1) Protocol Title

Antipruritic effect of topical ketamine, amitriptyline, and lidocaine

2) IRB Review History*

N/A

3) Objectives*

The purpose of this study is to examine the antipruritic efficacy of topical ketamine, amitriptyline, lidocaine, and a tri-combination of ketamine, amitriptyline and lidocaine (hereafter referred to as "KeAmLi") using non-histaminergic itch provocations in healthy volunteers. The primary outcome is itch reduction (AUC) between the vehicle and active treatment (KeAmLi-combo). Secondary outcomes include modality-specific analgesic properties of the topically applied ketamine, amitriptyline, lidocaine, and KeAmLi-combo to controlled quantitative thermal and mechanical stimuli, which can improve our understanding of the mechanism of action of these substances in the context of topical therapy.

4) Background*

Introduction

Chronic itch is a prominent symptom of many dermatological conditions such as atopic dermatitis (AD), urticaria, psoriasis and prurigo nodularis, as well as neuropathic itch conditions. Moreover, itch is also common in conditions such as biliary cirrhosis, renal deficiency as well as several infectious-, allergic-, neoplastic-, autoimmune-, and drug-induced conditions [1–3]. Itch almost inevitably leads to scratching of the affected area which can further exacerbate the skin inflammation and deterioration, leading to a vicious "itch-scratch cycle" [4,5] characterized by maintenance of skin aberrations, cutaneous infections and pain [5,6]. According to recent epidemiological studies, AD alone affects 5-20% of children worldwide with an estimated direct annual cost of nearly $3 billion in the USA alone [7]. Itch is usually the primary complaint of these patients and has been reported to occur in as much as 87% of adult AD patients [8]. The currently available treatment options are suboptimal for most chronic itch conditions [3,9].

Rational for investigation of compounds for topical application

Ketamine is a potent N-Methyl-D-aspartate receptor (NMDAR) antagonist and a weak activator of μ-opioid receptors. Apart from being

abundantly expressed in the central nervous system, NMDAR and the μ-opioid receptor are widely present in the periphery [10,11]. All sensory and motor unit axons are equipped with NDMAR and μ-opioid receptors are also expressed by C- and Aδ-fibers [11–13] Notably, NMDAR expression is increased in the peripheral processes of sensory neurons as a reaction to inflammation [10,14]. Activation of these receptors in rat skin resulted in mechanical allodynia and hyperalgesia. Pain behavior was attenuated following peripheral injection of appropriate antagonists [14–16]. Furthermore, there are several preclinical studies indicating that NMDA receptors and opioid receptors interact in the modulation of pain and that opioid-receptors play a pivotal role in regulation of both itch and pain [17,18].

Amitriptyline potently inhibits serotonin and norepinephrine reuptake, with preferential actions on the serotonin transporter, giving rise to its anti-depressant effect when administered systemically. However, as for other tri-cyclic antidepressants amitriptyline interacts with a range of receptors, including inhibition of 5-HT receptors, histamine receptor 1 as well as muscarinic and -adrenergic receptors, and sodium channels [19–21]. The exact mechanism by which topically (and systemically) administered amitriptyline inhibits pain remains to be elucidated with several candidate mechanisms suggested [19,20,22].

Lidocaine is a commonly applied local anesthetic that works by altering signal conduction in neurons by blocking the fast voltage-gated sodium-channels in the neuronal cell membrane normally responsible for signal propagation. When sufficiently blocked this incapacitates depolarization and inhibits action potential transmission. When applied to the skin epicutaneously or intradermally, lidocaine prominently inhibits traffic from nociceptive (and pruriceptive) primary afferents thus exerting its analgesic and antipruritic effect.

Several studies have shown a significant analgesic effect of topical amitriptyline combined with ketamine (and occasionally lidocaine [23]) in neuropathic pain, and in one instance itch [24], patients [11,19,25–27].

While itch is an unpleasant sensory modality, distinct from pain, it is principally transmitted by two parallel pathways of nociceptive afferent fibers: a subgroup of mechano-insensitive C-fibers (CMi) transmit histaminergic itch and a subgroup of polymodal C-fibers (PmC) transmit non-histaminergic itch [28,29]. The axonal branching for both of these fibers classes terminates in the stratum granulosum [30,31] of the epidermis principally making them a very appropriate target for topical therapy. Given the overlap in the neuronal substrate for pain and itch it is likely (and previously observed) that pharmaceuticals which interfere with pain transmission also have an effect on itch [32,33].

In light of the recently shown analgesic efficacy of topical ketamine, amitriptyline and particularly these applied in combination, often also with lidocaine, we hypothesize that the compounds may also have a significant antipruritic effect [11,25,26,34]. Since that the majority of poorly treatable itch conditions is characterized by being histamine-independent [35–37], we aim to assess whether cowhage evoked non-histaminergic itch can be alleviated by topical ketamine, amitriptyline or these two in combination with lidocaine. Moreover, sensory evaluations of the combined and individual specific anesthetic effects of the compounds will be conducted to provide mechanistic information and potential cellular and molecular targets for improved antipruritic and analgesic therapies.

5) Inclusion and Exclusion Criteria*

Study subjects will be healthy subjects with no itch and no known skin or systemic conditions. An approximately equal number of men and women between 18 and 50 years of age will be recruited using IRB-approved advertising.

Inclusion:

1) Healthy subjects (absence of disease) between 18 and 50 years of age.
2) Must be in general good health with no disease or physical conditions that would impair evaluation of itch and pain perception.
3) No history of chronic itch or pain.
4) Must abstain from the use of moisturizers on the arms the day of study visit.

Exclusion:

1) Individuals under 18 or over 50 years of age.
2) Inability to complete the required measures.
3) The presence of an itchy skin disease or a painful condition.
4) Diagnosis of diseased that would affect itch or pain perception (e.g. neuropathies).
5) Currently enrolled in any investigational study in which the subject is receiving any type of drug, biological, or non-drug therapy.
6) Use of oral, topical analgesics, or other medications known to interfere with itch or pain perception 48 hours prior to the study visits (e.g. antihistamines, anesthetics, anti-inflammatories, opioids, neuroleptics, etc.).

7) Use of emollients on the volar aspects of the forearms arms on the day of the study visit.
8) Use of anti-depressants, anti-psychotics, and illicit drugs.
9) Known history of neuropathy, uremia, uncontrolled thyroid disease, and diabetes mellitus.
10) Known history of anaphylactic shock, or allergy to cowhage and/or known adverse reactions to lidocaine (or other local anesthetics of the amide type), ketamine or amitriptyline.
11) Pregnant (females of childbearing potential will undergo an hCG pregnancy test).
12) Currently incarcerated.

6) Number of Subjects*

A priori power calculations were conducted in G*Power based on data from a previous test-retest study [38]. In order to have an effect 80% power with a size effect of 0.30 and alpha error probability of 0.05, we will need 36 subjects (10 within post hoc pairwise comparisons). We estimate that we will need to screen up to 42 individuals in order to enroll 36 healthy subjects.

We will seek to recruit a sample with an approximately equal gender distribution.

7) Study-Wide Recruitment Methods*

IRB approved flyers will also be placed around the University of Miami medical campus, and within the waiting rooms of the Department of Dermatology Out Patient Clinic at UMH (South Building, suites K-M) and at the Lennar Foundation Medical Center.

8) Study Timelines*

This projected is expected to last 8 months. The first 6 months will be dedicated to subject enrollment. During this time each subject will be a part of 2 study visits; one session lasting approximately 2.75 hours and one lasting approximately 2 hours. The second session must be completed within 7 days of the first session. The second 2 months will be dedicated to the sample analysis and the last month will be dedicated to dissemination of findings.

9) Study Endpoints*

The primary endpoints are visual analog scale (VAS) ratings of the perceived intensity of itch sensation elicited by epicutaneous application of cowhage spicules (non-histaminergic itch) on the volar aspects of the forearms comparing skin pretreated with vehicle cream with the KeAmLi-combination. Ratings will be plotted vs. time to generate a temporal itch intensity graph and the area under the curve (AUC) will be calculated for each subject. The secondary study endpoints are the individual itch peak intensity, itch peak time, and itch time duration comparing skin pretreated with vehicle cream with ketamine, amitriptyline, lidocaine, and KeAmLi-combination.

The exploratory outcomes (for all experimental interventions vs. the vehicle condition) are:

- Warmth detection threshold (°C needed to perceive warmth sensation)
- Heat pain threshold (°C needed to perceive heat pain)
- Mechanical detection threshold (mN needed to perceive touch)
- Mechanical pain threshold (mN needed to perceive pain)
- Development of hyperknesis (NRS ratings of itch evoked by touch before vs. after cowhage provocation)

10) Procedures Involved*

At the beginning of the first session, subjects will be asked to fill out an informed consent, and a form for demographic information including health information. They will also be asked if they have taken any allergy or pain medicine during the past 24 hours. If so they will be asked to come back at a later time since these drugs might interfere with the study. Females of childbearing potential will undergo an hCG pregnancy test. Finally, subjects will receive instruction on the use of the visual analog scale for rating the intensity of itch sensation. Below is a short procedural overview of the proposed study this is followed by sections introducing in details each of the methods applied.

Subjects will be seated comfortably in a chair with both arms resting on armrests or on a pillow. Using a block-randomized design, we will deliver either topical vehicle (PCCA Lipoderm®), topical ketamine 10%, topical amitriptyline 5%, lidocaine 5% or KeAmLi-combo (ketamine 10%, amitriptyline 5%, and lidocaine 5%) to four 4 x 4 cm predefined skin areas on the ventral forearms over two study visits. The 1st visit will involve applying 3 of the 5 treatments (randomized), to 3 of the 4 designated test sites in a randomized manner. The 2nd visit will involve applying the

remaining 2 treatments to 2 of the 4 designated test sites in a randomized manner. The subject and investigator will be blinded to the identities of the treatments (see blinding procedure below).

Application will be conducted with the investigator wearing nitrile gloves. The pre-treatment will occur under topical occlusion for 30 minutes to allow the ointment to be adsorbed. Following this, residual ointment will be removed and sensory testing, strictly within the pretreated area, will commence. For each of the five tested topical solutions the following procedures will be conducted (see also Fig. 1):

1) Demarcation of 4 x 4 cm treatment area (see Fig. 2)
    a) Application of 2 g of ointment (randomized substance)
        i) 30 min pretreatment
            (1) During this time a subject familiarization procedure for the quantitative sensory testing will be conducted
        ii) Removal of residual ointment
        iii) Local safety assessment
2) Thermal sensory thresholds
    a) HDT
    b) HPT
3) Mechanical sensory thresholds
    a) MDT
    b) MPT
4) Baseline sensitivity to touch-evoked itch (STI) assessment
5) Cowhage provocation
    a) 40-45 spicules rubbed into the epidermis
    b) Continuous VAS-recording of itch and pain for up to 15 minutes
6) Post-provocation STI assessment (assessment of hyperknesis)
    a) Spicules gently removed by 2-3 tape-strippings
        i) 0
7) Procedure is repeated with next topical intervention

The second session is conducted in a manner completely identical to session 1. However, the remaining 2 ointments not used in session 1are assessed in the second session. The second session must be completed within 7 days of the first session.

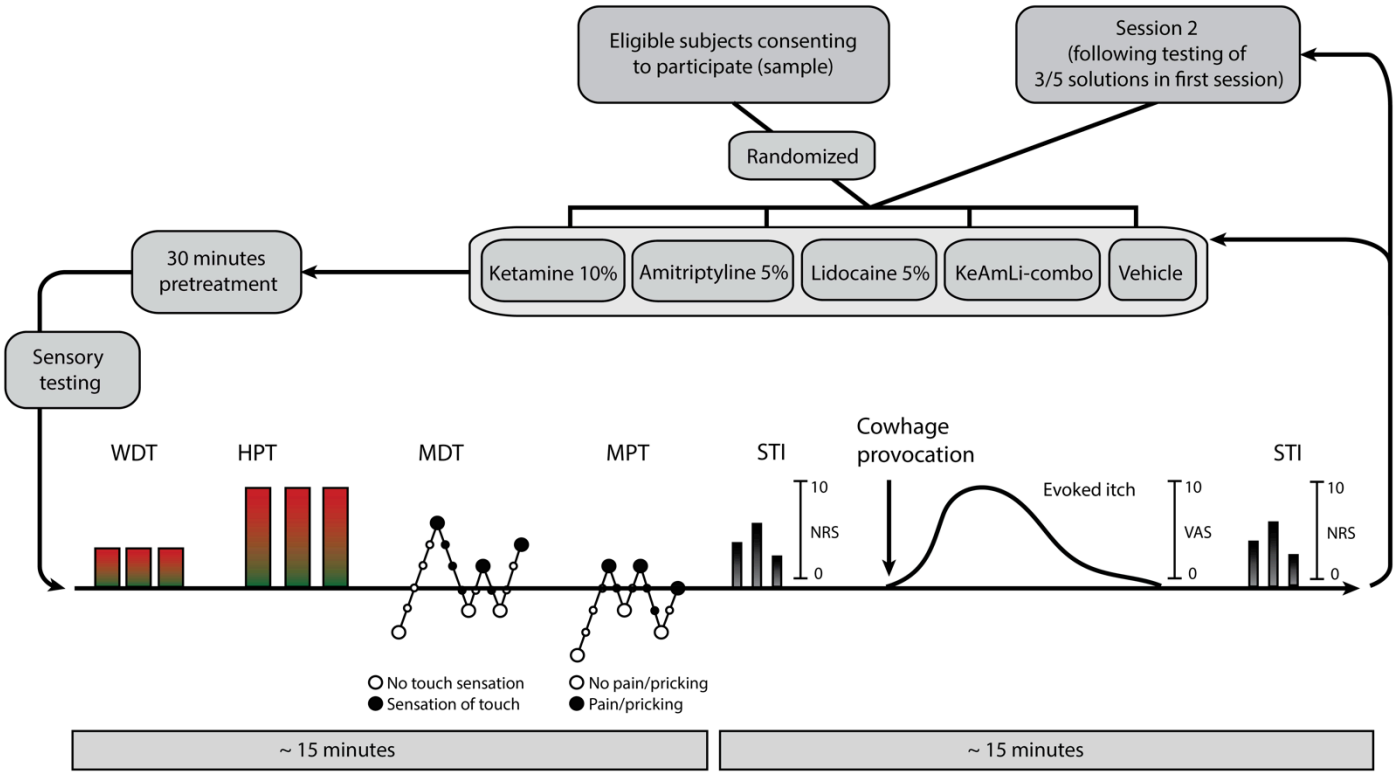

Figure 1 – Overview of the course of study procedures. Grey boxes in the top designate inclusion of subjects for sensory and skin parameters assessment in the depicted test-battery below. Thermal and mechanical sensitivity quantitative sensory tests are performed first, then assessment of STI is assessed and the itch provocation is conducted. Times estimates are derived from the DFNS protocol [39] and may vary between subjects mainly due to differences in the ease of understanding instructions. Abbreviations: WDT = warmth detection threshold; HPT = heat pain threshold; MDT = mechanical detection threshold, MPT = mechanical pain threshold; NRS = numeric rating scale; STI = Sensitivity to Touch-evoked Itch; VAS = visual analog scale;

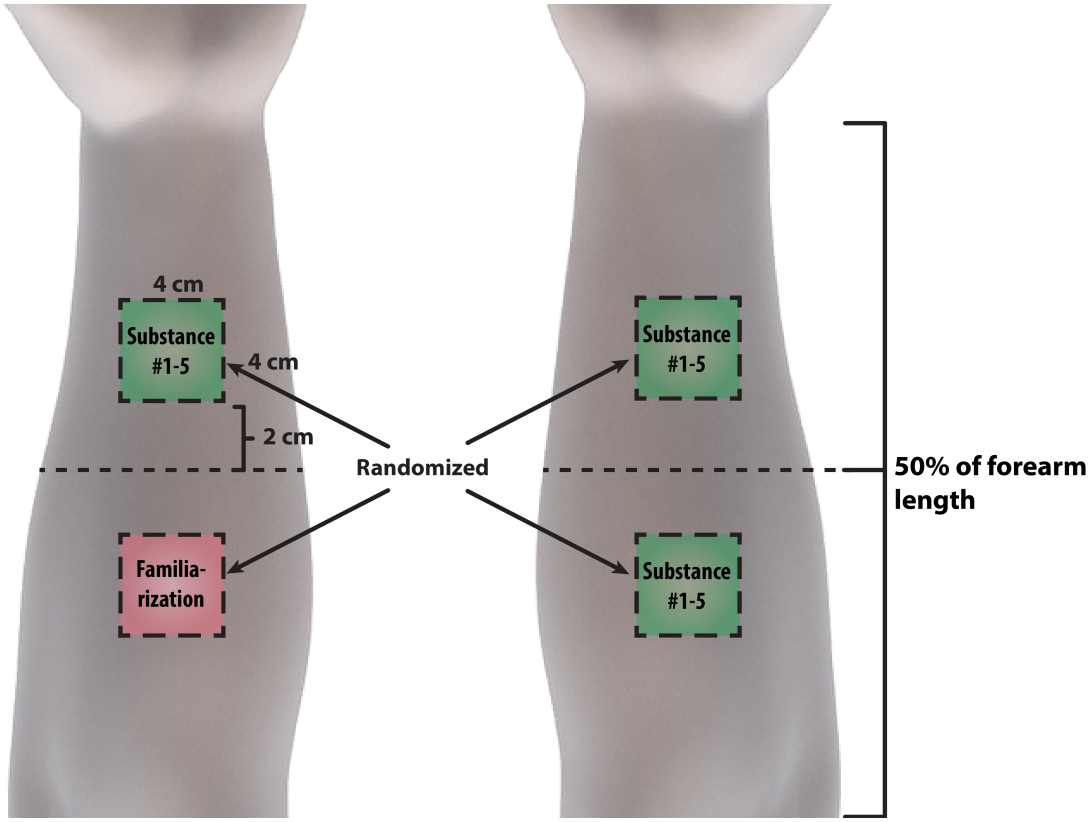

Figure 2 – Overview of application areas on the volar forearms. Notice that this is an exemplification. Actual substance application/familiarization will be randomized for order, and location. In session 1, only three of the four areas will be used. In session 2, only two of the four areas marked for substance application will be used.

Computerized Visual Analog Scale (VAS) Scoring: During induction of itch the subjects will be asked to rate the sensation of itch, stinging/pricking and burning on a VAS scale from 0-10 where 0 denotes "no itch" and 10 denotes "worst imaginable itch" and similarly for sensations of stinging/pricking and burning, both of which are frequently associated with the sensation of itch. The subjects will report the sensation using a horizontal electronic scroll bar giving a digital output in a frequent (i.e., every 15 seconds) discrete manner. Rating of the itch intensity will continue until ≥ 3 subsequent ratings are below 1 (assessed for itch) or after a maximum duration of 15 minutes. Based on similar studies this is typically between 5-15 minutes after the itch has been induced. Nociceptive sensations associated with non-histaminergic itch are usually subtle; typically rated 1-3 on a 0-10 VAS and "weak" on a generalized Labeled Magnitude Scale (gLMS) [38,40,41].

Itch scores are typically rated 3-8 on a 0-10 VAS scale and "moderate" to "strong" on a gLMS (depending on induction paradigm).

Application of cowhage spicules: Each 4 x 4 cm test area on the forearm will undergo itch induction with cowhage after a 5-minute rest from the thermal testing. Ratings of itch intensity will be taken on a VAS scale (0-10) continuously for 10 minutes. Subjects will be asked to not scratch the areas.

Cowhage itch will be induced by the application of cowhage spicules on the skin. Cowhage or the velvet bean (Mucuna pruriens var. pruriens) is a tropical plant whose pods are covered with short, fine hairs (also called trichomes or "spicules"). Cowhage spicules are 1-2 mm in length and have diameter of 1-3 um at their tip. Inserted into the epidermis, the spicules evoke a moderate to intense sensation of itch and occasionally mild sensations of burning and stinging pain. The insertion is associated with a light prick/sting lasting a few seconds followed by a latency period of no sensations of approximately 30 seconds and then a rapid onset of itch. The model is a well-established method for human non-histaminergic itch induction and has been thoroughly tested [38,41–43]. A number of 40 to 45 cowhage spicules will be applied to one area of the volar forearm. The spicules will be gently rubbed for 30-45 seconds onto the skin with a circular motion to facilitate contact until a substantial itch sensation is induced. After itch sensation is gone, the cowhage spicules will be removed using adhesive tapes.

Thermal stimuli (TSA-II, Medoc, Israel): A thermode surface probe of 3 x 3 cm is used to test skin areas for warmth detection and heat pain thresholds. The probe works by warming and cooling the skin by circulating fluid (water) close to a thin ceramic plate attached to the relevant skin area. Two standardized quantitative sensory tests are performed: 1) warmth detection threshold (HDT, assesses the threshold of which warmth sensation is first detected and reflect non-nociceptive warmth C-fiber function), 2) heat pain threshold (HPT, assesses the threshold at which heat pain sensation is first detected and reflect function of nociceptive heat-sensitive C-fibers). Ramp stimuli are delivered starting at 32°C, ramping upwards with 1°C/s and proceeding until the subject first experience warmth sensation (HDT) or pain (HPT) and presses a stop button to mark his/her perception threshold. Immediately as the subject presses the button the probe returns to the baseline temperature of 32 °C at a rate of 5°C/s and thus the subject will only experience brief pain just around his/her perception threshold. Both HDT and HPT are always assessed in triplicates (the final threshold is the arithmetic mean of 3 stimuli) and HDT is always assessed before HPT. The quantitative sensory assessment of HDT and HPT is based on the standardized and thoroughly tested methodology developed by the German Association for the Study of Neuropathic Pain (DFNS) and follows the published protocol [39,44].

Mechanical stimuli:

Mechanical detection thresholds (Von Frey Filaments, Stoelting, CA, US)

To assess mechanical detection thresholds standardized, CE-approved and commercially available von Frey filaments stimulators (North Coast Medical, Stoelting, CA, US) are used. The set consists of 20 calibrated nylon filaments with bending forces from 0.078 mN to 2.9 N (with slightly increasing exerted force surface). Starting with the lightest filament each probe is applied perpendicularly to the skin area for 1-2 seconds with a soft "landing" and removal in an ascending/descending order. The stimulators are designed to detect the mechanical detection threshold (MDT), which is considered the perceptual correlate of cutaneous Aβ-fiber sensitivity. The final threshold is the geometric mean of five series of ascending and descending stimuli. The quantitative sensory assessment of MDT is based on the standardized and thoroughly tested methodology developed by the German Association for the Study of Neuropathic Pain (DFNS) and follows the published protocol [39,44]. Due to the completely non-invasive nature of the procedure it is not associated with any risks.

Mechanical pain thresholds (The Pinprick, MRC, Germany)

To test mechanical pain sensitivity standardized, CE-approved and commercially available pinprick stimulators (The PinPrick, MRC, Germany) are used. This set consists of 7 pins with exponentially increasing force increments from 0.8 mN to 512 mN (0.8, 1.6, 3.2, 6.4, 128, 256, and 512 mN). The pinprick stimulators have a 0.2 mm blunted tips designed to deliver a sharp needle-like stimulus without penetrating the epidermis. Starting with the lightest (0.8 mN), each stimulator is applied in an ascending/descending order at a rate of 2s on, 5s off until the first perception of sharpness/pain is reached. This is denoted the mechanical pain threshold (MPT) and represents function of mechano-receptive C- and Aδ-nociceptors. The final threshold is the geometric mean of five series of ascending and descending stimuli. The quantitative sensory assessment of MPT is based on the standardized and thoroughly tested methodology developed by the German Association for the Study of Neuropathic Pain (DFNS) and follows the published protocol [39,44].

Statistical Analysis: Data will be entered into a database program, such as Microsoft Excel and/or IBM SPSS, and analyzed at using Prism GraphPad 6 and/or IBM SPSS. Basic descriptive statistics using mean (SEM), range, and n (%) will be calculated in Excel and presented. Statistical significance will be set at $p < 0.05$. Analysis will be performed within and between groups.

Normality assessment will be tested by visual inspection and Sharpiro-Wilks tests. A repeated measure (between–within) ANOVA will be used for the main analysis and post hoc comparisons will be conducted using the Bonferroni correction in a manner similar to Dunnett's test.

Blinding procedures: The study will be conducted under double-blinded conditions. The investigators responsible for the data collection, i.e. application, sensory tests, itch provocations, and data entry, will be blinded to the substance being applied. Blinding will be carried out by random allocation of letters "A" to "E" to labels on the pharmaceuticals (including the vehicle). This task is carried out at the compounding pharmacy. With delivery of the pharmaceuticals a list specifying the compounds corresponding to allocated letters is supplied to the PI. In case of serious adverse effects, the PI will breach the blinding. Otherwise unblinding will not occur until after the primary analysis has been conducted.

Local safety assessment: After pretreatment with each ointment (before any sensory testing is performed), and after each cowhage application a local safety assessment will be done. Erythema, edema, and any other local reaction will be recorded on a 5-point Likert scale (0=clear, 1=almost clear, 2=mild, 3=moderate, 4=severe).

11) Data Management*

All of the data, including records of subjects, source documents, and inform consent will be kept in the study center office under lock for 6 years after the study finished. The study center office is located at: 1600 NW 10th Ave, RMSB 7167, Miami FL 33136.

12) Provisions to Monitor the Data to Ensure the Safety of Subjects*

The study team will be responsible for protecting the safety, rights, and well-being of study participants. When testing is performed the subjects' vital signs (blood pressure, heart rate, and respiratory rate) will be monitored.

Recording and reporting of adverse events that occur during the course of the study will help to ensure the continuing safety of participants. Local and systemic adverse events will be monitored during the study. Dermatologic evaluations for rash, erythema (outside of administration area), urticaria, eruptions, and epidermal necrolysis will be performed throughout the study visit. Anaphylaxis and hypersensitivity (erythema multiforme) will also be monitored for.

Study subjects will be instructed to report any adverse events up to one week after the study is completed. In case an adverse event does take place, a documentation system will be used to record the following information: description of event, time/date of onset, duration of reaction, does/frequency/route of drug administration, reversibility or sequelae,

therapy (if any), and seriousness/severity (based on Common Terminology Criteria for Adverse Events v4). If a subject experiences an adverse event assessed at ≥ Grade 2 according to CTCAE v4, the subject will not receive additional treatments and will be followed until the adverse event resolves or stabilizes.

Study stoppage will occur if 36 subjects complete the study or if a severe adverse event occurs.

The PI will be responsible for monitoring ongoing activates to ensure compliance with regulator ad protocol requirements, data quality, and participant safety.

13) **Withdrawal of Subjects***

All subjects are free to withdraw from participation in this study at any time, for any reason, and without prejudice.

Subjects must be withdrawn from the study if:

1) A subject withdraws consent.
2) The Investigator believes it is in the best interest of the subject to be removed from the study.
3) If the subject experiences greater than a mild adverse event. (Subjects that experience a mild adverse event will be asked if they would like to continue the study. The occurrence of a severe adverse event will trigger the study to stop for safety purposes.)

If withdrawal occurs, subjects will receive prorated compensation. The data collected before the withdrawal will still be used in data analysis.

14) **Risks to Subjects***

1. **Discomfort.** The researchers will take care to minimize discomfort and pain during the measurement of pain thresholds and cowhage application. These procedures are already designed to limit magnitude and duration of discomfort.

2. **Cowhage application.** No specific serious risks or adverse effects are associated with the application of cowhage spicules to the skin. At the site of application, subjects may experience a mild to moderate burning/stinging sensation apart from the itch sensation itself, which may last from 1-7 minutes [40,41,45]. Cowhage is not an allergen and does not cause contact dermatitis, chemical irritation or erythema. No serious adverse reactions to cutaneous cowhage application have been described in the literature. We do not expect a tachyphylactic reaction or an additive

affect from multiple cowhage applications because we are not applying cowhage more than once to the same location during the same study visit. In the event of an allergic reaction, the study will be stopped and appropriate treatment administered (use of EpiPen).

Following use of EpiPen, the patient will be referred to the emergency room, where basic vitals including heart rate, blood pressure, and oxygen saturation in the blood will be monitored. A respiratory exam will also be given to assess the quality of breathing. Steroids to prevent additional airway closure and fluids to maintain blood pressure will be given as necessary. The patient will be monitored in the emergency room for 6 hours, and then sent home with an additional EpiPen to guarantee immediate access to self-injectable epinephrine. The patient will be instructed on EpiPen usage. The patient will also be educated to watch for symptoms of biphasic anaphylaxis, which may occur up to 72 hours after initial symptoms of anaphylaxis.

3. **Sensory testing of heat pain thresholds.** Heat pain will be assessed using a thermode probe raising the temperature of circulating water from 32ºC up to 50ºC. Since we only assess the pain perception threshold (i.e. the first temperature at which pain is experienced) this heat pain is marginally unpleasant and only persists for a few seconds. If the pain is unbearable, the thermode can be immediately removed to alleviate the painful sensation. Although the thermode is computer-controlled, it may potentially produce hot temperatures, which causes pain. In this case, the subjects are allowed to withdraw their arm from a thermode. The Medoc analysis station controls and monitors the thermode temperature for the TSA-II. This software has a built in safety feature, which has an automatic shut-off if the thermode reaches 50°C. This build-in shut-off, along with the ability for the investigator to monitor the thermode's temperature in real-time makes the chance of even superficial burns occurring minimal. Several large multicenter studies have safely used identical equipment and test paradigms without the occurrence of adverse events [39,44,46,47].

4. **Sensory testing of mechanical pain thresholds.** Mechanical pain thresholds will be assessed with 7 weighted pinprick stimulators in accordance with the manufacturer's directions. The assessment is associated with mild pain, but since we only assess the pain perception threshold (i.e. the first pin at which pain is experienced) the pain only persists for a few seconds. The pinprick stimulators do not penetrate the epidermis on normal skin. However, as a precautionary measure the pinprick stimulator tips are sterilized with 70% ethanol prior to each new test session. Several large multicenter studies have safely used identical equipment and test paradigms without the occurrence of adverse events [39,44,46,47].

5. **Topical administration of ketamine, amitriptyline and lidocaine.**

   a. **Ketamine.** In the present study topical ketamine 10% is applied. Topical ketamine has previous previously been applied in various concentrations and vehicles (usually to treat neuropathic pain) and is generally considered devoid of serious side effects [11,48,49]. Notably, in studies investigating plasma levels of ketamine following topical exposure the concentration has generally been below the assay detection limits and/or below physiologically significant levels [19,25,34,49]. Minor side effects occasionally include local skin rashes and hives, as well as dizziness and light-headedness [11,22,49]. Only one serious adverse event case has been described in the literature for topical ketamine and it concerns application of a non-prescribed compounded ointment containing ketamine in an infant [50]. In the present study ketamine will only be applied to intact skin, in adults, and to a relatively small area (4 x 4 cm) and as such the potential systemic exposure is negligible.

   b. **Amitriptyline.** In the present study topical amitriptyline 5% is applied. Amitriptyline has previous previously been applied in various concentrations, in different vehicle creams and combined with other topical agents (usually to treat neuropathic pain) [19,20,24,25,27]. It is generally considered safe and the systemic exposure associated with topical usage is many times lower than the therapeutic dosage for systemic therapy. In a clinical trial with 140 participants receiving 4% amitriptyline no serious adverse effects occurred [51]. Minor side effects include skin rashes, sedation and palpitations [20,22,24,27]. In the present study amitriptyline will only be applied in adults and to a relatively small area (4 x 4 cm) and as such the potential systemic exposure is negligible.

   c. **Lidocaine.** In the present study topical lidocaine 5% is applied. Lidocaine is an extremely well-studied and generally safe local anesthetic available for OTC use [26,48,52–54]. Common side effects include local blanching and erythema. Contact hypersensitivity to lidocaine is rare but has been described [52,54]. Systemic allergic (anaphylactic) reactions to lidocaine are exceedingly rare, particularly in response to percutaneous application [52,55,56]. A systematic review on the use of ointment containing lidocaine found single dose percutaneous administration to be safe in neonates [53]. In the present study lidocaine alone and in combination with amitriptyline and ketamine will only be applied in adults and to a relatively small area (4 x 4 cm) and as such the systemic exposure is negligible.

In the previous studies applying combinational use of above-mentioned substances no tendencies towards more frequent or more severe side effects have been reported.

15) Potential Benefits to Subjects*

There is no direct benefit to the subjects.

16) Vulnerable Populations*

No vulnerable populations will be enrolled in this study.

17) Sharing of Results with Subjects*

Results of this study will not be shared with the subjects, unless through scientific publication.

18) Setting

Potential subjects will be identified and recruited through IRB approved flyers.

Research procedures will be performed at the Miami Itch Center at the Lennar Foundation Medical Center or at the Department of Dermatology Research Clinic at UMH (West Building, rooms 504, 505, 506, &508).

19) Resources Available

The Principal Investigator Gil Yosipovitch, MD, is a Professor from the Department of Dermatology & Cutaneous Surgery at the University of Miami. Dr. Yosipovitch's primary research interest is in pruritus (itch) and the neurophysiology and innervation of skin in chronic itch. Dr. Yosipovitch has conducted research in the field of skin physiology, pharmacology, and neurophysiology of itch over the past 20 years. His research activities have centered on basic and clinical studies on itch neurophysiology and on clinical topics in dermatology, including topical and systemic formulations of anti-pruritics and skin barrier function and in itch in atopic eczema and psoriasis. Dr. Yosipovitch will assist with recruitment, study procedures, and assess adverse events.

Sub-Investigator Leigh A. Nattkemper, PhD, is an Instructor from the Department of Dermatology & Cutaneous Surgery at the University of Miami. Dr. Nattkemper uses advanced molecular biology and genetic approaches to understand the patho-mechanisms of chronic itch, with a special interest in the neurological processes. She has conducted research

in the field of chronic itch for over 6 years and will act as Study Coordinator.

Sub-investigator Lars Arendt-Nielsen, Prof, Dr. Med., PhD, is a research collaborator from Aalborg University, Denmark. Prof. Arendt-Nielsen has conducted research in the field of pain neurobiology, pharmacology and assessment for the last 28 years. His research activities have centered on basic, translational and clinical studies primarily in the field of pain neurophysiology, pharmacological proof-of-concept studies, quantitative sensory testing and development of human surrogate models of pain symptomatology. Prof. Arendt-Nielsen has been using cowhage itch provocations in several previous projects starting from 2013 as well as quantitative thermal and mechanical sensory testing starting from 1987. Prof. Arendt-Nielsen has conducted numerous pharmacological trials and early human mechanistic studies to test the efficacy of novel or off-label purported analgesic compounds. Prof. Arendt-Nielsen will assist with protocol design and development, data interpretation of non-identifiable data, as well as manuscript preparation.

Sub-investigator Hjalte H. Andersen, MS Med., is a PhD fellow, from Center for Sensory Motor Interaction, Aalborg University, Denmark. He will conduct research out of the Dermatology Department at University of Miami during a 6-month research stay. Hjalte H. Andersen has conducted cowhage itch provocations in several previous projects starting from 2013 as well as quantitative thermal and mechanical sensory testing starting from 2011. Hjalte H. Andersen will assist with protocol design and writing, study coordination, subject recruitment, research fieldwork, data collection, data analysis, as well as draft and revise the manuscript.

Pharmacist: Jack Korbutov, RPh, PharmD, FACA, is working out of the Art of Medicine Compounding Pharmacy located in Philadelphia. The pharmacy has been compounding the combination ointment KeAmLi for approximately 3 years. Jack Korbutov will compound the applied topical formulations, advise on its storage, and label the tubes for the purpose of blinding.

Student researcher Jeremy Hsiang, BS, is medical student at the University of Miami. He will will assist in subject recruitment, and study procedures, and data entry.

All study personnel will complete their CITI training and be adequately informed about the protocol and study procedures. All duties and functions are appointed and overseen by the PI.

20) **Prior Approvals**

Approved under FDA IND 134066.

21) Recruitment Methods

IRB approved flyers will be placed around the University of Miami medical campus, and within the waiting rooms of the Department of Dermatology Out Patient Clinic at UMH (South Building, suites K-M) and at the Lennar Foundation Medical Center.

Subjects are compensated $25 at the completion of each study visit for a total of $50 for 2 visits.

22) Confidentiality

We will not be accessing or adding to any medical records. Information will be collected directly from the subject, and will be used for research purposes only. Every attempt will be made to ensure any information gathered is kept secure. All study data will be kept in a locked office and kept under password protection on a computer that is only accessible by study personnel.

23) Provisions to Protect the Privacy Interests of Subjects

Study subjects will only be asked to provide personal information to approved study personnel, who will ensure the subject is at ease with the situation. Study personnel will clearly explain that the subject does not have to answer any questions or provide any sample they are uncomfortable about.

24) Compensation for Research-Related Injury

Treatment will be available if enrolled subjects get sick or injured. However, the subject or the subject's insurance will be responsible for these costs. Funds to compensate for pain, expenses, lost wages, and other damages caused by injury are not available.

25) Economic Burden to Subjects

There will be no charges to the subjects that agree in participating in this study.

26) Consent Process

The research team will follow the "HRP-090 SOP: Informed Consent Process for Research" to obtain informed consent and the "HRP-091 SOP: Written Documentation of Informed Consent" to document informed consent in writing.

Study personnel will meet with each potential subject to discuss the study in detail, answer questions, and allow the subject to read the entire consent form. The informed consent form explicitly states the rationale for the study and requirements for participation, both before and during the session. The informed consent form states that subjects may discontinue participation or be terminated from the study at any time.

All pertinent aspects of the study will be explained to the subject before he or she signs the informed consent form. A signed informed consent form will be obtained from the subject before any activity or treatment is undertaken as part of the study.

Non-English Speaking Subjects will not be enrolled. Our current research team does not have a member that fluently speaks Spanish. We are actively trying to recruit such a member and will submit translated consent forms once we hire such personnel. Subjects who are not yet adults (infants, children, teenagers), cognitively impaired adults, and adults unable to consent will not be enrolled.

27) Process to Document Consent in Writing

The research team will follow the "HRP--091 SOP: Written Documentation of Informed Consent" to document informed consent in writing.

28) Drugs

Cowhage:

Cowhage (Mucuna pruriens) is a tropical legume also known as Velvet bean or cowitch and it is found in Latin America, the Caribbean, India, Africa and Florida. M. pruriens is a climbing vine and considered variously as an annual or perennial. The plant consists of a vine with slender stems that bear numerous alternate, trifoliolate leaves approximately 10cm long. The flowers are typically purple, red or green-yellow. The fruit pods range from 3-10 cm in length, are curved and, when mature, are covered with dark hairs/spicules that provide a velvety appearance. The pods contain 4-6 seeds that are approximately 1 x 1 cm.

The dry pods are collected by cutting the stems from the plant and letting them fall directly into a clean, dry polythene bag. The spicules are separated from the pods by shaking the bag. Forceps are used to remove the pods and retain the spicules. The cowhage spicules are shipped at room temperature since they are stable unless exposed to extreme heat and pressure via autoclaving (>100°C), which renders them chemically inert.

Once it reaches the lab, Dr. Yosipovitch's staff documents on the tube the date of arrival. This batch is labeled the "master batch". The tube of cowhage is then stored under dry conditions in a 4°C refrigerator. Also

upon arrival, the spicules are examined under a microscope to confirm the material is cowhage. The dimensions of the spicules (2-4 mm length, 1-3 µm tip diameter) are measured [40].

The investigator then tests the spicules for the itch induction ability with the same application method used in studies [57]. Forty to forty-five spicules (a dose found to induce a consistent and reproducible itch sensation) are counted under the microscope and then rubbed in to a 4x4 cm area on the volar forearm for 45 seconds in a circular motion. Approximately 0.2 mm of the spicule tip enters the skin. Forty-five spicules of cowhage are equivalent to 45 µg of material, containing about 90-140 ng of mucunain [45]. The spicules usually induce itch after ~30-90 seconds of the application. If 2 minutes pass without itch being induced, the spicules are deemed inactive and the batch will not be used for itch induction in studies. If itch is induced, the itch sensation will last ~7 minutes and itch intensity is rated using a 0-10 numerical rating scale (NRS) every 30 seconds until the itching subsides. Cowhage spicules are removed from the skin using tape, which is disposed of in a biohazard trash bin. The itch subsides quickly once the spicules have been removed. Itch is only felt at the site of induction and mild temporary erythema at the induction site is common.

For studies using cowhage to induce itch, a small portion of the master batch of cowhage is aliquoted into smaller 5 ml tubes as a "working batch". The date of separation from the main batch is marked on the smaller tube. This tube then remains at room temperature for the duration of the study. The potency of the cowhage is preserved for many years when stored in a dehumidified area at room temperature or at 4°C, but the potency is checked every 3 months and before the start of any study.

<u>Drug Formula for Ketamine 10% - Amitriptyline 5% - Lidocaine 5% (Per 100gm of cream)</u>

- Ketamine HCl (Fagron Pharmaceuticals NDC 51552-0697-07): 10grams
- Amitriptyline HCl (Medisca NDC 38779-0189-09): 5 grams
- Lidocaine HCl (Medisca NDC 38779-0082-03): 5 grams
- Ethoxy Diglycol (Fagron Pharmaceuticals NDC 38779-1903-07): 5mL
- Liquigel (Medisca NDC 38779-2531-01): 3 grams
- Lipoderm Cream Base (PCCA NDC 51927-3338-00): 72 grams

<u>Drug Formula for Ketamine 10% (Per 100gm of cream)</u>

- Ketamine HCl (Fagron Pharmaceuticals NDC 51552-0697-07): 10grams
- Ethoxy Diglycol (Fagron Pharmaceuticals NDC 38779-1903-07): 4mL
- Liquigel (Medisca NDC 38779-2531-01): 3 grams
- Lipoderm Cream Base (PCCA NDC 51927-3338-00): 83 grams

Drug Formula for Amitriptyline 5% (Per 100gm of cream)

- Amitriptyline HCl (Medisca NDC 38779-0189-09): 5 grams
- Ethoxy Diglycol (Fagron Pharmaceuticals NDC 38779-1903-07): 3mL
- Liquigel (Medisca NDC 38779-2531-01): 3 grams
- Lipoderm Cream Base (PCCA NDC 51927-3338-00): 89 grams

Drug Formula For Lidocaine 5% (Per 100gm of cream)

- Lidocaine HCl (Medisca NDC 38779-0082-03): 5grams
- Ethoxy Diglycol (Fagron Pharmaceuticals NDC 38779-1903-07): 3mL
- Liquigel (Medisca NDC 38779-2531-01): 3 grams
- Lipoderm Cream Base (PCCA NDC 51927-3338-00): 89 grams

Drug Formula for Vehicle (Per 100gm of cream)

- Lipoderm Cream Base (PCCA NDC 51927-3338-00): 100 grams

Formulation Instructions:

1. Weigh powder reagents in weigh boats and set aside.
2. Tare 100mL unguator jar
3. Place all powders in jar, and top with wetting agent (Ethoxy Diglycol).
4. Mix with a spatula until a thick paste is formed
5. Place emulsifier/thickiner (Liquigel) on top of the paste.
6. Pour Lipoderm cream on top (total weight of ingredients should equal 100gm).
7. Using Mixing blade and Unguator e/s, Mix the compound for 2:30 at a mixing speed of 5.

8. Pour contents into jar/pump for patient use.

Labeling:

The formulations will be labeled “For Research Only” and will a coded in order to ensure double-blinding. Blinding will be carried out by random allocation of letters “A” to “E” to label the pharmaceuticals (including the vehicle). This task is carried out at the compounding pharmacy. With delivery of the pharmaceuticals and a list specifying the compounds corresponding to allocated letters is supplied to the PI.

Formation expirations:

As per USP 795 standards on compounding with a water-containing base, these formulations have an expiration of 30 days. The expiration date will be on the label.

Storage:

The formulations will be stored at room temperature in a locked cabinet in the Dermatology lab that only study personnel will have access with a key. Ambient temperature of the storage cabinet will be recorded once a day (except on weekends). Logs of formulation delivery and use per subject will be kept.

Application:

Application will be conducted with the investigator wearing nitrile gloves. The pre-treatment will occur under topical occlusion for 30 minutes to allow the ointment to be adsorbed. Following this, residual ointment will be removed and sensory testing, strictly within the pretreated area, will commence.

IND:

The holder of this IND is Gil Yosipovitch, MD (21 CFR 312). No electronic source data will be captured using an eCRF (21 CFR 11). The investigators declare no conflict of interests or financial disclosures and will be sure to maintain updated disclosures with the University of Miami (21 CFR 54). Pharmacist Jack Korbutov, RPh, PharmD, FACA at the Art of Medicine Compounding Pharmacy will compound the formulations under cGMP standards (21 CFR 211).

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