Background: ACH-1625 is a linear peptidomimetic inhibitor that non-covalently binds to HCV NS3 protease with high potency and specificity. Short-term monotherapy of HCV genotype-1 infection with ACH-1625 was found to be safe and resulted in ≥3.3 log10 IU/ml mean viral load reduction. These viral load decay data were analysed to compare HCV dynamics with prior reports and estimate the antiviral efficiency of ACH-1625.

Methods: Drug efficiency was estimated by analysing the viral decay following initiation of up to 5 days of monotherapy with ACH-1625 in 36 chronically infected HCV genotype-1 patients. During this monotherapy study, ACH-1625 was administered either twice-a-day for 4.5 days or once daily for 5 days at 5 different dose levels in 36 patients.

Results: A sharp viral decay during the first 48 h following the initiation of ACH-1625 treatment afforded high drug efficiency estimates (≥0.9934). In addition, an increase in the estimated drug efficiency was observed with increasing ACH-1625 dose. The observed anti-HCV response was fairly uniform in this proof-of-concept study across the population of 36 patients.

Conclusions: Estimates of the treatment-independent viral kinetics parameters were consistent with prior reports and the estimated drug efficiency of ACH-1625 monotherapy was very high (≥0.9934) in fasted and fed states.

Introduction

Approximately 3% of the world human population is infected with HCV. If untreated, 5–20% of HCV-infected individuals will develop cirrhosis over a period of 20–30 years, and 1–5% will die from the consequences of chronic infection, that is, liver cancer or cirrhosis. Chronic HCV infection is also the leading cause of liver transplants [1]. Due to lack of a proofreading mechanism, HCV exists as quasi-species. HCV has been divided into six genotypes by sequence similarity [2]. Infection with HCV genotype-1 (GT-1) is least susceptible to treatment with pegylated interferon-α and ribavirin (PR). Sustained response to PR therapy in GT-1-infected patients is achieved in only approximately 50% of patients and is also associated with serious side effects.

When combined with PR, direct-acting antiviral therapies have demonstrated improved sustained virological response rates in chronically-infected HCV patients. HCV NS3 serine protease is a validated target for anti-HCV therapies [3]. ACH-1625 is a linear peptidomimetic inhibitor that non-covalently binds to the HCV NS3 serine protease with high affinity and selectively inhibits this enzyme. It binds to the GT-1b:NS3 protease with an inhibition constant of 0.06 nM at steady state (K_{on}) and inhibits HCV replication in a cell line harbouring GT-1b/Con-1 subgenomic replicon with a 50% effective concentration (EC_{50}) of 11 nM [4]. Following oral dosing, ACH-1625 is distributed rapidly and selectively into the liver, which is partly due to transporter-mediated uptake [5].

ACH-1625 is safe and well tolerated at doses up to 2,000 mg/day for 4.5 days duration in healthy volunteers [6]. Subjects chronically infected with HCV GT-1 have been treated with ACH-1625 monotherapy or ACH-1625/PR combination therapy. Such patients were treated with PR in combination with a once-daily dose of 200 mg, 400 mg or 800 mg of ACH-1625 in a 4-week placebo controlled study. Treatment with ACH-1625/PR for 4 weeks resulted in undetectable viral load (<25 IU/ml) in ≥75% of patients by week 4 (rapid virological response [RVR]) as compared to 20% RVR in the placebo/PR arm. In a subsequent Phase II study, this therapy is being administered for 12 weeks in chronically infected HCV GT-1 patients.

In a randomized double blind placebo-controlled multiple-dose Phase I study, treatment-naive or
-experienced HCV GT-1-infected adults received ACH-1625 monotherapy for 4.5 or 5 days. In this proof-of-concept study, ACH-1625 administration at doses of 200, 500 or 600 mg twice daily for 4.5 days or 600 mg once daily for 5 days following a medium-fat meal resulted in mean maximum viral load drops of 3.63, 4.25, 3.94 and 3.81 log10 plasma HCV RNA IU/ml, respectively [4]. In addition, 400 or 600 mg once-daily dose administrations for 5 days in fasted state resulted in mean maximum viral load drop of 3.67 or 3.40 log10 plasma HCV RNA, respectively [7]. These suppressed levels of viral load did not return to the pretreatment level until 7 days post dosing (that is, until the end of the follow-up period in this study).

Here we describe an analysis of viral load reduction that resulted from monotherapy with ACH-1625. Quantitative analysis of the pharmacodynamic data obtained from these 36 patients allowed us to estimate the efficacy of ACH-1625 and other viral dynamics parameters at the five dosing levels of ACH-1625 monotherapy.

Methods

Patients in four groups of nine each were randomized to receive placebo or ACH-1625 at 200, 500 or 600 mg twice daily for 4.5 days or 600 mg once daily for 5 days following a medium-fat meal. Another two groups of six patients each received 400 or 600 mg once daily of ACH-1625 for 5 days in fasted state. Patients who received ACH-1625 were infected with HCV GT-1b. All trials were approved by local ethics committees and all patients gave written informed consent. HCV RNA data was collected as follows: at baseline (day 0); at the following time points during treatment: day 1 (2 h), day 2 (24 h), day 2 (36 h), day 3 (48 h), day 4 (72 h) and day 5 (96 h); and at the following time points post-treatment: day 5 (2 h), day 5 (9 h), day 6 (24 h), day 6 (36 h), day 7 (48 h), day 8 (72 h), day 9 (96 h) and day 12. On the cumulative time scale, HCV RNA data was collected as follows: at baseline (day 0); at the following time points during treatment: day 0.08, day 0.38, day 1, day 1.5, day 2.0, day 3.0 and day 4.0; and at the following time points post-treatment: day 5.08, day 5.38, day 6, day 6.5, day 7, day 8, day 9 and day 12. Serum HCV RNA was quantified using the Cobas Amplicor HCV Monitor (Pleasanton, CA, USA). The lower limits of detection and quantitation of this assay were 15 and 45 IU/ml, respectively.

HCV RNA data was analysed assuming a homogeneous virus population and by applying single population viral dynamics model described by Neumann et al. [8].

This mathematical model is described in Figure 1, and by Equations 1 and 2. It assumes constant number of uninfected cells and a steady state where infected cells produce virions at a constant rate (\( p \)), which are cleared from the serum at a constant rate (\( c \)). ACH-1625 specifically inhibits NS3 protease, therefore its efficiency was estimated as a factor reducing virion production rate in Equation 1. Estimates of drug efficiency, virion clearance rate constant and time delay in drug response were obtained by solving Equation 1 by non-linear fitting. These estimates were then placed into Equation 2 to estimate the infected cell death rate constant. Programming codes for viral kinetics modelling were developed in Matlab 7.9.0 (The Mathworks, Natick, MA, USA). Subsequently, the virion production rate (\( p \)) was computed by using the following equation: 
\[
V(t) = V_0[1-e^{-\lambda_2(t-\tau)}] e^{\lambda_1(t-\tau)}
\]
(1)

Where, \( V_0 \) is the initial viral load, \( c \) is the virion clearance rate constant, \( e \) is drug efficiency and \( \tau \) is the time delay.

\[
V(t) = V_0[Ae^{\lambda_3(t-\tau)}+(1-A)e^{\lambda_4(t-\tau)}] e^{\lambda_2(t-\tau)}
\]
(2)

where \( A = (\varepsilon \cdot \lambda) / (\varepsilon \cdot \lambda + \varepsilon \cdot \delta) \). \( \lambda_1 \) and \( \lambda_2 \) denote the slopes of phase 1 and phase 2 of viral decay, respectively, and \( \delta \) denotes the infected cell death rate constant.

Results

We mathematically analysed the viral decay profile observed following initiation of ACH-1625 monotherapy...
Rapid and sharp HCV decay on ACH-1625 monotherapy in subjects chronically infected with HCV genotype-1

Viral load did not return to the pretreatment level in any patient during treatment (that is, 4.5 days of twice-daily or 5 days of once-daily dosing) with ACH-1625 monotherapy in the six dosing cohorts. There was also prolonged suppression of viral load up to 7 days post-dosing and viral load did not return to baseline in this duration (Figure 2). In addition, the lower limit of detection (15 IU/ml) was reached by two patients in the highest dosing cohort, that is, 600 mg twice-daily dose group. Viral load decline in the first 0–48 h of therapy (first phase of viral decay) is primarily determined by drug efficiency and the virion clearance rate constant. Slope of this first phase of viral decay corresponds to the virion clearance rate constant for drugs whose efficiency is close to 1.

Table 1. HCV RNA log_{10} IU/ml decline in subjects chronically infected with HCV genotype-1 following ACH-1625 monotherapy

<table>
<thead>
<tr>
<th>Dose</th>
<th>Patients, n</th>
<th>Day 2 (48 h post first dose)</th>
<th>Day 5/hour 2 (2 h post last dose)</th>
<th>Maximum decline post-dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (sd)</td>
<td>Median</td>
<td>Mean (sd)</td>
</tr>
<tr>
<td>200 mg Twice daily</td>
<td>6</td>
<td>-3.0 (0.3)</td>
<td>-3.1</td>
<td>-3.4 (0.3)</td>
</tr>
<tr>
<td>500 mg Twice daily</td>
<td>6</td>
<td>-3.4 (0.4)</td>
<td>-3.4</td>
<td>-3.7 (0.4)</td>
</tr>
<tr>
<td>600 mg Twice daily</td>
<td>6</td>
<td>-3.3 (0.4)</td>
<td>-3.3</td>
<td>-3.4 (0.5)</td>
</tr>
<tr>
<td>600 mg Once daily</td>
<td>6</td>
<td>-3.2 (0.5)</td>
<td>-3.3</td>
<td>-3.7 (0.3)</td>
</tr>
<tr>
<td>600 mg Once-daily fasted</td>
<td>6</td>
<td>-3.4 (0.4)</td>
<td>-3.4</td>
<td>-3.3 (0.2)</td>
</tr>
<tr>
<td>400 mg Once-daily fasted</td>
<td>6</td>
<td>-2.6 (0.6)</td>
<td>-2.7</td>
<td>-3.4 (0.4)</td>
</tr>
</tbody>
</table>

Mean viral load drop observed in each cohort as a result of monotherapy with ACH-1625 at 200 mg twice-daily, 500 mg twice-daily, 600 mg twice-daily or 600 mg once-daily dose after a medium-fat meal and 600 mg once-daily or 400 mg once-daily dose in fasted state.

in subjects who carried chronic HCV infection. Our patient population was predominantly treatment-naive with high viral loads at baseline (Additional file 1).

Viral load did not return to the pretreatment level in any patient during treatment (that is, 4.5 days of twice-daily or 5 days of once-daily dosing) with ACH-1625 monotherapy in the six dosing cohorts. There was also prolonged suppression of viral load up to 7 days post-dosing and viral load did not return to baseline in this duration (Figure 2). In addition, the lower limit of detection (15 IU/ml) was reached by two patients in the highest dosing cohort, that is, 600 mg twice-daily dose group. Viral load decline in the first 0–48 h of therapy (first phase of viral decay) is primarily determined by drug efficiency and the virion clearance rate constant. Slope of this first phase of viral decay corresponds to the virion clearance rate constant for drugs whose efficiency is close to 1.

All dosing cohorts of ACH-1625 following a medium-fat diet or in fasted state demonstrated a sharp first-phase decline (mean declines >2.5 log_{10} IU/ml; Table 1) and the drug efficiency was estimated to be in
A range of 0.9934 to 0.9999 (Additional file 2). Specifically, mean ±sd ACH-1625 efficiency at 200 mg, 500 mg and 600 mg twice-daily dose levels was estimated to be 0.9977 ± 0.0023, 0.9991 ± 0.0005 and 0.9993 ± 0.0005, respectively (Table 2). The mean ±sd ACH-1625 efficiency estimates following initiation of 400 mg or 600 mg once-daily dosing in a fasted state or 600 mg once-daily dosing after a medium-fat diet were 0.9943 ± 0.0074, 0.9980 ± 0.0016 and 0.9987 ± 0.0015, respectively (Table 2).

The virion clearance rate constant (c) was estimated to be in the range of 6.2 to 9.1 days⁻¹ in six dosing cohorts. Estimated mean ±sd virion clearance rate constants following initiation of 200 mg, 500 mg or 600 mg twice-daily or 600 mg once-daily dosing after a medium-fat diet was 9.1 ± 1.6, 9 ± 1.5, 8.7 ± 1.5 and 8.1 ± 3.0 days⁻¹, respectively. Viral loads during once-daily dosing with 400 mg or 600 mg in a fasted state led to virion clearance rate constant estimates of 6.8 ± 1.9 and 6.2 ± 1.0, respectively. The mean ±sd virion production rate (b) was estimated to be 4.9 × 10¹⁰ ± 3.6 × 10¹⁰ virions per day in the six dosing cohorts.

The first phase of viral decay was followed by a slower viral load decline (second phase of viral decay) which is primarily dictated by the infected cell death rate constant, provided the drug efficiency is close to 1 (100%) [8]. Estimates of the infected cell death rate constant (δ) ranged from 0.0 to 0.52 days⁻¹ among 36 patients. Viral load decline during 200 mg, 500 mg or 600 mg twice-daily or 600 mg once-daily dosing after a medium-fat diet, and 400 mg or 600 mg once-daily dosing in fasted state resulted in mean ±sd δ estimates of 0.23 ± 0.10, 0.33 ± 0.13, 0.01 ± 0.02, 0.27 ± 0.20, 0.23 ± 0.03 and 0.35 ± 0.10 days⁻¹, respectively (Table 2, Figures 3 and 4, and Additional file 2).

**Discussion**

Early viral load decline in 0 to 48 h, following initial drug administration, is dose dependent and it reflects intrinsic viral susceptibility to the antiviral action of a drug. This viral load reduction followed a biphasic viral decay profile as described by Neumann et al. [8]. The viral kinetics observed during monotherapy with ACH-1625 was in accordance with this model. Response to ACH-1625 was dose dependent and fairly uniform within each dosing cohort. High efficiency estimates obtained from our viral kinetics modelling are further validated by virion clearance rate constant and infected cell death rate constant estimates that are consistent with literature reports.

An increase in the drug efficiency was observed with increasing twice-daily dose. The mean ±sd efficiency of 200 mg, 500 mg or 600 mg of ACH-1625 administered following a medium-fat diet was estimated to be 0.9977 ± 0.0023, 0.9991 ± 0.0005 and 0.9993 ± 0.0005, respectively. A similar trend was observed with once-daily dosing of ACH-1625 in fasted state. Estimated efficiency was higher (0.9980 ± 0.0016) in patients who received 600 mg once daily as compared to that of 400 mg once daily, 0.9943 ± 0.0074 in the fasted state. The efficiency of 600 mg once-daily dose of ACH-1625 was estimated to be 0.9987 ± 0.0015 when administered following a medium-fat diet. Estimated drug efficiency also suggested a correlation with drug response and dose levels included in this proof-of-concept study. Drug efficiency of 600 or 500 mg twice-daily doses was differentiated from that at 600 or 400 mg once daily in fasted state with a P-value of ≤0.09.

In addition to drug efficiency, virion clearance rate constant (c) was estimated by analysing viral load decay in 0 to 48 h following initiation of treatment with ACH-1625 monotherapy. We observed rather little interpatient variability in c and the mean ±sd was estimated to be 7.6 ± 1.9 day⁻¹ across all six dosing cohorts. Estimates of c have been reported in the range of 3 to 12.2 for interferon-α [8] and BILN 2061 [9]. A low interpatient variability in viral kinetics parameters estimated from phase I of viral decay has also been reported for anti-HCV treatment with interferon-α [8] and BILN 2061 [9].

<table>
<thead>
<tr>
<th>Dose, mg</th>
<th>200</th>
<th>500</th>
<th>600</th>
<th>600</th>
<th>600</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosing schedule</td>
<td>Twice daily</td>
<td>Twice daily</td>
<td>Twice daily</td>
<td>Once daily</td>
<td>Once daily</td>
<td>Once daily</td>
</tr>
<tr>
<td>Sample size, n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Efficiency/0–48 h (c)</td>
<td>0.9977 ± 0.0023</td>
<td>0.9991 ± 0.0005</td>
<td>0.9993 ± 0.0005</td>
<td>0.9987 ± 0.0015</td>
<td>0.9980 ± 0.0016</td>
<td>0.9943 ± 0.0074</td>
</tr>
<tr>
<td>Virion clearance rate</td>
<td>9.1 ± 1.6</td>
<td>9 ± 1.5</td>
<td>8.7 ± 1.5</td>
<td>8.1 ± 3</td>
<td>6.2 ± 1</td>
<td>6.8 ± 1.9</td>
</tr>
<tr>
<td>constant/0–48 h (c: d⁻¹)</td>
<td>4.4 ± 3.5</td>
<td>3.2 ± 2.6</td>
<td>3.3 ± 1.8</td>
<td>2.9 ± 3.7</td>
<td>3.4 ± 1.9</td>
<td>5.1 ± 5</td>
</tr>
<tr>
<td>Time delay/0–48 h (c: h)</td>
<td>0.23 ± 0.10</td>
<td>0.33 ± 0.13</td>
<td>0.01 ± 0.02</td>
<td>0.27 ± 0.2</td>
<td>0.23 ± 0.03</td>
<td>0.35 ± 0.1</td>
</tr>
<tr>
<td>Infected cell death rate</td>
<td>0.23 ± 0.10</td>
<td>0.33 ± 0.13</td>
<td>0.01 ± 0.02</td>
<td>0.27 ± 0.2</td>
<td>0.23 ± 0.03</td>
<td>0.35 ± 0.1</td>
</tr>
<tr>
<td>constant/0–5 days (δ: d⁻¹)</td>
<td>0.23 ± 0.10</td>
<td>0.33 ± 0.13</td>
<td>0.01 ± 0.02</td>
<td>0.27 ± 0.2</td>
<td>0.23 ± 0.03</td>
<td>0.35 ± 0.1</td>
</tr>
</tbody>
</table>

*Medium-fat diet. Data are mean ±sd.*
Figure 3. Observed HCV RNA levels and best-fit prediction by HCV kinetics model following twice-daily dosing of ACH-1625 monotherapy after a medium-fat diet.

Viral decay data fitted by using Levenberg–Marquardt algorithm implemented in Matlab 7.9.0 (The Mathworks, Natick, MA, USA) is shown along with HCV RNA quantified with the Cobas Amplicor HCV Monitor (Pleasanton, CA, USA). Viral decay during (A–F) 200 mg, (G–L) 500 mg and (M–R) 600 mg twice-daily dosing of ACH-1625 is shown. Each panel represents a patient.
Figure 4. Observed HCV RNA levels and best-fit prediction by HCV kinetics model following once-daily dosing of ACH-1625 monotherapy

Viral decay data fitted by using Levenberg–Marquardt algorithm implemented in Matlab 7.9.0 (The Mathworks, Natick, MA, USA) is shown along with HCV RNA quantified with Cobas Amplicor HCV Monitor (Pleasanton, CA, USA). Viral decay during (A–F) 600 mg once-daily dosing of ACH-1625 after medium-fat diet, and (G–L) 600 mg and (M–R) 400 mg once-daily dosing of ACH-1625 in fasted state is shown. Each panel represents a patient.
There was a significant variation in the infected cell death rate constant ($\delta$) estimated from the 4.5 or 5 day ACH-1625 monotherapy data. The mean ±SD infected cell death rate constant was estimated to be 0.24 ±0.15 in 36 patients dosed at six different levels with ACH-1625. This variability might be reflective of differences in the cellular immunity against HCV among patients, as suggested by Neumann et al. [8]. Our estimates of $\delta$ are consistent with the previously reported range, 0 to 0.92 [8–10].

Results described here were used in ACH-1625/PR combination treatment modelling and predicting RVR in a larger patient population (data not shown). These predictions aided in dose selection for further development of ACH-1625 in combination with PR. Subsequently, chronically infected subjects with HCV GT-1 were treated for four weeks with ACH-1625 doses of 200, 400 or 800 mg once daily in combination with PR. This 4-week treatment was safe and resulted in undetectable viral load (RVR<25 IU/ml) in ≥75% of subjects by week 4. In another ongoing study, this therapy was administered for twelve weeks in GT-1-infected subjects and resulted in 100% complete early virological response, and 67%, 80% and 100% RVR at ACH-1625 doses of 200, 400 and 800 mg once daily, respectively, in combination with PR.

This study estimated drug efficiency of ACH-1625 monotherapy to be very high (≥0.9934) in fasted and fed states, and the estimates of treatment independent viral kinetics parameters (infected cell death rate and virion clearance rate constants) were consistent with prior reports.

Acknowledgements

We thank the patients for their participation in this study.

Disclosure statement

All authors are employees of Achillion Pharmaceuticals, Inc. (New Haven, CT, USA) and declare no competing interests.

Additional files


References