Examples of Successful Applications from Past Grant Winners. Please note that the current application instructions and format differ from that used in these applications. These are to be used as examples only. Please refer to current instructions link for requirements.

Houston

Antimicrobial Activity of H. perforatum and M. chamomilla Against Skin bacteria in Austere Environment

Research-in-Training

Altitude Sickness Prevention with Ibuprofen Relative to Acetazolamide Trial for Efficacy (ASPIRATE)

SECTION A, SUMMARY OF PROJECT: Introduction & Background

The area of research regarding plant natural products is well explored. From the literature regarding herbs and plants, *Matricaria chamomilla* (chamomile) has been shown to display antimicrobial properties as an essential oil, and *Hypericum perforatum* (St. John's Wort) has even displayed efficacy against certain gut bacteria when using concentrations found in herbal teas (Boyanova. 2013) (Hans, et al. 2016). Both plants grow in the wild in North America. The prized parts of both plants are easily accessible (blossoms for SJW, and dried flower heads for Chamomile) without having to excavate the roots. However, in resource scarce environments there are no industrial methods to extract essential oils and water is usually the only solvent at hand. It is from this premise that we would like to base our following research model.

Hypothesis & Specific Aims:

Our specific aims are to test the antimicrobial efficacy of both Chamomile and St. John's Wort natural products prepared with methods that are feasible under resource scarce conditions. Our hypothesis is that both plants will display significant antimicrobial effects against growth of common, potentially infectious, skin pathogens.

Research Team Members:

[redacted]

[redacted]

[redacted]

[redacted]

Principal Investigator: [redacted]

Study Design & Methods

Natural Products

Both plants will be purchased from Rose Mountain Herbs in their dried, flower form. Three preparation methods will be performed to extract the natural plant products: tea, infusion, and decoction. Concentrations were chosen based off of Boyanova's since these had shown efficacy in her design, as well as being a reasonable amount one could expect to encounter in the wild. These concentrations are 5 mg/mL and 7.5 mg/mL for *M. chamomilla* and *H. perforatum*, respectively (Boyanova, 2013). To establish a range of concentrations, we will use 0 mg/mL (control), 3 mg/mL, 5 mg/mL, and 7 mg/mL concentrations of *M. chamomilla* for each of the three preparation methods, and 0 mg/mL, 5.5 mg/mL, 7.5 mg/mL, and 9.5 mg/mL concentrations of *H. perforatum* for each of the three preparation methods. For each concentration, the dried flower will be brought to a boil, and left to steep 10-20 minutes (tea), 8-10 hours (infusion), or left on heat to simmer at a boil for 20-30 minutes and allowed to sit 8-10 hours (decoction). Each successive method of preparation should extract higher amounts of plant alkaloids, with decoction producing the most.

Bacterial strains, maintenance, and cultivation

Staphylococcus aureus and *Streptococcus pyogenes* were selected because they are common skin bacteria that often play a major role in wound and skin infections. Moreover, the two bacteria are particularly lethal without access to modern medicine, making them ideal choices to test the efficacy of chamomile and St. John's wort.

Staphylococcus aureus subsp aureus Rosenbach (ATCC 12600) and *Streptococcus pyogenes* Rosenbach (ATCC 12344) will both be purchased from the American Type Culture

Collection. *S. pyogenes* will be cultivated on sterile Brain Heart Infusion (BHI) agar and *S. aureus* will be cultivated on nutrient agar (NA) and incubated at 37 degrees C.

Methods

We will place filter discs impregnated with either *M. chamomilla* or *H. perforatum* product, extracted by one of the three aforementioned methods at one of the 4 concentrations, onto plates inoculated with either *S. aureus* or *S. pyogenes*. Then plates will be placed into an incubator at 37 degrees C for time increments of 24, 48, and 72 hours. At each time increment the zone of inhibition (ZOI) will be measured using the disc agar diffusion bioassay, with the aid of electronic calipers. ZOIs will be compared with control groups. Each individual condition will be performed in replicates of 5 to establish statistical validity of results.

References:

Boyanova, L. 2013. Comparative evaluation of the activity of plant infusions against *Helicobactor pylori* strains by three methods. World J Microbiol Biotechnol (2014). 30: 1633-1637. doi: 10.1007/s11274-013-1589-5

Hans, V. Harpreet, S. Himanshu, D. Preeti, A. Antimicrobial Efficacy of Various Essential Oils at Varying Concentrations against Periopathogen *Porphyromonas gingivalis.* Journal of clinical and diagnostic research. 2016 Sep. Vol-10(9):16-19. doi: 10.786/JCDR/2016/18956.8435

Data analysis plan:

Bacterial death will be ascertained using ZOI for both strains of bacteria. Data will be analyzed using Student's t-test to compare mean ZOI by concentration for each of the three preparation methods compared to the control (0 mg/mL). Data will be presented as mean ± standard deviation and an alpha of $p<0.05$ will be used to determine statistical significance. Tables illustrating our outcome measures of interest are included below.

Example Table 1. Zone of inhibition of *streptococcus pyogenes* by preparation method for chamomile extracts

Preparation Method	0 mg/mL	3.0 mg/mL	5.0 mg/mL	7.0 mg/mL
Tea				
Infusion				
Decoction				

Example Table 2. Zone of inhibition of *staphylococcus aureus* by preparation method for St. John's Wort extracts

Preparation Method	0 mg/mL	5.5 mg/mL	7.5 mg/mL	9.5 mg/mL
Tea				
Infusion				
Decoction				

Facilities:

OSU-CHS Microbiology Lab, BSL-2 bacteriology containment facility, Lab-E439

Director: [redacted]

Timeline:

All of the collaborators on this project are current medical students at the Oklahoma State College of Osteopathic Medicine (OSU-COM) in Tulsa, OK. OSU-COM's summer break (May 12-August 4th) will be when the majority of the work regarding this project will take place. Prior to OSU-COM's summer break, we will conduct a literature review and refine our protocols in preparation for the 8 weeks of actual laboratory work.

	Tasks to be accomplished:
Weeks 1-11 **(March 1 - May 12)**	● Literature review ● Protocol establishment and refinement of extract concentrations ● Begin culturing 2 strains of bacteria ● Order necessary reagents and supplies ● Culture bacteria ● Analyze preliminary data ● Adjust protocol as needed
Weeks 12-13 **(May 15th - May 26th)**	
Weeks 14-15 **(May 29th - June 9th)**	● Culture bacteria and expose cultures to chamomile and St. John's Wort extracts ● Conduct time point experiments in lab
Weeks 16-17 **(June 12th - June 23rd)**	● Continue time point experiments allowing time for mis-cultured plates. ● Analyze additional data ● If time permits, evaluate additional doses of chamomile and St. John's Wort and add additional time points
Weeks 18-19 **(June 26th – July 7th)**	● Compile all data and analyze ● Draft/complete manuscript for publication ● If results are promising, evaluate efficacy of chamomile in *in vivo* models

SECTION B:

Significance of the project to the field of wilderness and environmental medicine:

This project is relevant to wilderness medicine because both chamomile and St. John's wort grow unattended in wilderness settings in North America. If their antimicrobial efficacy under our parameters is verified, then this would open the door to more research regarding the use of herbal plants in topical wound care and management in the wild, which is a hitherto relatively unexpanded field of research. Also, because the method of preparation we are proposing can be easily performed with a simple campfire and water, positive research results could also potentially provide an incentive to include basic plant identification and preparation in the education and training of wilderness medicine courses.

Altitude Sickness Prevention with Ibuprofen Relative to Acetazolamide Trial for Efficacy (ASPIRATE)

Introduction

The specific aim of the ASPIRATE Trial is to evaluate if ibuprofen will be equivalent to acetazolamide and superior to placebo in decreasing the incidence of Acute Mountain Sickness (AMS) in travelers to high altitude. It has been shown that ibuprofen taken 3 times a day 6 hours prior to ascent is effective for the prevention of AMS, with a number needed to treat of 4, decreasing the odds of getting AMS by a third. The efficacy appears to be similar to acetazolamide, with a NNT of 3 - 8, although these two medications have not been directly compared in prevention of AMS. Acetazolamide is a diuretic that is the only FDA approved AMS prophylactic medication and the most commonly used drug for AMS prevention. Although acetazolamide has been given a 1A indication, it has been shown to limit exercise capabilities at high altitude, and rapid ascent has been shown to attenuate its protective effects. It is unknown if a non-steroidal anti-inflammatory can provide protection from AMS equivalent to acetazolamide.

Background

Acute mountain sickness (AMS) is a constellation of symptoms including headache, sleep disturbance, fatigue, dizziness, and nausea, vomiting, or anorexia that commonly occurs in travelers ascending to altitudes above 2,500m. AMS incidence varies based on altitude and ascent profile with rates reported from 25 to 75% in tourists, trekkers, and mountaineers at North American altitudes. Symptom onset is typically six to twelve hours after arrival at high altitude. This disease can be debilitating when severe, and left unrecognized or untreated may progress to potentially fatal high altitude cerebral edema (HACE). While gradual ascent has proven effective

in preventing AMS this approach is often impractical to recreationalists, search and rescue, as well as military operations. While the exact pathophysiology of AMS is unknown, vasodilatation, inflammation, and blood brain barrier (BBB) permeability have been implicated. On exposure to high altitude, hypobaric hypoxia causes increased cerebral blood flow, cerebral blood volume, and ensuing hyper-perfusion of the cerebral parenchyma. Simultaneously, increased inflammatory mediators such as arachidonic acid and prostaglandins likely contribute to increased BBB permeability, and are known to sensitize meningovascular nociceptors and mediate vasodilation. The culmination of these mechanical and biochemical cerebral stressors seen at high altitude lead to increased cerebral hydrostatic pressures and formation of a mild vasogenic cerebral edema.

Ibuprofen is a widely available over-the-counter, non-steroidal anti-inflammatory drug used as the evidence-based standard for treatment of high altitude headache that occurs in AMS via cyclooxygenase inhibition that prevents the production of prostaglandins and thromboxanes. Ibuprofen's anti-inflammatory effects may be similarly protective against BBB damage in the hypoxic edematous state, preventing nociceptor irritation and endothelial breakdown.

Hypotheses

The primary hypothesis to be supported is that ibuprofen (600mg given three times a day) 4 hours prior to ascent will be superior to placebo and equivalent to acetazolamide (125mg given two times a day) in decreasing the incidence of AMS.

A secondary hypothesis of this study will look at the role of ibuprofen (600 mg given three times a day) 4 hours prior to ascent in comparison to placebo and acetazolamide (125mg given two times a day) in decreasing the incidence of sleep disturbance at altitude.

Study design and methods

The ASPIRATE Trial is designed as a double blind randomized placebo-controlled trial. Our study population is hikers who are ascending at their own rate under their own power in a true hiking environment at the White Mountain Research Station, Owen Valley Lab (OVL) and Bancroft Station (BAR), Bancroft Peak, White Mountain, CA. Advertisement of the study at sea level locals in and around Stanford University as well as email chains will attempt to gather a cohort of sea-level participants. Eligible participants will be randomized in a double blind fashion the morning of ascent to acetazolamide 125 mg PO bid and placebo tid, ibuprofen (600mg PO tid) and placebo bid, or two visually identical placebos (tid and bid). After informed consent is signed, participants will complete the baseline demographics sheet, Lake Louise Questionnaire (LLQ), have pulse oximetry readings, and perform End Tidal Cos (EtCO2) prior to taking the study medications. Participants will take the study capsules before they leave from OVL, in order to reveal any significant acute adverse reactions before they leave for higher altitude. Healthy participants without adverse reactions will drive to the Barcroft Gate at 11,700' and continue on foot to the Barcroft Research Station (BAR) at 12,500'. The study subjects will be allowed to hike at their own pace with minimal restrictions including no travel above 13,054' (Barcroft Summit). The participants will spend the night at BAR where they will be evaluated with 6 hours of their arrival and the morning after (LLQ, pulse oximetry, End Tidal CO2, Groningen Sleep Quality Questionnaire, side effects data, and compliance). Overnight, they will be wearing single axis actigraphs and data on total sleep time will be collected. After data collection the morning after arrival, the study will be completed for each participant. Participants with significant illness will be removed from the trial and appropriate safety measures taken. The highest scoring AMS data will be used for primary and secondary outcome analysis.

Inclusion criteria: It is expected that approximately 100 healthy male and female sea level-dwelling hikers between the ages of 18-65, without headache and not already taking NSAIDs, acetazolamide, or corticosteroids, will be enrolled in the study and hike to the 12,500' Barcroft Research Station. Questions concerning age, sex, baseline living altitude, prior visit to high altitude in the prior 1 week, regular medications, and prior history of altitude illness and disease will be posed at baseline to establish the subject's demographic profile and past medical history.

Exclusion criteria:

1. History of allergy to non-steroidal anti-inflammatory drugs, acetazolamide, or sulfa drugs.
2. Taken NSAIDs, acetazolamide, or corticosteroids within one week prior to study enrollment.
3. Hazardous medical conditions which precludes the ability to moderately hike to high altitude including: sickle cell anemia, asthma, severe anemia, or severe coronary arterial disease.
4. Pregnancy or suspected pregnancy.
5. Participants who are younger than 18 years of age and more than 75.
6. Sleeping above 4'000 elevation in the preceding 1 week.
7 Unable to complete a moderately strenuous hike at high altitude.
8. History of Chronic Kidney Disease

Data analysis plan

This study is designed to accommodate an intent-to-treat analysis, and appropriate statistical analysis will be completed using standard statistical software and discipline standard methodology. In addition to summary statistics and univariate comparisons, logistic regression will be utilized to examine multivariate risk factors for binary outcomes in measuring AMS. The study will be a two-sample comparison (drug to placebo) with a one-sided alternative hypothesis.

Native AMS severity with this identical ascent profile and methodology is 69% in placebo. Assuming that the trial will be evaluated by comparing the proportion of subjects with LLQ of $\geq$ 3 with a headache (diagnostic of AMS) to placebo, with total sample size of 100, the trial has 80% power when the (true) proportions at $\geq$ 3 are 65% and 38%, an effect size of at least 26%. Alpha will be set a 0.05. If the intervention reduces the proportion to by at least 28%, the number needed to treat is approximately 3.5.

Intent-to-treat analyses, Kaplan-Meier curve estimate, univariate comparisons (Pearson chi-square, Fisher's exact tests, t-tests) and multi-variate regression analysis will be examined. P values less than 0.05 are considered significant. All analyses will be conducted using R 2.6.0 software.

Time Frame

The study enrollment period will run for approximately 12 days over 4 weekends in August 2017. It is expected that participants will arrive on the Friday night of their chosen weekend, ascend to Barcroft Station on Saturday, and Sunday morning complete the study. Data analysis will be completed by October 31, 2017, with a goal for abstract submission and presentation of results to the 2018 Wilderness Medical Society Annual Meeting and submission for publication by June 2018.

Facilities

The White Mountain Research Center is run by the University of California and is publically accessible. The facilities to be used are the Owens Valley Station, located 4 miles east of Bishop, CA and Barcroft Station on White Mountain. The facilities provide comfortable lodging, full meals, oxygen concentrators if needed for symptomatic trial participants, and 24 hour truck access for rapid descent for severely symptomatic participants.

Significance of the project to the field of wilderness and environmental medicine

Ibuprofen holds promise as an alternative and potentially equally efficacious AMS prophylactic medication as compared to acetazolamide. Recent WMS Practice Guidelines have discussed the challenge in evaluating ibuprofen as prophylactic AMS medication, and awarded it a 2B rating, in part because of its lack of direct comparisons to acetazolamide. This will be the first study to directly compare these popular medications to determine efficacy. If ibuprofen proves to be more effective than placebo in minimizing the incidence of AMS and has demonstrated non-inferior efficacy versus acetazolamide in decreased incidence of AMS on ascent to high altitude, this will support a cornerstone in the evidence supporting a new class of drugs for both insight into the pathophysiology of AMS as well as potentially delivering a new medication with minimal side effects to those at risk for AMS on rapid ascent to high altitude.