Pharmacokinetics, Side Effects, and Anti-Hyperalgesic Efficacy of The Mglu5 Antagonist Fenobam.

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Abstract

Rates of opioid misuse, abuse, and addiction-related aberrant behaviors have been steadily rising in the past 20 years. The development of effective alternative pharmacologic therapies to treat acute and chronic pain would significantly reduce the need for opioid analgesic use. While metabotropic glutamate receptor 5 (mGlu5) has been shown to modulate nociception in animals, so far no mGlu5 antagonists have been developed commercially as analgesics. The mGlu5 antagonist fenobam [N-(3-chlorophenyl)-N'-(4,5-dihydro-1-methyl-4-oxo-1H-imidazole- 2-yl)urea] was originally evaluated for development as a non-benzodiazepine anxiolytic. Multiple studies have demonstrated that fenobam is analgesic in a variety of mouse models of inflammatory, neuropathic, and visceral pain, and acts exclusively via mGlu5 blockade. Furthermore, fenobam shows no signs of analgesic tolerance with up to two weeks of daily dosing. Here, we report a translational study of the analgesic effects of fenobam in human. We first established the pharmacokinetic properties of orally administered fenobam, and used this information to conduct a test of the analgesic effects of fenobam in an established experimental human pain model of cutaneous sensitization. While fenobam reduced sensitization in healthy volunteers at a single measurement time (at peak plasma fenobam concentration), we did not observe a statistically significant sustained analgesic (anti-hyperalgesic) effect of fenobam compared to placebo. We suggest that future studies testing possible analgesic effects of mGlu5 blockade should employ molecules with improved pharmacokinetic profiles. Prospective randomized clinical trials are needed to clarify the role of mGlu5 modulation in the development and maintenance of acute and chronic pain conditions in human.

Introduction

Thirty percent of adults in the US suffer from chronic pain(Johannes et al., 2010). For lack of better or equally effective alternatives, one in every five patients with chronic pain(Centers for Disease Control and Prevention. Annual Surveillance Report of Drug-Related Risks and Outcomes — United States and 2017), as well as almost all patients with acute pain following surgery or trauma are treated with opioid analgesics. Over 250 million opioid prescriptions are issued annually in the US(Volkow and McLellan, 2016). While most patients use opioid analgesics as prescribed, the rates of misuse, abuse, and addiction-related aberrant behaviors have been steadily rising in the past 20 years(Volkow and McLellan, 2016), significantly contributing to the current national epidemic of opioid addiction, and resulting in more than 30,000 opioid overdose deaths annually(Rudd et al., 2016). In this alarming scenario it is imperative that every effort is made to develop effective alternative pharmacologic therapies to treat acute and chronic pain.

Metabotropic glutamate receptor 5 (mGlu5) has emerged as a strong candidate for the development of a new class of analgesic drugs. An extensive literature demonstrates the efficacy of mGlu5 antagonists in a broad range of preclinical pain models been shown to modulate nociception in animals(Kolber, 2015; Chiechio, 2016) but, so far, to date no mGlu5 antagonists have been developed commercially as analgesics.

The investigational drug fenobam [N-(3- chlorophenyl)-N'-(4,5-dihydro-1-methyl-4-oxo-1H-imidazole- 2-yl)urea] was originally evaluated for development by Ortho-

McNeil (McN-3377) between the late 1970s and early 1980s as a non-benzodiazepine anxiolytic, with a then unknown molecular target(Itil T.M., 1978 ; Pecknold J.C., 1980; Lapierre, 1982; Pecknold J.C., 1982). While the commercial development of fenobam as an anxiolytic was not pursued, in 2005, Porter et al. characterized fenobam as a selective and non-competitive mglu5 antagonist(Porter et al., 2005). In agreement with earlier reports of the role of mGlu5 in nociceptive pathways(Walker et al., 2001) and the selectivity of fenobam for mGlu5, the analgesic efficacy of fenobam has been demonstrated in multiple mouse models of inflammatory, neuropathic, and visceral pain(Jacob et al., 2009; Montana et al., 2009; Crock et al., 2012; Lax et al., 2014), with no analgesic effect in mGlu5 knockout mice(Montana et al., 2009). In addition, two-week daily dosing did not result in tolerance to fenobam's analgesic effect in mice(Montana et al., 2011). These observations, coupled with the initial favorable data obtained in pre-clinical and clinical studies(Itil T.M., 1978 ; Pecknold J.C., 1980; Lapierre, 1982; Pecknold J.C., 1982; Bhave et al., 2001; Zhu et al., 2005; Berry-Kravis et al., 2009) , led us to consider the possibility of specifically targeting mGlu5 to treat pathologic pain conditions in humans.

In these prior studies, fenobam was administered to a limited number of human subjects, and its bioavailability is poorly characterized. We sought to establish the PK of orally administered fenobam, and use information from this initial study to conduct a test of the analgesic effects of fenobam in an experimental model of human pain in healthy volunteers. The main goal of the preliminary PK study was to test several doses of fenobam, and define t_{max} and C_{max} following oral administration to adult healthy volunteers. The highest dose that we administered (150 mg) was the highest reported in

the literature to be administered as a single oral dose in human and to be well tolerated(Itil T.M., 1978 ; Pecknold J.C., 1982; Berry-Kravis et al., 2009). After confirming lack of significant side effects and obtaining PK data for the 150mg oral dose, we set out to conduct the experimental pain study. Our aim here was to time the induction of hyperalgesia in the experimental pain model so that maximal painful stimulation would occur at the time of C_{max}.

The human experimental paradigm that we selected is the heat/capsacin sensitization model(Petersen and Rowbotham, 1999; Dirks et al., 2003). The main outcome of interest in this study was reduction of area of cutaneous sensitization. Our hypothesis was that, after exposure to the heat/capsaicin model of cutaneous sensitization, healthy volunteers treated with fenobam would show a significantly reduced area of cutaneous hyperalgesia and allodynia around the area treated with heat/capsaicin (area of secondary, centrally mediated sensitization(Petersen and Rowbotham, 1999)) as compared to healthy volunteers treated with placebo. Secondary outcomes of interest were: Heat Pain Detection Thresholds (HPDT) in normal and sensitized skin pre and post treatment with fenobam; acute pain scores at thermal stimulation on normal (untreated) skin with and without fenobam, and absence of significant side effects.

In consideration of the reported anxiolytic properties of fenobam(Itil T.M., 1978 ; Pecknold J.C., 1980; Lapierre, 1982; Pecknold J.C., 1982; Porter et al., 2005) we were also interested in the possible implications of anxiolysis on pain modulation; therefore mood and affect changes of the subjects were evaluated throughout the study by means of a combination of a short version(Mackinnon, 1999) of the brief Positive and

Negative Affect Scale (PANAS)(Watson et al., 1988) and Brief State Anxiety Measure (BSAM)(Berg et al., 1998). Moreover, taking into consideration pre-clinical studies reporting effects of mGlu5 modulation on working memory and cognitive performance(Ballard et al., 2005; Quarta et al., 2007; Semenova and Markou, 2007; Jacob et al., 2009; Mikulecka and Mares, 2009), subjects involved in the study underwent the Letter and Number Sequencing Assessment (LNS), a subtest of the Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV)(Wechsler, 2008) to evaluate working memory as a measure of cognitive function before and after administration of fenobam or placebo.

Methods

All studies were carried out in accordance with ethical principles of Good Clinical Practice and the Declaration of Helsinki and its guidelines, and approved by Washington University Human Research Protection Office (HRPO), following submission of an Investigational New Drug (IND) application to the FDA (IND#117,989). Subjects provided informed consent after the study procedures were explained and all questions were answered before study procedures were initiated.

Fenobam was manufactured under GMP guidelines by Scynexis, Inc. (Durham, NC) as "crude fenobam" and then compounded in gelatin capsules with lactose monohydrate at Washington University in St. Louis investigational Pharmacy under strict adherence to USP 795.

Study Setting

Both studies ("PK Study" and "Hyperalgesia Study") were conducted in the Washington University Clinical Research Unit (CRU) and/or the Anesthesiology Human Studies Lab at Washington University under the general supervision of the primary investigator (LFC); throughout the study sessions, subjects were continuously monitored by trained research personnel.

1. PK Study. Pharmacokinetics and Side Effects Following Oral Administration of the mGlu5 Antagonist Fenobam in Adult Healthy Volunteers

This was a randomized, double blind, single dose, parallel group, placebo controlled study to evaluate the pharmacokinetics and side effects of fenobam (Flow Chart - Fig.1A).

Screening Session/Pre-study Period

Potential candidates for the study were examined for qualification for study entry according to the established inclusion and exclusion criteria (Table 1A). During this session, informed consent was conducted. Study participants were asked to abstain from drinking alcohol for 24 hours before the study, and to abstain from eating and drinking after midnight on the night before the study to avoid interference with drug absorption. They were educated to the study procedures, and explained potential risks and benefits. The initial visit included a health self-assessment form, collection of medical history and physical examination. Vital signs were then recorded (heart rate, respiratory rate, blood pressure, temperature and oxygen saturation (SpO_2)). Each subject who qualified for entry into the study on the basis of inclusion/exclusion criteria, agreement with informed consent and pre-study evaluation was assigned the next available patient number.

Randomization

A computer generated randomization schedule assigned subjects to either 50 mg, 100 mg, 150 mg of fenobam or placebo administration after successful completion of the screening session. Therefore each subject received a single dose of fenobam or placebo.

Study Period

At arrival, subjects' vital signs were recorded and a urine pregnancy test was performed on women of childbearing potential. Subjects were excluded if pregnant.

A peripheral IV catheter was inserted in an arm for blood sampling, and venous blood samples were drawn at predefined time points before and after fenobam/placebo administration, for approximately 10 hours (at approximately 30, 60, 120, 180, 240, 300, 360 and 600 minutes) and one more time the next day. At the same time-points vital signs (HR, respiratory rate, BP and SpO2) were recorded and subjects were interviewed to evaluate for side effects (confusion, visual changes/blurred vision, dizziness, light-headedness, weakness, speech difficulties, abnormal cutaneous sensations, tingling, or numbness, nausea/vomiting, headache, metallic taste, hot flashes and any abnormal feelings).

One week after the study completion subjects were again asked to answer a questionnaire regarding any abnormal/unusual feeling they might be experiencing, including potential persistent changes in sensation at the site of cutaneous sensitization. The 1 week interview was conducted by phone.

Study Measurements

Blood samples were collected on day 1 and on day 2. The first blood sample on day 1 was used to test for complete blood cells count and comprehensive metabolic panel, including blood glucose, albumin, electrolytes, liver function tests, blood urea nitrogen and creatinine (CBC and CMP tests) at baseline, and subsequent blood samples on day 1 were collected to assess fenobam plasma concentration. On day 2 plasma concentration of fenobam, CBC and CMP tests were repeated. Side effects for all treatment groups were assessed as follows: 1) self-reporting from the participants and 2) via questionnaire regarding side effects to be administered by a study team member.

Data Analysis

Descriptive statistics were generated for reported measures, and difference in distribution of baseline characteristics among groups administered fenobam 50mg, 100mg, 150mg or placebo were analyzed with the Kruskal Wallis H test using SPSS statistical software. Proportions of patients with side effects in the different groups were compared using Fisher's exact test. For all analyses, careful attention was given to whether the data satisfied the distributional and model-specific assumptions of the procedures used.

Laboratory Analysis

Quantification of Fenobam in Plasma

All subjects had blood samples (each 5 ml, total approximtely 40 ml) collected from the non-dominant arm at the time points reported above. The chosen time points were expected to capture the peak plasma concentration of fenobam after oral administration(Cmax). Samples were centrifuged at 3000 rpm for 10 minutes at room temperature. Plasma was then transferred to micro-tubes and frozen at -20^0 C.

General Instrumentation

Fenobam was quantitated using LC/MS/MS, as previously utilized in mouse studies by our laboratory. (LC = Liquid Chromatography, MS = Mass Spectrometry). Calibrators were prepared in a matrix matching the samples (plasma). Midazolam was used as the internal standard. Instrumental analysis was performed on an API 4000QTRAP triple-quadropole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA),

equipped with a Turbo Ion Spray Source. The Agilent 1100 HPLC system (Waldbronn, Germany) includes a binary pump, a thermostatted well-plate autosampler, and a column thermostat. An external two-way Valco valve is utilized to direct HPLC flow to waste before and after column elution of analytes of interest. Chromatographic separation was performed on a SymmetryShield RP18 analytical (3.5µm, 2.1 x 30mm) column (Waters Corp., Milford, MA, USA) with a C18 guard (5µm, 2 x 10mm) column (Varian, Lake Forest, CA, USA) at 30oC. Before each injection, the needle was washed with methanol.

HPLC and Mass Spec Conditions for Quantification of Fenobam in Plasma

Mobile phase A is 0.1% formic acid in water and mobile phase B is 0.1% formic acid in acetonitrile. Mobile phase was delivered at an initial condition of 5% B and a flow rate of 0.4ml/min with the following time program: linear gradient between 5 and 60% B for 1.0 minute followed by a sharp gradient to 100% B for 0.2 minute and hold at 100% B for 0.4 min; mobile phase composition is then brought back down to initial condition of 5% between 1.6 and 2.1 minutes.The column was re-equilibrated with 5% B from 2.1 to 5.5 minutes. Under these conditions, the retention time for fenobam in previous experiments was 3.4 minutes and for midazolam 3.0 minutes. The injection volume previously utilized was 20 microliters.

Both Q1 and Q3 quadrupoles of the mass spectrometer were optimized to low and unit mass resolution respectively. The instrument was operated in positive ion mode with an ion spray voltage of 5100 volts. The curtain gas was set at 20 psi, ion source gas 1 and 2 at 40 and 50 psi respectively, and the collision gas on high.

Calibration and Sample Preparation

Plasma samples were homogenized and aliquots of 25 microliters transferred into a 96-well plate. Precipitation was performed using 100 microliters of acetonitrile, containing 50ng/ml of midazolam (internal standard).The plate was capped and vortexed, and then centrifuged at 3000 rpm for 10 minutes. The supernatants were then transferred to a 96-well autosampler plate, and 20 microliters were injected for analysis. Calibrators and quality control samples were prepared along with experimental samples.

Calibrators, Quality Controls and Internal Standard Samples

A methanolic solution of fenobam was prepared at 1mg/ml. Dilutions from this stock standard were prepared and used to make calibrator (6.0 to 16,000 ng/ml, 10 concentrations) and quality control (QC) samples (2 concentrations) in human plasma.

2. Hyperalgesia Study: Anti-Hyperalgesic Efficacy of a Single Dose of Fenobam on Heat/Capsaicin Induced Cutaneous Hyperalgesia in Adult Healthy Volunteers

This study was designed as a randomized, double-blinded, placebo-controlled, two-way cross-over trial with 32 healthy volunteers who received either 150 mg fenobam or placebo (lactose monohydrate) and were then tested for cutaneous hypersensitivity using the heat/capsaicin model of cutaneous sensitization (Flow chart - Fig 1.B).

The study consisted in an initial screening/training session to evaluate subjects, establish eligibility and explain the study procedures, and two subsequent study sessions (placebo or fenobam, randomized order) that were conducted one week apart. During the two study sessions, blood samples were collected hourly for 7 hours after

administration of fenobam, and fenobam plasma concentrations were then determined by mass spectrometry. Measures of hyperalgesia and hypersensitivity were taken at regular time points during each study session, and included the area of cutaneous sensitivity to calibrated von Frey (vF) filament and to brush stroke stimulation, and Heat Pain Detection Thresholds (HPDTs). Assessments of mood/affect and cognitive function also occurred at the same time points. A complete overview of the study timeline is shown in Fig 2.

Screening Session/Pre-study Period

Subjects who were potential candidates for the study were evaluated for study eligibility, according to the established inclusion and exclusion criteria (Table 1). During the *screening session*, informed consent was administered. Study participants were educated to the study procedures, explained potential risks and benefits and given the opportunity to discuss any concerns or questions with the study coordinator and PI. The initial visit included a health self-assessment form, collection of medical history and physical examination. Vital signs were then recorded (heart rate, respiratory rate, blood pressure, temperature and SpO2). Each subject who qualified for entry into the study on the basis of inclusion/exclusion criteria, agreement with informed consent and pre-study evaluation was assigned the next available patient number.

Study Period

Subjects participated in three sessions: one screening/training session and two study sessions, each scheduled approximately one week apart. The *training session* was held on the same day as the above mentioned screening session. In this session the

enrolled subjects were given a demonstration of the actual study procedures to be conducted during the study sessions (see below). The duration of the subjects' participation was approximately four weeks, with training session on day 1, the first study session within 14 days of the training session, and Session #2 approximately one week following Session #1. During the training session, subjects experienced the heat/capsaicin sensitization procedure and measurements of cutaneous sensitization as described in detail below. HPDTs greater than 47°C at baseline and lack of development of an area of measurable cutaneous sensitization following heat and capsaicin stimulation determined withdrawal from the study. The training session was intended to familiarize subjects with the experimental procedures so that communication between experimenter and subject about the subjective experiences of painful stimulation could be as precise as possible. No drug was administered during the training session.

During the study sessions subjects underwent a complete set of sensitization procedures; mapping of the area of sensitization; measurement of pain at thermal stimulation and HPDT at baseline, and then multiple times following rekindling of the sensitization via heat application.

Sample Size Estimates

Preliminary data with the heat/capsaicin model obtained from our group(Cavallone et al., 2013) enabled us to estimate the variability of the main outcome measure, so that we could calculate the sample size sufficient to detect a 20% difference in the area of hyperalgesia in the two treatment conditions (main outcome of interest).

In the population of 15 subjects that we studied, reproducing the heat/capsaicin model in the same subjects 1 week apart, the within day standard deviation ranged from 25.91 (within day St-dev of initial and final areas for the same subject on Session 2) to 29.62 (within day St-dev of initial and final areas for the same subject on Session 1). The standard deviation of the difference between the areas for the same subject between days (Session 1 vs Session 2) was 12.99.

Based on these standard deviations, we calculated the sample size needed to detect a 20% difference in the area of sensitization measured in the two treatment conditions (80% power, alpha = 0.05, two-sided). A total of 32 subjects were estimated to be sufficient for this two-treatment crossover study. To detect a 30% difference in size of area of hyperalgesia between treatments, 15 subjects would be sufficient. These sample size calculations agree with those suggested in the methodological study published by the researchers who originally validated the heat/capsaicin model, and the coefficients of variation of the areas of sensitization measured by our group between day and within day (0.36 to 0.44) are in the range the coefficients of variation previously reported for this model(Dirks et al., 2003)

Randomization

A computer-generated randomization schedule assigned subjects to fenobam 150 mg or placebo administration in random order over 2 sessions one week apart, after successful completion of the screening/training session. Therefore each subject received a single dose of fenobam or placebo, followed one week later by the treatment they did not receive the previous week.

Study Procedures and Measurements

Administration Protocol

The heat/capsaicin hyperalgesia model combines heat stimulation (heat ramps from 32°C to 45°C at a rate of 1°C per second and hold at 45°C for 5 min) applied to a 9 cm^2 area of skin on the forearm followed by topical low dose capsaicin (0.1% Capzacin-HP Cream) applied to the same area. The sensitization is then rekindled with subsequent applications of heat (40° for 5 min) at 35-45 minutes intervals. This procedure generates temporary pain and the sensory changes associated with peripheral and central sensitization for up to 4 hours(Petersen and Rowbotham, 1999). Thermal stimulations were applied in a precise and controlled manner using a Medoc Advanced Thermal Stimulator (Medoc, Israel and North Carolina, USA) driving a 9 cm^2 thermode. The thermode was a computer-controlled Peltier device that warms the skin from 32°C to a safety cutoff of 52°C in 1 C/sec increments. The heat-capsaicin model was used in numerous studies involving human subjects that demonstrate that it is safe and reliable and does not damage skin. All Medoc equipment received FDA clearance.

Measurement of Pain at Thermal Stimulation, Pain Thresholds and Areas of Hypersensitivity

The following methods were used to induce and quantify pain and sensitization: 1) the pain intensity and area (cm^2) of secondary hyperalgesia and allodynia induced by the heat/capsaicin model 2) heat pain detection threshold; 3) pain intensity produced by 1 minute 45°C thermal stimulation.

1) *Heat/Capsaicin Sensitization Procedure and Mapping an Area of Secondary Hypersensitivity*: Sensitization was established by heating the same surface of the dominant forearm on which HPDT was performed. Heat was applied using the thermode which ramped from 32°C to 45°C at a rate of 1°C per second and then held at 45°C for 5 min, followed by immediate application of 0.1% capsaicin cream covering the previously heated surface (9 cm2). The cream was left on for 30 min and then wiped off. Approximately 0.5 oz of cream were required to cover the entire surface completely. Subjects were asked to rate their pain on a Visual Analog Scale (VAS) at the start of the 30 min period and then for every 5 min until the cream was wiped off. At the end of the 30 min capsaicin application, measurements were performed to determine the areas of hypersensitivity and allodynia on the forearm. The borders of secondary mechanical allodynia and hyperalgesia were mapped using a 1 inch foam brush and a vF filament (26g bending force). Subjects were asked to close their eyes during these procedures. First, the brush was applied along four linear paths between the thermode outline and 1) the antecubital fossa, 2) the wrist joint, 3) the lateral aspect of the forearm in anatomical position, and 4) the medial aspect of the forearm (Fig. 3). Stimulation started distant from the heated area and worked closer in 5.0 mm steps at 1sec intervals. Subjects were asked to say when the stimulation first became painful, and that location was marked. *This procedure was then repeated with the vF hair*. The area of hypersensitivity was calculated as the distance between the farthest points on the rostral/caudal axis multiplied by the distance between the farthest points on the medial/lateral axis (in cm^2), then subtracting the area of the thermode.

2) *Heat Pain Detection Threshold (HPDT):* Thresholds for heat pain detection were determined by using a thermal ramp protocol on a marked location on the volar surface of the forearm. The temperature applied through the Medoc thermode was increased from 32°C to the 52°C safety cutoff at 1°C/s. Subjects were requested to turn off the heated thermode by pressing a button at “the lowest temperature that they perceive as painful.” Four thermal ramps were performed 10 seconds apart and the median value perceived as painful will be calculated. To avoid testing individuals whose pain threshold approached the safety cutoff, subjects with HPDTs greater than 47°C were excluded from the study.

3) Pain During Thermal Stimulation (PTS): Acute pain was induced by a 1 min 45°C heat stimulus on a marked location on the upper non-dominant arm (deltoid). Subjects were asked to rate their pain intensity during the 1 min heat stimulus continuously using an electronic Visual Analog Scale (VAS) ranging from 0 to 100 where 0 indicates “no pain sensation” and 100 indicates “the most intense pain imaginable.”

Rekindling Procedures

On drug study days (Session1 and 2), hypersensitivity was maintained by rekindling the site of heat/capsaicin application. This was accomplished by re-stimulating the previously treated skin four times at approximately 40-45 min intervals, with the thermode increasing from 32°C to 40°C) at a rate of 1° per second and held at 40°C for 5 min. Subjects rated their pain on a continuous visual analogue scale (VAS) during rekindling.

Measurement/Evaluation of Mood and Affect Changes

Using a combination of Brief Positive and Negative Affect Scale (PANAS) and Brief State Anxiety Measure (BSAM)(Mackinnon, 1999) we aimed to quantify changes from baseline in the subjects' mood and affect following administration of the drug and after sensitization.

Assessment of Cognitive Function

Changes in working memory (attention, concentration and mental control) were assessed with the Letter-Number Sequencing (LNS) test (Wechsler, 2008). Changes in performance from baseline following administration of the drug and after sensitization were recorded.

Data analysis

Independent samples t-test or non-parametric equivalent Wilcoxon rank sum test were used to explore for differences in distribution of continuous level characteristics and baseline measures between subjects randomized to Fenobam or Placebo in Session 1. Our primary outcome measure was vF area of sensitization.

A mixed model analysis (using Proc Mixed) using restricted maximum likelihood estimation for linear models with degrees of freedom adjusted using Kenward-Roger procedure was used for data analysis. The mixed model allowed controlling for potential confounders. The possibility of a carryover effect was explored by comparing baseline values between the two treatment Sessions, as well as by testing the sequence of randomization in the mixed model analysis. To address the carryover effect, we decided

to analyze separately data from Session 1 of the study: we used the mixed model approach to explore the difference in size of vF area of sensitization through different time points and compare area sizes between the two treatment groups. The same analytical approach was also used for brush area and HPDT measures.

Laboratory Analysis

Blood samples for fenobam quantification were centrifuged at 3000 rpm for 10 minutes at room temperature. Plasma was transferred to micro-tubes and frozen.

Quantification of Fenobam in Plasma

All subjects had blood samples (each 5 ml, total approximately 40 ml) collected from the non-dominant arm every hour for 7 hours following drug administration to obtain fenobam plasma concentration levels at the time of post-drug measurements.

General Instrumentation

Fenobam plasma concentrations were quantitated using LC/MS/MS, as described in detail for the PK Study.

Results

1. PK Study. Pharmacokinetics and Side Effects of the mGlu5 Antagonist Fenobam in Adult Healthy Volunteers

A total of 24 adult healthy volunteers were randomized to three groups to receive doses of 50, 100, and 150 mg of oral fenobam respectively. Subjects were both male and female, aged 20 to 49 (demographic characteristics presented in Table 2). Safety and tolerability assessments and PK analysis were performed.

Safety and Tolerability

Fenobam was well tolerated up to the highest oral dose of 150 mg. Adverse events included headache, nausea, metallic or weird taste, and fatigue. All adverse events were described as “mild” by the subjects. Adverse events associated with either fenobam or placebo administration are presented in Table 3. No serious adverse events occurred in any subject.

Pharmacokinetic Analysis: t_{max} and C_{max}

After oral administration of 50 mg fenobam, C_{max} was reached between 2 and 4 hours and ranged between 0.8 and 49.2 ng/ml; C_{max} values for the 100mg and 150mg oral doses were respectively between 1.46 and 7.84 ng/ml, and 0.64 and 113 ng/ml, with an observed t_{max} between 2 and 6 hours (Figure 4).

2. Hyperalgesia Study. Effects of 150 mg Orally Administered Fenobam on the Development of Central Sensitization in the Heat Capsaicin Test.

In this study 32 subjects were administered a single dose of 150 mg PO for the purposes of both assessing PK data and for the analysis of the analgesic effects of fenobam.

Demographic information and baseline characteristics of subjects who received the heat/capsaicin sensitization procedures are presented in Table 4.

Pharmacokinetic Analysis: t_{max} and C_{max}

Following oral administration of fenobam 150 mg, t_{max} was between 2 and 4 hours, and C_{max} ranged between 1.77 and 187 ng/ml. Both t_{max} and C_{max} showed large inter-individual variability (Fig.5), consistent with prior reports(Itil T.M., 1978 ; Berry-Kravis et al., 2009).

Anti-hyperalgesic Effects

The primary outcome measure was the size of vF area of sensitization. A mixed within–between subject linear model approach using SAS Proc Mixed procedure was used to analyze the data. The mixed model uses restricted maximum likelihood estimation and allows controlling for potential confounders. Type III tests of fixed effects were used to evaluate the main effects of treatment group, time and interaction of treatment group with time.

Taking into account the crossover design of the study the presence of a carryover effect was explored by comparing baseline area measures between the 2 treatment sessions, as well as by testing the sequence of randomization in the mixed model analysis (SAS Proc Mixed procedure). We found a significant and consistent reduction in vF and

brush area measures at the start of Session 2 as compared to Session 1, independent of treatment, suggesting a significant carryover effect intrinsic to the sensitization model. In the presence of a significant carryover effect, we focused our analysis on Session 1, with 17 subjects randomized to receive fenobam and 15 subjects randomized to receive placebo.

In Session 1, after controlling for area of sensitization to vF filament on the training day and alcohol use, which have been found to be significant confounders of the drug effect, the change of vF area size through different time points (M-test = immediately post sensitization, through M4= after the 4th rekindling procedure, as illustrated in Fig.2) was significant, with the lowest values noted at time M4. These area reductions did not differ significantly between the placebo and fenobam groups. The mean reduction of vF area at M4 compared to the area measured at M-test was 44.0 cm^2 (95% CI: 28.2 to 59.8) in the placebo group, and 35.4 cm^2 (95% CI: 20.4-50.4) in the fenobam group. Overall, subjects treated with fenobam had a vF area 0.76 cm^2 smaller than subjects treated with placebo; however this difference was not statistically significant (95% CI:-16.03 to 17.56).

A similar pattern was also observed in brush area measurements. After controlling for area of sensitization on training day and wrist circumference (confounding variables of the drug effect in this model), the mean reduction of brush area at M4 compared to M-test was 46.9 cm2 (95% CI: 34.3 to 59.4), and 44 cm2 (95% CI 32.0-55.9) in the fenobam group. Overall, subjects treated with fenobam had a brush area 7.5 cm^2 smaller than placebo group with no statistical significance (95% CI:-7.1 to 22.1).

When vF and the brush areas measured immediately post-sensitization, at the single time point closest to the mean C_{max} of fenobam (M-test in Session 1, Fig.5) were compared with areas obtained at M-test on the training day within the same group of subjects (but without fenobam or placebo administration), both areas were significantly reduced in size (p=0.025 and 0.028 respectively), while the areas measured at M-test of Session 1 in the placebo group were not different from training day (Fig.6A and 6B).

After controlling for HPDT at baseline (Session 1), over all time points, mean HPDTs in the fenobam group were 0.42 ^{o}C higher than the placebo group; however this difference was not significant (95% CI -0.38 -1.23)). At M-test there was no difference in HPDTs between the fenobam and placebo group.

Anti-nociceptive Effect

Overall after controlling for PTS at S1 baseline, there was no significant difference in maximum VAS (PTS MAX) scores recorded between the 2 groups and across all time points (data not shown).

PNAS/BSAM and LNS

In a multi-level model to determine whether affect varies with blood concentration over time we could not observe any statistically significant effect of fenobam compared to placebo (not reported). Measure of cognitive function (attention, concentration and mental control) were also not significantly different between groups (Fig.7).

Discussion

Our study of the **pharmacokinetic** properties and side effect profile of fenobam after oral administration in human subjects confirmed prior limited observations(Berry-Kravis et al., 2009) of variable, but dose-dependent plasma concentrations, with t_{max} between 2 and 6 hours with marked inter-individual differences. After single administration of doses between 50 mg and 150 mg, fenobam was well tolerated, with only mild side effects observed (not different from placebo).

We did not observe any persistent anti-hyperalgesic or anti-nociceptive effect of fenobam compared to placebo in this cutaneous sensitization experimental pain model. We observed a reduction of vF area size through different time points (M-test = immediately post sensitization, through M4= after the 4th rekindling procedure), with the lowest values noted at time M4. These area reductions did not differ significantly between the placebo and fenobam groups. This finding was consistent with previously reported progressive fading of areas of hyperalgesia over time(Cavallone et al., 2013). The presence of a strong carryover effect of the sensitization model from Session 1 to Session 2, and possibly unexplained interactions between the carryover effects of both the model and of the drug prevented us from taking advantage of the original cross-over design.

We were aware of potential issues with the heat/capsaicin model, its reproducibility and presence of a carryover effect, causing areas of sensitization in session 2 to be smaller than session 1 in a crossover design(Cavallone et al., 2013). However, based on our pre-clinical data, we anticipated that the magnitude of the anti-

hyperalgesic effect of fenobam would be sufficiently large to detect despite the carryover effect of the model. Additionally, despite the adequacy of the washout period for fenobam demonstrated by PK analysis, we cannot exclude the possibility that an effect of fenobam on cutaneous sensitization in Session 1 could have affected the sensitization process in Session 2, one week later, when the same subjects were receiving placebo. A parallel comparison of two groups receiving fenobam in the two consecutive sessions or placebo in both could have provided more information on the effect of the drug versus the effect of the repeated exposure to heat and capsaicin on skin sensitization. Unfortunately we are not able to administer repeated doses of fenobam to the same subjects due to regulatory constraints.

Once a strong carryover effect was detected, the decision was made to examine data from Session 1 separately, an approach that has precedent in the literature (Lehmacher, 1991; Curtin et al., 2002). However this approach decreases the statistical power of the study and has been deemed "at risk of bias"(Freeman, 1989; Curtin et al., 2002; Higgins, 2011). We recognize this as a significant limitation of our study.

With the limitations noted above, in the heat/capsaicin cutaneous sensitization model we found no difference between fenobam and placebo in the overall analgesic effect. However, our study design also took into account the possibility that there could be a narrow window around the time of C_{max}, to detect an effect of fenobam in our constrained experimental conditions, and that this effect would not last throughout the sensitization procedures. We realized that in order to increase our chances to capture a potentially brief effect of fenobam we needed to time the heat/capsaicin stimulation based on expected fenobam plasma levels. For this reason we performed the

preliminary PK study and planned the skin sensitization procedures so that the development of initial sensitization (time of maximum stimulation with heat and capsaicin) would occur around the time of peak plasma concentration.

Plasma samples collected during the experimental pain procedures confirmed that we were successful in timing the initial heat and capsaicin application with peak plasma fenobam concentrations. By analyzing sensitization data from the single time point closest to C_{max} (M-test) we found that mean vF and brush areas were significantly reduced in size compared to baseline areas obtained on the training day in the same subjects, when no drug was administered, while areas measured at M-test in the placebo group were not different form baseline. This observation suggests that another factor in our inability to capture a persistent anti-hyperalgesic effect of fenobam in our model might be the short $t_{1/2}$ of fenobam compared to the duration of the cutaneous sensitization.

Availability of validated models to reliably create experimental hyperalgesia in healthy volunteers is limited, but - from a safety standpoint -we considered necessary to test fenobam in human healthy volunteers first, before conducting a prospective randomized clinical trial in a vulnerable population of patients affected by a clinical pain condition. With these considerations in mind, we relied on the prior extensive use of the model, and our direct knowledge and experience with the heat/capsaicin sensitization procedures. However, given the experimental nature of fenobam and the unconfirmed safety profile, regulatory constraints did not allow us to administer multiple doses of fenobam during this early phase, “proof of concept”, clinical study. This limitation might

have impaired our ability to detect the effect of sustained elevated plasma concentrations on the progressive fading of the areas of sensitization over time.

In conclusion, fenobam, administered orally to healthy volunteers at a single dose of 50, 100 or 150 mg, did not cause any significant side effects (comparable to placebo). In our limited experimental conditions with human healthy volunteers, measures of cutaneous sensitization evaluated at peak plasma concentration of fenobam were reduced with respect to baseline measures in the same subjects (not observed with placebo at the same time point). However, we did not observe a statistically significant sustained analgesic (anti-hyperalgesic) effect of fenobam compared to placebo.

Given the significant limitations introduced by the highly variable plasma exposure of fenobam, we feel that attempts to assess the potential utility of mGlu5 modulation for pain necessitate the use of a compound with improved pharmacokinetics and known target engagement. Prospective randomized clinical trials with such an improved molecule are needed to clarify the role of mGlu5 modulation in the development and maintenance of acute and chronic pain conditions in human.

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Footnotes

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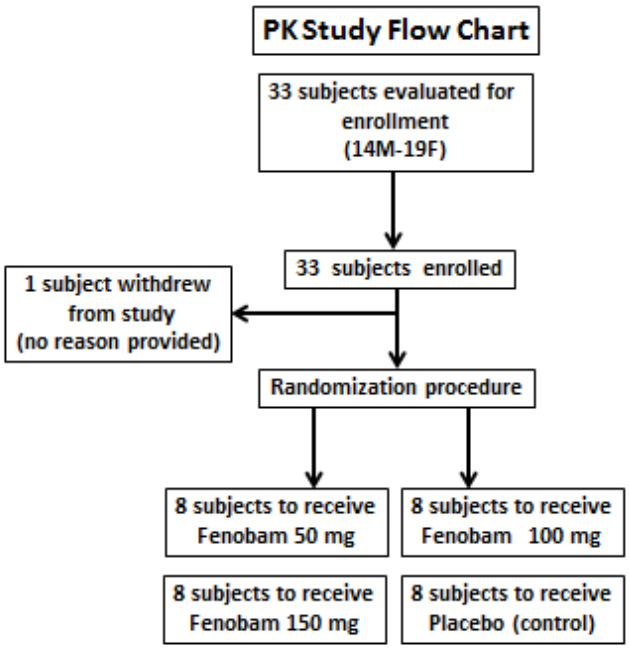
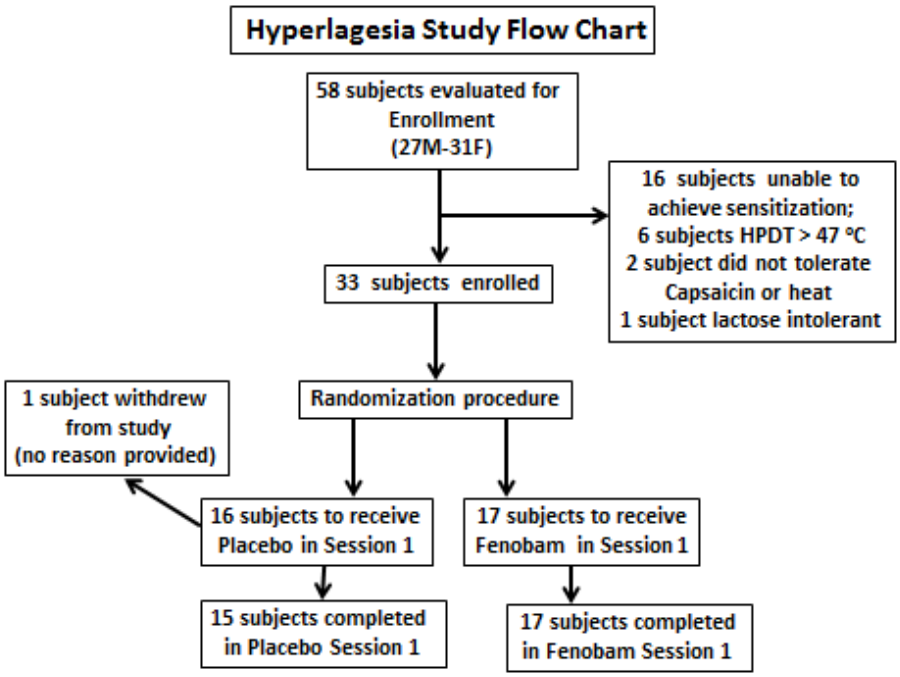

PK Study Flow Chart
33 subjects evaluated for enrollment (14M-19F)
33 subjects enrolled
1 subject withdrew from study (no reason provided)
Randomization procedure
8 subjects to receive Fenobam 50 mg
8 subjects to receive Fenobam 100 mg
8 subjects to receive Fenobam 150 mg
8 subjects to receive Placebo (control)

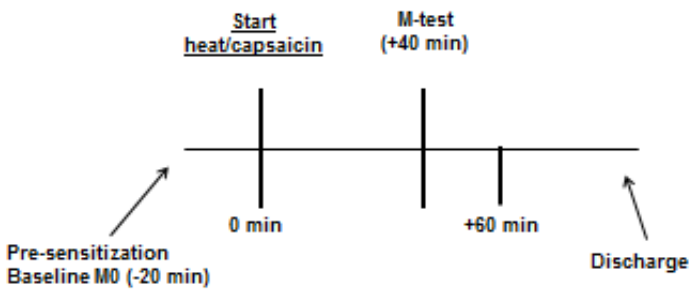
A


Hyperlagesia Study Flow Chart
58 subjects evaluated for Enrollment (27M-31F)
16 subjects unable to achieve sensitization; 6 subjects HPDT > 47 °C 2 subject did not tolerate Capsaicin or heat 1 subject lactose intolerant
33 subjects enrolled
1 subject withdrew from study (no reason provided)
Randomization procedure
16 subjects to receive Placebo in Session 1
17 subjects to receive Fenobam in Session 1
15 subjects completed in Placebo Session 1
17 subjects completed in Fenobam Session 1

B

Fig. 1

Timeline of Screening/Training Day Procedures



M = set of measurements (mapping of vF and brush areas of cutaneous sensitization; HPDTs; PTS)

Timeline of Study Day Procedures

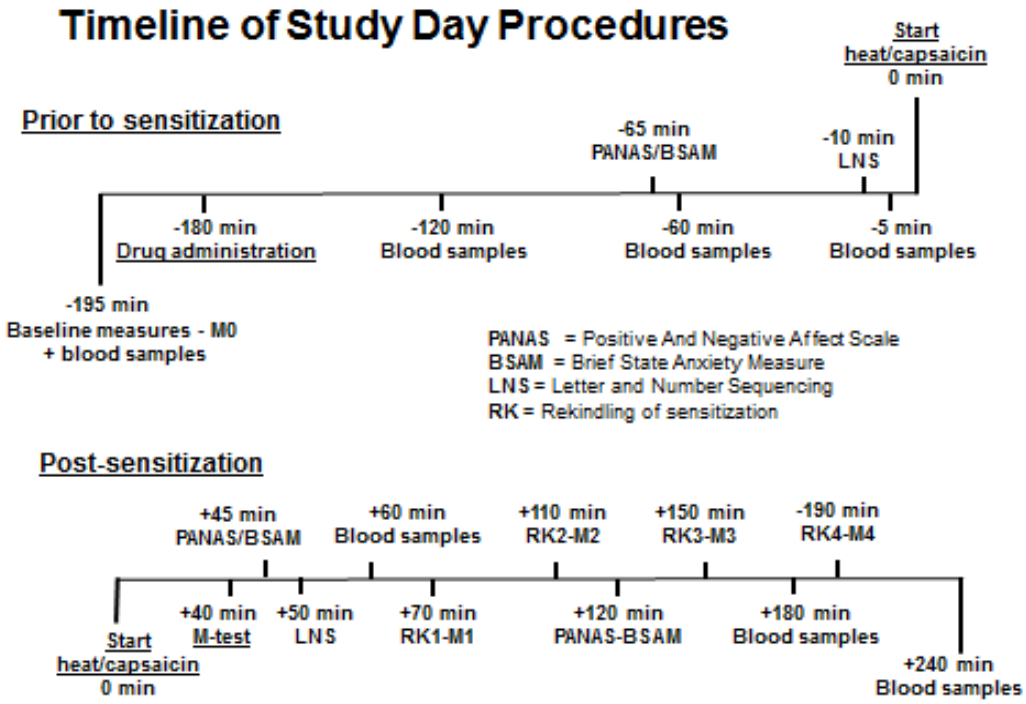


Fig. 2

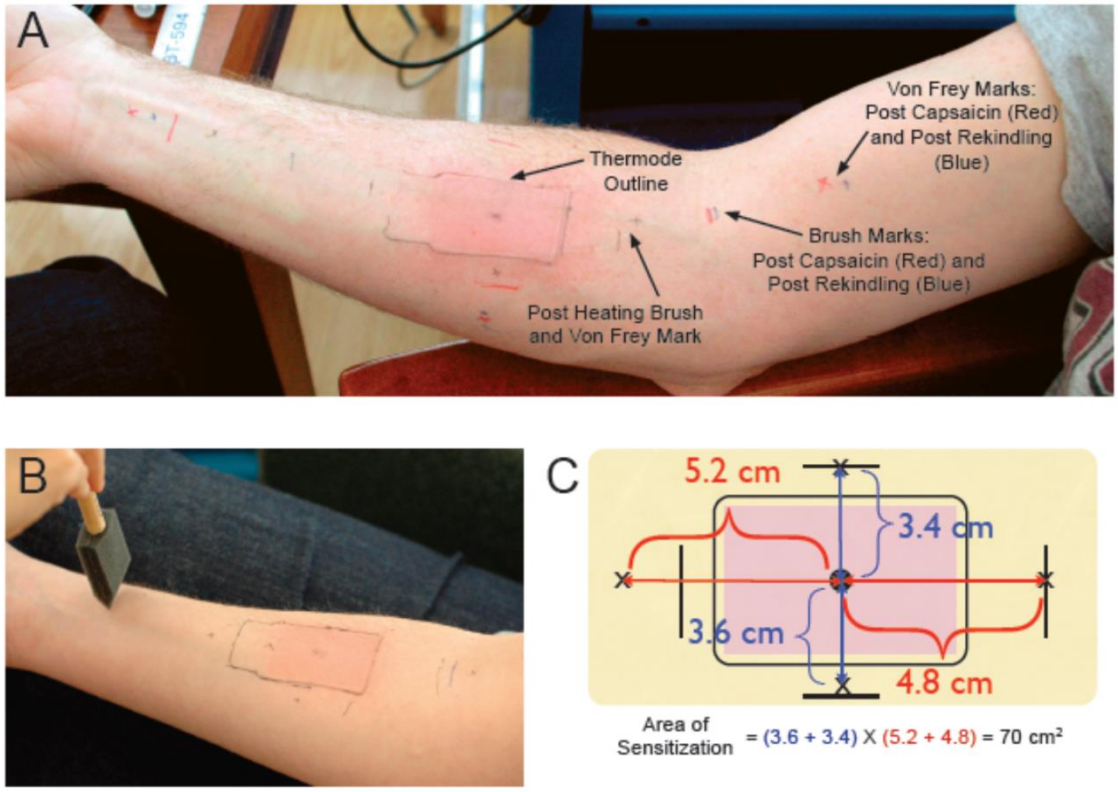
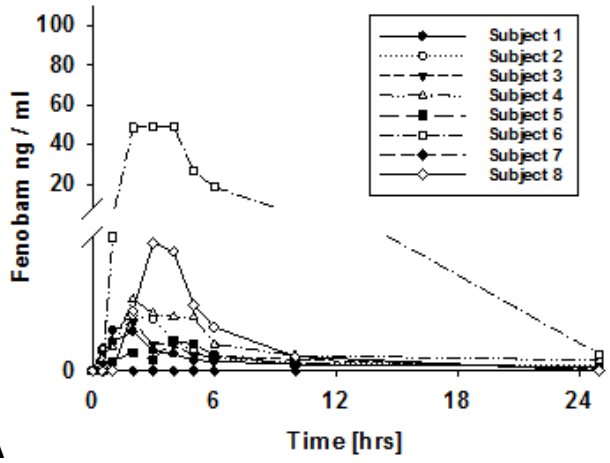
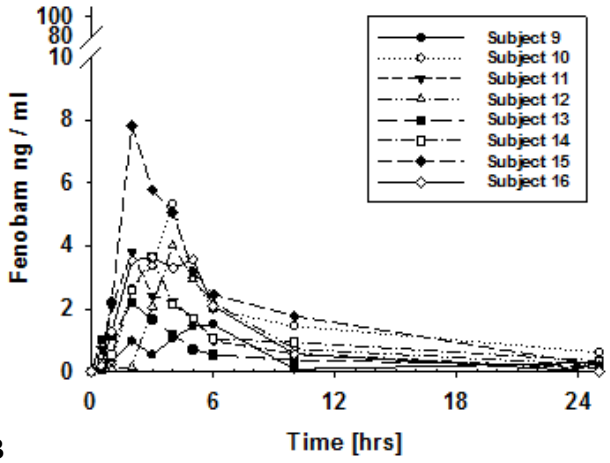


Fig. 3 Original heat/capsaicin model layout



A



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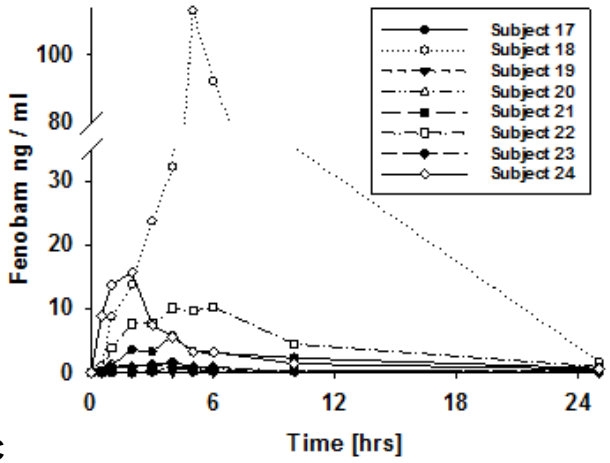


C

Fig. 4 PK Study: Fenobam plasma concentration following 50 mg oral dose (**A**);100 mg (**B**) and 150 mg (**C**). Curves of individual subjects are shown.

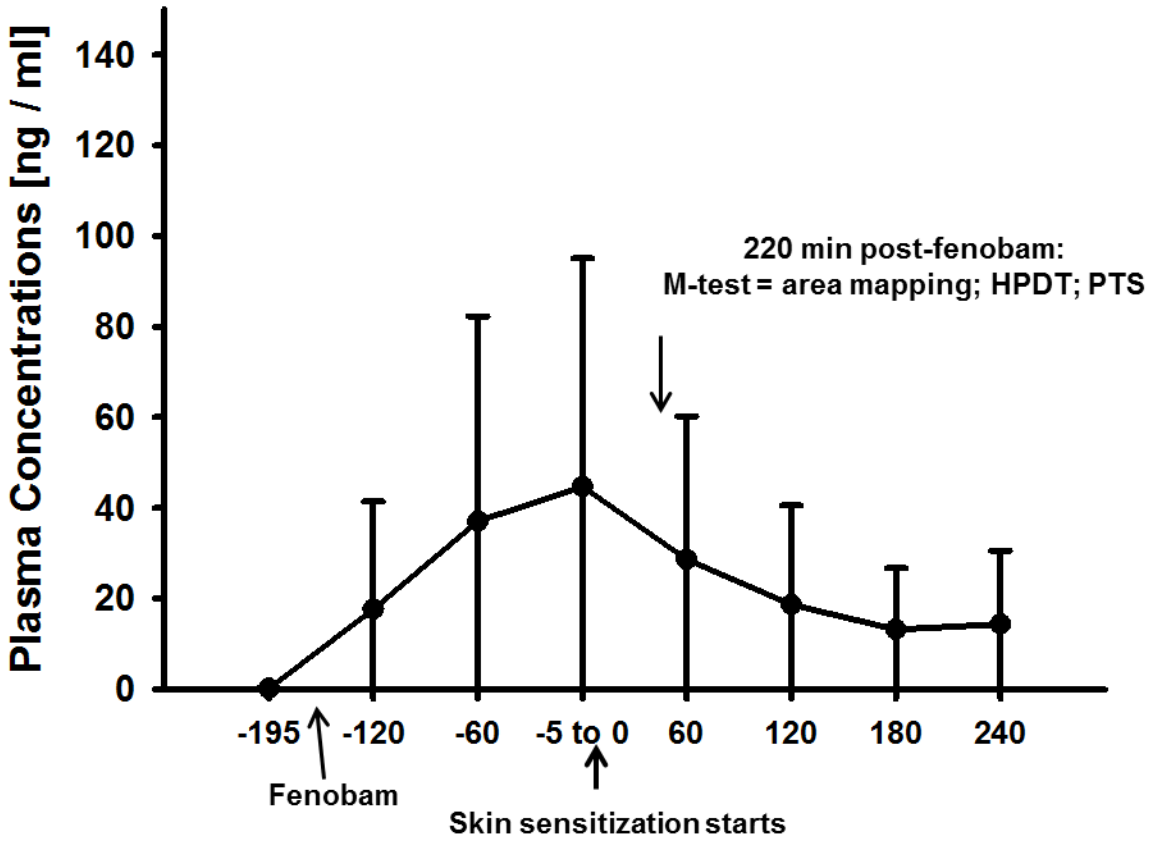
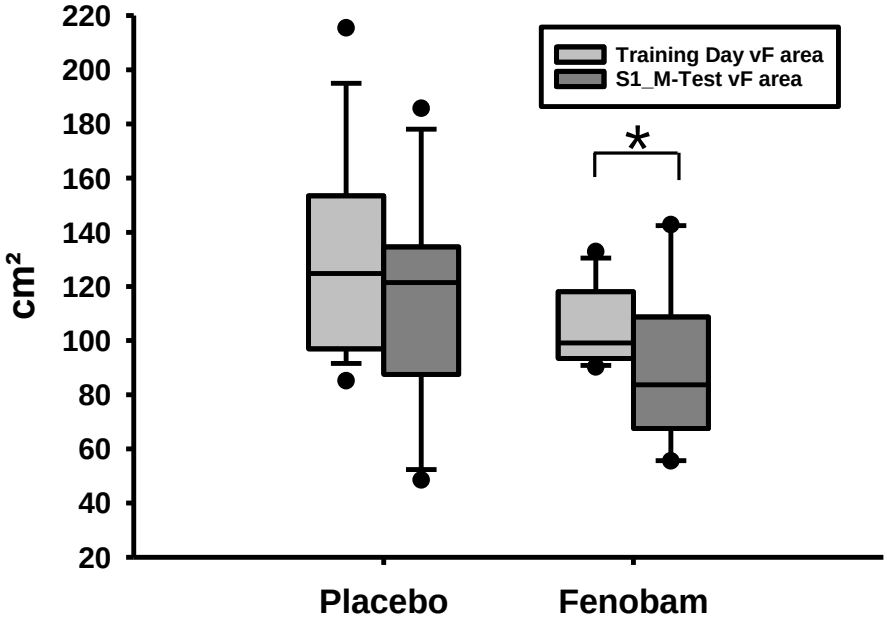


Fig. 5 Hyperalgesia Study: fenobam plasma concentration following 150 mg oral dose. The PK curve is shown in relation to the start of the skin sensitization procedures. The closest time point to the end of the sensitization procedure and to C_{max} at which areas of sensitization were measured (M-test) is also shown.

A



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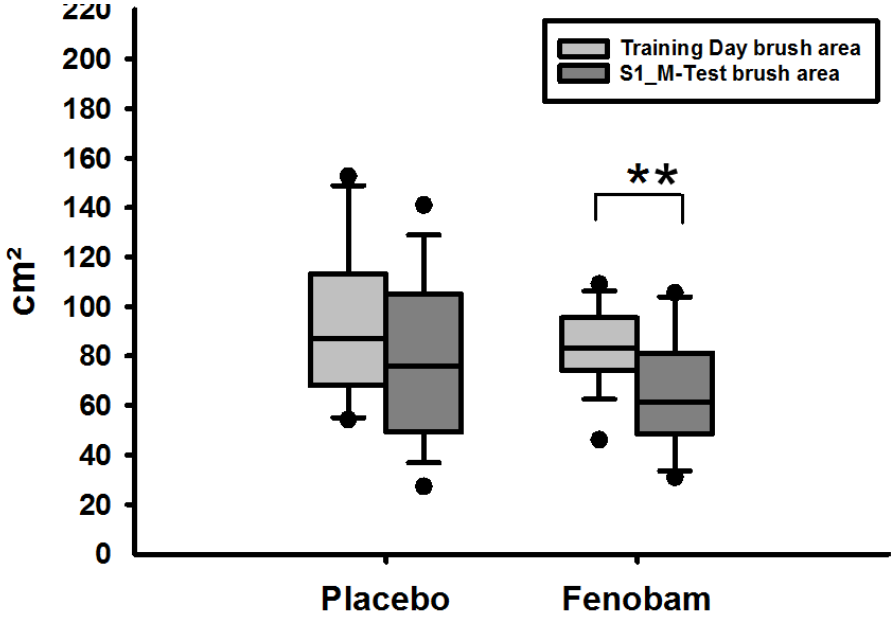

Fig. 6 For both groups of subjects receiving fenobam or placebo, **vF (A)** or **brush (B)** areas of sensitization **at M-test on Session 1 (S1)** are compared to areas on **Training Day.** Median; minimum; maximum values of areas; lower and upper quartiles are shown.

*** p = 0.025; **p = 0.028.**

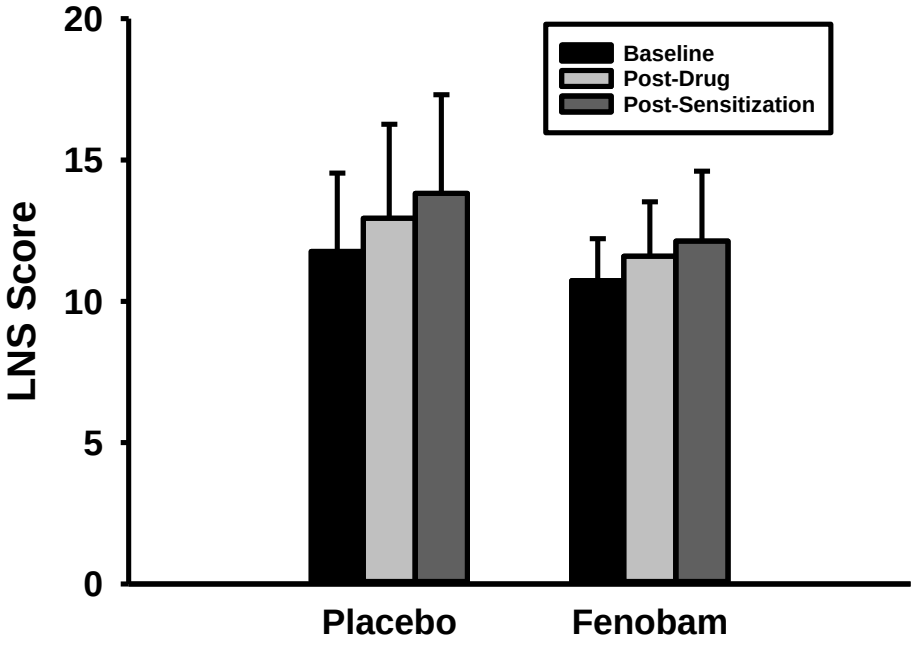

Fig. 7 Mean LNS score of subjects receiving fenobam or placebo in Session1 are shown. Both groups show a mild improvement in scores over time that could be explained by a learning effect with the repetition of letter and number sequences. No difference is detected between the two groups.

Tables

Inclusion Criteria

1) **18-50 years old**
2) **Good general health with no remarkable medical conditions (e.g., liver, kidney, heart, or lung failure) and BMI < 33.**
3) **Willing to comply with study guidelines as outlined in protocol**
4) **Willing to provide informed consent**

Exclusion Criteria

1) **Medication use (prescription or non-prescription medications, vitamins, herbals, dietary and mineral supplements and grapefruit products during or within 14 days prior to study participation; excludes contraceptives)^**
2) **History of addiction to drugs or alcohol (prior or present addiction or treatment for addiction)+**
3) **Pregnant and nursing females**
4) **History of lactose intolerance**
5) **Smokers**

Table 1.A PK Study: Inclusion and Exclusion Criteria

Inclusion Criteria

1) 18-50 year old

2) Good general health with no remarkable medical conditions (e.g. liver, kidney, heart, or lung failure).

3) BMI between 20-33

4) Willing to comply with study guidelines as outlined in protocol

5) Willing to provide informed consent

Exclusion Criteria

1) Anatomical malformation of upper extremities*

2) Status post recent trauma or chronic lesions on either forearm*

3) Medication use (includes vitamin, herbal, dietary and mineral supplements and grapefruit products during or within 14 days prior to study participation; excludes contraceptives)^

4) History of allergy or intolerance to capsaicin

5) History of multiple drug allergies

6) History of addiction to drugs or alcohol (prior or present addiction or treatment for addiction)+

7) History of chronic pain syndromes

8) Pregnant and nursing females

9) Smokers

Table 1.B Hyperalgesia Study: Inclusion and Exclusion Criteria

*Normal anatomy of the upper extremities and absence of lesions on either forearm are critical to the correct application of the protocol. The choice of a specific area of intact skin in the same position, with the center located medially on the forearm of each subject, midway between the antecubital fossa and the wrist, is essential to the proper evaluation of the data obtained.

^ To decrease potential confounders in the interpretation of the data (other drugs' effects) and avoid possible interactions with the study drug, use of medications other than contraceptives is considered not acceptable for the duration of the study.

+A history of drug or alcohol addiction could be related to persistent undiagnosed peripheral neuropathy and/or manifestation of drug seeking behaviors, both of which may compromise the results of the study.

	All subjects N=32	Placebo (n=8) Median (Min-Max)	Fenobam 50 mg (n=8) Median (Min-Max)	Fenobam 100 mg (n=8) Median (Min-Max)	Fenobam 150 mg (n=8) Median (Min-Max)	p-value
Gender						
Female	18 (56%)	7 (87.5%)	6 (75%)	2 (25%)	3 (37.5%)	0.03*
Male	14 (44%)	1 (12.5%)	2 (25%)	6 (75%)	5 (62.5%)	
Age	28 (20-49)	29.5 (20-49)	30.5 (21-35)	24.5 (21-46)	29 (21-40)	0.821
Height (cm)	170 (157-198)	170 (157-175)	169 (163-180)	176.5 (165-185)	174 (160-198)	0.405
Weight	77.5 (53-105)	82.5 (66-95)	71 (54-90)	77 (54-98)	78.5 (53-105)	0.588
BMI	25.55 (19.4-32.7)	27.55 (22.1-32.7)	24.9 (20.3-28.7)	25.25 (19.4-30.1)	24.8 (20.5-29.9)	0.206

Table 2. PK Study: demographic information and baseline characteristics.

The Independent Samples Kruskal-Wallis test was used for comparisons between groups.

Significance level: $p < 0.05$.

Reported Side Effects	Placebo (n=40)	Fenobam 50mg (n=8)	Fenobam 100mg (n=8)	Fenobam 150mg (n=40)
Headache	10%	50%	25%	10%
Nausea	7.5%	12.5%	12.5%	0%
Metallic /weird taste	2.5%	12.5%	12.5%	0%
Fatigue	2.5%	12.5%	0%	0%
Drowsiness	27.5%	0%	0%	25%
Lack of Concentration	5%	0%	0%	0%
Tingling	0%	0%	0%	2.5%
Dry mouth	7.5%	0%	0%	2.5%

Table 3. Rate of side effects in the groups **"Placebo"** and **"Fenobam 150 mg"** refer to subjects from both the PK study (8 subjects in each group) and hyperalgesia study (32 subjects in each group receiving fenobam or placebo).

Baseline Characteristics (on Training Day)	All subjects N=32	Placebo (n=15) Median (Min-Max)	Fenobam (n=17) Median (Min-Max)	p-value
Age		28 (22-34)	29 (19-45)	0.570
Height		173 (157-191)	170 (157-188)	0.314
Weight		71 (59-105)	79 (65-90)	0.316
BMI		25.5 (20.3-32.4)	27.3 (21.2-33.5)	0.100
Coffee cups		2 (1-4)	1 (0.5-3)	0.092
Waist		25.5 (23-33)	26.5 (23-30)	0.820
HPDT-nonDominant		43.8 (38.4-47.6)	44.1 (36.3-45.3)	0.940
HPDT-Dominant		42.8 (38.4-46.5)	43 (36.8-45.9)	0.806
HPDT-Post-nonDominant		42.1 (36.6-47.1)	43.7 (34.6-45.7)	0.558
HPDT-Post-Dominant		37.1 (35.4-38.3)	36.7 (34.6-38.7)	0.417
Brush Area		86.9 (54.3-152.6)	81.7 (46.2-109.1)	0.533
Von Frey Area		124.8 (85.1-215.5)	99.1 (90.2-132.9)	0.052
	n(%)	n(%)	n(%)	
Gender				
Female	19 (59%)	8 (53%)	11 (65%)	0.513†
Male	13 (41%)	7 (47%)	6 (35%)	
Daily caffeine intake				
Yes	21 (66%)	10 (67%)	11 (65%)	0.907†
No	11 (34%)	5 (33%)	6 (35%)	
Alcohol in the last 6 months				
Yes	29 (91%)	14 (93%)	15 (88%)	
No	3 (9%)	1 (7%)	2 (12%)	1.0†
Dominant Arm				
Left	6 (19%)	4 (27%)	2 (12%)	
Right	26 (81%)	11(73%)	15(88%)	0.383†

Table 4. Hyperlagesia Study: demographic information and baseline characteristics.

HPDT = Heat Pain Detection Threshold; Post- = post sensitization with heat and capsaicin

Wilcoxon rank sum test was used for continuous level variables.

(†)Fisher's Exact test was used for categorical variables. Significance level: $p < 0.05$.