The handle http://hdl.handle.net/1887/59753 holds various files of this Leiden University dissertation.

**Author:** Okkerse, P.
**Title:** The use of a battery of evoked pain models in early phase drug development
**Issue Date:** 2018-01-23
CHAPTER IV
Pharmacokinetics and pharmacodynamics of intrathecally administered Xen2174, a synthetic conopeptide with norepinephrine reuptake inhibitor and analgesic properties

British Journal of Clinical Pharmacology 2017;83:751-763.

ABSTRACT

Xen2174 is a synthetic 13-amino acid peptide that binds specifically to the norepinephrine transporter, which results in inhibition of norepinephrine uptake. It is being developed as a possible treatment for moderate to severe pain and is delivered intrathecally. The current study was performed to assess the pharmacodynamics (PD) and the cerebrospinal fluid (CSF) pharmacokinetics (PK) of Xen2174 in healthy subjects. This was a randomised, blinded, placebo-controlled study in healthy subjects. The study was divided into three treatment arms. Each group consisted of eight subjects on active treatment and two or three subjects on placebo. The CSF was sampled for 32 h using an intrathecal catheter. PD assessments were performed using a battery of nociceptive tasks (electrical pain, pressure pain and cold pressor tasks). Twenty-five subjects were administered Xen2174. CSF PK analysis showed a higher area under the CSF concentration-time curve of Xen2174 in the highest dose group than allowed by the predefined safety margin based on nonclinical data. The most common adverse event was post lumbar puncture syndrome, with no difference in incidence between treatment groups. Although no statistically significant differences were observed in the PD assessments between the different dosages of Xen2174 and placebo, pain tolerability in the highest dose group was higher than in the placebo group (contrast least squares mean pressure pain tolerance threshold of Xen2174 2.5 mg–placebo[95% confidence interval],/22.2%[-5.0%, 57.1%], p=0.1131). At the Xen2174 dose level of 2.5 mg, CSF concentrations exceeded the prespecified exposure limit based on the nonclinical safety margin. No statistically significant effects on evoked pain tests were observed.

INTRODUCTION

The majority of patients undergoing surgery experience moderate to severe pain in the postoperative period. Treatment consists of multiple pain relief agents and strategies. Significant side effects may occur with the use of opioids. Nonsteroidal anti-inflammatory drugs (NSAIDs) and paracetamol are not sufficiently effective against moderate to severe postoperative pain and should be administered in combination with opioids. Thus, there remains a clinical need for the development of new efficacious therapies with a beneficial side effect profile.

The venom of the marine cone snail genus Conus provides a rich source of pharmacologically active compounds. The peptide Mr1A, identified in the venom of Conus marmoreus, causes inhibition of norepinephrine (NE) uptake by the NE transporter (NET) in a selective, noncompetitive manner. Mr1A showed an antinociceptive effect after intrathecal administration in mice. This peptide has a relatively poor chemical stability in solution. To overcome this, Xen2174, modelled on Mr1A, was developed. Xen2174 is a synthetic 13-amino acid peptide that does not cross the blood-brain barrier and is being developed for the intrathecal treatment of moderate to severe pain. In vitro pharmacology studies have demonstrated that Xen2174 binds specifically to the NET, but not to other central nervous system molecular targets, resulting in selective inhibition of NE uptake by NET in a noncompetitive manner. Tricyclic antidepressants are also potent NE reuptake inhibitors (NRIs), but their poor specificity relative to other monoamine transporters and various G-protein-coupled receptors, results in dose-limiting side effects in clinical use. In vivo pharmacology studies in rat models of neuropathic pain have demonstrated that intrathecal administration of Xen2174 produces rapid and long-lasting antinociceptive effects, which were found to be greater in magnitude and duration than those of intrathecal morphine. Additional pharmacology studies have demonstrated that Xen2174 also provides long lasting antinociception in a rat model of postsurgical pain. In an inflammatory pain model in rats (inflammation induced by injecting Freund’s Complete Adjuvant) Xen2174 did not relieve pain after thermal latency or paw pressure tests (Investigator’s brochure Xen2174, Xenome Ltd., unpublished). Toxicology studies have shown that Xen2174 causes convulsions and seizures when administered at high doses in rats and dogs. In a beagle dog study in which Xen2174 was administered intrathecally at doses of 0, 1, 2, 4 and 8 mg (5 animals/gender/dose), seizures were observed in three dogs; one in the 1 mg and two in the 2 mg dose...
The use of a battery of evoked pain models in early phase drug development

PK and PD of intrathecally administered Xen2174 in healthy subjects

Materials and Methods

The study was approved by the Medical Ethics Committee of the Bebo Foundation (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

Subjects

Healthy male and female subjects between 18 and 45 years with a body mass index (BMI) of 18 to 30 kg m⁻² were enrolled. All subjects gave written informed consent. The subjects underwent a full medical screening to assess eligibility. Subjects with an abnormal electroencephalogram (EEG) at screening, a family history of epilepsy, a history of seizures, complaints of low back pain, regular use of any illicit drugs or history of drug abuse, a positive drug screen or other clinical significant abnormalities were excluded. Use of xanthine-containing products and alcohol was not allowed from 1 day prior to admission to the clinical research unit and during the stay at the research unit. Subjects were not allowed to use any medications from 2 weeks prior to the start of the study days.

Experimental design

This was a randomised, double-blind, placebo-controlled, serial-cohort, single ascending dose study of Xen2174 or placebo, administered intrathecally to healthy volunteers. At each dose stage, subjects were randomised to Xen2174 or placebo. Cohorts 1 and 2 consisted of eight subjects administered Xen2174 and three subjects receiving placebo. Cohort 3 consisted of eight subjects administered Xen2174 and two administered placebo. The three ascending doses of Xen2174 were 0.5 mg (cohort 1), 1.0 mg (cohort 2) and 2.5 mg (cohort 3). The maximum dose of 2.5 mg was chosen in order to have a threefold safety margin in the dose per kg body weight compared with the no-observed-adverse-effect level (NOAEL) in dogs. The lower dose of 0.5 mg was chosen based on the human equivalent dose of the median effective dose (ED₅₀) in rats exposed to the Brennan model of postsurgical pain.

Subjects arrived at the clinical research unit on the day before dosing and remained in-house for at least 56 h after study drug administration. The study drug was administered via a spinal needle at the L3-L4 or L4-L5 interspace, using a median approach. After administration, an intrathecal sampling catheter was left in place for the following 32 h. Subjects were asked to stay in bed in either a recumbent or supine position as much as possible during the period that the spinal catheter was in place, and up to 12 h after the spinal catheter had been removed.

Safety assessments were performed at specified time points and the occurrence of general symptoms was monitored continuously. The computer-generated randomization list was prepared by the statistician prior to the start of the study. Doses were prepared by a pharmacist/technician not involved in any of the study procedures.

Study drug

Xen2174 in glucose 5% was given intrathecally as bolus injection of 3 ml. Glucose 5% was used as placebo. Before drug administration, the skin on the lower back was anesthetised locally with 1-2 ml lidocaine. All intrathecal injections of the study drug were carried out by an experienced anaesthesiologist under aseptic conditions using a spinal catheter set. Owing to difficulties with Csf sampling, different spinal catheter sets were used during the course of the study: a Sprotte Special 21G needle with a 25G catheter (cohort 1) (Pajunk, Geisingen, Germany), a 19G needle with a 23G catheter (five subjects in cohort 2) (Pajunk, Geisingen, Germany) and a Spinocath 22G catheter (six subjects in cohort 2 and 10 in cohort 3) (B Braun, Melsungen, Germany). With the Sprotte Special cannula catheter set, the study drug was administered using the Sprotte needle (epidural introducer with an atrumatic modified pencil point) after which the sampling
catheter was left in place. The Sprotte needle had a directional bevel which was directed cranially. The study drug was administered directly through the epidural introducer. The catheter was placed after drug administration at the same level via the introducer. For the Spinocath set, first an introducer was inserted into the epidural space. After that, the study drug was administered into the intrathecal space using a 25G/27G pencil point needle. Thereafter, the sampling catheter was inserted into the intrathecal space through the epidural introducer. With both catheter sets, the sampling catheter was inserted 2-5 cm into the intrathecal space and left in place for the following 32 h. The Pajunk catheter had three lateral orifices at the distal end of the catheter. The Spinocath catheter had a central and lateral opening on the catheter tip. The intrathecal needle was placed with the subject in the sitting position. After insertion of the spinal catheter, the catheter was secured and subjects were placed directly in supine position afterwards. They were asked to stay in the supine or recumbent position while the catheter was in place.

Study assessments

The primary objectives of the study were to evaluate the effects of Xen2174 on evoked pain tasks and to assess the PK profile of Xen2174 in plasma and CSF. Nociceptive (pain) detection and tolerance thresholds were measured using a battery of evoked pain tasks. The battery takes approximately 25 min to complete. The evoked pain tasks (electrical pain, pressure pain and cold pressor tasks) were performed predose (twice) and 2, 4, 6, 8, 10, 48, 72 and 96 h after study drug administration. A training session was included as part of the screening examination to reduce learning effects during the study. All tests had previously been shown to be sensitive to the effects of analgesics in healthy adults.

Pain intensity was measured continuously for each nociceptive task using an electronic visual analogue scale (evas) scale ranging from 0 (no pain) to 100 (most intense pain tolerable). The equipment was programmed to cease giving stimuli if pain intensity reached the maximum possible score. For each task, the pain detection threshold (PDT), pain tolerance threshold (PTT) and area under the pain intensity-stimulation (-time for cold pressor) curve (AUC) were calculated.

Electrical stimulation task

For cutaneous electrical pain, Ag-AgCl electrodes (3M Red-Dot™, 3M Europe, Diegem, Belgium) were placed on the skin, 10 cm distal from the patella overltying the tibia. The electrical stimulus was delivered as two different paradigms by a computer-controlled constant current stimulator (DS5, Digitimer, Cambridge, UK). For the single stimulus, adapted from methods described previously13,14 (10 Hz tetanic pulse with a duration of 0.2 ms), current intensity increased from 0 mA in steps of 0.5 mA·s⁻¹ (cut-off 50 mA). For the repeated stimulus, adapted from methods described previously,15 each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) was repeated five times with a frequency of 2 Hz at the same current intensity with a random interval of 3-8 s between the repetitions. Current intensity increased from 0 mA in steps of 0.5 mA (cutoff 50 mA). The pain detection threshold was taken as the value (mA) when a subject indicated either that all five stimuli were painful or that the train of five stimuli, having started as feeling nonpainful became painful (VAS > 0). The pain intensity for each stimulation was measured using the evas slider, until the PTT was reached or a maximum of 50 mA was reached.

Pressure stimulation task

The method for inducing mechanical pressure pain was based on methods described previously, and was shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors.16,17 Briefly, an 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 kPa·s⁻¹. The pneumatic pressure was increased until the subject indicated maximum pain tolerance using the evas slider, or a maximum pressure of 100 kPa was achieved, at which point the device released pressure to the cuff.

Cold pressor task

The method of cold pressor pain was based on the methods described previously18,19 and is the most commonly used test to induce inhibitory conditioned pain modulation (ICPM, also known as ‘diffuse noxious inhibitory control’).20 Subjects placed their nondominant hand into a water bath (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 was inflated to 20 mmHg below resting diastolic pressure. At 2 min the subject moved that hand from the warm water bath, directly into a similar sized bath at 1.0 ± 0.5°C. The subjects were instructed to indicate when the PDT was reached as well as the pain intensity, by moving the evas slider. When the PTT or a time limit (120 s) was reached, subjects were instructed to remove their hand from the water.

**Conditioned pain modulation**

Conditioned pain modulation is the activation of the pain-modulatory mechanism, as part of the descending endogenous analgesia system.20 The degree of icpm was assessed by comparing the electrical pain thresholds for the single stimulus paradigm before and within 5 min after the cold pressor task.

**Measurements of drug concentrations in plasma and CSF**

Samples for the determination of Xen2174 in the plasma were obtained at baseline, and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 32, 48 and 72 h postdose. CSF samples were obtained using the intrathecal catheter at baseline, and at 0.5, 1, 2, 4, 8, 12, 24, and 32 h postdose. The potential for Xen2174 to adhere to components of the sampling material was tested prior to study execution. Acceptable recovery was obtained. First 0.2 ml CSF, representing the catheter dead-space, was sampled and discarded. Subsequently 0.3 ml was sampled with a new syringe, divided into two cryotubes and frozen at -70°C within 30 min of collection. Plasma was separated within 20 min of blood collection by centrifugation at 2000 g for 10 min. Samples were stored at -70°C until analysis. Plasma and CSF concentrations of Xen2174 were measured via high-performance liquid chromatography with tandem mass spectrometry detection. The lower limits of quantification were 1.0 ng ml⁻¹ and 10 ng ml⁻¹ for the concentrations of Xen2174 in plasma and CSF respectively. Sample analysis was performed by Pharmaceutical Product Development, Inc., Richmond, VA, USA.

**EEG**

All subjects received a standard 21-lead clinical EEG at the screening visit. The 1-h EEG recording was performed to detect subjects with abnormal EEG activity or with preseizure activity when stressed, through hyperventilation (for at least 3 min) and photic stimulation. Study EEG recording was initiated 1 h predose, and continued until 24 h postdose. Any change from the baseline EEG observed after dosing and interpreted in a blinded fashion by the clinical neurophysiologist as clinically significant, was reported as an adverse event (AE).

**Statistics**

No formal power analysis was performed. However, a previous study in which the electrical stimulation task was performed and where analgesia could be measured in healthy subjects used similar group sizes.13 The statistical analysis plan was part of the study protocol. For Xen2174 all PK parameters were analysed by noncompartmental methods. Summary statistics for each PK parameter were calculated for each dose group. The individual and median concentrations were plotted vs. time both on a linear and a logarithmic scale. Dose proportionality was assessed from dose-normalised AUC.

Residual q-q plots were produced to check the assumption of normality of the error term in the mixed effects models. This was done by visual inspection, the Shapiro-Wilk test statistic and the p-value for the test of normality. All PDT and PTT variables followed a log-normal distribution and were therefore log-transformed before analysis. Transformed parameters were back-transformed after analysis.

To assess the interaction effect of Xen2174 on nociceptive variables, the (transformed) variables were analysed with a mixed model analysis of variance, with treatment, time and treatment by time as fixed factor, subject as random factor and the (average) predose value as covariate. The contrasts calculated within the model were between the placebo and active treatments. Contrasts within the overall treatment effect and the time effect were estimated and reported, along with 95% confidence intervals. Subjects assigned to placebo within each cohort were treated as a single group. All calculations were performed using SAS for Windows v9.1.3 (SAS Institute, Inc., Cary, NC, USA).

**RESULTS**

A total of 33 healthy subjects (four females) participated in the study (Figure 1); subjects were aged 18-43 years (mean age 25.6 years) and had a BMI of 19.30 kg m⁻² (mean 24.4 kg m⁻²). The clinical phase of the study
started on 28 December 2011, and the last study visit was on 18 June 2012. One subject in cohort 3, in whom CSF sampling was not possible was replaced. The replacement subject was dosed in an unblinded fashion. Only PK assessments were performed in this subject.

Owing to sampling problems with the spinal catheter the study was amended. During the cohort 1 treatment the diameter of the spinal catheter was increased, and during the cohort 2 treatment, the type of spinal catheter was changed. Owing to a high incidence of postlumbar puncture syndrome in cohort 1, only male subjects with a BMI above 23 kg m⁻² were recruited in cohorts 2 and 3.

A large number of AES was observed in this study (Table 2). There was no clear difference in the severity or duration of AES between the different dosing groups and placebo. The most commonly reported AE was post lumbar puncture syndrome (25 out of 33 subjects). This AE was reported in all dose groups. In the majority of the subjects, complaints of headache as presentation of postlumbar puncture syndrome started after removal of the spinal sampling catheter. In two subjects, the severity of these complaints was mild, in 16 subjects moderate and in seven subjects severe. Subjects experiencing these complaints were treated with paracetamol and caffeine. Because of inadequate treatment response, 11 subjects were treated with an epidural blood patch; one subject was treated with two epidural blood patches. Evoked pain tasks were not performed subsequent to analgesic dosing for postlumbar puncture syndrome.

Other commonly reported AES were catheter site related reaction and back pain. This included a bruised feeling on the back, irritation, pain and stiffness. Paraesthesia was experienced by six subjects; in two during administration, and in four during the period when the catheter was in place. All these complaints were mild, and resolved shortly after spinal catheter removal.

One subject experienced a serious AE during the study. This subject continued to have headache complaints after treatment with the epidural blood patch. He was evaluated at the emergency room of the local university hospital to exclude severe pathology. No abnormalities were found on a computed tomography scan of the head, and the subject was discharged from the hospital the next morning. The headache complaints resolved without sequelae.

One subject reported persistent tinnitus after participation in the study, which persisted beyond the end of the clinical phase of the study. This subject was referred to an otolaryngologist for follow up.

No consistent clinically relevant abnormalities in vital signs, chemistry and haematology blood results, urinalysis, electrocardiograms or 24-h EEG registrations were observed.

**Evoked pain tasks**

The mean changes in the least squares means from baseline over 96 h following Xen2174/placebo administration for the different evoked pain task variables (AUCl, PDT, pTT) were evaluated. The summary statistics of the pTT are provided in Table 3. The time course for the mean change in the pTT from baseline in the first 48 h following Xen214/placebo administration for the different evoked pain tasks is shown in Figure 2.

Following treatment with Xen2174 2.50 mg, we observed an increase in the pTT over a prolonged period of time for the electrical stimulation tasks (single (overall treatment p-value, contrast least squares mean of the pTT Xen2174 2.5 mg – placebo [95% confidence interval], contrast p-value / p=0.1801, 17.1% [-10.4%, 53.2%], p=0.2372) and repeated stimulation (p=0.0713, 28.9% [-3.3%, 71.7%], p=0.0811)) and the pressure stimulation task (p=0.0328, 22.2% [-5.0%, 57.1%], p=0.1131). No clear differences in pTT between the different dose groups could be observed for iCPM (p=0.7615, 0.68 [-1.48, 2.84], p=0.5233) or the cold pressor task (p=0.5419, -3.4% [-27.8%, 29.2%], p=0.8091). AUClS and PDTS for the different pain tasks did not show any significant results. Seventeen subjects missed one or more nociceptive tests because of concurrent postlumbar puncture headache and treatments.

**Drug concentrations in CSF and plasma**

The mean PK concentration-time profiles and the corresponding PK variables of Xen2174 in CSF are shown in Figure 3 and Table 4, respectively. The mean half-life ranged between 4.27 h and 7.14 h in CSF. The AUCl (concentration-time) from time zero to infinity (AUCl⁻∞) values increased more than proportionally with dose in all dose groups.

The PK concentration-time profiles and variables of Xen2174 in plasma are shown in Table 5 and Figure 4. In general, concentrations were approximately 500- to 2000-fold lower in plasma than in CSF. Average plasma peak maximum concentration (cmax) increased from 5.49 ng ml⁻¹ at the 0.5 mg dose level to 9.75 ng ml⁻¹ at 1 mg and 15.4 ng ml⁻¹ at the 2.5 mg dose level. cmax appeared to increase slightly less than proportionally with dose.
between 0.5 and 2.5 mg. The average time to reach the plasma $c_{\text{max}}$ ($T_{\text{max}}$) was 194, 3.69 and 6.89 h, for the 0.5, 1, and 2.5 mg doses, respectively. $AUC_{0-\infty}$ increased proportionally to dose.

**DISCUSSION**

The present study showed that the 2.5 mg dose of Xen2174 administered intrathecally was able to influence pain thresholds in several evoked pain tasks. The pain tasks showed an increase in PTTs for the electrical pain tasks and the pressure pain task in favour of the highest dose of Xen2174 tested, although statistical significance was not reached.

In nonclinical experiments, intrathecal administration of Xen2174 produced anti-allodynic and antinociceptive effects in rats. The chronic constriction injury (CCI) model and the L5/L6 ligation model used in the study by Nielsen et al. are both models for neuropathic pain, while Obata and colleagues used a model of postincisional pain. The models used in the present study were mainly for acute nociceptive pain. Owing to the differences in etiology in these models, no direct translation can be made between the results in nonclinical results and the results in humans. Dosages in the present study were based on nonclinical data. The half maximal effective concentration ($E_{C_{50}}$) in a functional assay for the binding of Xen2174 to the NET, resulting in the inhibition of NE uptake by the transporter, was 185 nM, which corresponds to a concentration of 0.26 mg l⁻¹. The median effective dose ($ED_{50}$) concentration in CSF for antinociception in the Brennan model for postoperative pain in rats was 0.86 µg intrathecally (hypothetical concentration in CSF 3.2 mg l⁻¹). The $ED_{50}$ for anti-allodynia in the CCI model in rats was 15.7 nmol (22.1 µg, leading to a hypothetical CSF concentration of 81.9 mg l⁻¹). It was expected that dosages in the range of 1.0-2.5 mg would lead to CSF concentrations above the observed $E_{C_{50}}$ and $ED_{50}$, and thus induce nociceptive effects. The observed $c_{\text{max}}$ (after administration of 2.5 mg of Xen2174) in CSF of 33.2 mg l⁻¹ was above the $ED_{50}$ for the antinociception in the Brennan model, but below the $ED_{50}$ for anti-allodynia in the CCI model.

The Xen2174 1 mg intrathecal dose in dogs was determined as the NOAEL in dogs in nonclinical studies. The ratio of the $AUC_{0-\infty}$ measured in CSF in the Xen2174 2.5 mg dose group in humans compared with that in dogs after a 1 mg intrathecal injection was 1.43 (unpublished data). A preferred and expected safety margin for this $AUC_{0-\infty}$ ratio for single intrathecal doses of Xen2174 in dogs (expected ratio to be at least 10) was not reached, leading the sponsor to discontinue further development of this compound.

Xen2174 is one of a novel class of NRIs for the treatment of pain. It has been shown to exert its effects via spinal activation of $\alpha_2$-adrenoceptors subsequent to NE reuptake inhibition. Other NRIs include tricyclic antidepressants and tapentadol. The tricyclic antidepressant imipramine increases the PTT for pressure pain and for electrical stimulation. Tapentadol combines opioidergic activity with noradrenergic activity with both mechanisms accounting for the analgesic effects. It is efficacious in the treatment of moderate to severe acute pain compared with placebo. Furthermore, tapentadol caused activation of conditioned pain modulation in patients with diabetes in an experimental setting.

Several polymorphisms are known for the NET gene (SLC6A2). Patients carrying the homozygous SNP2 G/G variant of this gene reported a longer analgesic onset time after medication administration than heterozygous and A/A homozygous patients. Hypothetically, a larger overall analgesic effect could have been observed if SNP2 G/G subjects had been excluded from the study. An equipotent analgesic effect might have been achieved with lower CSF concentrations. Unfortunately, no genotyping for polymorphisms was performed in the present study.

In addition to local anaesthetics, which are used for spinal anaesthesia, there are several analgesic compounds that are intrathecally administered. Clonidine, an $\alpha_2$-adrenergic receptor agonist, showed analgesic action after intrathecal and epidural administration. Ziconotide, a synthetic equivalent of the venom of a marine snail, exerts its effect by binding and blocking voltage-sensitive calcium channels. Opioids show postoperative analgesia when administered intrathecally. Intrathecal NSAIDs have been tested for their analgesic efficacy in patients but are not used in current clinical practice. Only two studies have reported the use of evoked pain models after intrathecal drug administration. Intrathecal ketorolac, an NSAID, was tested in a study in healthy volunteers but did not show an effect on pain from acute heat stimuli. Clonidine caused an increase in heat pain tolerance after intrathecal administration. In the current study we confirmed that intrathecal drug administration in combination with performing a battery of evoked pain tasks is feasible, even with concurrent CSF sampling.

An increase of 28.9% in least squares mean (electrical repeat PTT) and 22.2% (pressure PTT) was observed after administration of Xen2174 compared with placebo. Similar effect sizes for electrical pain (42%) and pressure pain (22%) testing were observed after administration of an analgesic dose of alfentanil in previous research, suggesting that the difference we observed in pain tolerance was clinically relevant. The observed increase in
PTTs lasts for a long period (Figure 2), whereas the CSF concentration steadily drops (Figure 3). The prolonged analgesic effect cannot be explained by the CSF concentrations but it should be noted that such a measure is a surrogate for tissue concentration and receptor binding, and therefore may reflect a similar distribution to the effect site (reflected by half-life for equilibration, t½,ke0) to that observed with other analgesics and consequent clearance from the effect site. Although no mechanistic validation can be provided, the long duration of action has already been observed in nonclinical experiments, in which doses of intrathecal Xen2174 provided longer relief of tactile allodynia in CCI rats compared with morphine.

An increase in pain tolerance was observed in the electrical pain tasks and the pressure pain task, but no differences were observed in the cold pressor task. Earlier research with a centrally acting NRI, imipramine, also did not show an effect on the cold pressor task. The lack of effect on this task could suggest that administration of Xen2174 has only local effects, at and below the level of administration, but no effects at higher levels – for example, at the level of the brainstem. There was a difference in the level of administration of the study drug (L4-L5) and the dermatomes in which the cold pressor task was performed (C6-C8). In a study in which several amide local anaesthetics were compared, drug administration was performed at the second or third lumbar interspace, and the maximum level of sensory block to pinprick was level T2 in all dose groups. This might also be why no effects of Xen2174 on iCpm could be observed. The centrally acting NRI tapentadol has been shown to increase iCpm. Other explanations for the conflicting outcomes might include the fact that different methods were used to measure iCpm, or differences in patient populations.

Many studies employ evoked pain tasks to assess the analgesic effects of new drugs in healthy human subjects. Most of these studies test only one or two modalities of pain. The advantage of the method that was used in the current study was the combination of the different pain tasks in a standardised way. Earlier research has shown the advantages of multi-modal pain testing. Different evoked pain tests have different sensitivities for different analgesics. Using only one pain task could lead to a negative trial, while using a broad set of pain tasks could give a better understanding of how the different mechanisms that play a role in evoked pain tests are influenced, and therefore of the different pharmacological properties of a new compound. The models used in the present study represent only acute nociceptive pain models. No spontaneous, chronic or neuropathic pain was investigated. Therefore, caution should be exercised when interpreting our results.

While it has been shown that many different analgesics that are known to be effective in clinical acute and chronic pain management can affect the different tests that were used in this pain battery, the acute responses tested in the current study are not necessarily good models of chronic pain. Given the mode of action of Xen2174 to enhance descending inhibition, these acute measures may not adequately assess efficacy in clinical settings of chronic pain.

The limitation of multi-modal testing is the large number of different outcome variables. In the present study five PD tests, yielding 15 different variables were analysed without applying a correction for multiple testing. Only a weak signal for a dose-response relationship was observed in the study. Therefore, the multi-modal battery of pain tasks should be considered as a first screening tool for studying the analgesic properties of pain compounds in development. When the analgesic effect of a new drug on a certain pain mechanism has been established, predefining a primary outcome measure would prevent the need to correct for multiple testing. Furthermore, the present study was not formally powered for analgesic efficacy on the evoked pain tasks.

CSF sampling was limited in cohorts 1 and 2 because of catheter sampling difficulties. The introduction of a different type of intrathecal catheter improved the sampling success rate in the second part of cohort 2 treatment and in cohort 3. The total volume of CSF in humans is approximately 170 ml. Administration of 2.5 mg Xen2174 intrathecally would theoretically lead to a Cmax of 14.705 ng ml⁻¹. We found a Cmax of 33,200 ng ml⁻¹ after administration of 2.5 mg of Xen2174. This may suggest that the study drug was not completely mixed throughout the CSF at tmax. Alternatively, the CSF volume in which the drug can freely diffuse, even if proper mixing had occurred, was overestimated for yet unknown reasons. Describing the PK in CSF is different to that in plasma. Drugs administered intravenously are rapidly distributed within the central distribution volume. The PK of drugs administered in less ‘well-stirred’, oscillating fluid systems, like the CSF, is more difficult to predict; as such, it is difficult to predict drug concentrations at a particular level in the spinal column or intracranially. However, describing the dose-response relationship is more feasible if the site of injection of a drug is directly at the target site, which was the case in the present study.

No PK or PK/PD modelling was performed on the data. As discussed previously, the site of administration was the same as that of sampling. As a consequence, the drug concentrations of the CSF samples may have
been the sum of the concentration in CSF and that of the drug solution that had not yet fully distributed throughout the CSF, for which we could not quantitatively correct. The development of a PK model for these CSF data would have resulted in high uncertainty in parameter estimates and large values for variability, also contributed by the limited number of subjects. As a result, the parameter estimates were not expected to have physiological meaning, but merely to describe the observations in the lower spine. Moreover, Xen2174 has a high molecular weight and is therefore not expected to passively cross the blood-brain barrier to a large extent, apart from leakage. Finally, using the PK models that describe the CSF concentrations in the lower spine as the driving force for the PD would also have resulted in parameter estimates with high levels of uncertainty and large between subject variability – in our view, parameter estimates that have limited physiological meaning. The purpose of measuring CSF and plasma samples was to provide quantitative evidence of CNS exposure and limited plasma exposure which in our view, is sufficiently supported by the non-compartmental analysis. Given the lack of real physiological meaning that PK parameter estimates would have had, it was decided not to develop a PK model; similarly, the development of a PK/PD model would not have been logical.

Based on the literature, the incidence of postlumbar puncture syndrome was higher than expected. In a study in which the same intrathecal catheter was tested, CSF Xen2174 concentrations exceeded the required exposure limit vs. the expected value for this difference might be the age difference (63.3 years was higher than expected. In a study in which the same intrathecal catheter was tested, CSF Xen2174 concentrations exceeded the required exposure limit vs. the expected value.

REFERENCES


The use of a battery of evoked pain models in early phase drug development


Summary of previous clinical studies with Xen2174.

**Table 1**

<table>
<thead>
<tr>
<th>Study</th>
<th>Number (n) of subjects</th>
<th>Dose</th>
<th>Outcomes</th>
<th>Serious adverse events*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1 study of Xen2174 administered intrathecally in healthy male subjects</td>
<td>n=16 treated with Xen2174; n=8 placebo</td>
<td>0.025-40 μg kg⁻¹</td>
<td>No effects on nociceptive testing.</td>
<td>None</td>
</tr>
<tr>
<td>Phase 1-2 open-label study of Xen2174 administered intrathecally in oncology patients with chronic pain</td>
<td>n=16 treated with Xen2174</td>
<td>0.025-40 mg</td>
<td>No definitive conclusions regarding clinical benefit due to small number of patients per dose group and variation in type of pain. Each cohort contained at least one patient with ≥70% reduction in pain scores.</td>
<td>Confusion and dysphasia (0.25 mg), apnea, unresponsiveness, grand mal seizure (40 mg), acute drug-induced meningitis (40 mg)</td>
</tr>
<tr>
<td>Phase 2 study of Xen2174 administered intrathecally in adults prior to bunionectomy surgery (partially completed)</td>
<td>n=13 treated with Xen2174; n=3 placebo</td>
<td>1.0 mg</td>
<td>No final conclusion regarding clinical efficacy.</td>
<td>None</td>
</tr>
<tr>
<td>Phase 1-2 tic safety study of Xen2174 administered in healthy male and female subjects</td>
<td>n=8 treated with Xen2174; n=7 placebo</td>
<td>0.3-2.5 mg</td>
<td>No apparent effects on ECG.</td>
<td>None</td>
</tr>
</tbody>
</table>

*Considered related to the study drug. ECG, electroencephalogram

Summary of treatment emergent adverse events (AEs) by frequency ([n%]). AEs occurring more than once within one treatment are reported.

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo</th>
<th>Subjects with ≥1 AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with ≥1 AE</td>
<td>8 (100)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Number of different AEs</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Post Lumbar Puncture Syndrome</td>
<td>5 (62.5)</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>Headache</td>
<td>3 (37.5)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>Parasthesia</td>
<td>3 (37.5)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>3 (37.5)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Musclekeletal Stiffness</td>
<td>2.5 (31.25)</td>
<td>-</td>
</tr>
<tr>
<td>Syncope</td>
<td>2 (25.0)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>-</td>
<td>1 (12.5)</td>
</tr>
</tbody>
</table>
The use of a battery of evoked pain models in early phase drug development

PK and PD of intrathecally administered xen2174 in healthy subjects

Table 3
Least squares means for the pain tolerance thresholds and estimates of difference, 95% confidence intervals and p-values for main contrasts.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Xen2174 0.5 mg</th>
<th>Xen2174 1.0 mg</th>
<th>Xen2174 2.5 mg</th>
<th>Treatment P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold PTT (s)</td>
<td>39.94</td>
<td>33.18</td>
<td>34.84</td>
<td>38.58</td>
<td>0.541 (0.16%, 11.1%)</td>
</tr>
<tr>
<td>Electrical repeated PTT (ms)</td>
<td>10.01</td>
<td>10.39</td>
<td>14.19</td>
<td>0.0713</td>
<td>-0.7% (-7.7%, 28.4%)</td>
</tr>
<tr>
<td>Electrical single PTT (ms)</td>
<td>25.54</td>
<td>22.52</td>
<td>29.92</td>
<td>0.0802</td>
<td>-0.5% (-0.8%, -0.1%)</td>
</tr>
<tr>
<td>icPM: Delta Electrical Stair PTT (mA)</td>
<td>0.88</td>
<td>0.93</td>
<td>0.65</td>
<td>0.365</td>
<td>0.83 (0.25%, 1.60)</td>
</tr>
<tr>
<td>Pressure PTT (kPa)</td>
<td>60.52</td>
<td>54.36</td>
<td>53.68</td>
<td>73.95</td>
<td>-10.2% (-30.1%, 15.5%)</td>
</tr>
</tbody>
</table>

Table 4
Cerebrospinal fluid (CSF) pharmacokinetic parameters for Xen2174.

<table>
<thead>
<tr>
<th>Dose Xen2174 (mg)</th>
<th>Cmax (ng ml⁻¹)</th>
<th>Tmax (h)</th>
<th>t½ (h)</th>
<th>AUClast (h ng ml⁻¹)</th>
<th>AU0-∞ (h ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
</tr>
<tr>
<td>Xen2174 0.5 mg</td>
<td>40.00 ± 3.20</td>
<td>1.94 ± 0.40</td>
<td>6.32 ± 1.20</td>
<td>80.8 ± 16.4</td>
<td>45.3 ± 10.9</td>
</tr>
<tr>
<td>Xen2174 1.0 mg</td>
<td>55.00 ± 3.49</td>
<td>3.69 ± 0.79</td>
<td>9.65 ± 1.54</td>
<td>12.3 ± 2.11</td>
<td>6.7 ± 3.1</td>
</tr>
<tr>
<td>Xen2174 2.5 mg</td>
<td>134.0 ± 5.67</td>
<td>6.89 ± 1.23</td>
<td>18.6 ± 3.86</td>
<td>32.1 ± 6.54</td>
<td>15.9 ± 3.5</td>
</tr>
</tbody>
</table>

Table 5
Plasma pharmacokinetic parameters for Xen2174.

<table>
<thead>
<tr>
<th>Dose Xen2174 (mg)</th>
<th>Cmax (ng ml⁻¹)</th>
<th>Tmax (h)</th>
<th>t½ (h)</th>
<th>AUClast (h ng ml⁻¹)</th>
<th>AU0-∞ (h ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
</tr>
<tr>
<td>Xen2174 0.5 mg</td>
<td>5.49 ± 3.20</td>
<td>1.94 ± 0.40</td>
<td>6.32 ± 1.20</td>
<td>80.8 ± 16.4</td>
<td>45.3 ± 10.9</td>
</tr>
<tr>
<td>Xen2174 1.0 mg</td>
<td>9.75 ± 3.49</td>
<td>3.69 ± 0.79</td>
<td>9.65 ± 1.54</td>
<td>12.3 ± 2.11</td>
<td>6.7 ± 3.1</td>
</tr>
<tr>
<td>Xen2174 2.5 mg</td>
<td>15.4 ± 5.67</td>
<td>6.89 ± 1.23</td>
<td>18.6 ± 3.86</td>
<td>32.1 ± 6.54</td>
<td>15.9 ± 3.5</td>
</tr>
</tbody>
</table>

Figure 1
Disposition of subjects

- Female subjects after protocol amendment, n = 12
- BMI too low after protocol amendment, n = 7
- Abnormal screenings EEG, n = 7
- Medical history, n = 6
- Withdrew consent during screening period, n = 5
- Positive drug screen, n = 4
- Abnormal lab results, n = 3
- Family history of epilepsy, n = 1
- Reserve subject, n = 4
- Cancelled study participation, n = 3
- Assigned to study drug (Xen2174), n=25
- Assigned to placebo, n = 8
The use of a battery of evoked pain models in early phase drug development.

**Figure 2**
Time course of the mean change from baseline profile in least squares means for the pain tolerance threshold for electrical stimulation tasks (single [A] and repeated stimulus [B]), cold pressor task [C] and the pressure stimulation task [D] after administration of single doses of Xen2174 (0.5, 1.0 or 2.5 mg) or placebo. Vertical lines represent the 95% confidence intervals.

**Figure 3**
Mean CSF Xen2174 concentration-time by cohort. Vertical lines represent the standard deviation.
FIGURE 4
Mean Plasma Xen2174 concentration-time by cohort. Vertical lines represent the standard deviation.

0.5 mg  1.0 mg  2.5 mg