Direct-acting antiviral therapy for chronic hepatitis C

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Safety and antiviral activity of JTK-652: a novel HCV infection inhibitor


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ABSTRACT

Background: Standard treatment of chronic hepatitis C with pegylated interferon and ribavirin is associated with suboptimal virological response rates and substantial side effects. This study describes the in vitro and in vivo development of JTK-652, a novel pyrrolopyridazin-derived HCV infection inhibitor.

Methods: JTK-652 was evaluated in multiple cell lines using an in vitro HCV infection model consisting of HCV pseudotype vesicular stomatitis virus bearing HCV E1/E2 envelope proteins. Safety, tolerability, pharmacokinetics and efficacy of JTK-652 were tested in a randomized double-blind and placebo-controlled study in healthy male volunteers (n=36) and chronic hepatitis C patients. A total of 10 HCV genotype-1-infected patients (treatment-naive [n=2] and treatment-experienced [n=8]) with HCV RNA>1×10^5 IU/mL received an oral dose of 100 mg JTK-652 three times daily or placebo (8:2 ratio) for 4 weeks.

Results: JTK-652 showed potent inhibitory activity against HCV genotype 1a and 1b pseudotype viruses bearing HCV E1/E2 envelope proteins in HepG2 cells and in human primary hepatocytes. No significant clinical laboratory, vital sign, ECG or physical examination abnormalities were observed during the Phase I trial. JTK-652 was found to be well tolerated. No significant changes in HCV RNA levels compared with baseline were observed at the end of treatment.

Conclusion: Although results from the preclinical studies indicated that JTK-652 has well-established anti-viral properties and a Phase I clinical trial has showed that JTK-652 was safe and well tolerated at a 100 mg three times daily dose level, plasma HCV RNA levels in chronically HCV-infected patients did not decrease during 28 days of dosing at a 100 mg three times daily dose level.

INTRODUCTION

Hepatitis C results from infection with HCV through exposure to infected blood. Approximately 170 million people worldwide, 3% of the world’s population, are infected with HCV. Globally, it is estimated that 3–4 million people are newly infected each year, with 50–80% of HCV-infected patients developing chronic hepatitis. The most important sequelae of chronic HCV infection are progressive liver fibrosis leading to cirrhosis and hepatocellular carcinoma. Standard hepatitis C treatment consists of 24–48 weeks of combination therapy with pegylated interferon-α and ribavirin, which is dependent on HCV genotype, baseline HCV RNA titre and virological response. Antiviral treatment should be considered in all chronically HCV-infected individuals; however, suboptimal response rates, abundant side effects and high treatment costs are major disadvantages of the current standard of care.

Development of new specifically targeted antiviral therapy against hepatitis C is eagerly anticipated. The target cells of HCV are hepatocytes and the natural hosts are humans and chimpanzees. Because of the lack of a cell culture system that supports efficient production of infectious particles, studying HCV entry has been difficult; for this reason, several surrogate in vitro systems have been developed to study the process of HCV entry into host cells. Recombinant HCV envelope glycoproteins have been successfully used as surrogate models to study virus–host interaction. Development of these functional models (HCV-like particles, recombinant HCV envelope glycoproteins, HCV pseudotype particles and vesicular stomatitis virus [VSV]/HCV pseudotypes) to analyse HCV entry has led to the identification of several cell surface molecules (including CD81, scavenger receptor B type 1, claudin-1, occludin, heparin sulphate, glucosaminoglycans, DC-SIGN, L-SIGN and low-density lipoprotein receptor) that are involved in HCV entry. Recent data suggest that HCV entry requires a complex, tightly regulated interaction between the host and HCV. VSV can be efficiently propagated in animal cells and readily forms pseudotypes with the envelope proteins of different viruses, including HCV. VSV pseudotype viruses expressing HCV E1/E2 chimeric proteins, containing transmembrane and cytoplasmic domains of the VSV-G glycoprotein, have been developed as HCV pseudotype models to study HCV binding and entry.

JTK-652, a novel pyrrolopyridazin-derived HCV infection inhibitor, was identified by high-throughput screening of a large number of potential compounds (Central Pharmaceutical Research Institute, Japan Tobacco Inc., Osaka, Japan). Using the in vitro HCV infection model consisting of HCV pseudotype VSV bearing HCV E1/E2 envelope proteins, JTK-652 was evaluated in multiple cell lines.

This study describes the preclinical development of this first-in-class infection inhibitor, followed by clinical studies testing safety, tolerability, pharmacokinetics and efficacy of JTK-652 in healthy volunteers and chronic hepatitis C patients.
JTK-652: a novel HCV infection inhibitor - CHAPTER 2

PRECLINICAL DEVELOPMENT OF JTK-652

Pharmacology

To evaluate the inhibitory effect of JTK-652 on HCV infection, HepG2 cells, human primary hepatocytes and HuH7 cells were infected with HCV genotype 1a and/or 1b pseudotype VSV.\textsuperscript{18} The secreted alkaline phosphatase gene was inserted into the VSV genome, and its secretion from the infected cells into the culture supernatant was used to measure the viral infectivity. JTK-652, at various concentrations, was added at the same time as inoculation of target cells with HCV pseudotype virus. Subsequently, the secreted alkaline phosphatase activity in the culture supernatant was measured after cultivation for 24 h. Recombinant VSV bearing the G protein, the original envelope protein of VSV, was used as a control. HepG2 cells were infected with both HCV genotype 1a and 1b pseudotype virus and human primary hepatocytes were infected with HCV genotype 1b pseudotype virus. The effect of JTK-652 on infection of HuH7 cells with HCV genotype 1a pseudotype virus was evaluated using HCV pseudotype virus produced in HCV E1/E2 protein-expressing 293T cells.

To determine the influence of human serum proteins on the inhibitory effect of JTK-652, human serum was added to the inoculum of HepG2 cells infected with HCV genotype 1b pseudotype virus at a final concentration of 50%. To evaluate the cytotoxicity of JTK-652 in the different target cells, living-cell-derived ATP-dependent luciferase activity was measured after cultivation for 24 h. Using the assay system for infection of HepG2 cells with HCV genotype 1b pseudotype virus, the effect of the timing of adding JTK-652 on its inhibitory activity was investigated. JTK-652 was added to HepG2 cells at a final concentration of 30 nmol/l at 0, 0.5, 1, 2, 3, 4 and 5 h after inoculation with HCV pseudotype virus. The effect of JTK-652 on replication of HCV replicon RNA was investigated using HCV replicon-containing cells.\textsuperscript{19} JTK-652 was added at various concentrations and the total RNA from the cells was extracted after cultivation for 48 h. The amount of HCV replicon RNA was measured by real-time reverse transcriptase PCR to evaluate the effect of JTK-652. As a positive control, JTK-109, an HCV RNA-dependent RNA polymerase inhibitor, was used.\textsuperscript{20}

Clinical evaluation of JTK-652

Safety, tolerability and pharmacokinetics of JTK-652 in healthy volunteers

Safety, tolerability and pharmacokinetics of JTK-652 in healthy male volunteers was evaluated by two Phase I, randomized double-blind placebo-controlled ascending dose studies. A single ascending dose study in 18 healthy male volunteers was conducted. In an alternating panel design, two panels of nine volunteers were administered single oral doses of 100, 200, 400, 800 (fed and fasted) and 1,200 mg JTK-652 (six participants) or placebo (three participants), over three study periods. Subsequently, two dose cohorts (400 and 800 mg) each consisting of nine volunteers were randomized to receive JTK-652 (n=6) or placebo (n=3) three times daily for 14 days. The main inclusion criteria for both studies were being a healthy male between 18 and 55 years of age and having a body mass index (BMI) of 19–28 kg/m\textsuperscript{2}.

Safety, tolerability, pharmacokinetics and antiviral activity of JTK-652 in chronic hepatitis C patients

The primary objective in this Phase Ib trial was to determine the safety, tolerability and antiviral activity of JTK-652 in patients with genotype 1 chronic hepatitis C. Our secondary objective was to assess the pharmacokinetics of multiple oral doses of JTK-652. This randomized double-blind placebo-controlled study was conducted at two sites: Academic Medical Center (University of Amsterdam, Amsterdam, the Netherlands) and PRA International (Zuidlaren, the Netherlands). The Erasmus Medical Center (Erasmus University Rotterdam, Rotterdam, the Netherlands) and the VU Medical Center (VU University, Amsterdam, the Netherlands) referred patients for the study. The JTK-652 starting dose of 100 mg three times daily was based on the safety, pharmacokinetic and tolerability results of the 14-day multiple increase dose study in healthy volunteers and was considered to be a minimum dose level to reduce viral load in chronic hepatitis C patients. In the first planned cohort, 10 patients (8 active and 2 placebo) were randomized to receive JTK-652 100 mg or placebo every 8 h for 4 weeks. Placebo was included to ensure a controlled assessment of safety during the study. The 4-week treatment period included a 2-week in-house admission period and a 2-week ambulatory period with hospital visits on days 22 and 29. Follow-up included two visits for medical examinations on days 36 and 43. JTK-652 was supplied as 100 mg tablets for oral administration. Placebo formulation and dosing was identical to JTK-652 except for the active compound. The main inclusion criteria were being a male or a post-menopausal female (follicle-stimulating hormone level >40IU/mL or lack of menses for >12months) between 18 and 65 years of age with chronic genotype 1a, 1b or mixed 1a/1b HCV infection, having HCV RNA 21×10⁵ IU/mL, BMI of 18.5–32.0 kg/m\textsuperscript{2}, alanine aminotransferase concentrations ≤5 the upper limit of normal and no clinically significant deviations from the normal range for haematology or clinical chemistry values. Haemophilia patients were allowed to participate in this study. Plasma samples for JTK-652 pharmacokinetics were collected on days 1 and 14 before the morning dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 16 (only day 1) h after the morning dose. Pre-morning dose samples were also collected on days 2–13, 22 and 29. Safety assessments were performed at regular intervals during the treatment and follow-up period. Safety assessments included ECG, vital sign examination, laboratory testing (chemistry, haematology and urinanalysis), physical examination and adverse events registration. HCV RNA concentrations were determined using the COBAS® AmpliPrep/Cobas® TaqMan® (Roche, Basel, Switzerland) assay. The dynamic range of the assay was 43–6.9×10⁵ IU/mL. The limit of quantification of the assay was 43 IU/mL and the limit of detection was 15 IU/mL. For HCV RNA measurements, serum samples were collected before the morning dose of study drug on days 1–4, 7, 14 and 22 of the dosing period and on days 29, 36 and 43 after the end of study.

Ethical committees of the participating centres (Academic Medical Center and PRA International) approved the study and all participants provided written informed consent before participating in any study-related activity. All studies were conducted in full compliance with the guidelines of Good Clinical Practice and the Declaration of Helsinki and were in accordance with the principles of Good Laboratory Practice.
RESULTS

Preclinical development of JTK-652

Pharmacology

In vitro pharmacology studies demonstrated that JTK-652 inhibits infection of HepG2 cells with HCV genotype 1a and 1b pseudotype virus in a dose-dependent manner. The half maximal inhibitory concentrations (IC$_{50}$ ±SE) were 1.9 ±0.07 nmol/l and 3.1 ±0.49 nmol/l, respectively (Figure 1A and 1B). In the presence of 50% human serum, inhibition of infection with HCV genotype 1b pseudotype virus decreased approximately 40-fold to an IC$_{50}$ ±SE value of 127 ±12.4 nmol/l (Figure 1C). No inhibitory effect on infection with recombinant VSV bearing G protein of original envelope protein of VSV was seen. JTK-652 did not show cytotoxicity in HepG2 cells up to a concentration of 10 µmol/l (Figure 1).

Figure 1. Inhibitory effect of JTK-652 on viral infection of HepG2 cells and the effect of human serum on JTK-652.

Values shown are mean ±SE ($n=3$). (A) Inhibitory effect of JTK-652 on infection of HepG2 cells with vesicular stomatitis virus (VSV)-based pseudotype virus bearing HCV genotype 1a E1/E2 envelope proteins (HCVpv [1a]) or with recombinant VSV (VSVΔG/G). (B) Inhibitory effect of JTK-652 on infection of HepG2 cells with genotype 1b HCVpv (HCVpv [1b]) or with VSVΔG/G. (C) Effect of 10% fetal bovine serum (FBS) and 50% human serum (HS) on the inhibitory effect on JTK-652 on infection of HepG2 cells infected with HCVpv [1b]. *Cytotoxicity in HepG2 cells.

JTK-652 inhibits infection of human primary hepatocytes with HCV genotype 1b pseudotype virus with an IC$_{50}$ value of 5.8 nmol/l (Figure 2A). This inhibition was comparable to that observed in HepG2 cells. JTK-652 did not show cytotoxicity in human primary hepatocytes up to a concentration of 10 µmol/l (Figure 2A). JTK-652, up to a concentration of 10 µmol/l did not inhibit infection of Huh7 cells with HCV pseudotype 1a virus (Figure 2B).

Figure 2. Inhibitory effect of JTK-652 on viral infection of human primary hepatocytes and Huh7 cells.

Values shown are means ($n=2$). (A) Inhibitory effect of JTK-652 on infection of human primary hepatocytes with vesicular stomatitis virus-based pseudotype virus bearing HCV genotype 1b E1/E2 envelope proteins (HCVpv [1b]). (B) Inhibitory effect of JTK-652 on infection of Huh7 cells with genotype 1a HCVpv (HCVpv [1a]) produced in HCV E1/E2 protein-expressing 293T cells exhibiting different cell tropism. *Cytotoxicity in human primary hepatocytes. **Cytotoxicity in Huh7 cells.

When JTK-652 was added to the cell culture at the same time as inoculation with HCV genotype 1b pseudotype virus, JTK-652 showed a potent inhibitory activity of approximately 90%. The inhibitory effects were reduced and almost disappeared when JTK-652 was added ≥3 h after inoculation (Figure 3).

Figure 3. Effect of the timing of adding JTK-652 on HCV genotype 1b pseudotype virus infection.

JTK-652 showed no inhibition of replication of HCV replicon up to a concentration of 10 µmol/l whereas JTK-109, an HCV RNA-dependent RNA polymerase inhibitor, inhibited the replication of HCV replicon RNA with an IC$_{50}$ ±SE value of 0.43 ±0.01 µmol/l (YK, data not shown). Safety pharmacology studies indicated that JTK-652 had no effect on the central nervous, cardiovascular, gastrointestinal, renal/urinary or respiratory systems in rats and dogs. In the
phototoxicity studies, a positive reaction was observed dose-dependently at ≥300 mg/kg with single oral administration in mice (doses were 30, 100, 300 or 1,000 mg/kg).

Table 1. Patient baseline characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>JTK-652 100 mg*</th>
<th>Placebo (n=2)</th>
<th>All participants (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n (%))</td>
<td>8 (80.0)</td>
<td>2 (20.0)</td>
<td>10 (100.0)</td>
</tr>
<tr>
<td>White (n (%))</td>
<td>8 (80.0)</td>
<td>2 (20.0)</td>
<td>10 (100.0)</td>
</tr>
<tr>
<td>Mean age, years (range)</td>
<td>51 (48-61)</td>
<td>45 (40-49)</td>
<td>49 (40-61)</td>
</tr>
<tr>
<td>BMI (Mean, kg/m² ±SD)</td>
<td>24.8 (3.6)</td>
<td>24.7 (2.4)</td>
<td>24.8 (3.3)</td>
</tr>
<tr>
<td>Range, kg/m²</td>
<td>20.8-31.8</td>
<td>23.0-26.4</td>
<td>20.8-31.8</td>
</tr>
<tr>
<td>Median ALT level, U/l</td>
<td>113.0</td>
<td>85.5</td>
<td>101.0</td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a, n (%)</td>
<td>5 (50.0)</td>
<td>2 (20.0)</td>
<td>7 (70.0)</td>
</tr>
<tr>
<td>1b, n (%)</td>
<td>2 (20.0)</td>
<td>-</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td>Mixed type 1a/1b, n (%)</td>
<td>1 (10.0)</td>
<td>-</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>Mean HCV RNA load, log₁₀U/mL ±SD</td>
<td>6.51 (0.49)</td>
<td>6.06 (0.04)</td>
<td>6.42 (0.47)</td>
</tr>
<tr>
<td>Prior HCV treatment, n (%)</td>
<td>6 (60.0)</td>
<td>2 (20.0)</td>
<td>8 (80.0)</td>
</tr>
</tbody>
</table>

*Three times daily. ALT, alanine aminotransferase; BMI, body mass index.

Clinical evaluation of JTK-652

Safety, tolerability and pharmacokinetics of JTK-652 in healthy volunteers

Treatment with single oral doses of JTK-652 at doses ranging from 100 to 1,200 mg was safe and well-tolerated in all healthy male volunteers. Multiple oral doses of 400 and 800 mg JTK-652 three times daily during 14 days were safe and well-tolerated by five out of six and three out of six healthy male volunteers, respectively. Four participants, one after treatment with 400 mg JTK-652 three times daily and three after treatment with 800 mg JTK-652 three times daily, developed a rash of mild intensity at approximately day 9 or day 10 and were withdrawn. Except for the transient rash, there were no other clinically significant findings considered to be related to study drug administration with regard to clinical laboratory, vital sign, body weight, ECG or physical examination. After multiple dosing with 400 and 800 mg JTK-652 three times daily, maximum JTK-652 plasma concentrations were reached between 1.50 and 1.75 h post-dose (median time to reach the maximum plasma concentration [t_{max}]). For area under the curve (AUC_{0-8}) maximum concentration (C_{max}) and trough concentration (C_{trough}), a less than dose-proportional increase was observed for the dose range of 400–800 mg JTK-652. The mean half-life (t_{1/2}) for JTK-652 was approximately 21 h for both doses studied, with a steady state reached by day 5. The accumulation ratio (day 14/day 1) with respect to AUC_{trough} was, on average, 1.53 after treatment with 400 mg JTK-652 three times daily and 1.19 after treatment with 800 mg JTK-652 three times daily.

Table 2. Summary statistics of JTK-652 plasma PK parameters in patients.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>n</th>
<th>C_{max} ng/mL</th>
<th>t_{max} h</th>
<th>C_{trough} ng/mL</th>
<th>AUC_{0-8}</th>
<th>C_{max}</th>
<th>C_{trough}</th>
</tr>
</thead>
<tbody>
<tr>
<td>JTK-652</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td></td>
<td>1</td>
<td>2.061</td>
<td>1.25</td>
<td>842</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>(968-3363)</td>
<td>(1.00-2.00)</td>
<td>(532-1,409)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>three times daily</td>
<td>14</td>
<td>8</td>
<td>3.025</td>
<td>1.75</td>
<td>1,524</td>
<td>1.64</td>
<td>1.47</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1,981-4,770)</td>
<td>(1.20-2.44)</td>
<td>(1,04-2.64)</td>
<td>(1.47-2.58)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the maximum concentration (C_{max}), trough concentration (C_{trough}), AUC_{0-8} (area under the curve for 0-8 h) and the accumulation ratio, the geometric mean (range) is presented; for time to reach the maximum plasma concentration (t_{max}) the median (range) is presented. C_{trough} of day 1 and day 14 is the concentration at 8 h after dosing. PK, pharmacokinetic.

Safety, tolerability, pharmacokinetics and antiviral activity of JTK-652 in chronic hepatitis C patients

A total of 10 patients were enrolled and randomized into the study and 8 patients completed the study (7 active and 1 placebo). Demographic characteristics and baseline HCV RNA levels were similar in all dosed patients (Table 1). Of the 10 patients in the study, all were male and Caucasian. Premature discontinuation occurred in two patients (one active and one placebo) on day 13 and day 19, respectively, because of a rash of mild intensity. Most patients, 8 out of 10 (80%), had been treated previously for HCV infection. All 10 dosed patients were fully compliant with study drug dosing.

With regard to safety and tolerability, administration of multiple oral doses of 100 mg JTK-652 or placebo three times daily for 28 days was safe and well-tolerated by 8 out of 10 male participants with chronic hepatitis C. There were no serious adverse events reported during this study. All adverse events (AEs) were of mild intensity. Treatment was not tolerated by two participants and they were withdrawn during the course of the study because of an AE. During treatment with 100 mg JTK-652 three times daily, one participant developed a rash on day 17. The other participant developed a rash on day 12 during treatment with placebo three times daily. Both rashes were mild in intensity for these two participants and dosing was discontinued on day 19 and day 13, respectively. The rash disappeared within days after stopping JTK-652 administration and additional treatment was not necessary.

In total, 16 of the 46 AEs were considered as possibly related to the study medication. These related AEs were mainly skin and to a lesser extent gastrointestinal disorders. Except for the rash, there were no other clinically significant findings with regard to clinical laboratory, vital sign, body weight, ECG or physical examinations.

The pharmacokinetics of JTK-652 were studied and its appearance in plasma shortly after oral administration with regard to clinical laboratory, vital sign, body weight, ECG or physical examinations. The pharmacokinetics of JTK-652 were studied and its appearance in plasma shortly after oral treatment. The mean accumulation ratio (day 14/day 1) with respect to AUC_{trough} was 1.64 after treatment with 100 mg JTK-652 three times daily. The mean accumulation ratio (day 14/day 1) with respect to C_{max} and C_{trough} was 1.47 and 1.81, respectively (Table 2). JTK-652 C_{trough} remained almost steady after...
day 2 (Figure 4) and the range of $C_{\text{trough}}$ values on day 14 in patients was 780–2,369 ng/mL. The $C_{\text{trough}}$ values in all patients, therefore, exceeded the IC$_{50}$ value by >10-fold (127 nmol/l in the presence of 50% human serum; approximately 70 ng/mL).

**Figure 4.** Mean$^a$ plasma JTK-652 100 mg three times daily trough concentrations in patients.

![Graph showing plasma JTK-652 concentrations](image)

$^a$Geometric mean (n=8). For days 22 and 29, n=7.

An investigation into the efficacy of JTK-652 found that after multiple dosing with 100 mg three times daily, no decrease in viral load was observed at the expected therapeutic dose level (Figure 5). Individual maximum viral load change from baseline in all participants was within 0.5 log$_{10}$ IU/mL. Alanine aminotransferase levels were unaffected by treatment with multiple doses of 100 mg JTK-652 three times daily.

**Figure 5.** Mean$^a$ plasma HCV RNA values throughout the study.

![Graph showing plasma HCV RNA concentrations](image)

$^a$Arithmetic mean (placebo: n=1 on days 14, 22, 29 and 36; and 100 mg: n=7 on days 22, 29 and 36).

DISCUSSION

This study is the first to describe the in vitro development of an HCV infection inhibitor and its clinical evaluation in chronic hepatitis C patients. Firstly, we described the in vitro antiviral properties of JTK-652 against HCV pseudotype virus, using multiple cell lines as target cells. Secondly, we evaluated JTK-652 in chronic hepatitis C patients to assess safety, tolerability, pharmacokinetics and antiviral activity.

Although we are still far from understanding the details of HCV entry, recent data show that viral entry into target cells is a complex multistep process requiring the presence of several entry factors. Initial attachment of the virion involves the tetraspanin CD81, glucosaminoglycans, heparan sulphate and the low-density lipoprotein receptor, followed by the sequential interaction with the scavenger receptor class B type I and the tight junction proteins claudin-1 and occludin. Furthermore, unidentified factors might be involved in the HCV entry process. Previous studies described the crucial role of CD81 in the process of HCV infectivity. Binding of CD81 to E2 is crucial for an HCV viral particle to penetrate into the host cell; however, recent studies showed that cell lines with little or no CD81 were capable of direct cell-to-cell viral transmission, suggesting a CD81-independent HCV entry process. This indicates that there are at least two modes by which a virus can transmit from an infected cell to an uninfected cell: cell-free transmission and cell-to-cell transmission.

In this study, three different cell lines were used to evaluate the inhibitory effect of JTK-652 on infection with HCV genotype 1a and 1b pseudotype viruses: HepG2 cells, human primary hepatocytes and Huh7 cells. JTK-652 showed inhibition of infection of HepG2 cells with HCV genotype 1a or 1b pseudotype virus. HepG2 cells do not express CD81 on their cell surface; however, human fibroblast growth factor receptor 5 has been reported as a possible candidate for CD81-independent HCV entry in HepG2 cells. We then demonstrated a similar inhibition when human primary hepatocytes were used as target cells. These results suggest that JTK-652 possesses a potent inhibitory activity against infection of HepG2 cells and human primary hepatocytes with HCV genotype 1a or 1b pseudotype virus. JTK-652 showed no inhibition of infection of Huh7 cells with HCV genotype 1a pseudotype virus. These Huh7 cells were infected with HCV pseudotype virus produced in HCV E1/E2 protein-expressing 293T cells. A possible explanation for the lack of inhibitory effect in these cells could be because of the amount of E1 and E2 expressed on the cell surface or because of differences of glycosylation as described before.

The timing of adding JTK-652 on the viral inhibitory effect on HepG2 cells infected with pseudotype 1b virus was investigated. Maximum inhibition of viral infection was achieved when JTK-652 was added during inoculation with HCV pseudotype virus. The inhibitory effect was reduced upon delayed addition of JTK-652 to the assay system and little inhibitory effect was observed when JTK-652 was added ≥3 h after the inoculation. In addition, because JTK-652 showed no inhibition of the replication of HCV replicon RNA, JTK-652 had apparently no effect on the replication process of HCV.
Safety and tolerability experiments in animals showed a positive, dose-dependent skin reaction in the phototoxicity studies. Phototoxicity was observed in mice after drug exposure at a dose level of 1,000 mg/kg; therefore, when JTK-652 was administered to humans in clinical studies, safety measures were taken, such as avoiding sunlight exposure. Despite such measures being taken, 4 out of 12 healthy volunteers were withdrawn during the course of the study because of a rash of mild intensity. These events of rash were considered to be related to the study drug and as a consequence the study dose in the Phase Ib study in chronic hepatitis C patients was lowered. Because JTK-652 plasma pharmacokinetic parameters in healthy volunteers had revealed that 100 mg dosing appeared to be sufficient as far as C\text{max} \ t\text{max}, AUC\text{max} and t\text{max} were concerned, it was decided to reduce the dose for the first cohort of chronic hepatitis C patients from 400 to 100 mg three times daily.

Data of the Phase Ib study revealed that JTK-652 at 100 mg three times daily was well-tolerated in the patients; however, two patients (one placebo and one active) had to stop treatment prematurely because of a mild rash. As one of these patients had received placebo, the rash might have been caused by an excipient that was present in the placebo as well as in the active medication. JTK-652 could not be excluded as the causal factor in the rash developed by the participant on the active treatment. JTK-652 did not demonstrate HCV RNA decreases in eight chronic hepatitis C patients. It was decided to raise a higher dosing cohort because of the lack of HCV RNA decrease in the first dosing cohort (100 mg three times daily) and the observed rash in healthy volunteers and chronic HCV patients. Despite inhibition of infection of HepG2 cells and human primary hepatocytes with HCV genotype 1a and/or 1b pseudotype virus by JTK-652, no viral inhibitory effect of JTK-652 was seen during the Phase Ib trial in chronic hepatitis C patients. Therefore, it can be concluded that the in vitro viral inhibition of JTK-652 against HCV pseudotype virus infection might not represent the actual in vivo process of HCV infection. Other approaches to study HCV infection inhibition, such as lentiviral particles bearing HCV glycoproteins or HCV produced in cell culture or eventually small animal models, could potentially help to bridge the gap from in vitro models to effective HCV entry inhibitors.

In summary, JTK-652 monotherapy did not decrease HCV RNA in chronic hepatitis C patients. The majority of adverse events were mild and there were two premature discontinuations in the Phase Ib study because of rash. Further development of JTK-652 was discontinued. Nevertheless, HCV infection inhibition remains a potential target for antiviral therapy within the HCV life cycle.

REFERENCES


