Pain Medicine, 22(8), 2021, 1814–1826
doi: 10.1093/pm/pnab032
Advance Access Publication Date: 5 February 2021
Original Research Article

A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Crossover Study to Evaluate the Pharmacodynamic Effects of VX-150, a Highly Selective $Na_V1.8$ Inhibitor, in Healthy Male Adults

Hemme J. Hijma, MSc*,† Pieter S. Siebenga, MD, PhD*,† Marieke L. de Kam, MSc* and Geert Jan Groeneveld, MD, PhD*,†

*Centre for Human Drug Research, Leiden, The Netherlands; †Leiden University Medical Center, Leiden, The Netherlands

Correspondence to: H. J. Hijma, MSc, Zernikedreef 8, 2333 CL Leiden, The Netherlands. Tel: 0031 71 5246 400; Fax: 0031 71 5246 499; E-mail: HHijma@chdr.nl.

Funding source: The study was funded by Vertex Pharmaceuticals, Incorporated.

Conflict of interest: The authors report no conflicts of interest.

Trial registration: ToetsingOnline number NL63609.056.17 and EudraCT number 2017-003557-42.

Abstract

Objective. To evaluate the analgesic potential, safety, tolerability, and pharmacokinetics of VX-150, a pro-drug of a highly selective $Na_V1.8$ inhibitor, in healthy subjects. **Design**. This was a randomized, double-blind, placebo-controlled, crossover study in healthy subjects. **Subjects**. Twenty healthy male subjects with an age of 18–55 years, inclusive, were enrolled. Eligibility was based on general fitness, absence of current or previous medical conditions that could compromise subject safety, and a training assessment of pain tolerance across pain tests to exclude highly tolerant individuals whose tolerance could compromise the ability to detect analgesic responses. All dosed subjects completed the study. **Methods**. Subjects were randomized 1:1 to one of two sequences receiving a single VX-150 dose and subsequently placebo, or vice versa, with at least 7 days between dosing. A battery of pain tests (pressure, electrical stair, [capsaicin-induced] heat, and cold pressor) was administered before dosing and repetitively up to 10 h after dosing, with blood sampling up to 24 h after dosing. Safety was monitored throughout the study. Data were analyzed with a repeated-measures mixed-effects model. **Results**. VX-150 induced analgesia in a variety of evoked pain tests, without affecting subject safety. Significant effects were reported for the cold pressor and heat pain thresholds. Maximum median concentration for the active moiety was 4.30 μg/mL at 4 h after dosing. **Conclusion**. Results of this proof-of-mechanism study are supportive of the potential of VX-150, a highly selective $Na_V1.8$ channel inhibitor, to treat various pain indications.

Key Words: $Na_V1.8$-Selective Inhibitor; VX-150; Experimental Pain

Introduction

Pain is a protective mechanism designed to prevent tissue injury, but when persisting beyond its usefulness, pain results in one of the most common and incapacitating chronic disorders for which patients seek medical attention. Although a variety of treatment options are available, current pharmacological therapies suffer from poor efficacy or a high risk of adverse events [1]. For example, systemic lidocaine (a nonselective sodium channel blocker) may effectively reduce pain, but its utility is limited because of prominent side effects when given at dose levels that are required for pain relief [2, 3]. Opioids, though prominently and ever increasingly used in the treatment of pain, have a high abuse liability. Annual deaths due to opioid overdose numbered approximately 47,000 in the United States in 2018 and were estimated

to between 10,000 and 20,000 in Europe in 2014 [4, 5]. Moreover, with long-term use, opioids induce pain (i.e., hyperalgesia) instead of providing the intended pain relief.

The limited treatment options currently available—especially for patients suffering from chronic pain—and growing awareness of the risks that are associated with the standards of care underscore the need for new pharmacological treatment options to manage pain. Certain subtypes of the voltage-gated sodium channels (Na_V), which facilitate electrical signaling in neurons [6], have been identified as potential targets for selective analgesic drugs aimed at providing pain relief without unwanted side effects. The role these channels play in normal physiology, in pathological states arising from mutations in sodium channel genes and animal models, as well as the pharmacology of known sodium channel–modulating agents, together indicate that $Na_V1.3$, $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ can play critical roles in pain signaling [7–9]. Of these Na_V subtypes, $Na_V1.8$ is a sensory neuron–specific channel with preferential expression in the dorsal root ganglion and trigeminal ganglion neurons [10]. $Na_V1.8$ is highly expressed on nociceptors, where it mediates pain sensation and chronic pain [11]. As such, $Na_V1.8$ gain-of-function mutations are thought to directly cause chronic pain in patients with painful small-fiber neuropathy [12–14]. Moreover, $Na_V1.8$ has been found to quickly recover from inactivation and to exhibit a more depolarized voltage dependency of (in)activation than other named subtypes [15], which highlights its involvement in repetitive firing and neuronal excitability [11] and thus central sensitization and chronification of pain. Inhibiting $Na_V1.8$ has been found to result in analgesia [16, 17], a finding that supported the channel as a pharmacological target and showed that selective $Na_V1.8$ inhibitors may have the potential to treat pain in which the primary mechanism of pain is nociceptor hyperexcitability.

VX-150 is an orally bioavailable pro-drug that rapidly converts into its active moiety, which is a highly selective inhibitor of $Na_V1.8$ relative to the other sodium channel subtypes (>400-fold). VX-150 is being developed for the treatment of pain. To investigate the analgesic potential of novel compounds such as VX-150 in early-phase trials with healthy volunteers, evoked pain tests may be included in the design. A variety of pain tests related to different mechanisms that are involved in clinical pain have been developed to inform the investigator on the analgesic potential of a new investigational product. A comprehensive battery of different pain tests has been developed at our institution, which allows measurement of different mechanisms involved in nociception in an integrated manner and in a fixed and repeated fashion over time [18]. Previously, this pain test battery has been used to show the analgesic potential—and lack thereof—of a variety of analgesic compounds, including certain Na_V inhibitors [19, 20]. In the present study, we evaluated the analgesic potential of VX-150 in healthy males in a placebo-controlled crossover fashion, and we report the effects of VX-150 at a multitude of end points. As literature suggests that the pain perception of women may change across phases of the menstrual cycle [21–23], we limited our study to men only to reduce variability and increase the chance of demonstrating a treatment effect in a phase 1 trial setting.

Methods

The study was conducted at the Centre for Human Drug Research (CHDR) in Leiden, The Netherlands, and was executed in accordance with the Declaration of Helsinki (1964; amended most recently in 2008) of the World Medical Association and the Guideline for Good Clinical Practice. Before the start of the procedures, the study received Medical Ethics Committee approval from Stichting Beoordeling Ethiek Biomedisch Onderzoek (BEBO), Assen, The Netherlands. The study was registered under ToetsingOnline number NL63609.056.17 and EudraCT number 2017-003557-42.

Design

This was a Phase 1, randomized, double-blind, placebo-controlled, two-way crossover study to evaluate the analgesic effects of VX-150 in healthy adult male subjects (Figure 1). A randomized crossover design was chosen to enhance the power to detect treatment differences by reducing the variability, which is lower when a within-subject comparison is used than the between-subject variability of a parallel-arm study. Male subjects with an age of 18–55 years, inclusive, were screened for general fitness and current or previous medical conditions that could put the subject at risk or bias the study results (e.g., neurological, mental, or cardiovascular disease; [chronic] pain; significant allergies; malignancies or conditions affecting drug absorption). All participants voluntarily provided written informed consent before any of the study assessments. Any information, including illustrations, is as anonymized as possible to comply with privacy regulations.

Twenty male subjects were enrolled in a 1:1 ratio to one of the two treatment sequences (i.e., 10 subjects per sequence) to receive a single dose of VX-150 or placebo, in two treatment periods (Figure 1). A washout period of at least 7 days was used between the two periods. Screening procedures occurred within 28 days before admission to the clinical research unit on Day –1 of the first treatment period; a safety follow-up visit 5–9 days after the last dosing day completed study participation. Both treatment periods consisted of an in-house period of two nights and one full study day each. Blood sampling for pharmacokinetics and a panel of pain tests, as described in the *Study Procedures: Pharmacodynamic* section, was performed on Day 1 in both treatment periods.

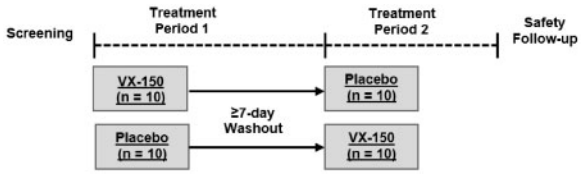

Figure 1. Study design. Twenty subjects were randomized and equally allocated to one of the two treatment sequences. n = number of subjects.

Study Drug VX-150 and Placebo Administration Procedures

During each treatment period, a single dose of VX-150 1,250 mg or placebo was administered as a capsule on the morning of Day 1 with the participant in a fasted state. Compliance to dosing was confirmed with a hand-and-mouth check. The 1,250-mg dose was chosen on the basis of previous studies with VX-150, where it was found to be safe and well tolerated. Results of those studies also indicated that maximum pharmacodynamic effects were expected to be observed for the present study when using this dose (unpublished data).

Study Procedures: Safety

Safety evaluations included adverse event monitoring, clinical laboratory assessments, clinical evaluation of vital signs, standard 12-lead electrocardiograms, and physical examinations.

Study Procedures: Pharmacodynamic

During each treatment period, nociceptive (pain) detection and tolerance thresholds were evaluated repeatedly over time with a validated battery of evoked pain models, in the following sequence: electrical stair pain test 1, pressure pain test, cold pressor pain test, electrical stair pain test 2, heat pain test on untreated skin, and heat pain test on capsaicin-treated skin. The heat pain test on capsaicin-treated skin and untreated skin were switched before dosing to allow for the pre-dosing heat pain test on capsaicin-treated skin to be performed 30 minutes after capsaicin administration while keeping remainder of the sequence intact (see details of the capsaicin model in the *Application of Capsaicin 1% Cream; Capsaicin-Induced Pain Test and Heat Pain Test* section). Before enrollment and as part of the screening procedures, subjects received a training session in order to minimize learning effects and to exclude from study participation any subjects who were too sensitive to or tolerant of the tests. Excessive tolerance was defined as achieving tolerance at more than 80% of the maximum input intensity for the cold pressor, electrical, or pressure pain tests. Subjects were allocated to separate rooms without any form of distraction, where they were asked to sit in a chair. For all but the thermal and capsaicin tests (see *Application of Capsaicin 1% Cream; Capsaicin-Induced Pain Test and Heat Pain Test*), subjects were given an electronic visual analog scale (eVAS) slider to hold, with which they could indicate their current perceived pain intensity. The eVAS had a range of 0–100, with eVAS = 0 defined as "no pain," eVAS > 0 defined as the pain detection threshold (PDT), and eVAS = 100 defined as the pain tolerance threshold (PTT; "worst pain tolerable"). When PTT was reached, the test automatically stopped and immediately relieved subjects from their pain. For each measurement, eVAS vs. time was used to calculate the area above the eVAS pain curve (AAC; for the cold pressor pain test) or area under the eVAS pain curve (AUC; for the pressure test, electrical stair pain test, and conditioned pain modulation response [CPM]). In both treatment periods, the complete test sequence was performed twice before dosing and at 1, 2, 4, 7, and 10 h after dosing.

Electrical Stimulation Pain Test

The method of electrical stimulation is based on that of Arendt-Nielsen et al. and was used in previous studies in which the same integrated pain test battery was used [19, 24–26]. The test has been shown to assess nociception generated primarily from the Aδ- and C-sensory afferent fibers, which pass nociceptive signals from the periphery to the spinal cord. The Aδ-fibers conduct the signal relatively rapidly, causing the sharp localization of pain and the rapid spinal response that is perceived during a transcutaneous electrical stimulus [27].

Two electrodes (Ag-AgCl) were positioned on clean (if needed, scrubbed) skin on the left tibial bone. The location of the first electrode was 100 mm distal to the caudal end of the patella; the second electrode was located 135 mm directly underneath the first. Resistance between the electrodes was less than 2 kΩ. The single (stair) stimuli that were given (10-Hz tetanic pulse, duration of 0.2 ms) were controlled by a computer-controlled constant current stimulator. The intensity of the current increased from 0 mA in steps of 0.5 mA/s. Pain intensity was measured with the eVAS until PTT or the maximum output of 50 mA was reached.

Pressure Pain Test

The method used to induce pressure pain in the present study has been shown to assess nociception generated primarily from the muscle with minimal contribution from cutaneous nociceptors [28], and it is based on previously described methods [29].

A constant pressure, increasing at a rate of 0.5 kPa/s (controlled by an electro-pneumatic regulator [ITV1030-31F2N3-Q, SMC Corporation, Tokyo, Japan], Power1401mkII analog-to-digital converter and Spike2 software [CED, Cambridge, UK]), was forced on the gastrocnemius muscle via an 11-cm-wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany). Pneumatic pressure increased until the subject indicated his PTT or a maximum pressure of 100 kPa was

achieved, at which point the device released pressure to the tourniquet.

Cold Pressor Pain Test

For the cold pressor test, an extremity (in the present study, a hand) is submerged in cold water. This assessment is used in clinical studies to investigate cardiovascular responses, nociception, and opioid-induced hyperalgesia and to induce a CPM response (previously termed diffuse noxious inhibitory control [DNIC]-like effects; see the *Conditioned Pain Modulation* section) [30, 31]. The method used here is based on methods described previously [19, 32, 33]. In summary, the subject was asked to put his nondominant hand into a water bath with circulating water (minimal depth 200 mm) at 35 ± 0.5°C for 2 min. At 1 min 45 s, a blood pressure cuff on the upper arm—placed there before the start of the test—inflated to 20 mm Hg below resting diastolic pressure, to minimize the return of warm blood to the hand. At 2 min, the subject moved his nondominant hand from the warm water bath to a similar-sized bath with circulating water at 1.0 ± 0.5°C. Subjects were instructed to indicate their PDT (i.e., first change in sensation from cold non-painful to painful), increase in pain intensity, and PTT (i.e., the cold sensation was no longer tolerable) by using the eVAS slider. When PTT was reached or when the nondominant hand had been in the 1.0°C water for 120 s, subjects removed their hand from the water, at which point the blood pressure cuff would also deflate. Time to reach PDT and PTT or the time limit of 120 s was used for analysis.

Conditioned Pain Modulation

The effects of VX-150 on the descending inhibitory control pathway were evaluated via the CPM paradigm [30]. By calculating the difference of PDTs and PTTs of the electrical stair pain test directly after the cold pressor pain test minus the electrical stair PDTs and PTTs before the cold pressor pain test, a possible modulatory effect was quantified.

Application of Capsaicin 1% Cream; Capsaicin-Induced Pain Test and Heat Pain Test

The capsaicin 1% model was included as a model for thermal allodynia by selectively sensitizing the transient receptor potential cation channel subfamily V member 1 (TRPV1) channel [34–36].

Capsaicin 1% cream, produced according to the Formulary of Dutch Pharmacists (Formularium der Nederlandse Apothekers [FNA]), was applied during screening procedures to evaluate whether subjects were hyper-responsive to the cream, and in both treatment periods it was applied 60 min before study drug administration. A 3 × 3-cm surface on the dominant volar forearm was used for the application of the 1% capsaicin cream, after which the area was covered by a cotton gauze. The nondominant volar forearm served as a nonstimulated control (i.e., not treated with capsaicin). Thirty minutes after application, the cream was wiped off toward the center of the application site.

Immediately afterward and subsequently at given time points, heat PDTs were determined on the capsaicin-treated skin (on the dominant volar forearm), as well as on normal (nonstimulated) skin (on the nondominant volar forearm). To evaluate these PDTs, a thermode (Q-Sense, Medoc, Ramat Yishay, Israel) with a contact area of 3 cm × 3 cm was placed on a marked area on the subject's nondominant volar forearm and on a marked area on the subject's dominant volar forearm (on which the capsaicin had been applied). Starting at 32°C, the temperature of the thermode increased by 0.5°C/s until the subject perceived the stimulus as painful (PDT) or a temperature of 50°C was reached. PDT was recorded by the subject by pushing a button on the handheld feedback control. The average of a triplicate measurement was used for analysis.

Study Procedures: Pharmacokinetics

Plasma pharmacokinetic parameters were assessed for the active moiety of VX-150 and its major circulating metabolite. Blood was sampled before dosing (0 h) and at 0.5, 1, 1.5, 2, 3, 4, 5, 7, 10, 12, and 24 (Day 2) h after dosing in both treatment periods.

Statistical Considerations and Analysis

Sample Size

The sample size of 20 subjects was chosen on the basis of known variability in the cold pressor and capsaicin pharmacodynamic assessments [19, 26] and was considered sufficient to meet the objectives of the study. For a one-sided significance level of 0.05, there was at least 80% power to detect a standard effect size of 0.6.

Statistical Analysis

Demographic and pharmacokinetic data are presented as mean ± standard deviation (SD). Analyses of plasma concentration vs. time data for the active moiety of VX-150 and its circulating metabolite were determined through the use of standard noncompartmental methods.

The period baseline value was defined as the average of the nonmissing pretreatment measurements for all pain tests, except the capsaicin-induced pain test. For the capsaicin-induced pain test, the second pre-dosing assessment served as the baseline, given that there was no capsaicin applied before this assessment taking place.

The change from period baseline in each primary end point was analyzed as a dependent variable with a repeated-measures mixed model, with sequence, period, treatment, time point within period, and treatment–by–time point interaction as fixed effects, and subject nested within sequence as a random effect. Denominator degrees of freedom for the *F* test for fixed effects were

estimated with the Kenward-Roger approximation. The least-squares mean and the 95% confidence interval (95% CI) of treatment difference at each post-dosing time point is given. For the secondary end points, a summary of raw values and the change from period baseline values is provided for each scheduled time point by treatment group and overall with descriptive statistics.

To calculate the effect size—defined as the estimate of the difference between the VX-150 and placebo—over the whole period, all repeatedly measured parameters were analyzed with a mixed-effects model in which treatment, time, and treatment by time were fixed factors; subject, subject by treatment, and subject by time were random factors; and the (average) period baseline measurement was the covariate. The Kenward-Roger approximation was used to estimate denominator degrees of freedom, and model parameters were estimated with the restricted maximum likelihood method. The cold pressor AAC, PDT, and PTT and the pressure PDT and PTT were log-transformed before analysis because of their log-normal distribution. Results were back-transformed and expressed as the percentage difference for the estimated difference between treatments.

The effect size calculation was performed post hoc to compare the study results with results of previous studies that had used the same pain test battery at an exploratory level [19, 20, 26]. All statistical inferences and *P* values were also exploratory. Therefore, no multiplicity adjustment was performed for any of the pharmacodynamic analyses.

Results

Baseline Characteristics

Twenty male subjects were enrolled and completed all study assessments. Mean age was 27.9 ± 8.6 years, most (70%) were of Caucasian descent, and mean body mass index was 23.18 ± 2.77. Demographic and baseline characteristics are given in Table 1.

Table 1. Demographic and other baseline characteristics, represented as mean (± SD) of total subject set

Demographic Category	Number (N = 20)
Sex, n (%)	
Male	20 (100.0)
Age, y	
Mean ± SD	27.9 ± 8.6
Race, n (%)	
Caucasian	14 (70.0)
Black or African American	3 (15.0)
Asian	1 (5.0)
Mixed	1 (5.0)
Other	1 (5.0)
Weight, kg	
Mean ± SD	74.6 ± 10.3
Height, cm	
Mean ± SD)	179.3 ± 6.6
Body mass index, kg/m^2	
Mean ± SD	23.18 ± 2.77

Pharmacodynamic Results

Primary End Points

Significant effects of VX-150 were observed on the cold pressor and heat pain tests (Table 2). For the cold pressor test PTT, least-squares mean changes from baseline were substantially higher in the VX-150 1,250 mg treatment group than in the placebo group from 2 h through 10 h after dosing; the largest treatment effect was observed at 4 h after dosing, although it also significantly differed at 2, 4, 7, and 10 h after dosing (least-squares mean difference for 95% CI [LSM 95% CI], placebo vs. VX-150 per time point: at 1 h, –1.92 to 5.31; at 2 h, 0.8 to 20.12; at 4 h, 12.74 to 42.72; at 7 h, 8.43 to 32.33; and at 10 h, 8.43 to 32.33). For heat PDTs on untreated skin ("normal heat PDTs"), thresholds in the placebo group were consistently lower than in the VX-150 group at each time point but significantly differed only at 2 h and 10 h after dosing (Table 2) (LSM 95% CI, placebo vs. VX-150 per time point: at 1 h, –0.4751 to 1.0208; at 2 h, 0.0806 to 1.5764; at 4 h, –0.0716 to 1.4242; at 7 h, –0.0234 to 1.4724; and at 10 h, 0.1822 to 1.7070). No significant effects were reported for capsaicin-induced PDT or for electrical stimulation, pressure, or CPM PTT.

Secondary End Points

For the electrical stimulation and cold pressor pain tests, PDTs were higher after VX-150 treatment than after placebo at each time point but did not greatly differ (Table 2). CPM and pressure PDT results did not evidently differ between treatments.

Treatment Effect over Time (Effect Size Analysis over 10 h)

Cold pressor PTT displayed the largest effect size (53.7%) (VX-150 vs. placebo) when analyzed over the full time course of 10 h, followed by pressure PTT (6.76%), electrical stair PTT (2.76%), capsaicin heat PDT (1.81%), and heat pain PDT (1.6%) (Figure 2 and Table 3). Effects were significant for both cold pressor PTT and AAC (PTT: $P < 0.001$, estimate of difference [ED] 53.7%, 95% CI 24.9% to 89.2%; AAC: $P = 0.002$, ED 43.7%, 95% CI 16.2% to 77.3%), as well as for heat pain PDT ($P = 0.01$, 95% CI 0.16 to 1.23) (Table 3). Results for other end points were not significant.

Safety

Overall, VX-150 was well tolerated with no significant findings during study execution. Adverse events that were reported were evaluated to be mild or moderate in severity. The incidence of adverse events was comparable in subjects receiving placebo or VX-150 treatment, and none led to study discontinuation. Most reported adverse events were headache and catheter site pain. There were

Table 2. Primary analysis for pain thresholds

	Pain Test End points			
	PDT		PTT	
Pain Modality	Placebo	VX-150	Placebo	VX-150
Capsaicin, °C				
Baseline	36.45 ± 2.25	35.69 ± 2.09		NA
1 h	39.71 ± 2.59	39.91 ± 2.86 (–0.56 to 2.48)		NA
2 h	40.17 ± 2.55	40.32 ± 3.17 (–0.73 to 2.55)		NA
4 h	40.14 ± 2.86	40.84 ± 3.09 (–0.19 to 3.12)		NA
7 h	40.98 ± 2.83	41.41 ± 2.90 (–0.42 to 2.82)		NA
10 h	41.23 ± 2.54	41.53 ± 3.12 (–0.53 to 2.64)		NA
Heat, °C				
Baseline	44.63 ± 2.81	44.50 ± 2.32		NA
1 h	44.09 ± 2.84	44.16 ± 2.33 (–0.48 to 1.02)		NA
2 h	43.7 0 ± 3.35	***44.39 ± 2.65 (0.08 to 1.58)***		NA
4 h	43.58 ± 3.14	44.15 ± 2.87 (–0.07 to 1.42)		NA
7 h	43.32 ± 3.20	43.94 ± 2.63 (–0.02 to 1.47)		NA
10 h	43.16 ± 3.67	***44.07 ± 3.20 (0.18 to 1.71)***		NA
Cold pressor, s				
Baseline	6.86 ± 4.83	6.09 ± 3.82	21.00 ± 12.19	21.48 ± 11.60
1 h	8.12 ± 6.26	7.02 ± 4.68	21.17 ± 11.06	23.43 ± 11.87 (–1.92 to 5.32)
2 h	6.53 ± 4.73	8.37 ± 8.02	23.21 ± 13.36	***34.78 ± 25.92 (0.80 to 20.12)***
4 h	6.56 ± 4.19	7.52 ± 7.98	20.46 ± 11.53	***48.63 ± 37.19 (12.74 to 42.72)***
7 h	6.22 ± 5.18	8.27 ± 7.71	18.52 ± 11.71	***40.59 ± 32.19 (8.43 to 32.33)***
10 h	6.05 ± 5.35	10.05 ± 13.68	20.00 ± 12.75	***40.89 ± 32.45 (6.16 to 33.03)***
Electrical, mA				
Baseline	7.56 ± 4.86	7.41 ± 3.88	18.45 ± 7.05	18.66 ± 7.39
1 h	6.98 ± 4.28	7.68 ± 5.20	18.71 ± 7.62	18.60 ± 7.64 (–1.90 to 1.29)
2 h	7.77 ± 4.05	8.09 ± 5.90	19.09 ± 7.12	19.39 ± 7.89 (–1.61 to 1.78)
4 h	8.28 ± 4.27	9.02 ± 6.75	18.38 ± 6.58	19.66 ± 8.77 (–1.11 to 3.14)
7 h	7.91 ± 4.22	9.27 ± 6.17	18.93 ± 7.20	20.54 ± 8.03 (–1.00 to 3.62)
10 h	8.62 ± 5.62	9.29 ± 6.69	20.46 ± 8.33	21.24 ± 7.61 (–2.36 to 3.21)
Pressure, kPa				
Baseline	21.27 ± 13.49	22.66 ± 12.78	48.31 ± 19.81	47.42 ± 15.37
1 h	23.63 ± 16.95	22.82 ± 13.76	49.97 ± 18.25	48.31 ± 17.27 (–7.55 to 5.32)
2 h	24.82 ± 15.15	24.70 ± 17.10	52.46 ± 20.80	53.04 ± 21.66 (–4.00 to 7.62)
4 h	24.79 ± 16.40	25.38 ± 18.49	53.48 ± 18.11	55.03 ± 22.07 (–3.87 to 9.65)
7 h	22.83 ± 14.89	25.02 ± 18.60	48.92 ± 16.99	53.92 ± 21.10 (–1.17 to 11.63)
10 h	22.35 ± 14.18	25.70 ± 17.90	49.28 ± 19.97	54.86 ± 24.78 (–3.11 to 15.72)

(continued)

Table 2. continued

	Pain Test End points			
	PDT		PTT	
Pain Modality	Placebo	VX-150	Placebo	VX-150
CPM, mA				
Baseline	0.44 ± 1.44	0.21 ± 2.51	0.64 ± 1.64	0.72 ± 1.29
1 h	1.38 ± 3.57	1.05 ± 2.21	1.19 ± 1.55	0.86 ± 1.36 (–1.72 to 0.84)
2 h	0.94 ± 2.56	1.24 ± 2.83	0.81 ± 1.60	1.50 ± 2.14 (–0.78 to 1.80)
4 h	0.13 ± 2.62	1.01 ± 2.36	1.14 ± 1.51	0.82 ± 1.94 (–1.61 to 0.96)
7 h	1.16 ± 2.29	0.71 ± 3.11	1.46 ± 1.79	1.07 ± 1.99 (–1.63 to 0.93)
10 h	0.95 ± 1.34	1.20 ± 2.77	1.14 ± 1.56	1.44 ± 2.39 (–1.14 to 1.45)

Values represent mean ± SD. LSM 95% CIs are presented in parentheses in the VX-150 column for the primary end points (i.e., capsaicin PDT, heat PDT, cold pressor PTT, electrical PTT, pressure PTT, and CPM PTT). Only descriptive analysis was performed for the other (secondary) end points. Numbers in boldfaced italic denote time points at which the LSM 95% CI between the placebo and VX-150 group excluded zero and represent the treatment that was favored (e.g., if in the right column, the interval favored VX-150). °C = degrees Celsius; CPM = conditioned pain modulation; h = hour; kPa = kilopascal; mA = milliampere; PDT = pain detection threshold; PTT = pain tolerance threshold; s = second; SD = standard deviation.

no clinically meaningful changes or trends in laboratory (chemistry, hematology, coagulation, and urinalysis) values, vital sign measurements, or electrocardiograms. Two subjects (10%), while in the VX-150 treatment group, received an analgesic (ibuprofen and paracetamol) as concomitant medication to treat headache. In both cases, the medication was administered well after the last pain test had been performed.

Pharmacokinetic Results

Mean plasma concentration-time profiles of the active moiety and its major circulating metabolite after the administration of single oral doses of 1,250 mg VX-150 are presented in Figure 3, and related parameters may be found in the Supplementary Data. After having increased to its peak concentration at 4.30 µg/mL at 4 h after dosing, VX-150 gradually decreased, which shows a pharmacokinetic profile that is common for a capsule formulation and in line with results from earlier studies evaluating the pharmacokinetics of VX-150 in a capsule formulation (unpublished data).

Discussion

This study was performed to evaluate the analgesic potential of a single dose of VX-150 in a panel of pain tests in healthy adult male subjects. Overall, VX-150 demonstrated an analgesic response at up to 10 h for a subset of pain tests without displaying any notable adverse effects, thereby favoring $Na_V1.8$ inhibitor VX-150 as a potential treatment for pain.

Despite the fact that selective voltage-gated sodium channel inhibitors have been considered as an important possible alternative to opioids in pain treatment [6, 8], they have yet to live up to that promise. As such, multiple studies could not report analgesic effects for various selective voltage-gated sodium channel inhibitors [6, 20, 37]. The present study is the first to report significant analgesic effects of a selective $Na_V1.8$ inhibitor in a human experimental pain study, favoring the use of selective $Na_V1.8$ inhibitors as analgesics. Here, we show that VX-150 primarily influenced cold pressor pain thresholds, most likely by indirectly modulating the activity of transient receptor potential subfamily M, member 8 (TRPM8). This nonselective ion channel is present on both Aδ- and C-fibers, where it is activated by cooling agents, such as menthol, and cold temperatures, as during the cold pressor pain test [38, 39]. TRPM8-mediated pain sensation occurs through increased calcium influx of voltage-gated calcium channels after activation of $Na_V1.8$. When this activation is blocked by VX-150, however, pain relief is achieved. The interplay between TRPM8 and $Na_V1.8$ has previously been described in both models of sensory neurons and breast cancer [40, 41]. Although $Na_V1.8$ is not directly affected by heat, it is essential for the propagation and sustenance of the pain signal that follows activation of heat-sensitive TRPV1 and -3 channels, which explains the significant effects reported for the heat pain test and the suggestive (nonsignificant) effects over time for capsaicin-induced hyperalgesia [42–44]. Effects of VX-150 on capsaicin-induced pain thresholds were also expected, as capsaicin induces an inflammatory-like hyperalgesia that can be attenuated only by blocking tetrodotoxin-resistant channels such as $Na_V1.8$ [45]. Given the sample size of the present study, the limited effect size and variability

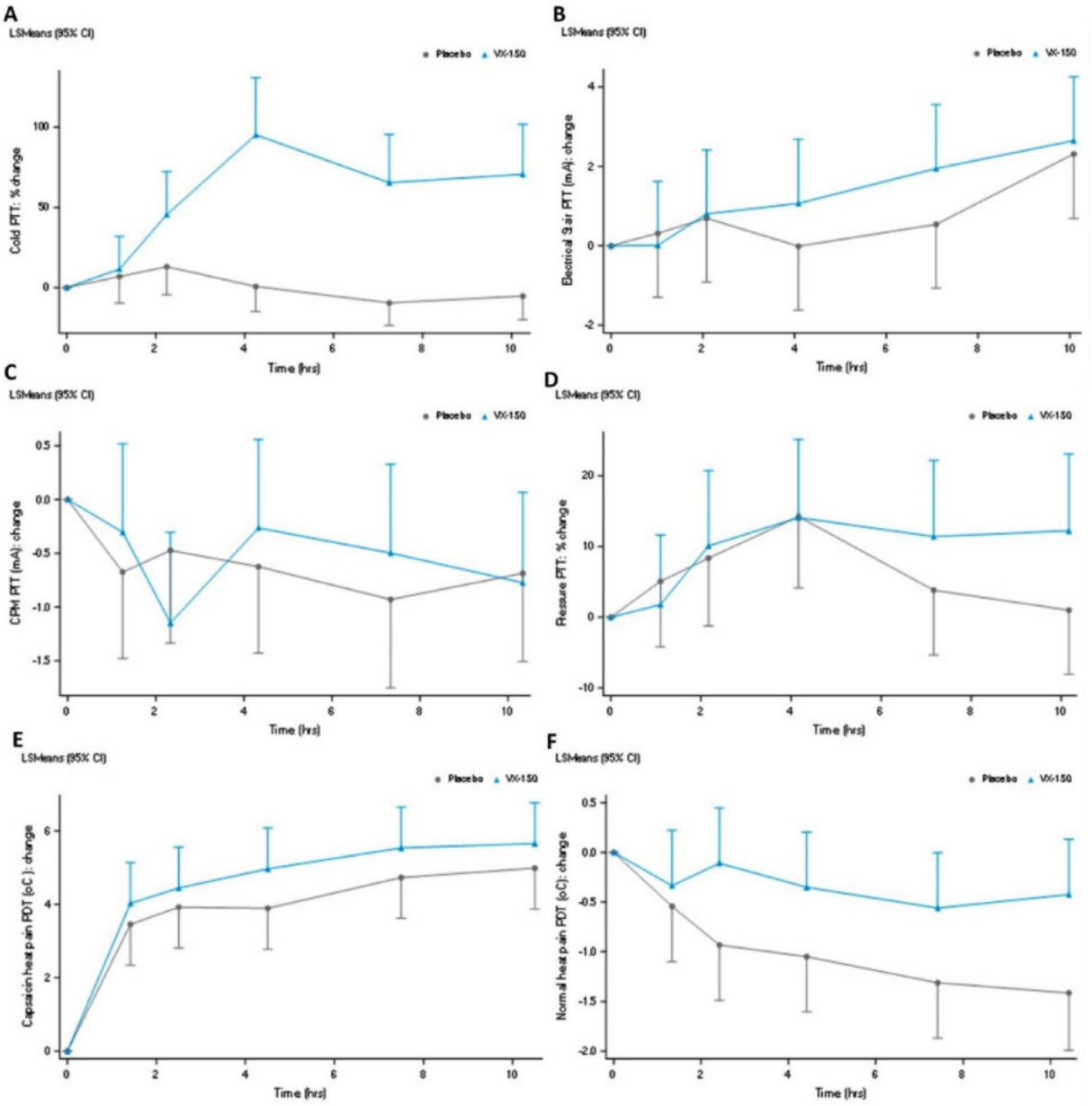

Figure 2. Primary evoked pain test endpoints, represented as change from baseline in percentages (%). Baseline has been defined as the average of two pre-dosing measurements of that occasion, except for the capsaicin-induced PDT. For this test, the second pre-dosing assessment served as baseline, given that there was no capsaicin applied before this assessment. Values on the y-axis represent the least-squares means change and the 95% CI. Time is shown in hours on the x-axis. **(A)** Cold pressor PTT. **(B)** Electrical stair PTT. **(C)** CPM PTT. **(D)** Pressure PTT. **(E)** Heat PDT on capsaicin-treated skin ("capsaicin heat PDT"). **(F)** Heat PDT on untreated skin ("normal heat PDT"). °C = degrees Celsius; CPM = conditioned pain modulation; mA = milliamperes; PDT = pain detection threshold; PTT = pain tolerance threshold.

observed within our test results (Table 2) may have prevented the VX-150–treated group from differing significantly from placebo (Figure 2). These assumptions also hold true for the electrical pain, CPM, and pressure pain paradigms, as no significant effects could be noted for these tests. Although reasons are speculative, plausibly the absence of effects on the electrical pain test—which induces pain by activating nerves directly, bypassing the sensory nerve endings—may be due to the test not specifically activating nociceptors and therefore not being modulated by alterations in $Na_V1.8$ signaling [24]. For CPM, it may be that VX-150 has an insufficient role in the inhibitory descending pain pathway, but, as stated, the nonsignificant response could just as likely be attributed to individual subject variability, given that CPM is particularly influenced by this [30, 46]. For pressure pain, the tolerance increase observed in the placebo group up to 4 h after dosing (Figure 2) may have diminished the treatment effect reported for VX-150. However, it may also be worth considering that a

Table 3. Evoked pain test results: effect size analysis from before dosing up until 10 h after dosing

	Pain Test Modalities (Contrast Placebo vs. VX-150)					
	Capsaicin	Heat	Cold Pressor	Electrical	Pressure	CPM
PDT	0.728 °C (*P* = 0.07) (–0.07 to 1.53)	*0.694 °C* *(P = 0.01)* *(0.16 to 1.23)*	14.8% (*P* = 0.488) (–23.9 to 73.0)	0.88 mA (*P* = 0.137) (–0.31 to 2.07)	–12.3% (*P* = 0.154) (–27.2 to 5.6)	–0.147 mA (*P* = 0.692) (–0.94 to 0.65)
PTT			*53.7%* *(P < 0.001)* *(24.9 to 89.2)*	0.53 mA (*P* = 0.428) (–0.84 to 1.89)	3.2% (*P* = 0.557) (–7.7 to 15.4)	0.080 mA (*P* = 0.748) (–0.439 to 0.60)
AAC/AUC			*43.5%* *(P = 0.002)* *(16.2 to 77.3)*	-61.80 mA*% (*P* = 0.333) (–192.47 to 68.87)	–197.28 % (*P* = 0.445) (–728.62 to 334.05)	24.38 mA*% (*P* = 0.4098) (–36.86 to 85.62)

Numbers represent estimates of the difference with *P* values and 95% CIs in parentheses. Values are presented in % for tests for which the data were log-transformed (i.e., cold pressor and pressure pain tests). Otherwise, data are given in the unit in which they were measured. Values in boldfaced italic denote nominal significance ($P < 0.05$). Estimates >0 favor VX-150. Estimates <0 favor placebo. °C = degrees Celsius; CPM = conditioned pain modulation paradigm; AAC/AUC = area above/under the eVAS pain curve; eVAS = electronic Visual Analogue Scale; mA = milliampere; PDT = pain detection threshold; PTT = pain tolerance threshold.

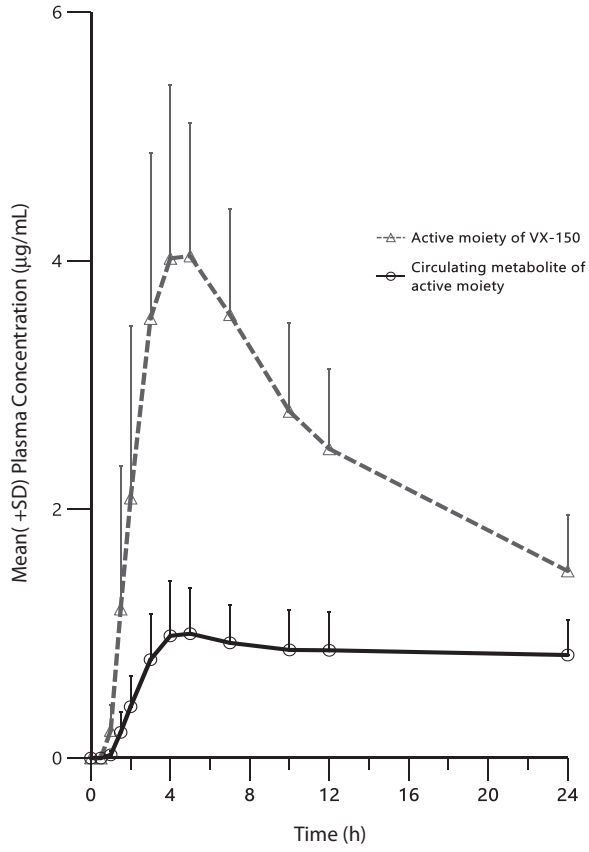

Figure 3. Pharmacokinetics results. Mean concentration of VX-150's active moiety and of its major circulating metabolite (in µg/mL, on the x-axis) after single oral doses of 1,250 mg VX-150 over time (in hours, on y-axis). Data are represented on a linear scale.

different mechanical pain test (e.g., assessment of secondary mechanical allodynia surrounding the capsaicin-treated skin by use of Von Frey filaments) may have been more applicable, given that there is preclinical evidence available describing a link between $Na_V1.8$ and mechanical allodynia in relation to neuropathic and inflammatory pain models, but not in regard to solely assessing pressure pain, as reported here [47, 48]. It must thus be noted that, a priori, we did not expect VX-150 to influence all pain tasks; as such, no (analgesic) drug is expected to influence all the tests we included. Rather, the integral combination of evoked pain models is used to profile the analgesic effects and magnitude of observed effects for each compound specifically. This allows for benchmarking of tested drugs, as briefly touched upon in the Introduction, as discussed in more detail previously [19, 26], and as discussed in the last paragraph of the Discussion.

After the discovery of $Na_V1.7$ deficiency underlying insensitivity to pain [49], $Na_V1.8$ has been studied as an analgesic target for conditions in which the mechanism of pain is related to peripheral nociceptor hyperexcitability. Nonclinical studies have reported that $Na_V1.8$ inhibitors, in addition to reversing cerebellar deficits in a rodent model of multiple sclerosis, showed potential to treat multiple pain conditions, including neuropathic and inflammatory conditions [50]. Specifically, the $Na_V1.8$ inhibitor A-803467 attenuated mechanical and thermal hyperalgesia in diabetic rats; reduced neuropathic pain in the L5/L6 spinal nerve injury model, in the chronic constriction injury of sciatic nerve model, and in the capsaicin-induced secondary mechanical allodynia model; and reduced thermal hyperalgesia in the Complete Freund's adjuvant model for inflammatory pain [51–54]. Here, we used an integral nociceptive test battery to confirm preclinical results and characterize the analgesic profile of VX-150 in an effort to bridge the gap to later-phase clinical trials. For the analgesic profile, each pain modality was plotted against the observed treatment effect size over the full 10 h time course (Table 3 and Figure 4). Again, the most pronounced

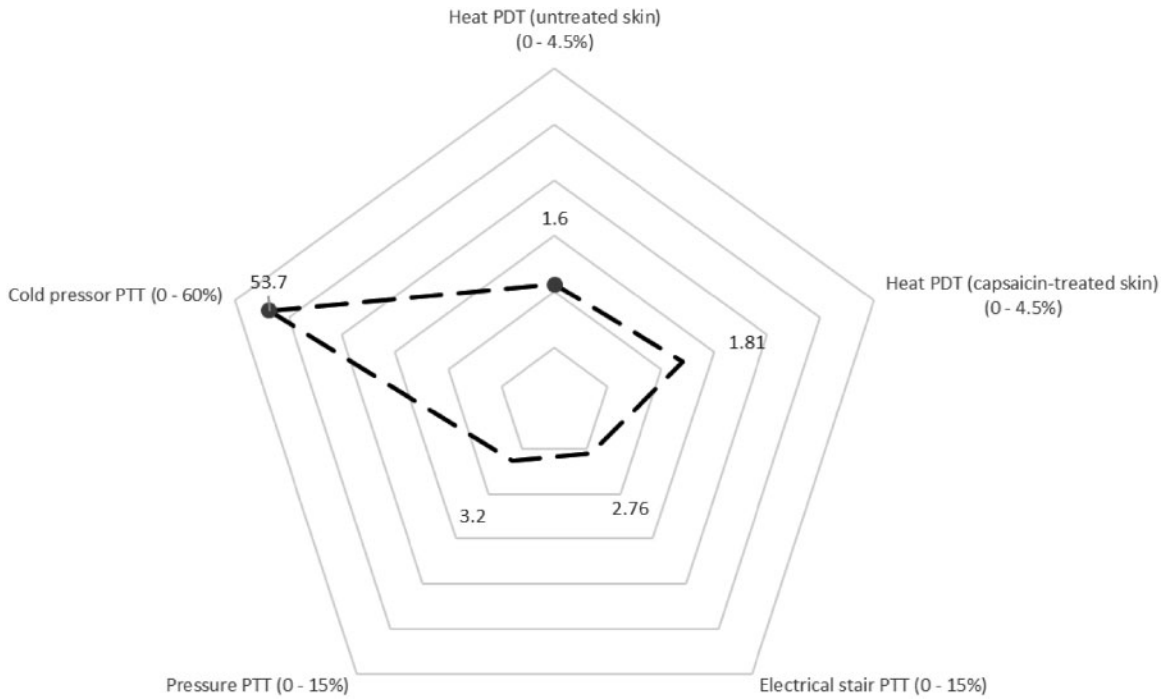

Figure 4. Analgesic profile of 1,250 mg VX-150. Visualization of the effect size of VX-150 for each pain modality, defined as the ED between the least-squares means of the contrast placebo—VX-150. Round markers for heat pain PDT and cold pressor PTT indicate a significantly different treatment effect of VX-150 vs. placebo over the complete time course, before dosing up until 10 h after dosing ($P < 0.05$). Percentage ranges provided in parentheses reflect the range of responses reported across a battery of analgesics summarized in an earlier report of this profile model, except for the cold pressor PTT, which had to be increased from 0–50% to 0–60% to reflect the larger effect size of VX-150 observed in this study [19]. For cold pressor PTT and pressure PTT, the ED as included in Table 3 was used, as the data for these end points were log-transformed for analysis and therefore already presented in percentages. For other end points, as those were not log-transformed, the ED was divided by the first least-squares mean of the contrast (i.e., of placebo) and multiplied by 100 to allow the effect size to be reported as percentages, as well.

effect was observed in the cold pressor pain test, which can be considered as a model for neuropathic pain. Recently, two clinical proof-of-concept trials were completed in which the efficacy of VX-150 was evaluated for two pain phenotypes. Not only did VX-150 relieve acute pain in patients who underwent bunionectomy surgery, it also reduced pain ratings in 46 patients with chronic pain caused by small-fiber neuropathy [55, 56]. Both studies align with the results here, i.e., the rapid onset of analgesia (acute pain) and the most pronounced results in the cold pressor pain test (model for neuropathic pain).

To the best of our knowledge, the present study is the first to report analgesic effectiveness of a selective Na_V inhibitor in an experimental pain study with healthy volunteers. Previously, we were not able to show any effects of a selective $Na_V1.7$ inhibitor (PF-05089771) when using the same pain test battery [20]. Although we cannot be certain, this plausibly may be due to one or a combination of the following reasons: Both compounds, though termed similar, represent a different class (i.e., $Na_V1.7$ vs. $Na_V1.8$ inhibitors). $Na_V1.7$ is thought to act as a threshold channel, whereas the contribution of $Na_V1.8$ to signal conductance lies with repetitive firing and neuronal excitability [11, 57], thereby arguably resulting in distinctive effects when either channel is inhibited. Results of the $Na_V1.7$ inhibitor study could, for example, have benefited from having included the TRPV1-sensitizing capsaicin model, given that in a later-phase clinical trial, PF-05089771 significantly reduced burning pain sensations in patients with diabetic neuropathy. This suggests a link between $Na_V1.7$ and TRPV1 on the peripheral nociceptor terminals [58]. Furthermore, the dose, potency, and the extent of blood–brain barrier penetration of the two compounds can significantly differ, thereby resulting in the discrepancy of results discussed here.

The results of the present study must be read with the following considerations. First, as literature suggests that the pain perception of women may change across phases of the menstrual cycle [21–23], we limited our study to men only to reduce variability and increase the chance of demonstrating a treatment effect in a phase 1 setting. Whether effects on pain thresholds are exerted in both women and men remains to be seen, but this is very likely in view of the identical role of $Na_V1.8$ in nociceptive nerve function in men and women. The electrical stair test after the cold pressor test was used to observe possible effects of the CPM response. Heat PDTs were quantified after this second electrical stair test (see *Study Procedures: Pharmacodynamic*) to increase the logistical

feasibility of including two baseline pain test sequences with application of capsaicin before VX-150 administration. The heat pain test therefore may have been influenced by an ongoing CPM response. The potential bias on heat PDTs, if present at all, will, however, have been limited, given that CPM effects are generally only short-lived [30, 59–62]. In addition, the effect of VX-150 on pain was quantified in a controlled setting in which pain tests were always performed in the same order, thereby affecting all results equally during each crossover period. Unadjusted multiple testing was performed to assess VX-150's temporal effect (primary analysis, Table 2) and the size of its total analgesic effect (Table 3). Although we acknowledge the increased risk of reporting erroneous inferences, the effect size analysis was performed as an add-on to allow for comparing the study results presented here with the results of other studies in which the same pain test battery was used [19, 20, 26, 63]. This was deemed reasonable given the experimental nature of the study.

Experimental pain studies are of major importance for the investigator, as the obtained results may aid in decision-making during the early phases of drug development. By repeatedly testing a fixed sequence of distinctive pain modalities over time, valuable data are collected that can inform on the active dose range and analgesic profile, as we are doing now for VX-150 and as has previously been done for a variety of other compounds with distinctive mechanisms of action [18, 19, 63–65]. Evoked pain models can thus provide confidence in advancing a compound to the next trial phase or can help evade questions about whether the right dose or patient population was chosen later on in development. For VX-150, the substantial response on the cold pressor PTT from 2 h up until the last time point at 10 h after dosing, with an over-time effect size of 53.7% (Figure 4), informs on robust acute analgesic effects in a model for neuropathic pain. VX-150 outperformed 300 mg pregabalin and 3 μg/kg fentanyl (both well-known analgesics for treating neuropathic and acute pain, respectively) on the cold pressor test (effect size of treatment vs. placebo of 46.4 and 17.1%, respectively) [19]. Combined with previous work, the translatability of $Na_V1.8$ models from nonclinical to experimental pain studies and eventually to the clinical stage seems to up the ante in the search for novel selective non-opioid analgesics.

Conclusion

VX-150 induced analgesia in a variety of evoked pain tests, without affecting subject safety. Results of this proof-of-mechanism study are therefore supportive of the analgesic potential of VX-150, a highly selective $Na_V1.8$ channel inhibitor.

Acknowledgments

The authors thank all subjects who participated in the study. Cynthia Barber contributed to study design, data analysis, and critical review of the manuscript. Lei Xu contributed to biostatistical study design, data analysis and validation, and critical review of the manuscript. Haili Ping contributed to clinical pharmacology study design, data analysis, and critical review of the manuscript. CB, LX, and HP are all employees of Vertex Pharmaceuticals and may own stock or stock options.

Supplementary Data

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

References

1. Rossi S, ed. Australian Medicines Handbook 2006. Sydney, Australia: Australian Medicines Handbook; 2006.
2. Petersen KL, Rowbotham MC. Will ion-channel blockers be useful for management of nonneuropathic pain? J Pain 2000;1(3):26–34.
3. Scriabine A. Discovery and Development of Major Drugs Currently in Use. In: Landau R, Achilladelis B, Scriabine A, eds. Pharmaceutical Innovation: Revolutionizing Human Health. Philadelphia: Chemical Heritage Press; 1999:222–3.
4. Hedegaard H, Miniño AM, Warner M. Drug overdose deaths in the United States. In: Key Findings Data from the National Vital Statistics System, Mortality. NCHS Data Brief No. 356, January 2020;1999–2018. Available at: https://www.cdc.gov/nchs/products/databriefs/db356.htm.
5. European Drug Report 2014: Trends and developments. 2014. Available at: http://www.emcdda.europa.eu/publications/edr/trends-developments/2014_en (accessed January 15, 2020).
6. Yekkirala AS, Roberson DP, Bean BP, Woolf CJ. Breaking barriers to novel analgesic drug development. Nat Rev Drug Discov 2017;16(8):545–64.
7. Rush AM, Cummins TR. Painful research: Identification of a small-molecule inhibitor that selectively targets Nav1.8 sodium channels. Mol Interv 2007;7(4):192–5.
8. England S. Voltage-gated sodium channels: The search for subtype-selective analgesics. Expert Opin Investig Drugs 2008;17(12):1849–64.
9. Krafte DS, Bannon AW. Sodium channels and nociception: Recent concepts and therapeutic opportunities. Curr Opin Pharmacol 2008;8(1):50–6.
10. Djouhri L, Fang X, Okuse K, et al. The TTX-resistant sodium channel Nav 1.8 (SNS/PN3): Expression and correlation with membrane properties in rat nociceptive primary afferent neurons. J Physiol 2003;550(3):739–52.
11. Hameed S. Nav1.7 and Nav1.8: Role in the pathophysiology of pain. Mol Pain 2019;15:1744806919858801.
12. Huang J, Yang Y, Zhao P, et al. Small-fiber neuropathy Nav1.8 mutation shifts activation to hyperpolarized potentials and increases excitability of dorsal root ganglion neurons. J Neurosci 2013;33(35):14087–97.
13. Faber CG, Lauria G, Merkies ISJ, et al. Gain-of-function Nav1.8 mutations in painful neuropathy. Proc Natl Acad Sci USA 2012;109(47):19444–9.
14. Han C, Vasylyev D, Macala LJ, et al. The G1662S NaV1.8 mutation in small fibre neuropathy: Impaired inactivation

underlying DRG neuron hyperexcitability. J Neurol Neurosurg Psychiatry 2014;85(5):499–505.
15. Dib-Hajj SD, Tyrrell L, Cummins TR, et al. Two tetrodotoxin-resistant sodium channels in human dorsal root ganglion neurons. FEBS Lett 1999;462(1–2):117–20.
16. Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. Nature 1996; 379(6562):257–62.
17. Amaya F, Decosterd I, Samad TA, et al. Diversity of expression of the sensory neuron-specific TTX-resistant voltage-gated sodium ion channels SNS and SNS2. Mol Cell Neurosci 2000;15 (4):331–42.
18. Hay JL, Okkerse P, Van Amerongen G, Groeneveld GJ. Determining pain detection and tolerance thresholds using an integrated, multi-modal pain task battery. J Vis Exp 2016;2016 (110):1–6. doi:10.3791/53800
19. Okkerse P, Amerongen G, Kam ML, et al. The use of a battery of pain models to detect analgesic properties of compounds: A two-part four-way crossover study. Br J Clin Pharmacol 2017;83 (5):976–90.
20. Siebenga P, Amerongen G, Hay JL, et al. lack of detection of the analgesic properties of PF-05089771, a selective Nav1.7 inhibitor, using a battery of pain models in healthy subjects. Clin Transl Sci 2020;13(2):318–24.
21. Teepker M, Peters M, Vedder H, Schepelmann K, Lautenbacher S. Menstrual variation in experimental pain: Correlation with gonadal hormones. Neuropsychobiology 2010;61(3):131–40. doi:10.1159/000279303
22. Kowalczyk WJ, Evans SM, Bisaga AM, Sullivan MA, Comer SD. Sex differences and hormonal influences on response to cold pressor pain in humans. J Pain 2006;7(3):151–60.
23. Hellström B, Anderberg UM. Pain perception across the menstrual cycle phases in women with chronic pain. Percept Mot Skills 2003;96(1):201–11.
24. Arendt-Nielsen L, Curatolo M, Drewes A. Human experimental pain models in drug development: Translational pain research. Curr Opin Investig Drugs 2007;8(1):41–53.
25. Arendt-Nielsen L, Yarnitsky D. Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera. J Pain 2009;10(6):556–72.
26. Siebenga PS, Amerongen G, Okkerse P, et al. Reproducibility of a battery of human evoked pain models to detect pharmacological effects of analgesic drugs. Eur J Pain 2019;23(6):1129–40.
27. Beaulieu P, Lussier D, Porreca F, Dickenson AH. Pharmacology of Pain. Seattle, WA: IASP Press 2010. DOI:10.1007/s12630-010-9327.
28. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Pressure-pain function in desensitized and hypersensitized muscle and skin assessed by cuff algometry. J Pain 2002;3(1):28–37.
29. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Computer-controlled pneumatic pressure algometry—A new technique for quantitative sensory testing. Eur J Pain 2001;5(3):267–77. doi:10.1053/eujp.2001.0245
30. Yarnitsky D, Bouhassira D, Drewes AM, et al. Recommendations on practice of conditioned pain modulation (CPM) testing. Eur J Pain 2015;19(6):805–6.
31. Oaks Z, Stage A, Middleton B, Faraone S, Johnson B. Clinical utility of the cold pressor test: Evaluation of pain patients, treatment of opioid-induced hyperalgesia and fibromyalgia with low dose naltrexone. Discov Med 2018;26(144):197–206.
32. Eckhardt K, Li S, Ammon S, Schänzle G, Mikus G, Eichelbaum M. Same incidence of adverse drug events after codeine administration irrespective of the genetically determined differences in morphine formation. Pain 1998;76(1-2):27–33.
33. Jones SF, McQuay HJ, Moore RA, Hand CW. Morphine and ibuprofen compared using the cold pressor test. Pain 1988;34 (2):117–22.
34. Bishop T, Ballard A, Holmes H, Young AR, McMahon SB. Ultraviolet-B induced inflammation of human skin: Characterisation and comparison with traditional models of hyperlagesia. Eur J Pain 2009;13(5):524–32.
35. Yang F, Zheng J. Understand spiciness: Mechanism of TRPV1 channel activation by capsaicin. Protein Cell 2017;8(3):169–77.
36. Roberts K, Shenoy R, Anand P. A novel human volunteer pain model using contact heat evoked potentials (CHEP) following topical skin application of transient receptor potential agonists capsaicin, menthol and cinnamaldehyde. J Clin Neurosci 2011;18(7):926–32.
37. Kingwell K. Nav1.7 withholds its pain potential. Nat Rev Drug Discov. 2019 (doi:10.1038/d41573-019-00065-0).
38. Andersen HH, Olsen RV, Møller HG, et al. A review of topical high-concentration L-menthol as a translational model of cold allodynia and hyperalgesia. Eur J Pain 2014;18(3):315–25.
39. Andersen HH, Poulsen JN, Uchida Y, et al. Cold and l -menthol-induced sensitization in healthy volunteers—A cold hypersensitivity analogue to the heat/capsaicin model. Pain 2015;156 (5):880–9.
40. Yapa K, , Deuis J, Peters AA, et al. Assessment of the TRPM8 inhibitor AMTB in breast cancer cells and its identification as an inhibitor of voltage gated sodium channels. Life Sci 2018;198 :128–35.
41. Gasperini RJ, Hou X, Parkington H, et al. TRPM8 and Nav1.8 sodium channels are required for transthyretin-induced calcium influx in growth cones of small-diameter TrkA-positive sensory neurons. Mol Neurodegener 2011;6(1):19.
42. Wang S, Davis BM, Zwick M, Waxman SG, Albers KM. Reduced thermal sensitivity and Nav1.8 and TRPV1 channel expression in sensory neurons of aged mice. Neurobiol Aging 2006;27(6):895–903.
43. Moon JY, Song S, Yoon SY, et al. The differential effect of intrathecal Nav1.8 blockers on the induction and maintenance of capsaicin- and peripheral ischemia-induced mechanical allodynia and thermal hyperalgesia. Anesth Analg 2012;114 (1):215–23.
44. Cao X, Cao X, Xie H, et al. Effects of capsaicin on VGSCs in TRPV1-/- mice. Brain Res 2007;1163(1):33–43.
45. Gold MS. Tetrodotoxin-resistant Na+ currents and inflammatory hyperalgesia. Proc Natl Acad Sci USA 1999;96(14):7645–9.
46. Firouzian S, Osborne NR, Cheng JC, et al. Individual variability and sex differences in conditioned pain modulation and the impact of resilience, and conditioning stimulus pain unpleasantness and salience. Pain 2020;161(8):1847–60.
47. Schuelert N, McDougall JJ. Involvement of Nav 1.8 sodium ion channels in the transduction of mechanical pain in a rodent model of osteoarthritis. Arthritis Res Ther 2012;14(1):R5.
48. Joshi SK, Mikusa JP, Hernandez G, et al. Involvement of the TTX-resistant sodium channel Nav 1.8 in inflammatory and neuropathic, but not post-operative, pain states. Pain 2006;123 (1):75–82.
49. Goldberg YP, Macfarlane J, Macdonald ML, et al. Loss-of-function mutations in the Nav1.7 gene underlie congenital indifference to pain in multiple human populations. Clin Genet 2007;71 (4):311–9.
50. Bennett DL, Clark XAJ, Huang J, Waxman SG, Dib-Hajj SD. The role of voltage-gated sodium channels in pain signaling. Physiol Rev 2019;99(2):1079–151.
51. Mert T, Gunes Y. Antinociceptive activities of lidocaine and the Nav1.8 blocker A803467 in diabetic rats. J Am Assoc Lab Anim Sci 2012;51(5):579–85.

52. Payne CE, Brown AR, Theile JW, et al. A novel selective and orally bioavailable Nav1.8 channel blocker, PF-01247324, attenuates nociception and sensory neuron excitability. Br J Pharmacol 2015;172(10):2654–70.
53. Shields SD, Butt RP, Dib-Hajj SD, Waxman SG. Oral administration of PF-01247324, a subtype-selective Nav1.8 blocker, reverses cerebellar deficits in a mouse model of multiple sclerosis. PLoS One 2015;10(3):e0119067–8.
54. Jarvis MF, Honore P, Shieh CC, et al. A-803467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. Proc Natl Acad Sci USA 2007; 104(20):8520–5.
55. Vertex Announces Treatment with the NaV1.8 Inhibitor VX-150 Showed Significant Relief of Acute Pain in Phase 2 Study | Business Wire. Available at: https://www.businesswire.com/news/home/20180214005740/en/Vertex-Announces-Treatment-NaV1.8-Inhibitor-VX-150-Showed (accessed March 26, 2020).
56. Vertex Announces Positive Phase 2 Data in Third Proof-of-Concept Study with the NaV1.8 Inhibitor VX-150 | Business Wire. Available at: https://www.businesswire.com/news/home/20181218005223/en/ (accessed March 26, 2020).
57. Rush AM, Cummins TR, Waxman SG. Multiple sodium channels and their roles in electrogenesis within dorsal root ganglion neurons. J Physiol 2007;579(1):1–14.
58. McDonnell A, Collins S, Ali Z, et al. Efficacy of the Nav1.7 blocker PF-05089771 in a randomised, placebo-controlled, double-blind clinical study in subjects with painful diabetic peripheral neuropathy. Pain 2018;159(8):1465–76.
59. Arendt-Nielsen L, Sluka KA, Nie HL. Experimental muscle pain impairs descending inhibition. Pain 2008;140(3):465–71.
60. Tuveson B, Leffler A-S, Hansson P. Time dependant differences in pain sensitivity during unilateral ischemic pain provocation in healthy volunteers. Eur J Pain 2006;10(3):225.
61. Campbell CM, France CR, Robinson ME, et al. Ethnic differences in diffuse noxious inhibitory controls (DNIC). J Pain 2008;9 (8):759–766.
62. Kakigi R. Diffuse noxious inhibitory control. Reappraisal by pain-related somatosensory evoked potentials following CO2 laser stimulation. J Neurol Sci 1994;125(2):198–205.
63. van Amerongen G, Siebenga P, de Kam MLL, Hay JLL, Groeneveld GJJ. Effect profile of paracetamol, Δ9-THC and promethazine using an evoked pain test battery in healthy subjects. Eur J Pain 2018;22(7):1331–1342.
64. Loudon P, Siebenga P, Gorman D, et al. Demonstration of an anti-hyperalgesic effect of a novel pan-Trk inhibitor PF-06273340 in a battery of human evoked pain models. Br J Clin Pharmacol 2018;84(2):301–309.
65. Hijma HJ, Moss LM, Gal P, et al. Challenging the challenge: A randomized controlled trial evaluating the inflammatory response and pain perception of healthy volunteers after single-dose LPS administration, as a potential model for inflammatory pain in early-phase drug development. Brain Behav Immun 2020;88: 515–28. DOI:10.1016/j.bbi.2020.04.033.