A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Crossover Study to Evaluate the Pharmacodynamic Effects of VX-150, a Highly Selective Na\textsubscript{V}1.8 Inhibitor, in Healthy Male Adults

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Abstract

Objective. To evaluate the analgesic potential, safety, tolerability, and pharmacokinetics of VX-150, a pro-drug of a highly selective Na\textsubscript{V}1.8 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 \textsuperscript{mg/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\textsubscript{V}1.8 channel inhibitor, to treat various pain indications.

Key Words: Na\textsubscript{V}1.8-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 (Nav), 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 Nav1.3, Nav1.7, Nav1.8, and Nav1.9 can play critical roles in pain signaling [7–9]. Of these Nav subtypes, Nav1.8 is a sensory neuron–specific channel with preferential expression in the dorsal root ganglion and trigeminal ganglion neurons [10]. Nav1.8 is highly expressed on nociceptors, where it mediates pain sensation and chronic pain [11]. As such, Nav1.8 gain-of-function mutations are thought to directly cause chronic pain in patients with painful small-fiber neuropathy [12–14]. Moreover, Nav1.8 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 Nav1.8 has been found to result in analgesia [16, 17], a finding that supported the channel as a pharmacological target and showed that selective Nav1.8 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 Nav1.8 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 Nav 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 (BEO), 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.
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 transcortaneous 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 reached.
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 cream, after which the area was covered by a cotton gauze. The nondominant volar forearm served as a non-stimulated 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.

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 10 h after dosing (Figure 2). 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, 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).

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

| Demographic Category                  | Number (N = 20) |
|--------------------------------------|-----------------|
| Sex, n (%)                           | 20 (100.0)      |
| Male                                 |                 |
| Age, y                               | 27.9 ± 8.6      |
| Mean ± SD                            |                 |
| 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                           | 74.6 ± 10.3     |
| Mean ± SD                            |                 |
| Height, cm                           | 179.3 ± 6.6     |
| Mean ± SD                            |                 |
| Body mass index, kg/m²               | 23.18 ± 2.77    |
| Mean ± SD                            |                 |

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.
Table 2. Primary analysis for pain thresholds

| Pain Modality       | PDT Test End points | PTT Test End points |
|---------------------|---------------------|---------------------|
|                     | 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        | NA                  |
| 2 h                 | 40.17 ± 2.55        | 40.32 ± 2.17        | NA                  |
| 4 h                 | 40.14 ± 2.86        | 40.84 ± 3.09        | NA                  |
| 7 h                 | 40.98 ± 2.83        | 41.41 ± 2.90        | NA                  |
| 10 h                | 41.23 ± 2.54        | 41.53 ± 3.12        | NA                  |
| **Heat, °C**        |                     |                     |                     |
| Baseline            | 44.63 ± 2.81        | 44.50 ± 2.32        | NA                  |
| 1 h                 | 44.09 ± 2.84        | 44.16 ± 2.33        | NA                  |
| 2 h                 | 43.70 ± 3.35        | 44.39 ± 2.65        | NA                  |
| 4 h                 | 43.58 ± 3.14        | 44.15 ± 2.87        | NA                  |
| 7 h                 | 43.32 ± 3.20        | 43.94 ± 3.63        | NA                  |
| 10 h                | 43.16 ± 3.67        | 44.07 ± 3.20        | 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       |
| 2 h                 | 6.53 ± 4.73         | 8.37 ± 8.02         | 23.21 ± 13.36       | 34.78 ± 25.92       |
| 4 h                 | 6.56 ± 4.19         | 7.52 ± 7.98         | 20.46 ± 11.53       | 48.63 ± 37.19       |
| 7 h                 | 6.22 ± 5.18         | 8.27 ± 7.71         | 18.52 ± 11.71       | 40.59 ± 32.19       |
| 10 h                | 6.05 ± 5.35         | 10.05 ± 13.68       | 20.00 ± 12.75       | 40.89 ± 32.45       |
| **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        |
| 2 h                 | 7.77 ± 4.05         | 8.09 ± 5.90         | 19.09 ± 7.12        | 19.39 ± 7.89        |
| 4 h                 | 8.28 ± 4.27         | 9.02 ± 6.75         | 18.38 ± 6.58        | 19.66 ± 8.77        |
| 7 h                 | 7.91 ± 4.22         | 9.27 ± 6.17         | 18.93 ± 7.20        | 20.54 ± 8.03        |
| 10 h                | 8.62 ± 5.62         | 9.29 ± 6.69         | 20.46 ± 8.33        | 21.24 ± 7.61        |
| **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       |
| 2 h                 | 24.82 ± 15.15       | 24.70 ± 17.10       | 52.46 ± 20.80       | (7.55 to 5.32)      |
| 4 h                 | 24.79 ± 16.40       | 25.38 ± 18.49       | 53.48 ± 18.11       | 53.04 ± 21.66       |
| 7 h                 | 22.83 ± 14.89       | 25.02 ± 18.60       | 48.92 ± 16.99       | (4.00 to 7.62)      |
| 10 h                | 22.35 ± 14.18       | 25.70 ± 17.90       | 49.28 ± 19.97       | (5.03 ± 2.07)       |

(continued)
Table 2. continued

| Pain Modality | Pain Test End points | Pain Test End points |
|---------------|----------------------|----------------------|
|               | PDT                  | PTT                  |
| CPM, mA       | Placebo              | VX-150               |
| Baseline      | 0.44 ± 1.44          | 0.21 ± 2.51          |
| 1 h           | 1.38 ± 3.57          | 1.05 ± 2.21          |
| 2 h           | 0.94 ± 2.56          | 1.24 ± 2.83          |
| 4 h           | 0.13 ± 2.62          | 1.01 ± 2.36          |
| 7 h           | 1.16 ± 2.29          | 0.71 ± 3.11          |
| 10 h          | 0.95 ± 1.34          | 1.20 ± 2.77          |

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 boldface 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.

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 Nav1.8 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 NaV1.8 inhibitor in a human experimental pain study, favoring the use of selective NaV1.8 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 Nav1.8. When this activation is blocked by VX-150, however, pain relief is achieved. The interplay between TRPM8 and Nav1.8 has previously been described in both models of sensory neurons and breast cancer [40, 41]. Although Nav1.8 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 Nav1.8 [45]. Given the sample size of the present study, the limited effect size and variability
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 NaV1.8 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

**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.
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 NaV1.8 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 NaV1.7 deficiency underlying insensitivity to pain [49], NaV1.8 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 NaV1.8 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 NaV1.8 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

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  | 0.694 °C | 14.8%       | 0.88 mA    | -12.3%   | -0.147 mA |
| (P = 0.07)                                        | (P = 0.01) | (P = 0.488) | (P = 0.137) | (P = 0.154) | (P = 0.692) |
| PTT                                               | 53.7%     | 53.7% | (-0.07 to 1.53) | (-0.31 to 2.07) | (-27.2 to 5.6) | (-0.94 to 0.65) |
| (P < 0.001)                                       | (P = 0.428) | (P = 0.557) | (P = 0.748) |
| AAC/AUC                                           | 43.5%     | -61.80 mA*% | (-0.07 to 1.53) | (-197.28 %) | 24.38 mA*% |
| (P = 0.002)                                       | (P = 0.445) | (P = 0.4098) |
| (16.2 to 77.3)                                    | (-192.47 to 68.87) | (-728.62 to 334.05) | (-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 boldface 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.
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\(V\) inhibiotor in an experimental pain study with healthy volunteers. Previously, we were not able to show any effects of a selective Na\(V\)\(_{1.7}\) 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\(V\)\(_{1.7}\) vs. Na\(V\)\(_{1.8}\) inhibitors). Na\(V\)\(_{1.7}\) is thought to act as a threshold channel, whereas the contribution of Na\(V\)\(_{1.8}\) 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\(V\)\(_{1.7}\) 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\(V\)\(_{1.7}\) 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\(V\)\(_{1.8}\) 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 pain) 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.

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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 NaV1.8 channel inhibitor.

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Supplementary Data

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

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