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A Phase I, Randomized, Double-Blind, Laser-Evoked Potential Study to Evaluate the Analgesic/Antihyperalgesic Effect of ASP9226, a State-Dependent N-Type Voltage-Gated Calcium Channel Inhibitor, in Healthy Male Subjects

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Abstract

Objective. Evaluate the analgesic/antihyperalgesic effects of ASP9226, a state-dependent N-type voltage-gated calcium channel inhibitor, in healthy male subjects.

Design. Randomized, double-blind, double-dummy, placebo- and active comparator–controlled crossover study.

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

Subject. Healthy male subjects aged 18–55 years.

Methods. Twenty-four eligible subjects were randomly assigned to one of four treatment sequences and received single doses of ASP9226 (30 mg or 50 mg), pregabalin (150 mg), or placebo during four treatment periods. Laser-evoked potentials (LEP) and postlaser pain visual analog scales (VAS) on capsaicin-treated skin were assessed during main assessment days (the first day of each study period). Primary and secondary end points were the differences in LEP N2-P2 peak-to-peak (PtP) amplitudes and VAS score, respectively, in all subjects.

Results. Overall, treatment with pregabalin resulted in a significantly lower LEP N2-P2 PtP amplitude vs placebo (–3.30 µV, $P < 0.0001$). There were no clinically relevant differences in N2-P2 PtP amplitudes between placebo and either ASP9226 dose (–0.31 µV and –0.27 µV). Furthermore, subjects reported significantly lower VAS pain scores with pregabalin vs placebo (–9.90%, $P < 0.0001$) in contrast to ASP9226 30 mg (–2.1%) and ASP9226 50 mg (1.2%) vs placebo. Subgroup analysis of LEP and VAS pain in participants with positive prestudy capsaicin response (n = 13) were in keeping with results in all subjects.

Conclusions. ASP9226 was well tolerated; however, there was no improvement in LEP and VAS pain scores with ASP9226 at either dose vs placebo.

Key Words. ASP9226; Pregabalin; LEP; VAS; Capsaicin/Neuropathic Pain

Introduction

Voltage-dependent calcium channels (VDCCs) regulate entry of calcium ions into cells in response to membrane depolarization and have important roles in the regulation of a variety of cellular functions, including membrane excitability, muscle contraction, synaptic transmission, and gene expression [1]. Electrophysiological and pharmacological studies have identified at least five distinct types of calcium channels, designated L-, N-, P/Q-, R-, and T-type [2]. These calcium channels represent a potential target for the management of pain conditions. Agents that interact with or inhibit VDCCs have been shown to exhibit therapeutic properties in peripheral neuropathic pain (PNP) [3,4]. For example, pregabalin, an approved gabapentinoid for the treatment of PNP, interacts with the VDCC and consequently reduces the influx of calcium ions through the channels to produce analgesic actions. Pregabalin has been shown to be effective in several models of neuropathic pain in preclinical and clinical studies; it is active in normal healthy volunteer experimental models of pain, including those that invoke central sensitization using intradermal injections of capsaicin and electrical stimulation. In these healthy volunteer models, pregabalin is pharmacologically active at doses that are consistent with approved doses for PNP, with single-dose administrations demonstrating antinociceptive/antihyperalgesic effects within two hours of administration that are sustained for over six hours [3,5–13]. However, clinically, treatment with pregabalin is commonly associated with side effects such as sedation, dizziness, peripheral edema, and dry mouth [4,14]. Therefore, new treatment options are needed with improved tolerability and better efficacy.

The human hyperalgesia model with topical capsaicin, which delivers a constant nociceptive input at the spinal level, helps to evoke and assess pain in healthy volunteers under controlled conditions [15,16]. Thus, the antinociceptive/antihyperalgesic properties of a new compound can be investigated without confounding biases (e.g., multimorbidity, comedications) often seen in patient studies. Topical application of capsaicin on normal skin stimulates TRPV1 expression of cutaneous nociceptors to evoke mixed peripheral and spinal sensitization [15,17,18]. The evoked responses in these models can be assessed using an *objective* method such as laser-evoked potential (LEP) and/or a *subjective* visual analog scale (VAS) method of assessment [15,16]. The application of laser technology, as a relatively novel, alternative, or complimentary technique in human pain research, has resulted in a major advance in our ability to generate and interpret well-defined thermonociceptive sensations (also dependent and interacting in their mechanisms with applied skin types). The LEP model has demonstrated construct and predictive validity for neuropathic pain across numerous compounds with various mechanisms of action [13,19]. Therefore, the healthy volunteer LEP model of hyperalgesia and pain is a valuable tool as it can be used to make predictions to inform investigators about dose/regimen and efficacy for the purpose of de-risking a proof of concept (PoC) clinical trial in patients.

ASP9226 is a novel and state-dependent N-VDCC inhibitor. Here, the results from a phase I study, conducted in healthy male subjects, to evaluate the analgesic/antihyperalgesic effects of two different single-dose administrations (30 mg and 50 mg) of ASP9226 vs an active control (pregabalin 150 mg, which has shown single-dose efficacy in the LEP model in healthy subjects [13]), are reported.

Methods

Study Overview

This was a phase I, randomized, double-blind, double-dummy, placebo- and active comparator–controlled, single-dose, four-period crossover study conducted at the Human Pharmacodynamic Research (HPR) Dr. Schaffler GmbH site in Munich, Germany (EudraCT number 2014–000492-79). The study protocol was approved by the local Independent Ethics Committee (BLÄK, Munich, Germany) and the Competent Federal Authority (BfArM, Bonn, Germany) prior to study initiation. An Independent Ethics Committee–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 in its actual revision status, Good Clinical Practice (GCP), International Conference on Harmonization (ICH) guidelines, and applicable regulations and guidelines governing clinical study conduct and ethical principles.

Study Design

The study consisted of a 21-day screening assessment period (days −21 to −1) followed by four consecutive study periods (Williams square design), each consisting of seven days (Figure 1). During the screening period, subjects underwent standard screening procedures, were genotyped for CYP2D6 polymorphism, and had their individual laser pain threshold (LPT) measured by day −7. The LPT was determined on normal untreated skin by the application of CO_2 laser (radiant-heat) stimuli of increasing intensity, which was then kept constant for each subject for all LEP nociceptive sessions, throughout the study. Following LPT determination, subjects received their first capsaicin application. Capsaicin response was measured via stimuli with ascending weighted needle impact (weight in mN, standard impact range = 1–512 mN) on the restricted area of secondary flare (Quantitative Sensory Testing) until feeling pain; results were then used as an exploratory marker to investigate and optimize the predictive value of the capsaicin response during screening. Subjects who
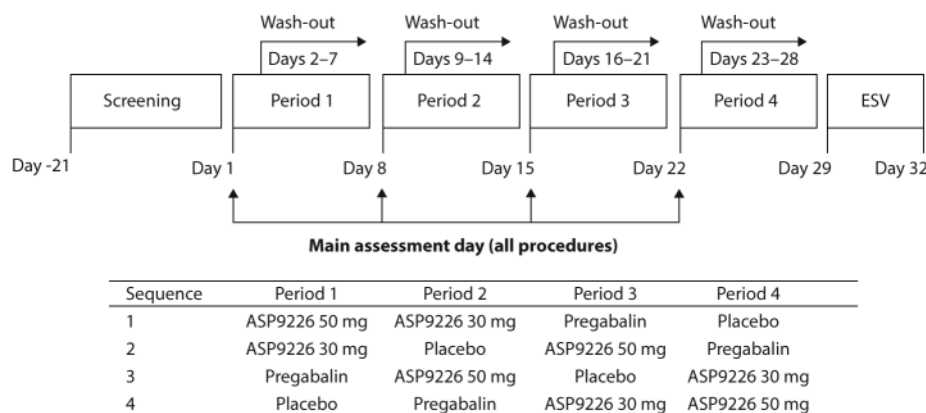
Figure 1 Study design. ESV = End-of-study visit

demonstrated positive capsaicin skin effects (capsaicin-positive subgroup) were defined as participants who showed higher precapsaicin weighted needle test results compared with postcapsaicin application.

Eligible subjects were randomly assigned to one of four treatment sequences and received single doses of 30 mg ASP9226, 50 mg ASP9226, 150 mg pregabalin, or matching placebo (double-dummy) in four periods. The first day of each study period was the main assessment day and was followed by a six-day washout period. Several LEP and VAS pain testing sessions were performed on capsaicin-treated skin during the main assessment days to assess the analgesic/antihyperalgesic effects of ASP9226 and the positive control (pregabalin). Single-dose (150 mg) pregabalin administration orally has demonstrated pharmacologic activity in the LEP model (in capsaicin, UV, and normal skin conditions) vs placebo [13]. Upon the completion of the four study periods, or after early withdrawal, subjects had their end-of-study visit (ESV). All study participants and the study investigator were blinded to treatment. To maintain blinding, all subjects received the same number of tablets and capsules, and a double-dummy approach was used during all study periods.

Study Subjects

Twenty-four healthy male subjects (aged 18–55 years) of Caucasian origin with a body mass index of ≥18.5–$30.0\,kg/m^2$, body weight of $\geq50\,kg$, type II–IV skin (Fitzpatrick classification)-covering 90% of the appearance in the Middle European population-and a CYP2D6 extensive or intermediate metabolizer genotype, were eligible for enrollment. Subjects and their female spouse of childbearing potential were required to be using highly effective contraception (consisting of two forms of birth control/double-barrier) at screening, throughout the study period, and for 90 days after the final study drug administration. Subjects were not required to be naïve to LEP application.

Subjects were excluded if they had known or suspected hypersensitivity to ASP9226, pregabalin, capsaicin, or any components of the formulations used. Subjects were also excluded if they had an abnormal liver function test above (1.5×) the upper limit of normal, a clinically significant history of allergic conditions, or history or evidence of any clinically significant disease or malignancy, as judged by the medical investigator. In addition, subjects with febrile illness or symptomatic, viral, bacterial, or fungal (noncutaneous) infection within one week of the first assessment day were excluded. Furthermore, the following subjects were excluded: those with acne, eczema, scars, or tattoos at the site of exposure to laser and/or capsaicin; those who used topical drugs or cosmetics on the sites where laser and/or capsaicin were applied; and who used any prescribed or nonprescribed drugs in the two weeks prior to period 1 day 1, except for the occasional use of paracetamol for pain (up to 2 g/d, but not within 24 hours before screening or main assessment days).

Study End Points

The primary end point was the difference in LEP N2-P2 peak-to-peak (PtP) amplitudes (μV) between ASP9226 and placebo, on capsaicin-treated skin. The secondary end point was the difference in postlaser pain visual analog scales (VAS pain; 0–100 mm expressed as %). Additional measurements included pharmacokinetic (PK) analysis, as well as safety and tolerability of ASP9226 and pregabalin.

Study Assessments

For LEP assessments, random sites on the skin of the back were pretreated with topical capsaicin (standardized 1% alcoholic solution, Extrakt Chemie, Stadtbergen, Germany), applied in an occlusive mode for 25 minutes, after which the dressing was removed and the skin dried. During the interval between capsaicin application and dosing, two (thermal) rekindling

sessions were given with the CO_2 laser (Pulsed SYNRAD infrared gas LASER model E48-/- 10 W, SYNRAD Inc., North Bothell, WA, USA)-inducing a "spinal wind-up" (acting as an additional hyperalgesia enhancement by delivering added spinal nociceptive input on the primary capsaicin application area). During each laser session, at least 13 stimuli (each lasting 80 msec) were applied to the treated skin. The first stimulus of the LEP recording was generally rejected, as the subject becomes acclimated to the procedures and was not used for online/real-time data processing. Between each single stimulus, the laser was moved 2–3 mm so that the same skin area was not stimulated twice. The time interval between two individual nociceptive stimuli randomly varied between four and eight seconds to avoid habituation to, and expectation of, the laser stimuli in subjects. During each session, to avoid influences of external disturbing noise, 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 perform an attention-focusing permanent pursuit tracking task on a computer screen (i.e., subjects continuously followed a randomly moving target on-screen by a joy-stick-controlled pursuer, to keep the distance between both signals as minimal as possible). In each period, baseline LEP was measured before dosing, and in hourly sequences (up to eight hours) postdose, by the Vertex-electroencephalogram (EEG leads were taken from Vertex/C_z vs right mastoid/C_{br} and evaluated after automatic rejection of eye blinks, facial electromyography influences, and EEG baseline drifts; the filter setting was 0.15–30 Hz; data sampling was done after analog signal amplification by a bioprocessor with a digitization rate of 512 Hz). Immediately following each laser session, each subject provided a subjective VAS pain estimation assessment using an electronic 100 mm VAS on a tablet PC.

Blood (plasma) samples were collected at predose and hourly (up to eight hours) postdose, after completion of LEP and VAS sessions, for PK assessment of ASP9226 and pregabalin. Safety and tolerability were assessed through spontaneous reporting of adverse events (AEs; MedDRA version 15.1), concomitant medication, vital signs, clinical laboratory assessments, physical examination, and electrocardiogram parameters.

Statistical Analyses

Based on the results of a previous study (ASP8477; EudraCT-number 2011–005122-22), using the same LEP model, a difference of at least 5.37 μV in the primary end point between ASP9226 treatment and placebo was expected; the residual standard deviation was assumed to be 12.6 μV, and the subject standard deviation was assumed to be 1 μV. Under the aforementioned assumptions, a total of 24 subjects was planned (six per treatment sequence). This would allow the study to have greater than 98% power to detect a difference between ASP9226 treatment and placebo at the

two-sided significance level of 5% (with no adjustment for multiple comparisons).

In a meta-analysis of LEP phase I studies comparing peak amplitude mean values of several analgesics with proven efficacy (using regression of LEP amplitude vs VAS pain) in capsaicin-treated skin [19], conducted to define a threshold of clinical efficacy, we identified that all active analgesics investigated clustered above a (N2-P2) PtP amplitude reduction of >2.5 μV. Thus, based on this finding, the noninferiority margin for this study was prospectively set as 2.5 μV. This was assumed to have approximately 77% power to evaluate noninferiority of ASP9226 compared with pregabalin, at a significance level of 5%, in 24 subjects.

All subjects who were randomized, received at least one dose of study drug, and provided a valid baseline value and at least one postbaseline value for the LEP N2-P2 PtP amplitude, were included in the full analysis set. All subjects who had sufficient plasma concentration data available to facilitate derivation of at least one PK parameter, and for whom the time of dosing on the day of sampling was known, were included in the PK analysis. All subjects who took at least one dose of study drug were included in the safety analysis.

Demographic and other baseline characteristics data, as well as PK and safety data, were summarized using descriptive statistics.

Analyses of treatment differences vs placebo in LEP N2-P2 PtP amplitudes (μV) from capsaicin-treated skin of subjects and VAS pain, were performed using the statistical software package SAS™ (version 9.3; SAS Institute, NC, USA). Analysis of the primary end point was based on a linear mixed model including sequence (four levels), period (four levels), and treatment (four levels) as fixed effects, and subject within sequence as a random effect. All primary response variables were summarized by treatment, and continuous variables were summarized using descriptive statistics. A secondary analysis of the primary end point was also performed, in which the primary analysis model was modified to include additional fixed effects for session (nested within period; eight sessions per period) as well as the treatment by session interaction. The secondary end point was analyzed with VAS pain as the dependent variable. The subgroup analysis for subjects who demonstrated positive capsaicin skin effects (n = 13) was conducted using the same statistical approach as described above for the respective end points for all subjects (n = 24).

Results

Subject Disposition and Baseline Characteristics

A total of 24 subjects were assessed for eligibility and randomized to the four treatment sequences (six subjects per group). All subjects received at least one single dose of the study drug and were evaluable for efficacy,

Table 1 Summary of baseline characteristics in the safety population

Parameter	ASP9226 50 mg ASP9226 30 mg Pregabalin Placebo, Mean (SD) (n = 6)	ASP9226 30 mg Placebo ASP9226 50 mg Pregabalin, Mean (SD) (n = 6)	Pregabalin ASP9226 50 mg Placebo ASP9226 30 mg, Mean (SD) (n = 6)	Placebo Pregabalin ASP9226 30 mg ASP9226 50 mg, Mean (SD) (n = 6)	Total, Mean (SD) (n = 24)
Age, y	30.7 (8.2)	36.8 (12.1)	35.0 (13.7)	31.5 (10.1)	33.5 (10.8)
Weight, kg	75.5 (10.1)	81.5 (12.7)	78.6 (5.6)	77.6 (6.4)	78.3 (8.8)
BMI, kg/m^2	23.6 (3.1)	24.1 (3.7)	25.2 (2.1)	23.8 (0.9)	24.2 (2.6)

BMI = body mass index; SD = standard deviation.

Table 2 Pairwise comparisons of LEP N2-P2 PtP amplitudes

Medication 1	Medication 2	Difference,* μV	Standard Error	Confidence Interval	*P* Value
ASP9226 50 mg	Placebo	−0.27	0.77	95% (−1.8 to 1.2)	0.726
ASP9226 30 mg	Placebo	−0.31	0.77	95% (−1.8 to 1.2)	0.684
Pregabalin	Placebo	−3.30	0.77	95% (−4.8 to −1.8)	<0.0001
ASP9226 50 mg	Pregabalin	3.03	0.76	80% (2.1 to 4.0)	<0.0001
ASP9226 30 mg	Pregabalin	2.99	0.76	80% (2.0 to 4.0)	<0.0001
ASP9226 30 mg	ASP9226 50 mg	−0.04	0.76	95% (−1.5 to 1.4)	0.954

LEP = laser evoked potentials; PtP = peak-to-peak.
*Difference reported is for medication 1 vs medication 2.

safety, and PK analysis. Of the 24 subjects randomized, two were later identified as not satisfying entry criteria. Both had elevated total bilirubin at screening and were identified after receiving placebo (treatment sequence: placebo–pregabalin–ASP9226 30 mg–ASP9226 50 mg) or after receiving ASP9226 30 mg (treatment sequence: ASP9226 30 mg–placebo–ASP9226 50 mg–pregabalin). These subjects were not withdrawn from the study because the elevated total bilirubin values did not meet the exclusion criteria for liver safety monitoring. Baseline characteristics were comparable between the treatment sequences (Table 1), and none of the randomized subjects discontinued treatment or the study. All subjects were genotyped as CYP2D6 extensive or intermediate metabolizer.

Difference in LEP N2-P2 PtP Amplitude and VAS Pain

Overall, treatment with pregabalin resulted in a significantly lower LEP N2-P2 PtP amplitude value (26.7 μV) compared with each dose group of ASP9226 (29.7 μV) and placebo (30.0 μV; *P* < 0.0001; differences to placebo are shown in Table 2). The differences in LEP N2-P2 PtP amplitudes between placebo and either dose of ASP9226 treatment were below the clinically relevant margin of 2.5 μV. In keeping with LEP results, subjects reported a significantly lower VAS pain score with pregabalin treatment (36.6%) compared with ASP9226 30 mg (44.5%), ASP9226 50 mg (47.8%), or placebo

(46.6%; *P* < 0.0001) (Supplementary Data). The analgesic effect of pregabalin on LEP N2-P2 PtP amplitudes and VAS pain was observed at one to two hours postdose and was maintained until seven to eight hours postdose (Figure 2). There was no difference in VAS pain score between ASP9226 and placebo.

Of the 24 randomized subjects, 13 (54%) were subjects who demonstrated a positive capsaicin skin response (capsaicin-positive subgroup, as defined earlier). Results of this secondary analysis of LEP N2-P2 PtP amplitudes, and VAS pain score in the capsaicin-positive subgroup, were similar to the full analysis population. Active control with pregabalin treatment was observed with significantly lower LEP N2-P2 PtP amplitudes and VAS pain values compared with ASP9226 30 mg, ASP9226 50 mg, or placebo, and there was no difference in LEP N2-P2 PtP amplitudes and VAS pain score between treatments with either dose of ASP9226 and placebo (Supplementary Data).

PK Profiles of ASP9226 and Pregabalin

ASP9226 was rapidly absorbed, with a median time to reach maximum concentration (t_{max}) of two hours for both doses, and a mean maximum concentration (C_{max}) of 40 ng/mL and 67 ng/mL for ASP9226 30 mg and 50 mg, respectively (Table 3). There was a dose-proportional increase in C_{max} and area under the
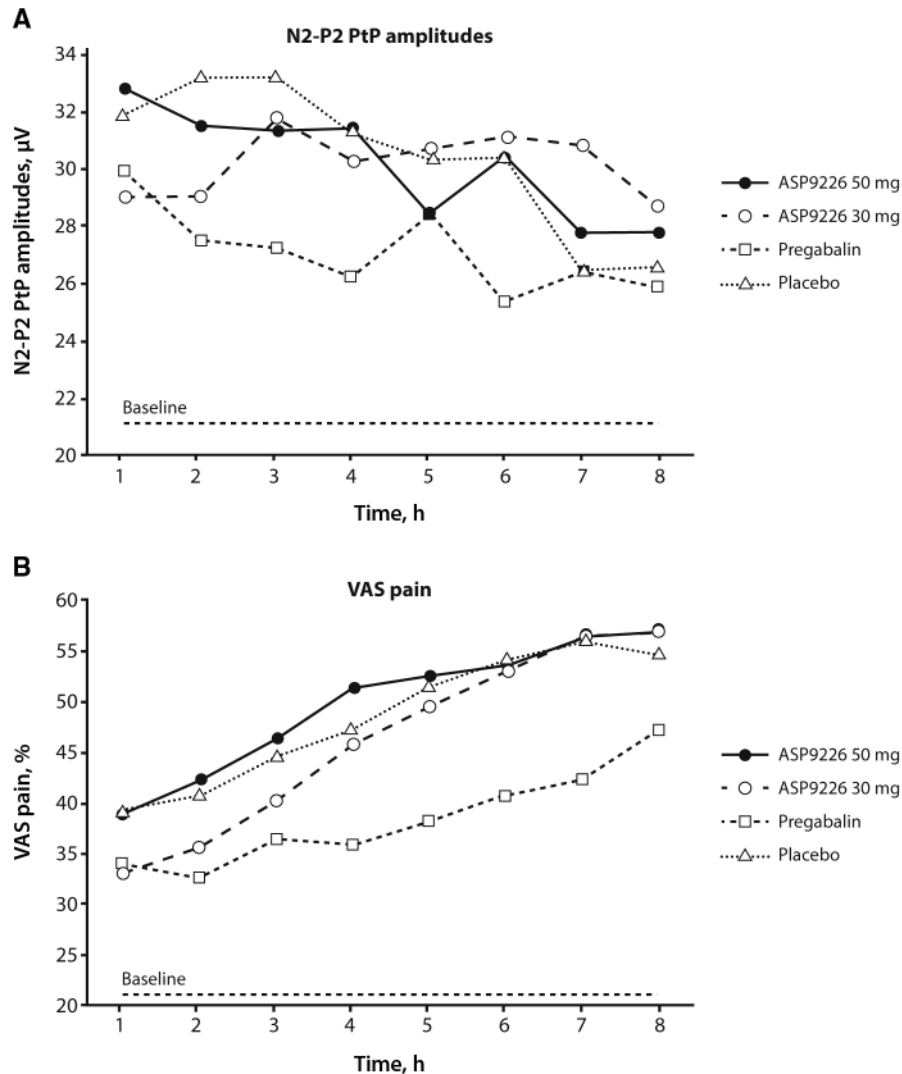
Figure 2 Mean laser-evoked potential N2-P2 (**A**) PtP amplitude and (**B**) VAS pain score: Time course by treatment group. The dotted horizontal line represents the overall predose baseline. PtP = peak-to-peak; VAS = visual analog scale.

concentration-time curve from the time of dosing to 8 hours postdose (AUC_8) (geometric means) from the ASP9226 30 mg to 50 mg dosing; however, given the intersubject variability in the concentration–time profiles, there was an overlap in the ranges of individual C_{max} and AUC_8 values of the ASP9226 30 mg and 50 mg dose levels (Supplementary Data). Pregabalin was rapidly absorbed, with a median t_{max} of one hour and a mean C_{max} of 3,484 ng/mL (Table 3).

Safety and Tolerability of ASP9226

No deaths, serious AEs, or treatment-emergent AEs (TEAEs) leading to drug discontinuation or study discontinuation were reported in this study. Two subjects (8%) reported TEAEs during treatment with ASP9226 50 mg, and 12 subjects (50%) reported TEAEs during treatment

with pregabalin, all of which were considered related to study treatment. No TEAEs were reported during treatment with ASP9226 30 mg (Table 4). All TEAEs reported during this study were either of mild or moderate intensity. Overall, the most commonly reported TEAE was dizziness (seven subjects, 29%; six during treatment with pregabalin and one during treatment with ASP9226 50 mg). There were no clinically significant measurements in clinical laboratory findings, vital signs, or 12-lead electrocardiogram results.

Discussion

Summary of Study End Points

In this study, the analgesic and antihyperalgesic effects of single doses of ASP9226 were evaluated and

Table 3 PK parameters for ASP9226 and pregabalin

Study Treatment	C_{max}, ng/mL	t_{max}, h	AUC_8, ng*h/mL
ASP9226 (30 mg; n = 24)			
Median (min, max)	40.8 (10.6, 65.7)	2.08 (1.05, 4.05)	180 (65, 361)
%CV	39	34	39
GM	36.8	NA	189.1
ASP9226 (50 mg; n = 24)			
Median (min, max)	57.4 (30.5, 123.0)	2.05 (1.05, 3.10)	318 (144, 650)
%CV	40	33	40
GM	62.7	NA	325
Pregabalin (n = 24)			
Median (min, max)	3,322 (2,508, 5,258)	1.08 (1.05, 2.12)	15,442 (11,778, 18,339)
%CV	19	25	11
GM	3,428	NA	15,290

AUC_8 = Area under the concentration-time curve from the time of dosing to 8 h postdose; C_{max} = maximum concentration; CV = coefficient of variation; GM = geometric mean; NA = not assessable; PK = pharmacokinetic; t_{max} = time to reach maximum concentration.

Table 4 Summary of adverse events

Parameter, No. (%)	ASP9226 30 mg (n = 24)	ASP9226 50 mg (n = 24)	Pregabalin (n = 24)	Placebo (n = 24)
Any TEAE*	0	2 (8)	12 (50)	2 (8)
Drug-related TEAE	0	2 (8)	12 (50)	2 (8)
Serious TEAE	0	0	0	0
Incidence of TEAEs occurring in all subjects				
Dizziness	0	1 (4)	6 (25)	0
Nausea	0	1 (4)	1 (4)	0
Fatigue	0	0	4 (17)	0
Headache	0	0	0	2 (8)
Somnolence	0	0	1 (4)	0
Vertigo	0	0	3 (13)	1 (4)

AE = adverse event; TEAE = treatment-emergent adverse event.
*TEAE was defined as an AE that started or worsened in severity after first study drug intake.

compared with placebo and pregabalin in a healthy human subject hyperalgesia pain model using topical capsaicin. Treatment with ASP9226 (30 mg or 50 mg), compared with placebo, did not produce a difference greater than the 2.5 μV criteria that was predefined as the minimal clinically meaningful reduction in LEP N2-P2 PtP. However, treatment with pregabalin 150 mg showed differences vs placebo in LEP N2-P2 PtP amplitudes well above 2.5 μV, consistent with previous findings in healthy subjects [13], and confirmed the validity of the study design and method. In keeping with LEP results, subjects treated with pregabalin reported significantly lower postlaser VAS pain ratings compared with either dose of ASP9226 or placebo. The additional analysis of the LEP N2-P2 PtP amplitudes and VAS pain score in the capsaicin-positive subgroup had no significant impact on the results of the efficacy end points. In the smaller number of subjects who belonged to the capsaicin-positive subgroup, treatment with pregabalin

also produced significantly improved (reduced) LEP N2-P2 PtP amplitudes and VAS pain outcomes compared with ASP9226 or placebo. There was no difference in LEP N2-P2 PtP amplitudes between either dose of ASP9226 and placebo in the capsaicin-positive subgroup of subjects. The study was not powered to make comparisons between capsaicin-positive and capsaicin-negative subgroups.

Predictiveness and Validity of the Human Hyperalgesia Pain Model

In this study of ASP9226 with the capsaicin/LEP model, pregabalin performed as expected. Subjects treated with pregabalin showed a difference compared with placebo in LEP N2-P2 PtP amplitudes being well above the 2.5 μV value that was predefined as clinically relevant [19]. The significant difference from placebo was also observed in the VAS postlaser pain results, and

both LEP and VAS pain results were sustained up to the end of the daily experimental observation period. The LEP N2-P2 PtP profile of pregabalin in this study was similar to previous data in healthy subjects that showed reductions in PtP amplitudes of LEPs starting one to two hours postadministration and maintained for more than six hours postdose [13].

The study was approached by the investigators from the standpoint of using the LEP model as a developmental decision tool. Development of new drug compounds for treating neuropathic pain has a very high failure rate, due to positive findings in animal models not always being translated into clinical efficacy in patients. Compared with a typical PoC phase II study, the LEP model is sensitive in a small cohort of healthy subjects, is relatively inexpensive, and is swift to conduct and may prevent exposing a larger number of patients with PNP to an ineffective compound over longer treatment periods (including treatment wash-outs). The LEP model has successfully been used for early clinical development of pain medications [16,20], and a reduction in PtP amplitude has been reported as a reliable measure of the magnitude of antinociceptive/anti-hyperalgesic drug effects [19,21,22]. Given the success of the LEP model and the study design used here in identifying compounds with analgesic effects, combined with the confirmed effect of pregabalin in this study, the LEP results with ASP9226, at the dose levels tested, predict that a clinically relevant benefit in pain relief would not be demonstrated if a phase II PoC study with ASP9226 in patients with PNP were to be conducted. Despite this, the current results with pregabalin underline the predictive validity of the LEP model in healthy human subjects and further emphasize the value of this model as a potential decision tool for compounds in early clinical development to de-risk (i.e., probability of success, dose/regimen) potentially costly and resource-intensive PoC clinical trials in patients.

Although unpublished promising observations from animal studies of ASP9226 could not be translated into the present LEP study in healthy subjects, it is important to note that preclinical studies of pain in animal models are fundamentally behavioral measures of reaction time to heat or other stimuli, which may be one of the reasons for the failure of translation of pain studies in animals to humans [23]. Animal behavioral models of pain may not have construct or predictive validity when used to evaluate novel target mechanisms for neuropathic pain. Indeed, currently available drugs for PNP, such as pregabalin and duloxetine, were primarily developed for other indications (epilepsy and depression, respectively) prior to approval in PNP. As such, their efficacious dose range and knowledge of target modulation in patients had been demonstrated prior to assessing for therapeutic effects in neuropathic pain.

Future preclinical models may utilize more objective measures, such as electrophysiological responses or imaging, rather than behavioral measures, to improve model predictivity.

Similarly, using this model, it is difficult to speculate on the potential efficacy of ASP9226 for spontaneous pain. ASP9226 was evaluated using the whole-cell patch clamp method, demonstrating that it inhibited resting and inactivated N-channels in a concentration-dependent manner. As ASP9226 is an N-VDCC inhibitor, similar in many ways to pregabalin, and is active following single-dose administration, it has been inferred from in vitro and in vivo nonclinical studies that the onset of action is fairly rapid (i.e., within 15–30 minutes of administration). All animal models employ evoked or stimulated pain behavioral responses; therefore inferences on spontaneous pain using this model in healthy subjects are difficult compared with traditional animal experimental models.

PK and Safety Results

ASP9226 and pregabalin were rapidly absorbed. The intersubject variability in C_{max} and AUC_8 was similar for the 30-mg and 50-mg dose levels of ASP9226. Although the individual C_{max} and AUC_8 values of both dose levels of ASP9226 overlapped, there was a dose-proportional increase in the geometric mean values. Overall, single oral doses of ASP9226 30 mg and 50 mg were well tolerated in healthy male subjects in this study, with a higher incidence of TEAEs reported during treatment with pregabalin compared with ASP9226. Although the safety profile of ASP9226 is consistent with previous unpublished findings, further studies with longer duration, repeated doses, and in patients with PNP are needed to fully assess the safety of ASP9226.

Limitations

Although ASP9226 did not meet the predefined criteria for a clinically relevant response vs placebo or pregabalin treatment in this study, one possible reason for the lack of response to ASP9226 in the LEP model may be the doses of ASP9226 chosen for the present study. Although the doses used were the highest possible, based on the preclinical safety profile of the compound, the LEP response was still negative. However, the plasma concentrations of ASP9226 exceeded the predicted efficacious concentrations based on in vivo and in vitro preclinical models. Therefore, there were probably adequate ASP9226 concentrations available to inhibit activity at the N-channel target within the peripheral nervous system. Higher doses of ASP9226 were not administered in this study due to toxicity findings from previous nonclinical studies. The findings for ASP9226 in this study are unlikely to be a false negative owing to the several lines of evidence (e.g., in LEP *and* pain VAS) that support the observed lack of analgesic activity with single-dose ASP9226 administration in this model. ASP9226 inhibited the resting and inactivated states of N-VDCCs in a concentration-dependent manner, and inhibited the inactivated state of this channel with more potency than at resting state. Importantly, single doses of ASP9226 administered to in vivo animal models of evoked pain showed good analgesic activity (unpublished

findings), thus providing support that the single-dose administration of ASP9226 in the current human model of pain would be acceptable to identify potential analgesic activity. A further consideration is that preclinical models of PNP are not predictive of responses in patients with neuropathic pain, and it would be interesting if the human LEP results from this study could be replicated in an animal version of the LEP model. However, the primary value of this translational healthy volunteer experimental pain model is to make validated predictions in patients with pain, rather than to validate the predictability of the animal models.

Conclusion

Single oral doses of 30 mg and 50 mg ASP9226 were well tolerated in healthy male subjects. Treatment with ASP9226 did not induce a response that was considered to be a clinically meaningful reduction in LEP and VAS pain. Therefore, there was little confidence that further development of ASP9226 for the treatment of PNP would be successful. Subjects treated with pregabalin, the active control, showed a positive response-confirming the validity of this study. The results of this study further support the utilization of human phase I LEP models in a small number of healthy subjects to make validated predictions prior to PoC clinical studies in patients with, among other types of pain, PNP [13,19–22].

Supplementary Data

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

Authors' Contributions

KS conducted this study as the Principal Investigator, and JP was involved in the trial design and interpretation of the results. All authors provided guidance in the analysis and interpretation of the data, and provided contributions to the writing, revising, and review of the manuscript.

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