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METHODOLOGY, MECHANISMS & TRANSLATIONAL RESEARCH SECTION

Original Research Article

A Randomized, Double-Blind, Placebo- and Active Comparator-Controlled Phase I Study of Analgesic/Antihyperalgesic Properties of ASP8477, a Fatty Acid Amide Hydrolase Inhibitor, in Healthy Female Subjects

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Abstract

Objectives. To evaluate the analgesic/antihyperalgesic effect of ASP8477.

Design. Randomized, double-blind, double-dummy, cross-over, placebo- and active comparator-controlled study.

Setting. HPR Dr. Schaffler GmbH, Munich, Germany.

Subjects. Healthy female subjects aged 18–65 years.

Methods. Eligible subjects were randomly assigned to one of six treatment sequences and received multiple ascending doses of ASP8477, duloxetine, and placebo over three treatment periods (each consisting of 21-day dosing separated by 14-day washout periods). On the last day of each dose level, laser evoked potentials (LEPs) and visual analog scales (VAS pain) on capsaicin-treated skin at baseline and at multiple postdose time points were assessed. The primary end point was the difference in LEP N2-P2 peak-to-peak (PtP) amplitudes for ASP8477 100 mg vs placebo.

Results. Twenty-five subjects were randomized. In all subjects, LEP N2-P2 PtP amplitudes were numerically lower for ASP8477 100 mg vs placebo ($P = 0.0721$); in subjects who demonstrated positive capsaicin skin effects, a greater mean difference of $-2.24 \,\mu V$ ($P = 0.0146$) was observed. Across all doses, LEP N2-P2 PtP amplitudes were lower for duloxetine compared with ASP8477 (mean difference $-3.80 \,\mu V$; $P < 0.0001$) or placebo (mean difference $-5.21 \,\mu V$; $P < 0.0001$). The effect of ASP8477 (all doses) on down-scoring the VAS pain score was significant compared with placebo (mean difference -2.55%; $P < 0.0007$).

Conclusions. ASP8477 was well tolerated in this study. Analysis of all subjects did not demonstrate a significant difference in LEP for ASP8477 100 mg over placebo but did in subjects who demonstrated positive capsaicin skin effects.

Key Words. ASP8477; FAAH; Duloxetine; Neuropathic Pain; LEP; VAS

Introduction

The fatty acid amide hydrolase (FAAH) enzyme catalyzes the degradation of endocannabinoids, for example, N-arachidonoyl-ethanolamine (AEA, anandamide), palmitoylethanolamide (PEA), and oleoylethanolamide (OEA) [1,2]. A wide range of preclinical models have shown how inhibition of the FAAH enzyme produces antinociceptive effects via an indirect increase in the levels of AEA, PEA, and/or OEA [3–5]. Furthermore, FAAH inhibition can also produce an opposite (nociceptive) effect through the activation of vanilloid receptors (TRPV1), narrowing the therapeutic window of endocannabinoids such as AEA [6]. The effects observed from FAAH inhibitors are dependent on the dose and/or concentration of the compound [6]. A recent study showed that inhibition of spinal FAAH leads to TRPV1-mediated analgesic and antihyperalgesic effects in neuropathic pain (NP) animal models, but with a decrease in spinal AEA levels [7], further emphasizing the paradoxical nature of FAAH inhibition.

Currently approved treatment options for the management of NP, including pregabalin, gabapentin, and duloxetine [8–10], are associated with side effects (mostly central nervous system–related) such as dizziness, fatigue, somnolence, vertigo, and nausea [8,11–13]; therefore, there is a need for new treatment options with improved tolerability and better efficacy. Inhibition of FAAH may be a promising novel therapeutic target for patients with NP. Inhibition of FAAH has shown anti-inflammatory and antihyperalgesic effects in a wide range of animal models, and the beneficial effects are predominantly mediated through the activation of cannabinoid subtypes 1 or 2, although noncannabinoid mechanisms of action can also play contributory or even primary roles [3,14,15]. Other reports have also suggested that the inhibition of FAAH may avoid the side effects associated with the use of a direct cannabinoid receptor-1 agonist, and the abuse associated with cannabinoids is less likely to occur with FAAH inhibitors [2,16].

Experimental methods to evoke and assess pain in healthy volunteers under controlled conditions provide a means of investigating the antinociceptive/antihyperalgesic properties of a new therapy without the superimposed interventional biases such as multimorbidity and comedications often seen in patient studies [17]. Topical application of capsaicin on normal skin stimulates TRPV1-expressing cutaneous nociceptors causing peripheral and spinal sensitization, allowing assessment of the effect of analgesic compounds on hyperalgesia and pain [18,19]. Reports in the published literature suggest that response to topical application of capsaicin may vary between individuals with different neuropathic conditions [20,21]; hence, the ability to measure response to capsaicin is important when applying the model in a clinical study. The evoked sensations/responses following topical application of capsaicin and the administration of the study drug can be assessed using a *subjective* method such as the visual analog scale (VAS) or an *objective* method such as laser evoked potentials (LEP) [17,22].

ASP8477 is a novel, potent, selective inhibitor of FAAH in development for the symptomatic treatment of pain associated with osteoarthritis (OA) and peripheral NP. Here, results from a phase I study evaluating the analgesic/antihyperalgesic effects of multiple-ascending doses of ASP8477 in healthy female subjects compared with an active control (duloxetine, which has shown efficacy in the LEP model [23] and is approved for the clinical treatment of NP and also known to work in OA [24,25]) are reported.

Methods

Study Overview

This was a phase I, randomized, double-blind, double-dummy, three-period cross-over, placebo and active comparator (duloxetine)–controlled, single-center study to evaluate the analgesic/antihyperalgesic effects of multiple ascending doses of ASP8477 using LEP and VAS pain scores on capsaicin-treated skin. The aim was to obtain a first indication into the analgesic and antihyperalgesic effects of ASP8477 and to determine the dose range and dose regimen of ASP8477. The study used multiple doses of ASP8477, because it was unknown whether a FAAH inhibitor would have analgesic and antihyperalgesic effects after a single dose. As the efficacious dose range of ASP8477 was not known at the time of protocol development, three multiple-ascending doses of ASP8477 were selected based on the safety and tolerability and pharmacodynamic results (anandamide concentrations in plasma) of previous clinical studies (unpublished data). Duloxetine was selected as an active comparator to serve as an intra-assay validator; although pregabalin is the standard treatment for NP, it does not have an effect on more inflammatory types of pain (i.e., pain from OA) and therefore would not have been an appropriate comparator. Although the mechanism of action of duloxetine (a potent and balanced serotonin–norepinephrine inhibitor [24]) differs from that of ASP8477, duloxetine is registered for the treatment of pain from OA and NP.

The study was conducted at the Human Pharmacodynamic Research (HPR) Dr. Schaffler GmbH site in Munich, Germany (EudraCT-No.: 2011–005122-22), and the study protocol was approved by the local Independent Ethics Committee (IEC) in Munich, Germany, and the Competent Authority in Bonn, Germany, prior to study initiation. An IEC-approved written informed consent was obtained from each subject prior to the initiation of any study-specific procedures. This study was conducted in accordance with the Declaration of Helsinki and its actual revisions, Good Clinical Practice (GCP), International Conference on Harmonization (ICH) guidelines, and applicable laws and regulations.
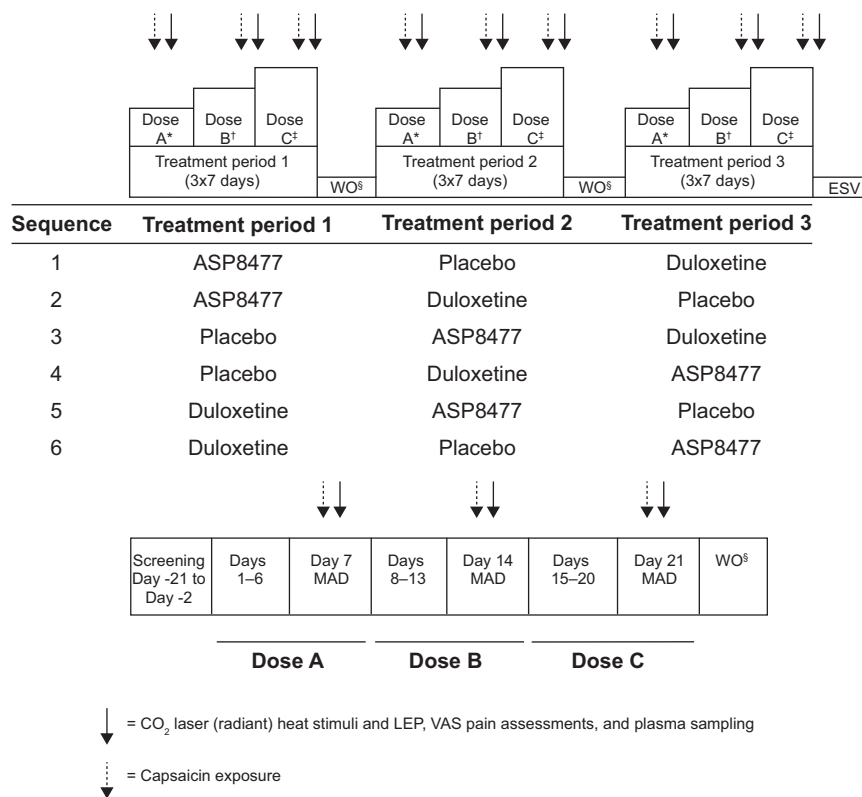
Figure 1 Study design. *ASP8477 low-dose 20 mg once daily, duloxetine low-dose 30 mg once daily; †ASP8477 mid-dose 60 mg once daily, duloxetine mid-dose 60 mg once daily; ‡ASP8477 high-dose 100 mg once daily, duloxetine mid-dose 60 mg once daily; §washout for 14 days following each treatment period. ESV = end of study visit; LEP = laser evoked potential; MAD = main assessment day; VAS = visual analog scale; WO = washout.

Study Design

The study consisted of a screening assessment period (between days –21 and –2) and three treatment periods (each treatment period consisted of three repeated dose periods of seven days each) (Figure 1). During the screening assessment, subjects underwent standard screening procedures and had their laser pain threshold (LPT) measured. The LPT was determined on normal untreated skin by the application of CO_2-laser (radiant-heat) stimuli of increasing intensity, and was kept constant for each subject for all LEP sessions throughout the study. Following screening assessment, eligible subjects were randomly assigned to one of six treatment sequences and received multiple doses of ASP8477, duloxetine, and placebo over three seven-day treatment periods. Each of the three treatment periods consisted of 21 days (3 × 7 days) of dosing with multiple ascending doses; for ASP8477, dose A (20 mg), dose B (60 mg), and dose C (100 mg); and for duloxetine, dose A (30 mg) and doses B and C (60 mg each). To maintain the double-dummy design and to minimize the risk of undesirable adverse events (AEs), the duloxetine 60-mg dose was maintained for two periods, rather than increased to 120 mg. Each of the three treatment periods (A, B, C) was separated by a 14-day washout period (Figure 1).

All study participants, the investigator, and clinical sponsor staff were blinded to the randomized treatment sequences as well as to the actual treatment received. The ASP8477 placebo-to-match tablets were indistinguishable from the ASP8477 tablets, and the duloxetine placebo-to-match capsules were indistinguishable from the duloxetine capsules. Eligible subjects returned to the clinic on day 1 to receive the first dose of study drug, with each dose period lasting seven days. On the last day of each dose level (main assessment day; days 7, 14, and 21 of each period), subjects returned to the clinic for safety, compliance (e.g., alcohol, CO-smoking breathalyzer tests, urine drug screen), pharmacodynamic (LEP/VAS pain), and pharmacokinetic (PK) assessments. LEP and VAS pain testing (described later) were performed on capsaicin-treated skin at baseline and at multiple postdose time points to assess the analgesic/antihyperalgesic effects of ASP8477. Upon completion of all three treatment periods, each subject attended an end of study visit (ESV) seven to 14 days after the last dose or, if discharged earlier, underwent ESV procedures (safety assessments).

Study Subjects

Healthy female subjects (aged ≥18–65 years) of Caucasian origin, with a body mass index (BMI) of ≥18.5 kg/m^2 to <30.0 kg/m^2 and a body weight of ≥50 kg, were eligible for enrollment. Female subjects were selected due to effects observed on male fertility with ASP8477 in nonclinical toxicity studies in rats (unpublished data). Subjects of child-bearing potential were required to be practicing a highly effective hormonal or nonhormonal method of birth control (i.e., a double-barrier method) from the day of first dosing until 30 days after the last dose. Subjects who were postmenopausal, surgically sterilized, or had a medical history of hysterectomy were also eligible for enrollment.

Subjects were excluded if they had known or suspected hypersensitivity to ASP8477, duloxetine, capsaicin, or any components of the formulations used. Subjects were also excluded if they had any abnormal liver function tests above the upper limit of normal, any clinically significant history of allergic conditions, history or evidence of any clinically significant disease or malignancy, as judged by the medical investigator, history of smoking (>10 cigarettes or an equivalent amount of tobacco per day) within three months prior to study start, history of drinking (>14 units of alcohol per week) within three months prior to study start, use of any prescribed or nonprescribed drug (including vitamins, hormone replacement therapy, except prescribed oral contraceptives), natural and herbal remedies (e.g., St. John's wort) in the two weeks prior to study start (except for the occasional use of paracetamol, up to 2 g/day), history of drug/chemical/substance abuse within the past two years prior to screening and/or within three months prior to study start, and regular use of any inducer of metabolism (e.g., barbiturates, rifampin). Subjects who were pregnant within six months or who were breast feeding within three months prior to screening were also excluded.

Furthermore, subjects with febrile illness or symptomatic, viral, bacterial (including upper respiratory infection) or fungal (noncutaneous) infection within one week prior to the first clinic check-in were excluded. Subjects with acne, eczema, scars, or tattoos at the sites of exposure to laser or capsaicin, or any actual evidence of sunburn on skin, were also excluded. Subjects who were unwilling to abstain from using topical drugs or cosmetics to the sites of exposure to laser or capsaicin or had any other condition, that in the opinion of the investigator, precluded the subject's participation in the trial were excluded.

Study End Points

The primary efficacy end point was the difference in LEP N2-P2 peak-to-peak (PtP) amplitudes (μV) from capsaicin-treated skin between the highest dose of ASP8477 (100 mg, day 21) and placebo (day 21). Secondary end points included differences in LEP N2-P2 PtP amplitudes (μV) from capsaicin-treated skin between ASP8477 and duloxetine (across all doses), ASP8477 (all doses) and placebo, and duloxetine (all doses) and placebo.

Other secondary end points included differences in VAS pain between ASP8477 (all doses) and placebo, and percent change from the day 7 predose value over N2-P2 PtP amplitudes (μV) and VAS pain scores (mm presented in %) following treatment with ASP8477, duloxetine, or placebo on days 7, 14, and 21. Exploratory end points included assessment of the efficacy end points (magnitude and duration of response to ASP8477 relative to controls) in all subjects vs subjects who demonstrated positive capsaicin skin effects (capsaicin-positive subgroup analysis, defined later), PK analysis, and safety and tolerability of ASP8477.

Study Assessments

For LEP assessment, nociceptive stimulation was applied by repeated CO_2 laser (radiant-heat) stimuli (Pulsed CO_2-Laser, SYNRAD infrared gas LASER model E48-/- 10 W, SYNRAD Inc., North Bothell, WA, USA) to a random site of normal skin only at screening, and to the capsaicin-treated skin at predose and hourly for up to eight hours after study drug administration. Nociceptive/hyperalgesic stimulus processing was measured via LEP from vertex-electroencephalogram (EEG; scalp leads Cz vs mastoid right, Cb1). On each assessment day, skin on the back of the subject's body (site and area were chosen randomly) was topically pretreated with capsaicin (standardized 1% alcoholic capsaicin extract, Extrakt Chemie, Stadtbergen, Germany), applied in an occlusive mode for 25 minutes, after which the occlusive dressing was removed and the skin was dried. During the interval between capsaicin application and dosing, two (nonevaluated) "rekindling" sessions were given with the CO_2 laser ("spinal wind-up"). LEP sessions were conducted predose (baseline, directly following removal of capsaicin; i.e., 90 minutes prior to dosing) and hourly (up to eight hours) postdose. In the absence of baseline assessment prior to treatment with any study drug, the predose assessment on the first assessment day (day 7 of each treatment period of 21 days) was used as baseline.

During each laser session, at least 13 stimuli (each of 80 msec duration, adjusted to 50% above LPT determined at screening), using a beam diameter of 1.5 mm, were applied to the treated skin. The first stimulus of LEP recording of each session was principally rejected in the respective data processing procedure. Between each single stimulus, the laser was moved 2–3 mm so that each skin area was not stimulated twice. Additionally, the time interval between subsequent individual stimuli randomly varied between four seconds and eight seconds to avoid development of habituation/tolerance to, and expectation of, the laser stimuli in subjects. During each session, to avoid influences of external disturbing noise, to raise and stabilize vigilance, and

distract subjects from pain stimulation and pain sensation expectancy, subjects were exposed to "white noise" via earphones (with a sound pressure of 85 dBA) and had to absolve an attention-focusing permanent pursuit tracking task on a computer screen (i.e., subjects continuously followed a random moving target on a computer screen by a joy-stick-controlled pursuer to keep the difference between both signals as minimal as possible). Subjective pain impression ("postlaser pain" rating) was retrospectively assessed, using an electronic 100-mm VAS on a tablet PC, immediately after each LEP session. The VAS pain scores were expressed as a percentage (corresponding to 100 mm).

An exploratory analysis of the efficacy end points in the capsaicin-positive subgroup (subjects who demonstrated hyperalgesic response to topical capsaicin) was performed. The rationale for this analysis, which was independent of drug treatment, was to specifically demonstrate the antihyperalgesic effect of ASP8477/duloxetine, which would otherwise be impossible in subjects with no hyperalgesic response to topical capsaicin. To demonstrate the hyperalgesic response to topical capsaicin, the area under the effect curve (AUEC) in LEP from baseline (predose D7) was calculated. Subjects with a positive $AUEC_{0-8h}$ on days 7, 14, and 21 when receiving placebo (positive $AUEC_{0-8h}$ suggests that the average LEP over an eight-hour period was higher than baseline) were defined as those demonstrating hyperalgesic response to capsaicin and were included in the exploratory capsaicin-positive subgroup analysis. Those with a negative $AUEC_{0-8h}$ on days 7, 14, and 21 (i.e., no hyperalgesic response to capsaicin) were excluded from the capsaicin-positive subgroup analysis.

Blood (plasma) samples were collected on days 7, 14, and 21 at predose and two, four, six, and eight hours postdose, after completion of LEP and VAS assessments, for PK assessment of ASP8477 and duloxetine. AUC_{0-8h}, maximum serum concentration (C_{max}), trough serum concentration (C_{trough}), and time to maximum concentration (t_{max}) were determined. Safety and tolerability were assessed through AE monitoring (MedDRA version v14.0), clinical laboratory evaluations, vital sign measurements, 12-lead computer electrocardiogram measurements, physical examinations, and questionnaires (Bond-Lader [26] and Bowdle [27] VAS series, Columbia-Suicide Severity Rating Scale [28], and physician withdrawal checklist [29]).

Statistical Analyses

A meta-analysis performed (using regression LEPs vs VAS pain) to cluster diverse analgesics with known clinical efficacy, in order to define the threshold of clinical efficacy, demonstrated that a difference of at least 1.86 μV in the primary end point between an active treatment and placebo might indicate an acceptable effect between the reductions in LEP and in VAS [30]. Under the aforementioned assumptions, a total of 24

subjects was planned (four per treatment sequence). This would allow the study to have an 80% power to detect a difference between active treatment and placebo at the two-sided significance level of 5% (with no adjustment for multiple comparisons).

All subjects who received at least one dose of study medication were analyzed–according to the actual treatment received–and were included in the safety analysis. All subjects, who received active treatment for whom at least one quantifiable plasma concentration of ASP8477 or duloxetine was obtained and for whom the dosing and sampling history was recorded, were included in the PK analysis set. All subjects who took at least one dose of study medication after randomization, and who provided both valid baseline and postbaseline values for the primary efficacy variable or at least one of the secondary efficacy variables, were included in the efficacy analysis (full analysis set [FAS] population).

Continuous baseline variables were summarized using descriptive statistics, by treatment sequence and overall, for the safety analysis. The primary end point was assessed using a repeated measures linear mixed-effects model. The model was fitted to LEP N2-P2 PtP amplitude as the dependent variable, and the classification variables included were treatment (ASP8477, duloxetine, or placebo), assessment day (1, 2, or 3; nested within treatment), treatment period (1, 2, or 3), treatment sequence (1–6) as fixed effects, and subject as a random effect. A secondary analysis of the primary end point was also performed, where the primary analysis model was modified to include additional effects for sessions (nested within assessment day, eight sessions per assessment day) as well as the treatment by session interaction. For all secondary end points, models similar to the primary and secondary models were fitted and appropriate contrasts derived with 95% confidence intervals (CIs). PK analysis was performed by Kinesis Pharma B.V. (Breda, Netherlands) using Phoenix software version 6.2 or higher (Pharsight Cooperation, Mountain View, CA, USA). All safety and tolerability data were summarized using descriptive statistics.

Results

Subject Disposition and Baseline Characteristics

A total of 29 subjects were screened; 25 of these met the inclusion criteria and were randomized to the six treatment sequences and received at least one dose of study drug. Randomized subjects were equally distributed across treatment sequence, and baseline characteristics were comparable among the treatment sequences (Table 1). Of the 25 subjects randomized, a total of three discontinued treatment. One subject randomized to the ASP8477–duloxetine–placebo treatment sequence discontinued treatment due to AEs of nausea, vomiting, and asthenia during treatment period 3 (placebo), and another subject in the same treatment group withdrew consent without any known reason

Table 1 Summary of baseline characteristics and disposition of subjects (SAF)

Parameter	ASP8477–Duloxetine–Placebo (n = 5)	ASP8477–Placebo–Duloxetine (n = 4)	Duloxetine–ASP8477–Placebo (n = 4)	Duloxetine–Placebo–ASP8477 (n = 4)	Placebo–ASP8477–Duloxetine (n = 4)	Placebo–Duloxetine–ASP8477 (n = 4)	Total (n = 25)
Baseline characteristics, mean (SD)							
Age, years	35.2 (9.7)	37.8 (10.8)	35.8 (14.8)	39.0 (14.1)	36.5 (8.4)	37.3 (11.4)	36.8 (10.4)
Weight, kg	66.5 (16.8)	72.2 (7.0)	57.3 (5.3)	59.8 (6.2)	70.3 (17.4)	69.3 (18.2)	65.9 (13.1)
BMI, kg/m²	23.1 (4.8)	24.8 (3.3)	20.5 (1.7)	21.6 (2.1)	24.3 (4.2)	24.6 (5.9)	23.1 (3.9)
Study discontinuation, n (%)							
Yes	2 (40)	0	0	0	0	1 (25)	3 (12)
No	3 (60)	4 (100)	4 (100)	4 (100)	4 (100)	3 (75)	22 (88)
Primary reason for discontinuation, n (%)							
Adverse event	1 (20)	0	0	0	0	1 (25)	2 (8)
Withdrawal by subject	1 (20)	0	0	0	0	0	1 (4)

BMI = body mass index; SAF = safety analysis set; SD = standard deviation.

during period 1, day 11 of treatment (after completing treatment with ASP8477 20 mg). The third subject, randomized to the placebo–duloxetine–ASP8477 treatment sequence, discontinued treatment due to AEs of headache, acute tonsillitis, and sinusitis during treatment period 2 (duloxetine 30 mg).

Efficacy

There was a trend for lower mean LEP PtP amplitudes in ASP8477 100 mg (day 21) compared with placebo (day 21); however, the difference was not statistically significant ($P = 0.0721$; Table 2). When additional effects for session and treatment by session interactions were included in a secondary analysis, the result was consistent with the aforementioned primary analysis results.

Overall, the application of topical capsaicin led to peripheral and spinal sensitization as shown by a rise in N2 and P2 amplitudes vs baseline. Analysis of capsaicin skin effects showed that six subjects had a negative LEP $AUEC_{0-8 h}$ on days 7, 14, and 21 when receiving placebo, indicating that LEP over an eight-hour period was lower than baseline (i.e., these subjects were not sensitive to capsaicin and consequently did not develop hyperalgesia). Thus, these subjects were excluded from the exploratory capsaicin-positive subgroup analysis. Furthermore, two of the three subjects who discontinued the study were excluded from the subgroup analysis due to lack of LEP data in response to duloxetine and/or ASP8477. Analysis of differences in LEP N2-P2 PtP amplitudes in the capsaicin-positive subgroup population showed a mean difference of $-2.24 \mu V$ (95% CI = -4.04 to -0.44, $P = 0.0146$), indicating a significant difference in LEP N2-P2 PtP amplitudes between ASP8477 100 mg (day 21) and placebo (day 21) in subjects who met the positive capsaicin skin effects criteria.

In all subjects, across all doses, there was a statistically significant difference in LEP N2-P2 PtP amplitudes between ASP8477 (28.51 μV) and placebo (29.92 μV; mean difference = -1.41, 95% CI = -2.40 to -0.42, $P < 0.01$; Figure 2A). Furthermore, the difference in LEP N2-P2 PtP amplitudes between duloxetine (24.71 μV) and ASP8477 or placebo were statistically significant; for duloxetine vs ASP8477, the mean difference was $-3.80 \mu V$ (95% CI = 2.81 to 4.78, $P < 0.0001$), whereas for duloxetine vs placebo, the mean difference was $-5.21 \mu V$ (95% CI = -6.19 to -4.22, $P < 0.0001$). When additional effects for session and treatment by session interactions were included in the analysis, the results were consistent with the aforementioned primary analysis results.

Comparing the mean VAS pain scores of all three doses of ASP8477 (overall) with placebo in all subjects showed that the effect of ASP8477 (42.23%) on decreasing VAS pain was statistically significant compared with placebo (44.78%; mean difference = -2.55%, 95% CI = -4.01 to -1.08, $P < 0.0007$; Figure 2B). The results were

Table 2 LEP N2-P2 PtP amplitudes of ASP8477 100 mg (day 21) vs placebo (day 21) in all subjects

Analysis Statistics	ASP8477 100 mg (n = 24)	Placebo (n = 24)	Mean Difference (95% CI)	P Value
Primary analysis*				
LS mean PtP (SE), μV	28.31 (2.75)	29.88 (2.75)	−1.58 (−3.30 to 0.14)	0.0721
95% CI	(22.92 to 33.69)	(24.50 to 35.27)		
Secondary analysis†				
LS mean PtP (SE), μV	29.66 (2.80)	31.24 (2.80)	−1.58 (−3.30 to 0.15)	0.0729
95% CI	(24.18 to 35.15)	(25.75 to 36.73)		

CI = confidence interval; LEP = laser evoked potential; LS = least squares; PtP = peak-to-peak; SE = standard error.
*Linear mixed regression model with session as replications.
†Linear mixed regression model with session as a random effect.
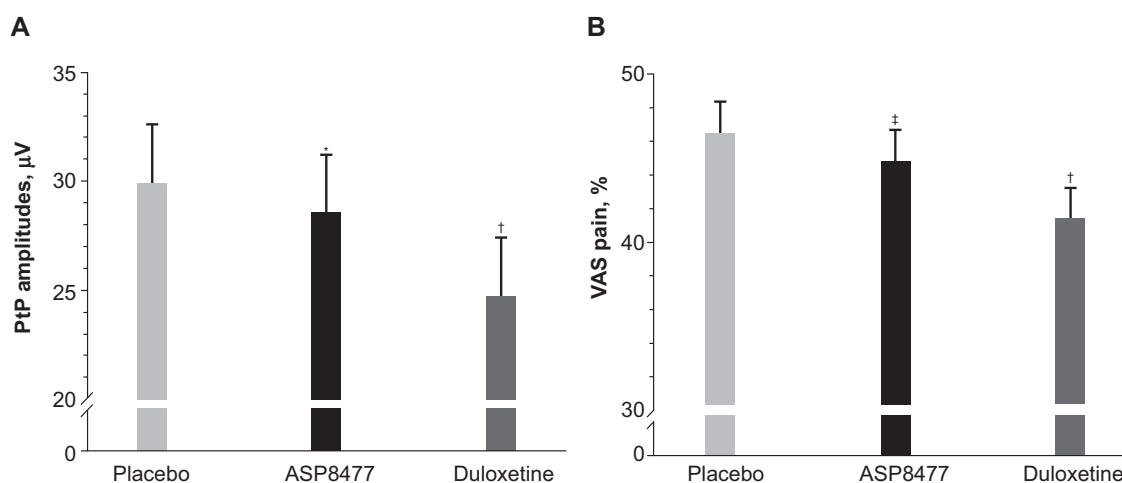
Figure 2 Overall postadministration mean plus 95% confidence interval for laser evoked potential (LEP) N2-P2 peak-to-peak (PtP) amplitudes and visual analog scale (VAS) pain by treatment group in all subjects (FAS). Overall postadministration mean plus 95% confidence interval for (**A**) LEP N2-P2 PtP amplitudes and (**B**) VAS pain by treatment groups in all subjects. *$P = 0.0054$ relative to placebo. †$P < 0.0001$ relative to ASP8477 or placebo. ‡$P < 0.0007$ relative to placebo. FAS = full analysis set.

consistent when additional effects for session and treatment by session interactions were included in the analysis.

Following capsaicin treatment and radiant-heat stimuli, there was a hyperalgesia development (increase in nociception over time) across all doses of ASP8477, duloxetine, and placebo. The percentage change in LEP N2-P2 PtP amplitudes from day 7 predose (baseline) was lowest for all doses of duloxetine (indicating the greatest level of antihyperalgesic effect), followed by all doses of ASP8477 and then placebo (Figure 3A). The times to maximum response of ASP8477 and duloxetine were comparable and were reached approximately one to four hours after dosing. The time course of action of ASP8477, duloxetine, and placebo in VAS pain scores from day 7 predose (baseline) to postdose sessions on days 7, 14, and 21 were consistent with the LEP N2-P2

PtP amplitude results (Figure 3B), with the percent change from baseline being lowest for all doses of duloxetine, followed by all doses of ASP8477, and then placebo.

The cumulative magnitude of responses for LEP N2-P2 PtP amplitudes, assessed by comparing the percent change in LEP N2-P2 PtP amplitudes from predose day 7 (baseline) ASP8477 and duloxetine, showed no apparent dose-response relationship for duloxetine and ASP8477 in the one to eight hours postdose. The maximum postdose response was attained at 20 mg for ASP8477 and 30 mg for duloxetine. The magnitude of response in all subjects by days 7, 14, and 21 was greater for duloxetine compared with ASP8477 when measured in the two to eight hours postdose (Figure 4A). In the exploratory capsaicin-positive subgroup analysis, the magnitude of response (AUC) with duloxetine increased from
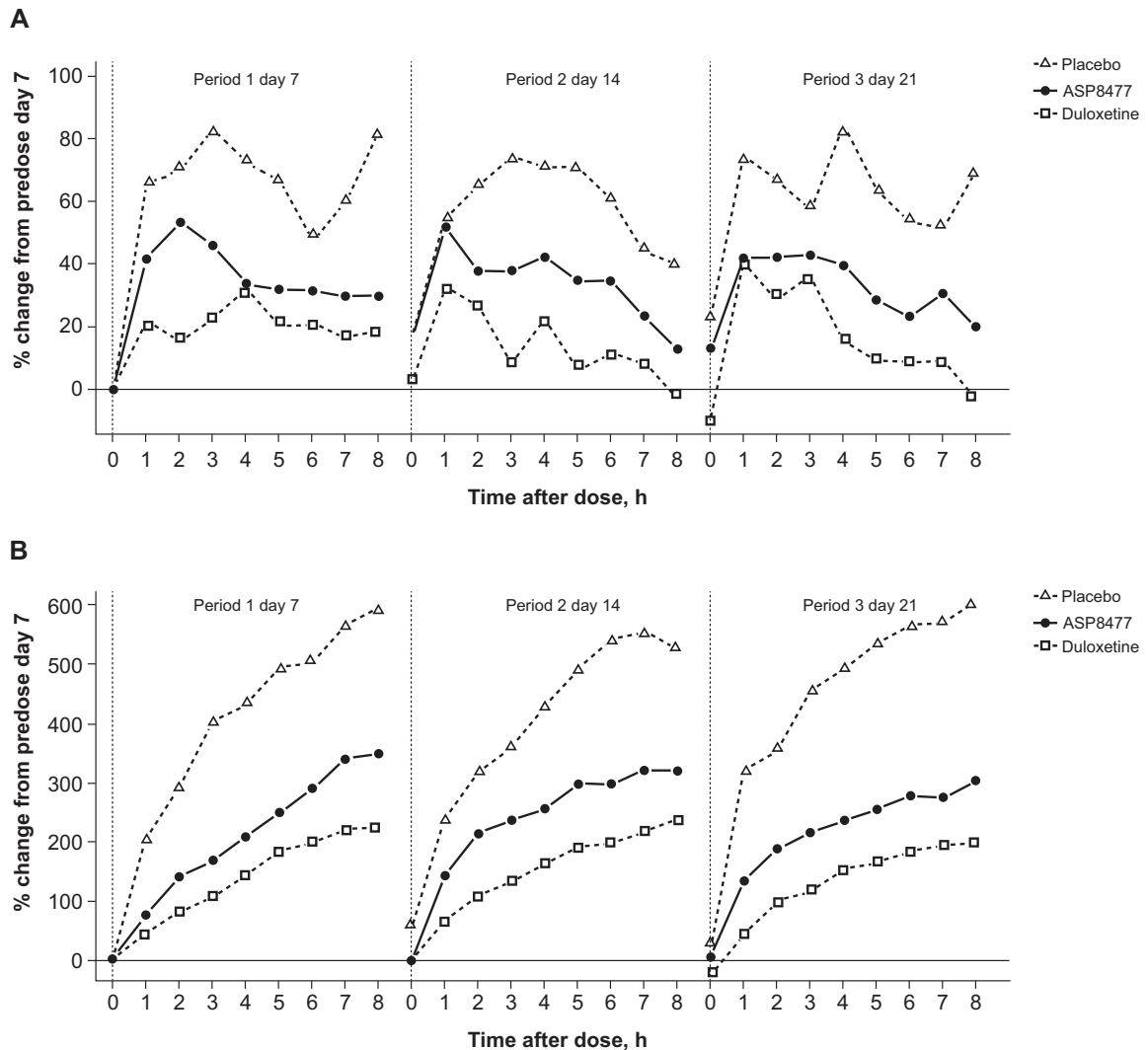
Figure 3 Time course of action of the treatment groups in laser evoked potential (LEP) N2-P2 peak-to-peak (PtP) amplitudes and visual analog scale (VAS) by assessment days (FAS). Time course of action of the treatment groups on (**A**) LEP N2-P2 PtP amplitudes and (**B**) VAS pain by assessment days. Time after dose = 0 refers to predose assessment on days 7, 14, or 21, assessed directly following removal of capsaicin. By convention, the predose value day 7 (at steady state) was defined as 0% change. FAS = full analysis set.

approximately 360 (for all subjects) to approximately 500 on days 7, 14, and 21 (capsaicin-positive subgroup), whereas for ASP8477 the magnitude of response increased by at least 100% from approximately 200 (all subjects) to approximately 400, 420, and 470 (capsaicin-positive subgroup) on days 7, 14, and 21, respectively. Overall, the difference in response between ASP8477 and duloxetine became smaller (Figure 4B), and the cumulative AUC for ASP8477 100 mg (day 21) in the exploratory capsaicin-positive subgroup analysis was similar to that of duloxetine.

In all subjects, the mean day 14 predose response for LEP N2-P2 PtP amplitudes was 26% of the maximum

response for duloxetine and 4% of the maximum response for ASP8477; the day 21 predose response was 44% of the maximum response for duloxetine and 19% of the maximum response for ASP8477 (Figure 5A), suggesting a dose-response relationship for duloxetine and ASP8477. In the exploratory capsaicin-positive subgroup analysis, the day 14 predose for duloxetine increased from 25% to 40%, and the predose effect on day 21 remained around 40%; however, for ASP8477, the day 14 predose effect increased from 8% to 25%, and on day 21 increased from 18% to 40% of the maximum achievable effect (Figure 5B). The day 21 predose effect for ASP8477 was similar to that of duloxetine. In all subjects, the day 14 predose response for VAS pain
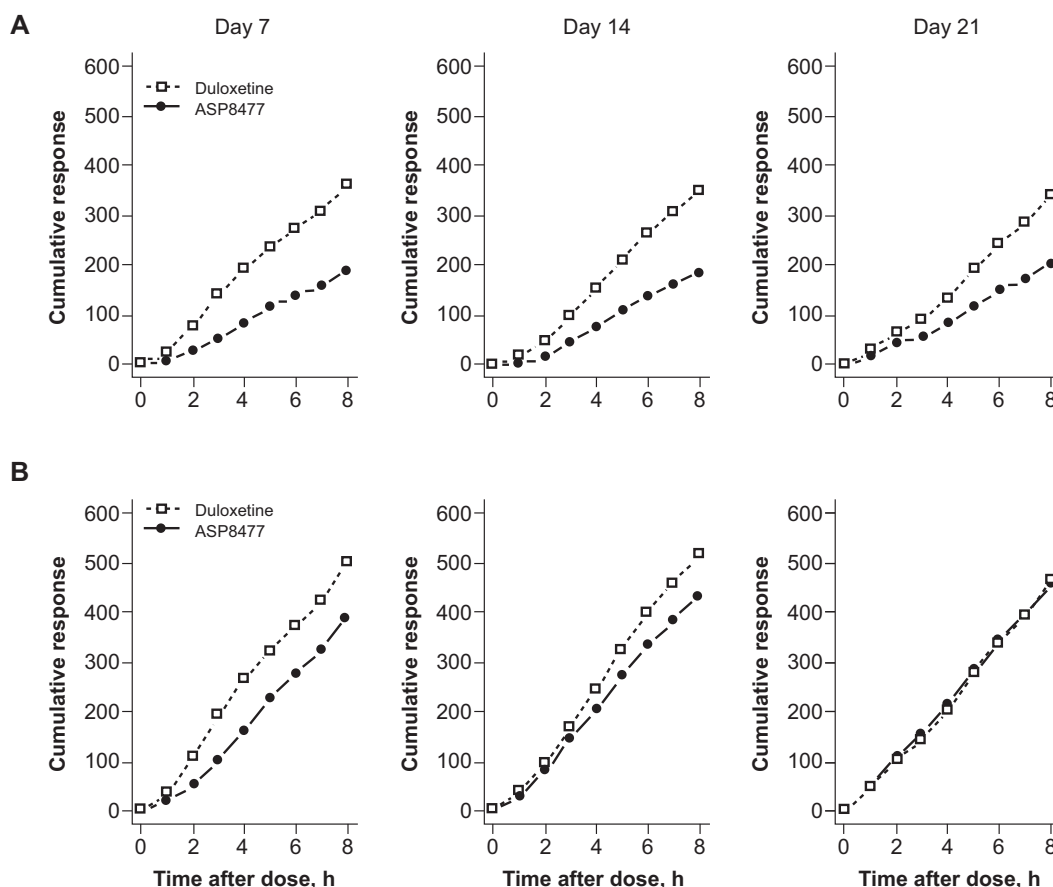
Figure 4 Laser evoked potential (LEP) N2-P2 peak-to-peak (PtP) cumulative magnitude of response (AUC) for ASP8477 vs duloxetine. LEP N2-P2 PtP cumulative magnitude of response (AUC) for ASP8477 vs duloxetine in (**A**) full analysis set (all patients treated) and (**B**) capsaicin subgroup analysis. AUC = area under curve.

scores was 17% of the maximum response for duloxetine and 23% of the maximum response for ASP8477; the day 21 predose response was 10% of the maximum response for duloxetine and 4% of the maximum response for ASP8477 (Supplementary Data).

Pharmacokinetics

Following multiple ascending doses of ASP8477, the PK parameters of ASP8477 increased in a more than dose-proportional manner between 20 mg and 100 mg ASP8477 (Supplementary Data). The deviation from dose proportionality was more pronounced between 20 mg and 60 mg ASP8477 than between 60 mg and 100 mg ASP8477. Following oral dosing, ASP8477 was rapidly absorbed with comparable median t_{max} for all doses (range = 2.1–4.1 hours). The intersubject variability on C_{max} and AUC_{0-8h}, expressed as %CV, ranged between 28% and 48%. The mean plasma concentration–time profiles of ASP8477 are presented in the Supplementary Data.

Following multiple dosing of duloxetine once daily for seven days, the PK parameters of duloxetine 60 mg approximately doubled compared with PK parameters of duloxetine 30 mg; the PK parameters on days 14 and 21 were comparable (Supplementary Data). The intersubject variability on C_{max} and AUC_{0-8h}, expressed as %CV, ranged between 55% and 67%. The mean plasma concentration–time profiles of duloxetine are presented in the Supplementary Data.

Safety and Tolerability

No deaths or serious AEs were reported during this study. A total of 20 subjects (83%) reported a treatment-emergent adverse event (TEAE) during treatment with ASP8477, 21 (86%) with duloxetine, and 15 (63%) with placebo. Most of the reported AEs were considered related to study treatment. All TEAEs reported during this study were of mild or moderate intensity. The most common TEAEs reported during ASP8477 treatment were headache, fatigue, and vertigo (seven

A

B
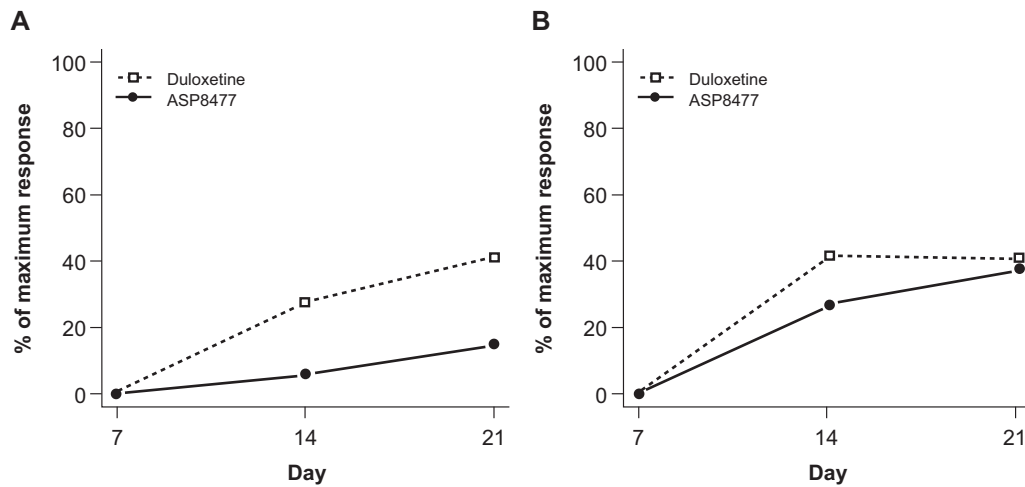
Figure 5 Laser evoked potential (LEP) N2-P2 peak-to-peak (PtP) duration of response (AUC) for ASP8477 vs duloxetine. LEP N2-P2 PtP duration of response (AUC) for ASP8477 vs duloxetine in (**A**) full analysis set (all subjects treated) and (**B**) capsaicin subgroup analysis. Results shown are predose values on days 7, 14, and 21. AUC = area under curve.

subjects each; 29%), the most common TEAEs reported during duloxetine treatment were nausea and fatigue (seven subjects each; 29%), and the most common TEAE reported in those receiving placebo was headache (nine subjects; 38%). The TEAEs of higher incidence during treatment with ASP8477 overall compared with placebo were fatigue (29% vs 17%, respectively), vertigo (29% vs 4%, respectively), dry mouth (21% vs 8%, respectively), disturbance in attention and illusion (13% vs none, respectively), memory impairment (13% vs none, respectively), and disorientation (8% vs none, respectively). Incidence of TEAEs occurring in at least 5% of all subjects are summarized in the Supplementary Data.

Overall, two subjects discontinued the study due to TEAEs. One subject randomized to the placebo–duloxetine–ASP8477 treatment sequence experienced AEs of headache (considered as possibly related to the study drug), and acute tonsillitis and sinusitis (both considered unrelated to the study drug) during treatment with duloxetine 30 mg (day 12 of treatment). The AEs of acute tonsillitis and sinusitis were reported as resolved after 12 days of treatment. The second subject, randomized to the ASP8477–duloxetine–placebo treatment sequence, experienced AEs of nausea, vomiting, and weakness (all of which were considered possibly related to the study drug) during treatment with placebo (day 20 of treatment). These AEs were reported as resolved by day 23 without treatment.

There were no clinically significant treatment- or dose-related trends in laboratory safety findings, vital signs, or 12-lead electrocardiogram results. One subject randomized to the placebo–ASP8477–duloxetine treatment sequence reported an aspartate aminotransferase level of

108 U/L (>3× upper limit normal) during treatment with ASP8477 (day 14 of treatment sequence), which had returned to normal levels upon re-examination on day 20.

Discussion

In this phase I study, the analgesic/antihyperalgesic effects, as well as the PK profile and safety of ASP8477, were evaluated in a human hyperalgesia model with topical capsaicin. Duloxetine, an approved medication for NP (and also effective in OA treatment), was chosen as the intra-assay validator (also known to be effective in a single-dose paradigm [23]). Duloxetine showed a statistically significant effect on both LEP PtP amplitudes and VAS for pain intensity, supporting the validity of the methods used in this study. Administration of ASP8477 (across all doses) showed a statistically significant reduction in LEP N2-P2 PtP amplitudes and VAS pain relative to placebo; however, the study failed to meet its primary end point. The difference in LEP PtP amplitudes between the highest ASP8477 dose of 100 mg (day 21) and placebo (day 21) was not statistically significant. Across all doses, the percentage change in LEP N2-P2 PtP amplitudes from baseline (hyperalgesia development) was greatest for placebo, followed by ASP8477 and then duloxetine. The maximum achievable effect of ASP8477 was reached following multiple dosing of 20 mg ASP8477 once daily.

Reports in the published literature suggest that response to topical application of capsaicin may vary between individuals with different neuropathic conditions [20,21], although the variability can be attributed to the differential skin penetration of topical capsaicin that is not usually observed with injectable capsaicin with a superior spatial resolution [31]. It is also well known that

certain patients are unresponsive to topical capsaicin treatment [32]. Given the known variability or lack of response to topical capsaicin, and in order to specifically demonstrate the antihyperalgesic effects of ASP8477, an exploratory subgroup analysis of the primary end point, independent of study drugs, was conducted in subjects who showed a hyperalgesic response to capsaicin (capsaicin-positive subgroup analysis). The results of this analysis showed a significant benefit of ASP8477 100 mg (day 21) compared with placebo (day 21) in LEP PtP amplitudes, in contrast to the primary analysis in the FAS population. In all subjects, the magnitude of the effect (maximum difference to placebo) of ASP8477 was smaller compared with duloxetine; however, capsaicin-positive subgroup analysis showed that overall, the difference in response between ASP8477 and duloxetine became smaller and the cumulative AUC for ASP8477 100 mg (day 21) in the capsaicin-positive subgroup analysis was even similar to that of duloxetine. Furthermore, analysis of the response duration of ASP8477 by LEP N2-P2 PtP amplitudes in the capsaicin-positive subgroup suggests that, at steady-state, duration of response increases with dose, remaining at 40% of the maximum achievable effect, which is a desirable property for an analgesic compound.

The time to maximum effect for duloxetine (five hours, approximately) was in keeping with the PK of duloxetine [33]; the effect of ASP8477 reached a plateau at maximum one to two hours postdose.

When assessed using VAS, the time to maximum effect of ASP8477 increased over time, unlike the observations in LEPs. As previously reported, in contrast to the LEP sessions, a progressive increase in VAS pain score was observed over an eight-hour experimental period in subjects receiving placebo regardless of their skin condition [22]. Although this apparent discrepancy between the objective LEP and the subjective VAS pain self-assessment score cannot be fully explained, it should be noted that the objective LEPs preferably reflect components of nociceptive processing, influenced mainly by the intensity of the nociceptive stimulation, whereas the subjective VAS pain score is a composite of pain perception, as well as of cognitive, emotional, and vigilance states, which are potential confounding factors. For example, the augmenting effect of negative emotions on experimental pain has been described [34]. Therefore, it cannot be excluded that negative emotions, resulting from repeated exposure to unpleasant experimental laboratory procedures, could contribute, at least in part, to the observed time-dependent increase in VAS pain score in subjects receiving placebo.

The maximum effect seems not to increase with dose, but the increase in percentage of maximum response observed predose on days 14 and 21 (Figure 5) indicates that the duration of the effect increases with dose. Higher doses were not tested due to potential tolerability issues. Data have suggested that AEA activations of CB1 and TRPV1 receptors play opposing roles

in modulating pain [35], and that the potency of AEA toward cannabinoid receptors is about 10 times higher than that of AEA toward TRPV1 receptors [36]. It has also been suggested that increasing the dose of an FAAH inhibitor, which increases AEA concentration (at least in plasma), may evoke rather than decrease the hyperalgesic effect [36]; therefore, it is unclear how these mechanisms may have affected the effects of ASP8477 in this study.

Overall, ASP8477 at ascending doses of 20 mg, 60 mg, and 100 mg once daily was well tolerated in healthy female subjects. All TEAEs reported were of mild or moderate intensity. In this study, the results of the exposure to ASP8477 are in keeping with previous findings for ASP8477 (unpublished data), and those for duloxetine are in keeping with the package insert content and actual reports in the literature [23,33].

A limitation in this study may have been the lack of baseline assessments prior to treatment with any study drug. As a result, the predose assessment on the first assessment (on day 7 of each period) was normalized to zero and was used as baseline, which may have had an impact on the observed efficacy results.

In conclusion, although the difference in LEP (in all subjects) between ASP8477 100 mg and placebo did not reach a statistically significant level, other results in this study showed that ASP8477 was well tolerated at all doses and also showed potential analgesic and antihyperalgesic properties of ASP8477 in humans. Furthermore, in the capsaicin-positive subgroup analysis, ASP8477 showed better efficacy compared with placebo, and the magnitude of ASP8477 effects was similar to that of duloxetine, supporting further investigation of ASP8477 for treatment of PNP. Based on the results of this study, a daily dose of 100 mg (either 50 mg BID or 100 mg QD) ASP8477 was recommended for the subsequent phase IIa study in patients with PNP. However, due to a strong drug-drug interaction potential at this dose level, a dose of 30 mg BID was chosen [37]. This study in patients with PNP employed two treatment periods: a single-blind treatment period, followed by a double-blind randomized withdrawal period. Although nearly 60% of patients reported a reduction of ≥30% in their pain during the single-blind period with ASP8477, no difference between ASP8477 and placebo was demonstrated in the double-blind randomized withdrawal period. To date, trials of FAAH inhibitors for chronic pain have all failed their primary end point [38,39]. Future development of compounds targeting FAAH inhibition and their respective trial designs need to be closely examined if the potential of these compounds for the treatment of chronic pain is to be revealed.

Authors' Contributions

KS conducted this study as the Principal Investigator, AY conducted the subanalysis of study data, PR was

Supplementary Data

Supplementary Data may be found online at http://painmedicine.oxfordjournals.org.

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