Direct-acting antiviral therapy for chronic hepatitis C

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Pharmacokinetics and antiviral activity of PHX1766, a novel hepatitis C virus protease inhibitor using an accelerated Phase 1 study design


*These authors made an equal contribution to this work

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ABSTRACT

Background: PHX1766 is a novel HCV NS3/4 protease inhibitor with robust potency and high selectivity in replicon studies (50% maximal effective concentration 8 nM). Two clinical trials investigated the safety, tolerability, pharmacokinetics and antiviral activity of PHX1766 in healthy volunteers (HV) and chronic hepatitis C patients, by use of a dose-adaptive overlapping clinical trial design.

Methods: Two randomized, double-blind, placebo-controlled clinical trials were conducted. Single doses of PHX1766 or placebo were administered to 25 HV and six HCV genotype 1-infected patients (50 mg once daily –1,000 mg once daily, 250 mg twice daily and 100 mg of a new formulation of PHX1766 once daily). Multiple doses of PHX1766 or placebo were administered to 32 HV and seven HCV genotype 1-infected patients (50 mg once daily –800 mg twice daily).

Results: Oral administration of PHX1766 was safe and well tolerated at all dose levels with rapid absorption (time at which concentration maximum is reached of 1–4 h) and with mean terminal half-lives of 4–23 h. Multiple doses of PHX1766 800 mg twice daily in HCV patients produced an area under the plasma concentration–time curve from time of drug administration to the last time point with a measurable concentration after dosing accumulation ratio of 2.3. The mean maximal observed HCV RNA decline was 0.6 log_{10} IU/mL in the first 24 h in the single-dose protocol and 1.5 log_{10} IU/mL after 6 days of PHX1766 dosing.

Conclusion: An overlapping, dose-adaptive single-dose and multiple-dose escalating design in HV and HCV-infected patients proved to be highly efficient in identifying a therapeutic dose. Although in vitro replicon studies indicated a robust HCV RNA viral decline of PHX1766, the study in HCV patients demonstrated only modest viral load reduction.

INTRODUCTION

Hepatitis C is a blood-borne virus infection with an estimated 170 million infected individuals worldwide and a prevalence of 3%. Chronic HCV infection carries an increased risk of developing liver cirrhosis and hepatocellular carcinoma and is, at present, the leading cause of end-stage liver disease. The current standard of care consists of combination therapy with pegylated interferon (peg-IFN)-α2a/b plus ribavirin. Current virological response rates vary from 41 to 84% after 24–48 weeks of therapy, mainly depending on the HCV genotype. Current antiviral treatment is associated with frequent and severe side effects and has a negative effect on patients’ quality of life. Therefore, adherence can be a challenge and physician-directed dose reductions (peg-IFN and/or ribavirin) are not uncommon, both of which have been shown to result in lower sustained virological response rates. Thus, there is an urgent need for a more effective, more tolerable and shorter regimen of antiviral therapy.

Current developments in HCV therapy focus on direct acting antivirals (DAAs). These drugs inhibit viral replication in a direct manner by blocking or modulating specific steps in viral replication. The HCV-encoded serine protease NS3/4A is essential for viral replication and is therefore one of the targets of DAA research. The small molecule BILN-2061 was the first selective inhibitor of the NS3/4A protease that demonstrated antiviral activity in reducing HCV RNA in human. This proof of concept in humans led to further development of NS3/4A protease inhibitors such as telaprevir (VX-950) and boceprevir (SCH 503034). These compounds have been shown to induce impressive HCV RNA declines, and are at present investigated in Phase III studies. However, it should be anticipated that DAAs still require combination therapy with peg-IFN and ribavirin as monotherapy is associated with frequent selection of resistant HCV variants. Triple therapy has been shown to improve sustained virological response rates but also induced additional side effects to the standard of care; rash is seen more often with the addition of telaprevir and anaemia is more severe when peg-IFN and ribavirin are combined with boceprevir.

PHX1766 is a novel NS3/4A HCV protease inhibitor discovered by Phenomix Corporation (San Diego, CA, USA; Figure 1). In the replicon system and biochemical assays, PHX1766 was shown to be a potent, tight-binding, reversible and highly selective HCV NS3/4A protease inhibitor (50% maximal effective concentration [EC50] 8 nM, Ki 0.05 nM). In vitro synergy was observed when PHX1766 was administered in combination with IFN-α and NS5B inhibitors in decreasing HCV replication in the replicon system. In vitro studies also showed that PHX1766 was active against genotypes 1a, 1b, 2a and 3a and common HCV mutants selected by telaprevir and boceprevir therapy (A156, R155, V36, T54, D168 and V170). These preclinical data indicated that certain clinically relevant mutations that have reduced susceptibility against telaprevir and boceprevir would be expected to remain sensitive to PHX1766. The 50% inhibitory concentration values of PHX1766 against these mutants and the relative fold shift compared with wild-type HCV 1b were generally several fold lower than the 50% inhibitory concentration values of telaprevir and boceprevir, although with PHX1766 they were not lower than 1 nM. In the HCV 1b replicon, analysis for mutations selected for in the presence of PHX1766 demonstrated the NS3 protease...

We report the safety and tolerability of two Phase I studies conducted with PHX1766 in healthy volunteers (HV) and HCV-infected patients using an accelerated trial design. We also present the antiviral activity and pharmacokinetic profile of PHX1766. A dose adaptive and overlapping design was applied to accelerate therapeutic dose finding because the results of the single-dose study guided dose selection for the multiple-dose study and vice versa. This study design seemed highly informative in a short period of time and the most cost-effective approach without compromising the safety of the participants.

**Figure 1. Structure of PHX1766.**

**METHODS**

**Study design**

Preclinical studies indicated that PHX1766 has a low clearance and a high volume of distribution in humans with a predicted terminal half-life between 12 and 24 h, which supported the potential of once-daily dosing. Animal studies suggested a high human liver-to-plasma concentration ratio and a low risk of drug–drug interactions because PHX1766 did inhibit the CYP3/4A at high concentrations (50% inhibitory concentration 13.7 μM) but did not show inhibition of other major human CYP isoenzymes. Regarding serum binding, preclinical studies have shown a high plasma or serum protein binding in human, rat, monkey and dog (99.3%, >99.6%, 99.3% and 97.6%, respectively). The human starting dose of 50 mg/day was calculated on the basis of 1/10th of the level of no observed adverse effect in animals.

A single-dose clinical protocol and a multiple-dose clinical protocol were designed to provide initial safety and tolerability information of PHX1766 in HV and chronic hepatitis C patients. Additionally, viral load decline data from chronic hepatitis C patients were intended to assist in identifying an anticipated therapeutic dose range. Therefore, the starting dose was defined at the initiation of the protocols, with the subsequent dosages to be guided by the viral load decline results of the previously dosed hepatitis C patients.

**Figure 2. Single-dose and multiple-dose protocols.**

(A) Single-dose protocol. All participants dosed with PHX1766 received Formulation A, unless otherwise denoted. Cohorts 1 and 2 were dosed with three increasing doses in an alternating manner with at least 7 days between dose escalations. Cohort 6 was dosed with two different dosages with at least 7 days between the doses. The last dosing period of cohort 1 was not dose escalation, but exploration of the food effect (fed state). Ratio of dosing PHX1766 and placebo: 6:2 for cohorts 1 and 2; and 7:2 for cohort 6. Cohorts 3–5 were dosed with PHX1766, no placebo.

(B) Multiple-dose protocol. All participants dosed with PHX1766 received Formulation A, unless otherwise denoted. Ratio of dosing PHX1766 and placebo: 6:2 for cohorts 1 and 2; and 7:2 for cohort 6. Cohorts 3–5 were dosed with PHX1766, no placebo.

The single ascending dose protocol was a randomized, double-blind, placebo-controlled study, in HV, alternated by an open-label single-dose trial in chronic hepatitis C patients infected with genotype 1. The multiple ascending dose study was a randomized and placebo-controlled study, in which HV cohorts and cohorts of chronic hepatitis C patients were alternately dosed (see Figure 2A and 2B). A safety review by the ethics committee was conducted and approval of the ethics committee was received prior to each dose escalation step. The trials were carried out in accordance with the Declaration of Helsinki version 2008 and in compliance with the current regulations and standards of Good Clinical Practice. The single-dose and multiple-dose...
clinical trials were conducted from October 2008 to April 2009 and from January 2009 to May 2009, respectively. Both studies were conducted at three sites in the Netherlands. All volunteers signed the informed consent form before participating in any study-related activity. The studies were conducted as in-house studies, with all patients admitted to the clinical facility 1 day prior to first dosing and discharged 48 h (single-dose protocol) or 24 h (multiple-dose protocol) after the last dose of the study drug.

Participants were dosed with oral capsules of PHX1766 or, not discernable from the active study drug, oral capsules of placebo. The capsules of PHX1766 were produced in a 75:25 ratio of the pharmacologically inactive substances to PHX1766 (Formulation A). To investigate whether a different formulation would lead to a higher gastrointestinal absorption of PHX1766, Formulation B was developed with a 90:10 ratio for the excipients of PHX1766.

**Dosing regimen**

The single-dose study was carried out in three cohorts of HV (cohorts 1, 2 and 6) and three cohorts of hepatitis C patients (cohorts 3, 4 and 5). The multiple-dose study was carried out in four cohorts of HV (cohorts 7, 8, 9 and 10) and two cohorts of hepatitis C patients (cohorts 11 and 12). The design was adaptive regarding dose selection; the first cohort of HCV-infected patients was enrolled at 100 mg once daily and the subsequent single and multiple dose levels were based on HCV RNA decline data of the previous cohort.

**Inclusion and exclusion criteria of healthy volunteers and chronic hepatitis C patients**

In the study male and female HV and chronic hepatitis C patients, aged 18–65 years, with a body mass index of 18–32 kg/m² were included. Chronic hepatitis C patients had to be chronically infected with HCV genotype 1, with a baseline HCV RNA viral load ≥1×10^5 IU/mL, be treatment-naïve or treatment-experienced to previous IFN-based therapy and have never been treated with any NS3/4A inhibitor. Female participants had to be postmenopausal by history or surgically sterile. A negative pregnancy test at screening and on day -1 was required when postmenopausal status was documented by history. In addition, increased follicle-stimulating hormone >40 mIU/mL at screening was required to confirm postmenopausal status. Key exclusion criteria were decompensated liver disease (findings consistent with Child Pugh B/C liver cirrhosis), any uncontrolled or active major systemic disease and coinfection with HIV or HBV. Patients with chronic stable haemophilia or on stable methadone substitution treatment were eligible for the study.

**Safety assessments**

HV and patients were monitored for safety and tolerability at regular intervals from the start of dosing throughout the study and follow-up. Safety parameters included vital signs, physical examination, laboratory parameters (biochemistry, haematology and urinalysis), electrocardiograms and the recording of all adverse events (AEs). In the single-dose study, the assessments were made at least four times on the dosing days. Afterwards, they were performed on day 2 and day 3 and at the day of follow-up, which was days 7–9 counted from the last dose. In the multiple-dose study, safety assessments were made at screening, admission to the clinic and in the dosing period on a daily basis. After dosing, safety assessments were made on the day of discharge in HV and 17 ±3 days or 20 ±3 days after the first dose of study drug in healthy volunteers and HCV-infected patients, respectively.

**Pharmacokinetic assessments**

Plasma samples for measurement of PHX1766 levels were collected in each treatment group of the single-dose and multiple-dose studies just before dosing, at various time points of each dosing day and at follow-up (days 7, 8 or 9 from the last dose in the single-dose study; days 7–10 from the last dose in the multiple-dose study with addition of days 17 and 20 for the HCV-infected patients). Plasma samples were analysed by Tandem Labs (Salt Lake City, UT, USA) using a validated HPLC tandem mass spectrometry method. The pharmacokinetic parameters were estimated using a non-compartmental model with the WinNonlin® Pro software (Pharsight Corporation, Mountain View, CA, USA). The PHX1766 concentration maximum and time at which the concentration maximum is reached were obtained by calculation of the plasma concentration data.

**Pharmacodynamic assessments**

In the single-dose study, samples for HCV RNA measurement were collected between 3 h and 30 min before the morning dose and at various time points on the dosing day, 48 and 72 h after dose and during follow-up. In the multiple-dose study, HCV RNA measurements were performed 3 h until 30 min before dose, after dose throughout day 1 and day 6, on days 2–5, and during follow-up. Plasma HCV RNA levels were determined using the Roche Ampliprep/Cobas Taqman HCV/HPS assay (Roche Molecular Systems Inc., Branchburg, NJ, USA). The lower limit of detection of this assay was 15 IU/mL and the linear range was (43–6.90) ×10^5 IU/mL. The Truegene assay was used to determine the genotype of all patients.25

**RESULTS**

**Baseline demographics of healthy volunteers and hepatitis C patients**

A total of 25 HV (cohorts 1, 2 and 6) and six chronic hepatitis C patients (cohorts 3, 4 and 5) were enrolled in the single-dose protocol. One HV (cohort 2) was discontinued after the second dose because of an intercurrent febrile illness. One HV was only available for one of the two dose groups, so cohort 6 required another HV for the second dose group and resulted in nine HV.

In the multiple-dose protocol, 32 HV (cohorts 7–10) and seven hepatitis C patients (cohorts 11 and 12) were enrolled. No patient was discontinued from the study. In both clinical protocols, the hepatitis C patients were infected with genotype 1a or 1b. Of the 13 hepatitis C patients infected with genotype 1a, all patients were infected with genotype 1b.
enrolled, nine were experienced and four were naïve to an IFN-based regimen. Baseline demographics are summarized in Table 1. There were no significant differences in baseline characteristics between the HV in the different dose groups and HCV-infected patients.

**Table 1.** Baseline characteristics of healthy volunteers and HCV-infected patients that participated in the single-dose and multiple-dose protocols.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy volunteers (n=57)</th>
<th>HCV-infected patients (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohorts</strong></td>
<td>1, 2, 6, 7, 8, 9, 10</td>
<td>3, 4, 5, 11, 12</td>
</tr>
<tr>
<td><strong>Doses</strong></td>
<td>50 mg once daily – 400 mg twice daily</td>
<td>100 mg once daily – 800 mg twice daily</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>29 (51)</td>
<td>12 (92)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White/Caucasian, n (%)</td>
<td>56 (98)</td>
<td>11 (85)</td>
</tr>
<tr>
<td>Asian, n (%)</td>
<td>1 (2)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Black, n (%)</td>
<td>0</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>55 (18-65)</td>
<td>49 (22-60)</td>
</tr>
<tr>
<td>Median BMI, kg/m² (range)</td>
<td>26 (19-32)</td>
<td>28 (21-32)</td>
</tr>
<tr>
<td>Median ALT, U/L (range)</td>
<td>74 (26-149)</td>
<td></td>
</tr>
<tr>
<td>HCV subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a, n (%)</td>
<td>9 (69)</td>
<td>4 (31)</td>
</tr>
<tr>
<td>1b, n (%)</td>
<td>6.4 (5.3-7.0)</td>
<td></td>
</tr>
<tr>
<td>Mean baseline HCVRNA IU/mL log_{10} (range)</td>
<td>Mean baseline HCVRNA IU/mL log_{10} (range)</td>
<td>Mean baseline HCVRNA IU/mL log_{10} (range)</td>
</tr>
<tr>
<td>Prior IFN treatment experienced, n (%)</td>
<td>9 (69)</td>
<td></td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; BMI, body mass index; IFN, interferon.

**Safety and tolerability in healthy volunteers and hepatitis C patients**

A total of 33 AEs were reported by 16 HV and seven AEs by three hepatitis C patients who received PHX1766 or placebo during the single-dose protocol. Only two AEs were considered related to PHX1766. Both AEs concerned myalgia, which were considered mild in intensity and resolved spontaneously during follow-up (Table 2). There was one serious AE during the study that led to the death of one of the HV. The patient was administered 100 mg of PHX1766 once daily and 300 mg of PHX1766 once daily (cohort 2) consistent with the protocol. During follow-up, 2 weeks after the second dose, the patient presented with a febrile illness for which study treatment was discontinued. One week later, all safety assessments were normal. Two days after that follow-up visit, the HV underwent an elective eyelid and abdominal wall correction as an outpatient and died suddenly 1 day after surgery. Autopsy determined the cause of death as postoperative multiple pulmonary emboli and the event was considered not related to the study treatment. Among the remaining 24 HV and six hepatitis C patients, no clinically important changes were seen concerning vital signs, laboratory assessments, physical examination and electrocardiogram from the start of the study to the end of the study.

In the multiple-dose protocol, 37 AEs were reported by 17 HV and 20 AEs by five HCV patients who received PHX1766 or placebo. A total of 12 AEs were considered to be related to PHX1766; they were all considered mild in intensity and resolved after treatment cessation (Table 2). There were no clinically significant changes in the vital signs, electrocardiogram or physical examination from the start until the end of study.

**Table 2.** Adverse events among healthy volunteers and HCV-infected patients that were considered related to the active study drug PHX1766.

<table>
<thead>
<tr>
<th>Trial/patient</th>
<th>Dose of PHX1766</th>
<th>Adverse event</th>
<th>Intensity</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteer</td>
<td>100 mg OD</td>
<td>Myalgia</td>
<td>Mild</td>
<td>Paracetamol</td>
<td>1,000 mg</td>
</tr>
<tr>
<td>Healthy volunteer</td>
<td>100 mg OD</td>
<td>Myalgia</td>
<td>Mild</td>
<td>Paracetamol</td>
<td>500 mg QD</td>
</tr>
<tr>
<td>Healthy volunteer</td>
<td>50 mg OD</td>
<td>Feeling cold</td>
<td>Mild</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>Healthy volunteer</td>
<td>400 mg OD</td>
<td>Erythema</td>
<td>Mild</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>Healthy volunteer</td>
<td>400 mg OD</td>
<td>Myalgia</td>
<td>Mild</td>
<td>Paracetamol</td>
<td>500 mg QD</td>
</tr>
<tr>
<td>Healthy volunteer</td>
<td>400 mg BID</td>
<td>Headache</td>
<td>Mild</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>Healthy volunteer</td>
<td>400 mg BID</td>
<td>Headache</td>
<td>Mild</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>HCV-infected patient</td>
<td>400 mg BID</td>
<td>Dizziness</td>
<td>Mild</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>HCV-infected patient</td>
<td>800 mg BID</td>
<td>Fatigue</td>
<td>Mild</td>
<td>No</td>
<td>Resolved</td>
</tr>
<tr>
<td>HCV-infected patient</td>
<td>800 mg QD</td>
<td>Upper abdominal pain</td>
<td>Mild</td>
<td>No</td>
<td>Resolved</td>
</tr>
</tbody>
</table>

**Pharmacokinetics**

Following single doses with PHX1766, plasma concentrations were detectable within 30 min after dosing and the median time at which the concentration maximum is reached ranged from 1 to 3 h. In HV, the systemic exposure of PHX1766 in Formulation A increased dose-dependently from 50 to 1,000 mg, with the mean concentration maximum increasing from 10.6 to 699.7 ng/mL and the mean area under the plasma concentration–time curve from time of drug administration to the last point with a measurable concentration after dosing (AUC0–last) from 40.4 to 2,443.5 h•ng/mL, indicating that the increase was more than dose proportional in the tested dose range. PHX1766 exhibited a moderate terminal half-life, with the means...
ranging from 7 to 16 h in HV dosed Formulation A and 18 h in HV dosed Formulation B. HCV-infected patients showed a somewhat longer terminal half-life (means 17–23 h). A fed state increased the systemic exposure but was not statistically significant. Formulation B enhanced the systemic exposure in terms of AUC0–last by 3.7-fold as compared with Formulation A. The individual maximal Cmin at 24 h after dosing was 0.255 ng/mL with Formulation A 100 mg once daily and 0.847 ng/mL with Formulation B 100 mg once daily. The systemic exposure of PHX1766 appeared to be higher in HCV-infected patients than in HV (Tables 3 and 4).

Table 3. Summary statistics of PHX1766 plasma pharmacokinetic parameters in the single-dose study.

<table>
<thead>
<tr>
<th>PHX1766 treatment</th>
<th>Cmax ng/mL (n=29)</th>
<th>Tmax h (n=29)</th>
<th>AUC0–last ng.h/mL (n=29)</th>
<th>T1/2 h (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg</td>
<td>10.57 (3.28-26.50)</td>
<td>3.00 (1.00-4.00)</td>
<td>40.38 (15.1-72.1)</td>
<td>7.45 (7.29-10.52)</td>
</tr>
<tr>
<td>100 mg</td>
<td>18.44 (4.77-35.30)</td>
<td>2.00 (1.00-3.00)</td>
<td>63.00 (11.7-114.3)</td>
<td>9.63 (6.06-17.52)</td>
</tr>
<tr>
<td>200 mg</td>
<td>76.99 (42.3-203.0)</td>
<td>2.52 (1.00-4.02)</td>
<td>189.29 (81.0-388.9)</td>
<td>10.61 (5.20-14.05)</td>
</tr>
<tr>
<td>HCV infected patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 mg Fasted</td>
<td>59.80 (15.3-129.0)</td>
<td>3.00 (2.00-4.00)</td>
<td>187.08 (71.6-354.7)</td>
<td>17.7 (10.25-32.01)</td>
</tr>
<tr>
<td>200 mg Fed</td>
<td>97.9 (28.8-216.0)</td>
<td>3.00 (2.00-4.03)</td>
<td>300.99 (111.6-505.7)</td>
<td>15.82 (10.11-21.94)</td>
</tr>
<tr>
<td>300 mg</td>
<td>90.39 (62.0-159.0)</td>
<td>2.01 (2.00-6.00)</td>
<td>271.19 (170.1-381.9)</td>
<td>8.92 (5.84-14.32)</td>
</tr>
<tr>
<td>500 mg</td>
<td>262.881 (70.3-441.0)</td>
<td>3.00 (2.00-3.09)</td>
<td>872.36 (358.5-1,341.6)</td>
<td>12.10 (8.16-19.13)</td>
</tr>
<tr>
<td>1,000 mg</td>
<td>699.71 (375.0-1,500.0)</td>
<td>3.00 (3.00-4.00)</td>
<td>2,443.5 (1,656.2-4,388.7)</td>
<td>14.89 (11.63-17.67)</td>
</tr>
<tr>
<td>Formulation B</td>
<td>- (32.90-37.20)</td>
<td>(2.00-4.00)</td>
<td>(119.4-151.3)</td>
<td>(16.36-17.81)</td>
</tr>
<tr>
<td>50 mg</td>
<td>- (275.0-2,170.0)</td>
<td>(3.00-4.00)</td>
<td>(977.3-8,548.5)</td>
<td>(13.9-32.80)</td>
</tr>
<tr>
<td>250 mg</td>
<td>- (177.0-227.0)</td>
<td>(20.0-20.0)</td>
<td>(1,828.4-2802.2)</td>
<td>(7.13-26.86)</td>
</tr>
</tbody>
</table>

For the concentration maximum (Cmax), area under the plasma concentration–time curve from time of drug administration to the last time point with a measurable concentration after dosing (AUC0–last) and terminal half-life (T1/2), the geometric mean (range) is presented; for the time at which Cmax is reached (Tmax), the median (range) is presented.

With the multiple-dose protocol, steady state was reached within 3 days of dosing. There was no apparent accumulation in systemic exposure at steady state versus the first dose in HV (Figure 3A). By contrast, there was approximately two-fold accumulation in systemic exposure at steady state versus the first dose in chronic hepatitis C patients (Figure 3B), suggesting a difference in uptake. The average accumulation ratio with respect to AUC0–last was 1.5 in HV (400 mg twice daily) and 2.3 in HCV-infected patients who were dosed 800 mg twice daily. The highest average trough plasma concentration of PHX1766 in HV was 21.0 ng/mL in the 400 mg twice daily dose group at 96 h after dose (Figure 3A) and was lower than the EC50 of 8 nM (~49 ng/mL) in the replicon system. Among the HCV-infected patients, the highest average trough level was measured at 120 h after dose in the 800 mg twice daily dose group (163.5 ng/mL) and was >3 times higher than the EC50 (Figure 3B).

Table 4. Summary statistics of PHX1766 plasma pharmacokinetic parameters in the multiple-dose study.

<table>
<thead>
<tr>
<th>PHX1766 treatment</th>
<th>Cmax ng/mL (n=29)</th>
<th>Tmax h (n=29)</th>
<th>AUC0–last ng.h/mL (n=29)</th>
<th>T1/2 h (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg once daily</td>
<td>- (105.5-698.0)</td>
<td>(1.00-3.00)</td>
<td>(394.5-2071)</td>
<td>(3.86-4.50)</td>
</tr>
<tr>
<td>100 mg once daily</td>
<td>- (139.0-952.0)</td>
<td>(1.00-3.00)</td>
<td>(560.0-3105.1)</td>
<td>(4.38-4.41)</td>
</tr>
<tr>
<td>400 mg twice daily</td>
<td>1,048.3 (762.0-1,260.0)</td>
<td>1.01 (1.00-3.00)</td>
<td>2,833.7 (1,927-4,088)</td>
<td>3.61 (2.50-4.77)</td>
</tr>
<tr>
<td>800 mg twice daily</td>
<td>1,844.1 (715-1,980)</td>
<td>1.00 (1.00-4.00)</td>
<td>6,413.7 (6,242-7,673)</td>
<td>7.25 (4.86-13.0)</td>
</tr>
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For the concentration maximum (Cmax), area under the plasma concentration–time curve from time of drug administration to the last time point with a measurable concentration after dosing (AUC0–last) and terminal half-life (T1/2), the geometric mean (range) is presented; for the time at which Cmax is reached (Tmax), the median (range) is presented.
Figure 3. Geometric mean plasma concentration–time profile of PHX1766.

Viral response

In the single-dose study, there was a transient decrease in plasma viral load with a mean maximal HCV RNA decline of 0.6 log₈ IU/mL (±0.50), which was achieved during the first 24 h after PHX1766 administration (100–500 mg once daily; 250 mg twice daily). The individual maximal HCV RNA decline was 1.5 log₈ IU/mL and was observed in the 500 mg once daily dose group (cohort 4). In three out of six hepatitis C patients, the maximal viral load decline occurred during the first 12 h (including the individual with the highest viral load drop observed) with return to baseline values at 16 h after dose. In the multiple-dose study, all HCV patients treated with PHX1766 showed a transient decrease in plasma viral load with, after 6 days of dosing, a mean maximal HCV RNA decline of 1.5 log₈ IU/mL (±0.49; Figure 4). The individual maximal HCV RNA decline was 1.5 log₈ IU/mL within the first 24 h and 2.2 log₈ IU/mL after 6 days dosing, both in the 800 mg twice daily dosing group (cohort 12). All patients demonstrated a similar return of viral load to baseline at the follow-up visits. No significant changes in HCV RNA levels were observed in patients who received placebo (Figure 4).

Figure 4. Individual viral load decline of the seven dosed HCV-infected patients during the multiple PHX1766 dose study.

The baseline value was determined just before dosing. Dose levels presented on the right-hand side of the graphs. Patients received PHX1766 400 mg twice daily, 800 mg twice daily or placebo for 6 days.

DISCUSSION

This study is the first to describe the in vitro and in vivo profile of the novel HCV NS3/4A protease inhibitor PHX1766. A trial design, adaptive to dose selection, was used to explore the safety and pharmacokinetic and pharmacodynamic profile of PHX1766 in a short period of time in both HV and hepatitis C patients. The primary objectives of these clinical trials were to investigate the safety and tolerability of oral doses of PHX1766 in HV and chronic hepatitis C patients. Treatment with PHX1766 was generally well tolerated. Regarding both single-dose and multiple-dose protocols, one HV was discontinued from the study and this patient developed a serious AE. The cause of death was investigated by autopsy and considered unrelated to PHX1766 dosing. There were no other study discontinuations or serious AEs. The most frequently reported AEs that were considered related to PHX1766 concerned flu-like symptoms that were all mild in intensity and resolved...
spontaneously. Overall, administration of PHX1766 in single doses up to 1,000 mg and multiple
doses up to 800 mg twice daily in HV and HCV-infected patients was safe and well tolerated.
The secondary objectives were to investigate the pharmacokinetic profile and antiviral activity
of PHX1766. First of all, there was a clear discrepancy in the terminal half-life and accumulation
between HV and HCV-infected patients in favour of the HCV-infected patients, suggesting a
difference in uptake of PHX1766. Liver functions such as drug metabolism and biliary excretion
might be impaired in chronic hepatitis C patients. PHX1766 is cleared partially via biliary
excretion, which explains the longer terminal half-life and accumulation ratio of PHX1766
in HCV-infected patients when compared with the HV. Viral load suppression with single
oral doses of PHX1766 did not last for 24 h and this suggested a twice daily or three times
daily dosing regimen. However, with the current standard of care, the aim was to develop a
highly effective antiviral agent with the potential of once daily dosing. Thereby, twice daily
dosing for 6 days resulted in an only modest average viral load drop in HCV-infected patients.
Nevertheless, in vitro, PHX1766 was a selective protease inhibitor with an EC50 of 8 nM, low
human clearance and a high volume of distribution with a predicted human terminal half-life
between 12 and 24 h, which supported the potential of once daily dosing. In the multiple-dose
protocol, the highest dose group (800 mg twice daily) produced a trough-level 12 h after
dose of only three times the EC50. This is suboptimal when compared with other new DAAs
like TMC435 but would be sufficient if viral inhibition was strong. Mutations selected for in
the presence of PHX1766 have been demonstrated in the HCV 1b replicon. Because HCV RNA
suppression was shown to be incomplete in the clinical trials, it can be anticipated that viral
mutations would have occurred. With the suboptimal viral suppression achieved with PHX1766,
sequencing of possible viral mutations did not seem worthwhile and has not been performed.
Regarding kinetics, there was accumulation in the HCV-infected patients, a moderate human
terminal half-life but an, apparently, insufficient trough-level to produce vigorous and lasting
viral inhibition with once daily or twice daily dosing. Because replicon studies showed PHX1766
to be a potent protease inhibitor (EC50 8 nM) and animal studies suggested a high human-
to-plasma concentration ratio, there is a clear discrepancy between the results of the clinical
trials and the earlier described preclinical results concerning the pharmacokinetic profile and
antiviral effect.

New compounds that are introduced to clinical research often follow the different phases of
clinical trials chronologically. Our design accelerated dose finding because it implemented an
adaptive dose regimen with the investigation of safety and tolerability of PHX1766 in HV and
HCV-infected patients. The overlapping design of the single-dose and multiple-dose protocols
has proven to be a time-efficient and safe way to investigate the new protease inhibitor
PHX1766. Thereby, with this design, fewer patients are exposed to the experimental protease
inhibitor, which is of great importance in two ways. Firstly, it minimizes possible health risks
because fewer participants are dosed. The second reason is that less exposure reduces the
occurrence of mutations and, subsequently, resistance, because this phenomenon is inevitable
with the administration of a protease inhibitor as monotherapy. To conclude, time efficiency
and the requirement of fewer patients and fewer study drugs result in fewer costs. Taking

these arguments together, this design should be considered in studies of antiviral compounds
in order to rapidly assess initial safety, tolerability as well as antiviral effect.

Several limitations of both Phase I studies should be noted. First, the number of hepatitis C
patients that were dosed was small. These patients were randomized into different dose groups
making a solid statistical analysis of the pharmacokinetic and pharmacodynamic results difficult.
Second, the conducted trials did not investigate combination therapy of PHX1766 with peg-IFN
(and ribavirin). The in vitro studies showed a synergistic effect of PHX1766 in combination with
IFN-α, which encourages research into the antiviral effect of combination therapy.

In conclusion, single and multiple doses of PHX1766 in HV and HCV-infected patients were safe
and well tolerated. The overlapping and dose-adaptive clinical trial design proved to be a safe
and highly efficient way to investigate the molecule PHX1766 in healthy volunteers and HCV-
infected patients and should be considered for future Phase I clinical trials. PHX1766 dosing in
humans resulted in only a modest HCV RNA decrease, in contrast to the in vitro replicon results,
which indicated a robust viral load decline. Consequently, further development of PHX1766
was not pursued.
REFERENCES


