching significance for diastolic, F (1, 21) = 4.162, p = .054 (Figures 5-6). The main effect for systolic blood pressure between treatments was the 6.5 mm/Hg difference at 100 minutes post-dose via paired samples t-test, $t_{(21)}$ = -3.68, p = .001. By block, systolic and diastolic blood pressure followed similar trends regardless of treatment condition, though the INSCOP condition had lower means for both measures beginning at 25 minutes post-dose and continuing through the rest of the time points. There was no difference between treatments for temperature.

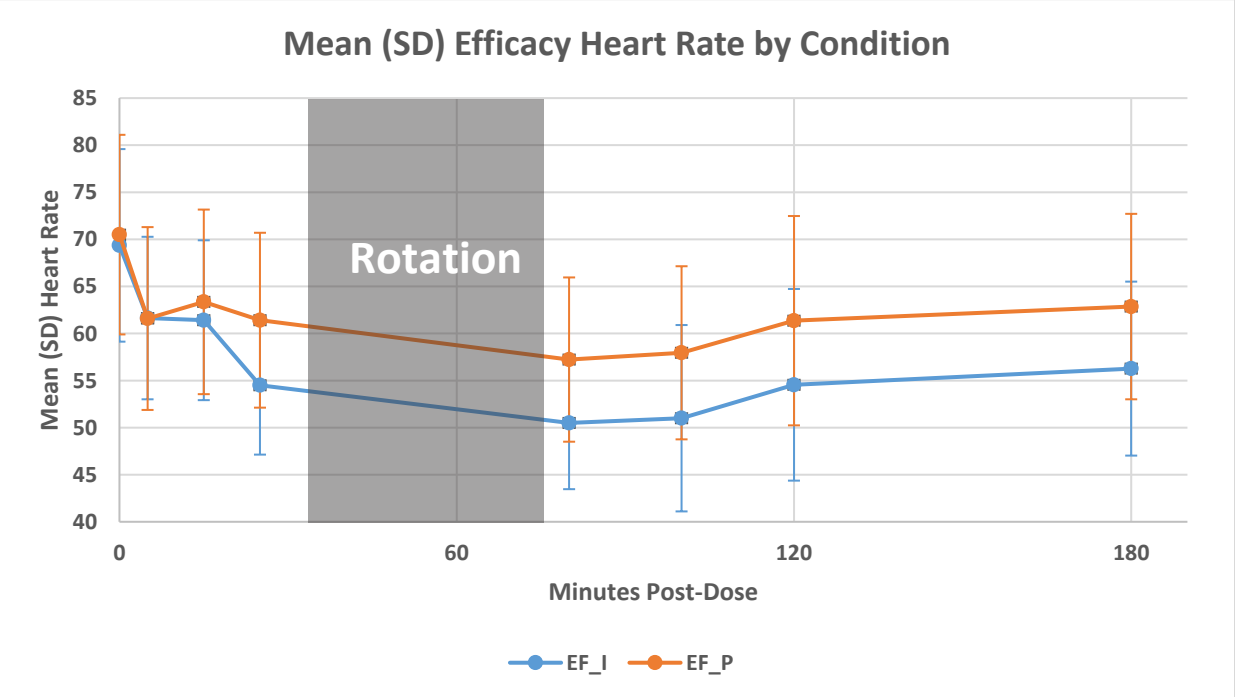

Figure 5. Mean Efficacy Heart Rate by Condition.

Subjective Alertness (KSS) Results

PK. All 13 subjects were included in the KSS analysis. There was a significant main effect on KSS scores by time, F (2.72, 32.6) = 4.08, p = .017, driven by the difference between the baseline scores ($\bar{X}$ = 3.5 ± 1.9) and the mean of the five post-dose scores ($\bar{X}$ = 4.9, SD = 1.8) [Figure 7]. The nadir ($\bar{X}$ = 5.4 ± 1.9) occurred at 185 minutes post-dose; however, this was only 0.18 deviation from the 125 minutes post-dose mean score of 5.2 ± 1.6.

Efficacy. All 22 subjects were included in the KSS analysis. There was no difference in KSS scores between treatment conditions or lots. In the repeated measures ANOVAs conducted within each condition, Mauchley's test indicated that the assumption of sphericity had been violated for overall effect for the INSCOP condition, $\chi 2(9) = 32.85, p = 0.000,$ and the placebo condition, $\chi 2(9) = 54.10, p = 0.000$; therefore the Greenhouse-Geisser correction for degrees of freedom was used. There was a significant effect of time on both the INSCOP condition, F (2.19, 45.89) = 17.24, p = .000, and the placebo condition, F (1.98, 41.54) = 8.88, p = .001. The effect of time was driven by the difference in pre- and post-rotation scores, with mean INSCOP sleepiness scores increasing from 3.8 to 5.2 and mean placebo scores from 3.7 to 4.9.

Cognitive Data Results

PK. All 13 subjects were included in the cognitive analysis. For CDS, analyses found a significant main effect of time on throughput (F(4, 48) = 3.79, p = .009), driven by the baseline score ($\bar{X}$ = 66.8 ± 8.88) compared with the mean of all post-dose scores ($\bar{X}$ = 61.3 ± 8.55). For CDD, analyses found a significant main effect of time on throughput (F (4, 48) = 10.06, p < .000), the driving force being between the mean scores at 125- and 165-minutes post-dose ($\bar{X}$ = 44.8 ± 8.8) compared to the mean of all other scores ($\bar{X}$ = 59.3 ± 12.8) [Figure 6]. There were no other significant main effects and no difference between medication lots.

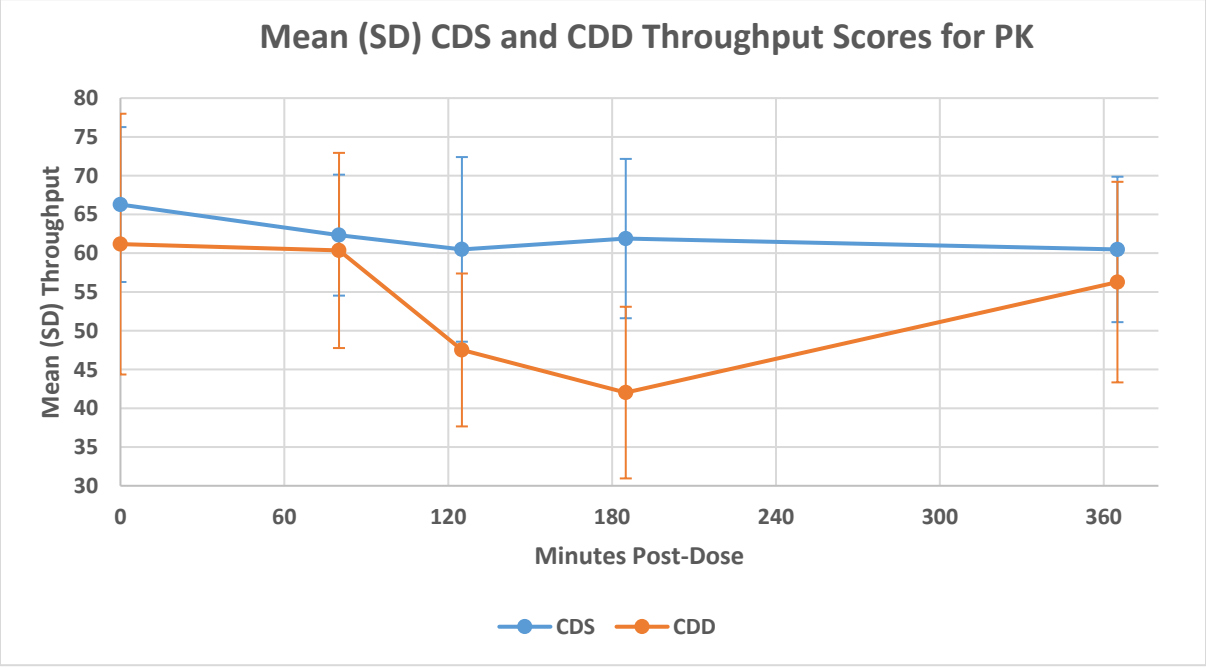

Figure 6. Mean PK CDS and CDD Scores.

Efficacy. All 22 subjects were included in the cognitive analysis. There were no significant differences between treatment conditions or between lots for any of the six cognitive tests.

For CDS, Mauchley's test indicated the assumption of sphericity had been violated for overall effect for the INSCOP condition, *χ2(5) = 15.09, p = 0.010.* There was a significant effect of time for INSCOP, F (2.04, 42.86) = 7.42, *p* = .000, and placebo, F (3, 63) = 8.71, *p* = .000. A post hoc pairwise comparison using the Sidak correction found baseline throughput scores significantly higher pre-rotation compared to all post-rotation scores in both conditions (Figure 7). For CDD, there was a significant effect of time for INSCOP, F (3, 63) = 22.65, *p* = .000, and placebo, F (3, 63) = 24.5, *p* = .000. A post hoc pairwise comparison using the Sidak correction found throughput scores had significant decreases at each time point in both INSCOP and placebo conditions (Figure 7).

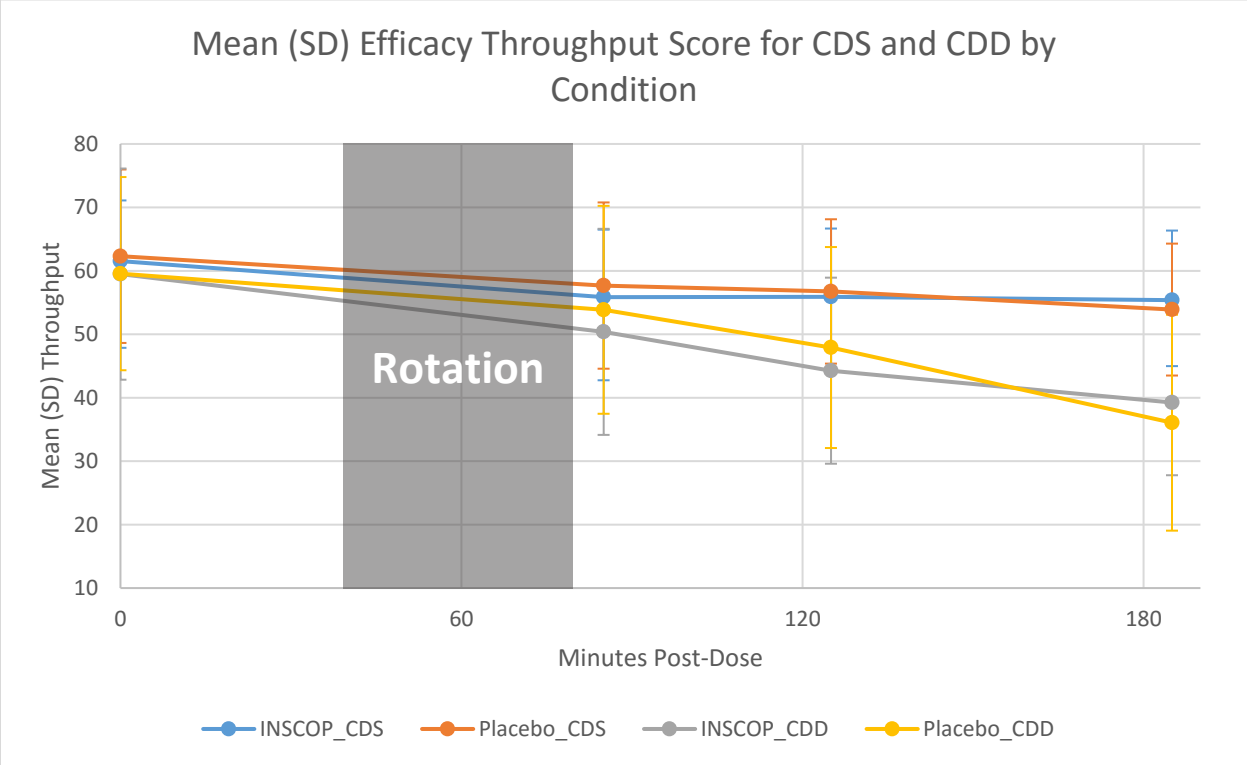

Figure 7. Mean Efficacy CDS and CDD Scores by Condition.

For CPT, Mauchley's test indicated the assumption of sphericity had been violated for overall effect for the INSCOP condition, *χ2(9) = 34.12, p = 0.000*. There was a significant effect of time for INSCOP, F (2.20,46.18) = 12.41, *p* = .000. A post hoc pairwise comparison using the Sidak correction found throughput scores significantly higher pre-rotation compared to the three post-rotation scores in both conditions (Figure 8). There was no similar effect of time for placebo. In the analysis for interaction, Mauchley's test indicated the assumption of sphericity had been violated for interaction between CPT and treatment condition, *χ2(9) = 24.31, p = 0.004.* There was a significant interaction between treatment condition and time, F (2.56, 53.7) = 4.49, *p* = .010. The interaction, and the significant effect by time, was driven by 21 (95%) subjects in the INSCOP condition's throughput scores decreasing between the pre- and post-rotation time points, whereas only 12 (55%) subjects' scores decreased in the placebo condition, resulting in a mean drop of 10.9 points in the INSCOP condition compared to only 3.1 in placebo.

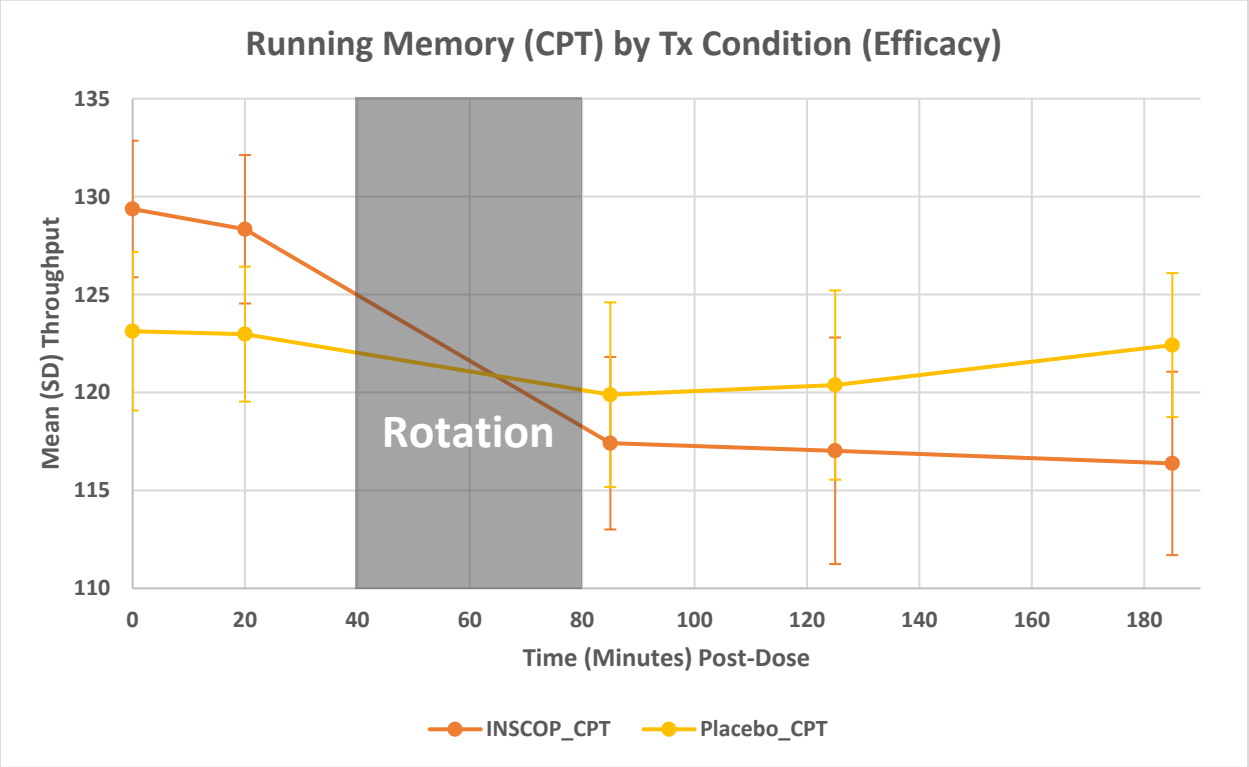

Figure 8. Mean Efficacy CPT Scores by Condition.

For LRS, there was no significant effect of time for either condition, or by lot.

For M2S, there was a significant effect of time for INSCOP, F (4, 84) = 7.74, p = .000, and placebo, F (4, 84) = 3.14, p = .019. A post hoc pairwise comparison using the Sidak correction found that the significant difference in the INSCOP condition was between the highest throughput score at 20 minutes post-dose compared to the two final throughput scores at 125- and 185-minutes post-dose. The significant difference in the placebo condition was between the highest throughput score at 20 minutes post-dose and the nadir at the final time point.

For SRT, Mauchley's test indicated the assumption of sphericity had been violated for overall effect for the INSCOP condition, $\chi 2(9) = 22.04, p = 0.009$. There was a significant effect of time for INSCOP, F (2.66, 55.77) = 5.24, p = .004, but not for placebo. A post hoc pairwise comparison using the Sidak correction was approaching significance between the first and final time point in the INSCOP condition (p = 0.57). However, the main effect by time for the INSCOP condition was driven by the 1.5 point decrease in mean score between the first and second time points, whereas the mean score for placebo condition rose 4.9 points between the first and second time points.

Adverse Events

Adverse events for PK are listed in Table 6 and for Efficacy in Tables 7 (INSCOP condition) and 8 (placebo condition). No critical adverse events were reported. One subject in Efficacy inadvertently withdrew the active medication vial from nose before the dose had fully dispersed, resulting in some droplets entering the subject's left eye, leading to observed eye dilation and blurred vision beginning at 80 minutes post-dose. There was no significant difference in the number or type of adverse events between conditions in Efficacy.

Table 6: PK Adverse Events (n=13)

Baseline	
Systolic BP <100	1
5m Post-Dose	
Sore Throat/Discomfort	2
Systolic BP >140	2
Dazed/Lightheaded	1
Increased Alertness	1
Line Failure	1
15m Post-Dose	
Sore Throat/Discomfort	1
Systolic BP >140	2
Systolic BP <100	2
Increased Alertness	1
30m Post-Dose	
Systolic BP >140	2
Systolic BP <100	1
Dazed/Lightheaded	1
Line Failure	1
45m Post-Dose	
Systolic BP >140	1
Systolic BP<100	3
Dazed/Lightheaded	1
60m Post-Dose	
Systolic BP <100	1
Dazed/Lightheaded	2
Elevated Temperature	1
120m Post-Dose	
Systolic BP <100	1
Line Failure	1
180m Post-Dose	
Systolic BP >140	2
Line Failure	1
240m Post-Dose	
Systolic BP <100	2
360m Post-Dose	
Systolic BP <100	1
Elevated Temperature	1
Fatigue	1
480m Post-Dose	
Systolic BP >140	1
Elevated Temperature	1
Fatigue	1
Line Failure	1

Table 7: Adverse Events (n=22) for Efficacy: INSCOP Condition

Baseline	
Elevated temp	1
Head fullness	1
5m Post-Dose	
Distinct taste	1
Elevated temp	1
Systolic BP >140	1
Throat irritation	1
15m Post-Dose	
Cold	2
Elevated temp	1
Increased salivation	1
Systolic BP <100	2
Systolic BP >140	1
Throat irritation	1
25m Post-Dose	
Elevated temp	2
Fatigue	2
Lightheaded	1
Systolic BP <100	2
Systolic BP >140	1
80m Post-Dose	
Abnormal	1
Cold	2
Distinct taste	1
Dizziness	6
Eye dilation/ blurred vision	1
Fatigue	4
Lightheaded	1
Nausea	1
Systolic BP <100	1
Systolic BP >140	1
100m Post-Dose	
Abnormal	1
Cold	2
Decreased temp	1
Dizziness	3
Elevated temp	1
Eye dilation/blurred vision	1
Fatigue	5
Hunger	1
IV Failure	1
Lightheaded	1
Nausea	2
Systolic BP <100	1
120m Post-Dose	
Cold	1
Dizziness	1
Elevated temp	1
Eye dilation/blurred vision	1
Fatigue	4
IV failure	1
Lightheaded	1
Nausea	1
Systolic BP <100	2
180m Post-Dose	
Eye dilation/blurred vision	1
Fatigue	3
Nausea	1
Systolic BP <100	1

Table 8: Adverse Events (n=22) for Efficacy: Placebo Condition

Baseline	
Elevated temp	1
Fatigue	1
Head fullness	1
Systolic BP <100	1
5m Post-Dose	
Systolic BP <100	1
Systolic BP >140	2
Throat irritation	3
15m Post-Dose	
Abnormal	1
Elevated temp	1
Lightheaded	1
Systolic BP <100	1
Systolic BP >140	3
Throat irritation	1
25m Post-Dose	
Elevated temp	2
Systolic BP <100	2
Systolic BP >140	2
80m Post-Dose	
Dizziness	5
Fatigue	2
Headache	1
Hiccups	1
Nausea	2
Systolic BP <100	3
Systolic BP >140	1
100m Post-Dose	
Abnormal	1
Cold	1
Dizziness	2
Elevated temp	1
Fatigue	2
Nausea	2
Systolic BP <100	1
Systolic BP >140	2
120m Post-Dose	
Abnormal	1
Cold	1
Elevated temp	1
Fatigue	2
Systolic BP <100	1
Systolic BP >140	1
180m Post-Dose	
Elevated temp	1
Fatigue	1
IV Failure	1
Systolic BP <100	1

Discussion

The purpose of this study was to examine the bioavailability and efficacy of a low-dose aqueous INSCOP spray. In Efficacy, as hypothesized, most subjects tolerated more head tilts after INSCOP administration when compared to placebo. These results are similar to previous INSCOP efficacy studies. A 2001 study rotated 20 subjects for 210 seconds at 45 rpm with head tilts every 4 seconds and self-reported seasickness scores (SKS) as the dependent variable. Subjects receiving 0.2% INSCOP spray reported significantly lower SKS compared to receiving either placebo or 50 mg oral dimenhydrinate (12). A 2008 study found subjects receiving either 0.2 or 0.4 mg of INSCOP gel tolerated off-axis vertical rotation significantly longer than in a placebo condition (2). Using a protocol similar to the current study, our predecessor lab subjected 16 MS-susceptible active duty military personnel to mechanical rotation with paced head tilts in both INSCOP gel and placebo conditions; subjects tolerated an average of 45.19 (SD = 81.93) more head tilts in the INSCOP gel condition compared to placebo (21). The same study also found a significantly positive relationship between higher doses of INSCOP and head tilts, r=0.462, $p<0.05$, whereas our study approached significance in a negative relationship between higher doses and head tilts, r = -.433, $p=.056$. Only a single study has failed to identify a significantly positive treatment effect via INSCOP (22). Fifty-four subjects were assigned to three different treatment conditions: 0.8 mg oral scopolamine, 0.4 mg INSCOP gel, and placebo. There were no significant differences between groups in the number of head tilts tolerated, though study authors noted a lack of power due to no within-group comparisons, as subjects participated in only one condition.

There were differences in pharmacokinetic properties both between this study and the pilot study, and between the PK and Efficacy parts in this study. For PK, there was a large difference in mean C_{max} and AUC between this study (117.5 ± 56.6 pg/mL and 320.5 ± pg/ml * h^{-1}) and the pilot study (165.6 ± 55.7 pg/mL and 491.5 ± 150 pg/ml * h^{-1}). This appears to derive from a difference between lots; the pilot study used only lot 2011 whereas our study used both lots. Figures 9 and 10 display plasma concentrations by lot and study part. The mean C_{max} and AUC for the 5 PK subjects receiving lot 2011 in our study is 156.3 ± 47.5 pg/mL and 444.8 ± 151.1 pg/ml * h^{-1}, respectively, compared to 85.2 ± 44.2 pg/mL and 203 ± 96.4 pg/ml * h^{-1} for the 6 subjects receiving lot 2014. Due to wide variance in pharmacokinetic parameters among individuals, it is possible the lower plasma levels with lot 2014 was due to individual differences, though additional evidence would suggest a potential manufacturing error in the production of lot 2014. In the Efficacy part, there was also a large difference in plasma parameters between lots. For the 15 subjects receiving lot 2011, C_{max} and AUC were 158.5 ± 56.6 pg/mL and 308.9± 139.9 pg/ml * h^{-1}, compared to 225.44 ± 56.9 pg/mL and 434.2 ± 125.2 pg/ml * h^{-1} for the 5 subjects receiving lot 2014. These differences between lots are the reciprocal of the PK part, with the mean lot 2011 concentrations lower than lot 2014's. The two lots were manufactured at a single university pharmacology department, though at different times and with different staffs. The lot 2014 clinical trial material for the PK part and the Efficacy part were manufactured and shipped at different dates, further suggesting errors in production. However, pharmacokinetic parameters for lot 2011 remained steady across studies and over a four-year period, suggesting a correctly-produced formulation is highly stable.

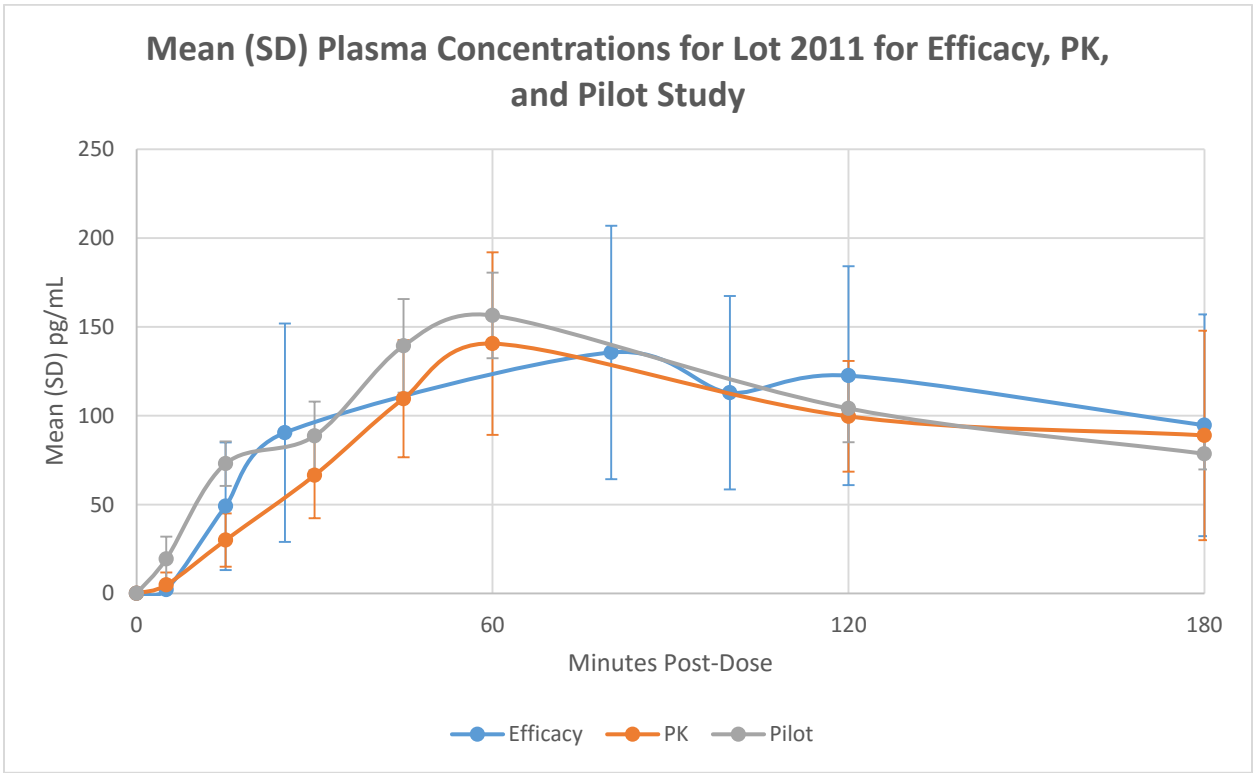

Figure 9. Mean Plasma Concentrations for Lot 2011.

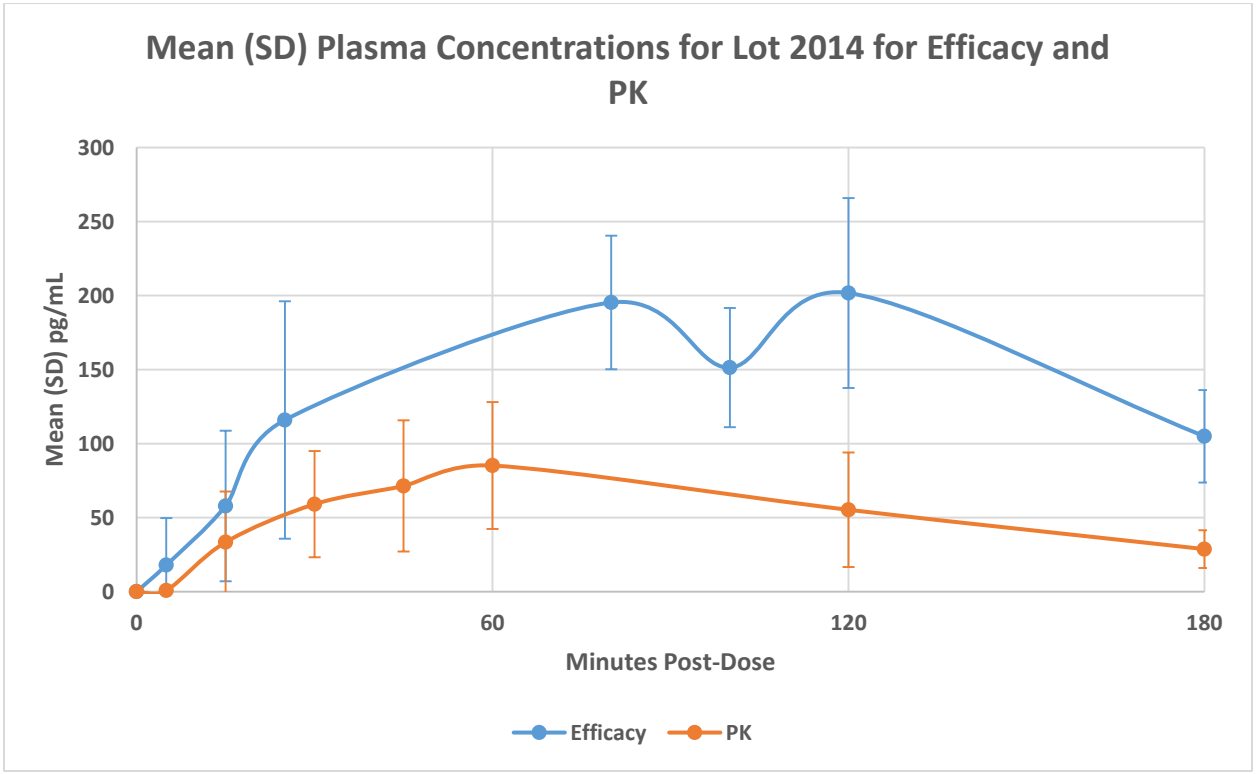

Figure 10. Mean Plasma Concentrations for Lot 2014.

In the Efficacy part, there is an uncharacteristic drop in plasma concentrations for most subjects at 100 minutes post-dose, followed by a subsequent rise 20 minutes later at 120 minutes post-dose. The drop occurs 20 minutes after the rotation period has ended, when subjects are served food and

liquids. It is most likely this drop is a result of food and drink intake; the PK part also offered food to subjects, but blood draws occurred at wider intervals in the second half of PK than in Efficacy, preventing identification of a similar decrease in the PK plasma levels.

Vital signs trends followed known courses of anticholinergic activity. Low doses of scopolamine (0.1-0.2 mg) are known to cause temporary heart rate decreases, whereas higher doses can cause temporary tachycardia (16). Both this study and the pilot study identified transient bradycardia. Scopolamine is also a known vasodilator, and the significant differences in systolic and diastolic blood pressures in both PK and in Efficacy INSCOP conditions were expected. The significant drops in temperature in both parts never reached clinical significance. Previous research has shown that motion sickness drops core body temperature (3), and the lab where blood and vitals were taken was noticeably colder than other testing areas, as openly stated by multiple subjects.

Both PK and Efficacy saw significant declines in cognitive testing scores by time, although there was no significant difference between conditions in Efficacy, and there was no correlation between any cognitive test score and plasma levels. It can be considered that the largest contributor to the decline in scores was boredom in both parts and feelings of illness in the Efficacy part, as scores in both conditions across all tests had their largest drop between pre- and post-rotation testing. The interaction between time and condition for CPT scores warrants special attention due to the difference in scores by lot received (Figure 11). In the INSCOP condition, scores dropped by a mean of 10.9 points from pre- to post-rotation, compared to only 3.1 in placebo. When considering the drop in scores by lot, however, subjects receiving lot 2011 dropped a mean of 8.2 points whereas subjects receiving lot 2014 dropped by a mean of 18.17 points. Indeed, lot 2011 subjects' scores post-rotation are not significantly different from all subjects' scores post-rotation in the placebo condition. However, it should be noted that the mean CPT baseline score for the INSCOP condition was 129.4 ± 16.3, compared to a mean of 123.1 ± 19 for placebo; 20 minutes post-dose the mean INSCOP score had dropped by 1.1 points compared to 0.1 points for placebo. The scores in both conditions immediately post-rotation were not significantly different, 117.4 ± 20.7 for INSCOP and 119.9 ± 22.1 for placebo, and there was no significant decrease in scores across time for PK, $F(2.454, 29.445)$, $p = .123$. These factors suggest that rotation may be the primary cause behind the drop in CPT scores, though CPT will be a necessary testing component in future clinical trials to ensure the drop is not a direct property of INSCOP.

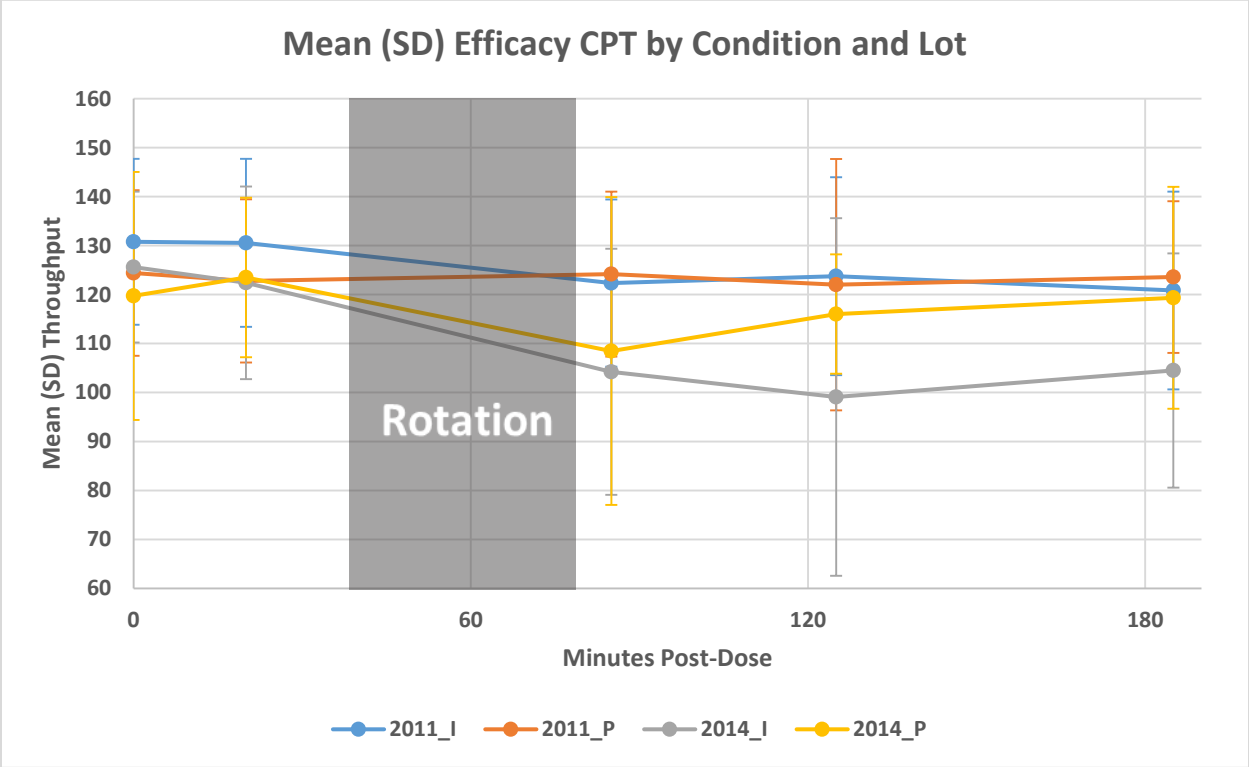

Figure 11. Mean Efficacy CPT by Condition and Lot.

Previous research of the transdermal scopolamine patch has assumed that concentrations of 50 pg/mL are the minimum threshold level for MS prophylaxis – a level that 23% of subjects fail to reach within 8 hours post-application and 7% never reach (14, 15). Gil et al. (2005) found that persons who developed MS even while wearing a transdermal patch had lower mean plasma concentrations (97.0 ± 73.3 pg/mL) compared those who responded positively (156.8 ± 77.0 pg/mL). In this study, we found no correlation between plasma levels at any time point and the number of head tilts tolerated. However, there was a positive correlation between plasma levels just prior to rotation (at 25 minutes post-dose) and the difference in head tilts between conditions, r(20)=.490, p = .028. Of the 20 Efficacy subjects with plasma results, the mean plasma level at 25 minutes post-dose was 116.6 ± 72.4 pg/mL for the 13 subjects who tolerated more head tilts in the INSCOP condition than placebo, compared to a mean of 60.2 ± 24.6 pg/mL for the 7 subjects who either tolerated more (n=4) or an equal number (n=3) of head tilts in the placebo condition. Though there was no additional positive correlation between the difference in head tilts and plasma levels at any other time point, subjects who responded positively to INSCOP had higher mean plasma concentrations post-rotation at 80 minutes post-dose (165.9 ± 79.5 pg/mL) compared to those who did not (122.0 ± 36.9 pg/mL). This trend continues through the remaining three time points. These results parallel Gil et al.'s (2005) results, suggesting that multi-dosing may increase INSCOP's prophylaxis capabilities.

Future Studies

There are several potential future studies. As persons are likely to experience motion sickness on multiple consecutive days, a repeat dose laboratory trial is necessary. Additionally, a Part III field trial among ship-board military personnel comparing INSCOP's efficacy against the transdermal patch is warranted. Only one previous study has examined the efficacy of the intranasal formulation after the appearance of motion sickness symptomology (Chinn et al., 1955). A study could administer INSCOP within a pre-specified time frame of specific symptom onset, possibly through use of electrogastric myography. Also, though both the gel and spray formulations have proven efficacious,

and their pharmacokinetics have been examined independently, an avenue of future research would be to compare the two formulations' pharmacokinetics within subjects.

Conclusion

INSCOP is rapidly absorbed, lacks the cognitive deficits and side effect profiles seen in other administrative routes, and improves capability to tolerate provocative motion. Further research examining multi-dose usage is needed. The next step is to conduct Part III clinical trials examining multiple use INSCOP spray in both operational and laboratory settings. Depending on the results of those trials, INSCOP spray could well provide the services with a MS countermeasure that is fast acting, highly effective, field expedient, easy to administer, and compatible with operational settings.

Military Significance

MS can have debilitating effects no matter what mode of transportation is used across the services. Unfortunately, once exposure to MS-inducing environments occurs it is too late to take a prophylactic. The most effective prophylactic has been scopolamine. Through its common modes of application, (oral and transdermal), the side effects seem to outweigh the benefits. Intranasal scopolamine in the spray formulation could provide rapid absorption and efficacy against MS. A just-in-time treatment would prevent mission disruption due to illness during military operations.

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Appendix A

INSCOP III Motion Sickness Susceptibility Questionnaire (MSSQ)

Date: _____

This questionnaire is designed to find out how susceptible to motion sickness you are, and what sorts of motion are most effective in causing that sickness. Sickness here means feeling queasy or nauseated, or actually vomiting.

Participant Screening Number:

☐☐☐

Gender *(check one)*: ☐ Male ☐ Female

Subject # _____

Age: ☐☐ *years*

Height: ☐☐ *ft.* ☐☐ *in.*

Weight: ☐☐☐ *lbs*

Ethnicity/Race: *(check*

☐ White ☐ Black ☐ Asian-American/ Pacific Islander ☐ Hispanic/Latino(a) ☐ Other

Please indicate the time of your *last* 1.5 mile run during PRT:

☐☐ *min* ☐☐ *sec*

Do any of your family members experience symptoms of motion sickness?

☐ Yes ☐ No **If Yes**, please indicate which family members and the severity of their symptoms.

☐ Mother	☐ Father	☐ Sister	☐ Brother	☐ I don't know.
☐ Mild	☐ Mild	☐ Mild	☐ Mild	
☐ Moderate	☐ Moderate	☐ Moderate	☐ Moderate	
☐ Severe	☐ Severe	☐ Severe	☐ Severe	

 Please continue on next page...

Appendix A

YOUR CHILDHOOD EXPERIENCE ONLY (BEFORE 12 YEARS OF AGE):

Check the appropriate boxes for each section:

	Experience Level What was your experience with each motion stimulus?				Level of Motion Sickness How often did you feel motion sick?			
Cars	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Buses or Coaches	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Trains	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Aircraft	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Small Boats	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Ships (e.g., Channel Ferries)	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Swings in Playgrounds	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Roundabouts in Playgrounds	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Big Dippers, Funfair Rides	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
				†	0	1	2	3

Did any of these experiences make you vomit while traveling?

☐ Yes ☐ No ***If yes, which experiences made you vomit?***

☐ Cars ☐ Buses or coaches ☐ Trains ☐ Aircraft ☐ Small boats ☐ Ships ☐ Swings in

playgrounds ? Roundabouts in playgrounds ? Big dippers, funfair rides

Please continue on next page...

Appendix A

OVER THE LAST 10 YEARS:

Check the appropriate boxes for each section:

	Experience Level What was your experience with each motion stimulus?				Level of Motion Sickness How often did you feel motion sick?			
Cars	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Buses or Coaches	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Trains	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Aircraft	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Small Boats	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Ships (e.g., Channel Ferries)	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Swings in Playgrounds	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Roundabouts in Playgrounds	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
Big Dippers, Funfair Rides	☐ High	☐ Medium	☐ Low	☐ Never Traveled	☐ Never	☐ Rarely	☐ Sometimes	☐ Frequently
				†	0	1	2	3

Did any of these experiences make you vomit while traveling?

☐ Yes ☐ No ***If yes, which experiences made you vomit?***

- Cars
- Buses or coaches
- Trains
- Aircraft
- Small boats
- Ships
- Swings in playgrounds
- Roundabouts in playgrounds
- Big dippers, funfair rides

Please stop and wait for further instructions.

Appendix A

Intranasal Scopolamine (INSCOP) III

CONFIDENTIAL MEDICAL QUESTIONNAIRE

Screening Number:_____ Subject Number: _____ Date: _____

Gender (check one): Male ☐ Female ☐

Age: _____ Height: _____ Weight: _____

Part 1- Directions: **Circle "Yes"** if you currently suffer from or have ever been diagnosed with the condition **AND** explain below the question.

Circle "No" if they don't apply.

These questions are being asked to ensure your safety in this study.

ALL ANSWERS WILL BE KEPT CONFIDENTIAL

1.	Do you have any drug allergies?	Yes	No
2.	Do you currently or have you ever been diagnosed with asthma?	Yes	No
3.	Do you have a history of or currently suffer from severe allergies?	Yes	No
4.	Have you ever been diagnosed with sleep apnea?	Yes	No
5.	Have you ever been diagnosed with a seizure disorder?	Yes	No
6.	Do you currently or have you ever suffered from liver/kidney problems?	Yes	No
7.	Do you have a history of urinary retention?	Yes	No
8.	Have you ever been diagnosed with heart/circulatory disease?	Yes	No
9.	Do you currently suffer from high blood pressure?	Yes	No
10.	Have you ever been diagnosed with glaucoma?	Yes	No

11.	Have you ever been diagnosed with emphysema?	Yes	No
12.	Have you ever been diagnosed with an enlarged prostate?	Yes	No
13.	Do you have a history of gastrointestinal disorders? (e.g. bowel distention, irritable bowel syndrome)	Yes	No
14.	Have you have been diagnosed with epilepsy?	Yes	No
15.	Have you ever suffered from pneumonia?	Yes	No
16.	Do you have a history of alcohol and drug dependency?	Yes	No
17.	Have you used any tobacco products in the last 6 months?	Yes	No
18.	Have you donated blood or plasma in the past 30 days?	Yes	No
19.	Have you, in the past or at present, experience discomfort in confined spaces?	Yes	No
20.	Do you take any prescribed medication on a regular basis?	Yes	No
21.	Have you taken a prescribed medication within the past 7 days?	Yes	No

Females:

22.	Are you currently pregnant or lactating?	Yes	No
23.	Do you tend to suffer regularly from premenstrual syndrome (PMS)?	Yes	No
24.	Are you taking prescribed Birth Control?	Yes	No

25. First day of menstrual cycle? _____

26. Length of cycle? _____ (days)

27. Duration of Menstruation? _____ (days)

28. On what date did your last menstrual period begin? _____

Appendix A

Part II- Directions: Note any medication to which you currently or have ever had an allergic reaction or sensitivity to.

Scopolamine (Scopace)	Yes	No
Atropine	Yes	No
Other(s)	(Please list each medication)	

_____

_____

Part III- Directions: Answer the following questions to the best of your ability.

1. Are you in your usual state of fitness? (*circle one*) Yes No

a. If not, please indicate the reason: _____

2. Have you been ill in the past week (circle one) Yes No

a. If yes, please indicate the nature of the illness (e.g., flu, cold, etc.) _____

b. The severity of the illness (*Circle one*):

Very mild----------1----------2----------3----------4----------5----------**Very Severe**

c. Length of the illness Hours:_____ Days:_____

d. Major Symptoms: _____

e. Are you fully recovered? Yes No

3. Indicate all medication you have used in the past 24 hours.
(circle all that apply)

a. None
b. Sedatives/Tranquilizers
c. Aspirin/Tylenol/any analgesic
d. Antihistamines

e. Decongestants

f. Other (please specify)

_____

4. Do you take any over the counter medications (e.g., antacids, Benadryl, Tylenol, etc.) two (2) or more times a month? Yes No

5. How many hours did you sleep last night? _____

Was this amount sufficient? Yes No

Appendix A

INSCOP III **CONFIDENTIAL** Exclusionary Behavior Questionnaire (CEB)

Subject Number: _____ **Date:** _____

Screening Number: _____

Gender: *(please check one)* **Male** ☐ **Female** ☐ **Age:** _____

Ethnicity *(please check one) **Used only to determine the diversity of the subject pool***

Caucasian	African-American	Hispanic	Asian/ Pacific Islander	Other
☐	☐	☐	☐	☐

Directions: Answer the following questions to the best of your ability. Some questions relate to past experiences.

1a.	Within the past 7 days, have you had any significant motion experiences? (e.g., amusement park rides, small aircraft, watercraft rides, etc.)	Yes No
1b.	How experienced are you at riding in motor vehicles? (e.g., small boats, large ships, small and large planes, helicopters, trains, buses, cars, amusement rides, etc.) Please circle **one**	None Slightly Moderately Very
2a.	How many hours did you sleep last night?	_____
2b.	Is this your usual sleep pattern?	Yes No
2c.	"The quality of my sleep last night was very good". *Please circle the number that best reflects this statement.*	Strongly Disagree 1 2 3 4 5 Strongly Agree
3.	Have you eaten regular meals today?	Yes No
4a.	Did you consume alcohol in the last 24 hours?	Yes No
4b.	If yes, how many alcoholic drinks did you consume?	_____ (Please give number)

4c. Total number of alcoholic drinks in the past week? _____
(Please give number)

5. In the past 7 days, have you taken any prescription or over the counter medications? Yes No

6a. In the past 7 days, have you used any tobacco products? Yes No

6b. If yes, how much of each tobacco product have you used? _____
(Please give number and type of product)

(State the number of cigarettes, "dips", "chew", or times a pipe was smoked) _____
(Please give number and type of product)

7. In the past 7 days, have you consumed any herbal products, vitamins, or performance enhancing drinks? Yes No

_____
(Please list products and amounts)

_____
(Please list products and amounts)

8a. In the past 7 days, have you had any grapefruit juice? Yes No

8b. If yes, how much? _____
(Number of 8 oz. cups)

9. On average, how much caffeine do you drink in a day? _____
(Number of 8 oz. cups)

FEMALES

10a. Are you currently experiencing symptoms related to your monthly cycle? Yes No

10b. If yes, please list symptoms:

_____ _____
(Please list symptom) (Please list symptom)

11. Date of your last menstrual cycle: _____

Thank you for your participation!