CLINICAL RESEARCH PROTOCOL

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

DATE: August 17, 2018 **CLINICAL PROTOCOL NO**.: 14-DK-0084

TITLE: Evaluation of Safety, Tolerability, and Antiviral Activity of Chlorcyclizine HCl Alone or in Combination with Ribavirin in Patients with Chronic Hepatitis C

SHORT TITLE: Chlorcyclizine HCl for Chronic Hepatitis C

IDENTIFYING WORDS: Chlorcyclizine HCl, Ribavirin, Direct Acting Antivirals, Chronic Hepatitis C

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ESTIMATED DURATION OF STUDY: 3 Years

NUMBER AND TYPE OF PATIENTS: 50 patients with chronic hepatitis C, ages above 18 years, both male and female

SUBJECTS OF STUDY:

Number of patients: 50 Sex: Male & Female Age Range: Above 18 years

Volunteers: None

PROJECT USES IONIZING RADIATION: Yes, for medical indications only.

PROJECT USES "DURABLE POWER OF ATTORNEY": No

OFF-SITE PROJECT: No **MULTI-INSTITUTIONAL PROJECT**: No

Précis

Up to 50 patients with chronic hepatitis C, who are treatment naïve or relapsers to any interferon/ribavirin regimen will be enrolled into this pilot study evaluating chlorcyclizine HCl with or without ribavirin (RBV) as antiviral therapy. Adult patients (≥18 years of age) with evidence of active chronic hepatitis C infection (all genotypes) with detectable HCV RNA in serum >10,000 IU/mL without contraindications to chlorcyclizine HCl or ribavirin or evidence/history of hepatic decompensation will be enrolled. Patients will be monitored for at least two months with regular testing for ALT and HCV RNA quantitative levels before treatment and will undergo admission to start therapy, which includes a thorough medical evaluation and timed blood sampling. Patients will be randomized to one of two treatment groups; one with chlorcyclizine HCl (75 mg twice daily) and the other with RBV+ chlorcyclizine HCl (75 mg twice daily). For all genotypes, RBV will be dosed based on weight (1000 mg daily <75 kg and 1200 mg daily ≥75 kg). At each clinic visit, patients will be questioned about side effects and symptoms, undergo a focused physical examination, and have blood taken for complete blood counts, HCV RNA, PT/INR and routine liver tests (ALT, AST, alkaline phosphatase, direct and total bilirubin, and albumin). At the end of 28 days of treatment, patients will undergo a repeat thorough medical evaluation inclusive of a complete physical exam, symptom scale evaluation, complete blood counts, routine liver tests, and HCV serology panels. The primary endpoint of therapy will be a decline in quantitative HCV RNA viral levels after 28 days of treatment as compared to baseline viral titers and between groups. Several secondary endpoints will be measured, including side effects of therapy, ALT levels, quantification of chlorcyclizine HCl and its metabolites in serum,

and quality of life. Therapy will be stopped for intolerance to RBV and/or chlorcyclizine HCl (which will be carefully defined).

Background

A. Chronic Hepatitis C and Current Therapy

Chronic infection with hepatitis C virus (HCV) is a global health problem. The World Health Organization (WHO) estimates that approximately 3% of the world's population is infected with HCV which amounts to an estimated 170 million to 200 million chronic HCV infections worldwide.[1,2] The prevalence varies with geographic region, with the highest regions being Southeast Asia and Africa.[3-5] The Centers for Disease Control estimates that approximately 3.2 million people are chronically infected with HCV in the United States.[6] Chronic HCV infection is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma. A recent analysis suggests that the number of cases of cirrhosis in people who were infected in the 1970s and 80s will not peak until 2020.[7] Without therapy, a significant proportion of these people will develop complications of their disease.

Prior to 2011, the recommended therapy for chronic hepatitis C (CHC) genotype 1 infection included a 48-week course of combination therapy with peginterferon (1.5 µg/kg [PegIntron] or 180 µg [Pegasys] once weekly) and ribavirin (1000 to 1200 mg daily based upon body weight).[8] In 2011, two new agents, boceprevir and telaprevir, were approved for use in CHC genotype 1 infection.[9] Both agents are direct acting antiviral (DAAs) agents which inhibit the HCV NS3/NS4A protease and must be used in combination with peginterferon and ribavirin. When used in combination with peginterferon and ribavirin (triple therapy), both agents significantly improve the sustained virological response (SVR) rate compared to standard peginterferon and

ribavirin therapy. With triple therapy in HCV genotype 1 patients, the SVR rates increased from 40-45% with standard combination therapy to 69-75% with triple combination therapy.[10,11]

Currently, the two FDA approved protease inhibitors appear to have similar overall rates of response, but differ greatly in when and for how long they are administered. Telaprevir is administered during the first 12 weeks of peginterferon and ribavirin therapy. Thereafter, peginterferon and ribavirin is continued without the protease inhibitor for a total of either 24 or 48 weeks depending upon the virologic response during the triple combination ("response guided therapy").[9,11] In contrast, boceprevir is started 4 weeks into the course of peginterferon and ribavirin therapy. It is then continued for either a total duration of therapy of 28 to 48 weeks based upon virologic response.[9,10] Side effects can occur with either protease inhibitor and appear to worsen the known side effects of peginterferon and ribavirin. Skin rash, anemia and ano-rectal discomfort have been dose-limiting side effects of telaprevir,[11] whereas anemia and dysgeusia have been the major adverse events associated with boceprevir.[10] Response rates vary greatly by virologic, host and disease characteristics, such as viral level (higher rates with lower baseline HCV RNA concentrations), race (lower in African Americans, higher in Asians), and disease severity (lower in patients with cirrhosis or advanced fibrosis).[10,11]

For patients chronically infected with HCV genotype 2 and 3, the recommended therapy is a 24-week course of the combination of peginterferon and a reduced dose of ribavirin (800 mg daily).[8] The optimal regimen for patients with genotypes 4, 5 and 6 is yet to be defined.[8]

Although treatment of CHC has greatly improved over the last two decades, more than half of patients with genotype 1 HCV infection treated with a course of peginterferon and ribavirin fail to achieve SVR to peginterferon and ribavirin.[12-15] Subjects who fail combination therapy are a heterogeneous group and include, persons who experienced *virological breakthrough* (detectable HCV RNA in serum during therapy after an initial response was achieved), *virological relapse* (reappearance of HCV RNA in serum after treatment was discontinued and an end of-treatment response was achieved), as well as individuals who failed to achieve an initial virological response- *partial responders* (≥ 2 log IU/mL decline in HCV RNA from baseline to treatment week 12 but with detectable HCV RNA at week 24) and *null responders* (≤ 2 log IU/mL reduction in HCV RNA level from baseline to treatment week 12). Boceprevir and telaprevir result in a two- to three-fold increase in SVR rates compared to peginterferon and ribavirin in previous HCV genotype 1 treatment failures.[16,17] Consequently, boceprevir or telaprevir in combination with peginterferon and ribavirin now represents the new standard of care for re-treatment of HCV genotype 1 relapsers, prior partial responders and null responders.[16,17]

Whether a subject should be re-treated now or wait for potentially better therapy in the future depends on many factors including the individual's desire to be re-treated, the reasons underlying failure, such as inadequate drug dosing or side effect management, the severity of underlying liver disease, prior response to therapy and risk of disease progression over the next three to five years. Currently, more effective therapies with fewer side effects that do not include interferon are under investigation and likely represent the future of HCV therapy.

B. Ribavirin therapy in HCV infection

Ribavirin is a nucleoside analogue with broad activity against both RNA and DNA viruses *in vitro*. Ribavirin has been used in several viral diseases and is approved for use in respiratory syncytial virus disease. In hepatitis C, ribavirin was found to lower serum alanine aminotransferase (ALT) levels and to improve liver histology, but it had little effect on HCV RNA levels in blood or liver. Addition of ribavirin to interferon therapy greatly increased the response rate. Multiple studies using both standard and pegylated interferon have shown that ribavirin increases SVR rates by two to three-fold.[14] Ribavirin affects SVR rates in two ways: first, a higher proportion of patients become HCV RNA negative on therapy (usually ~10% more than with peginterferon monotherapy) and, second and more importantly, ribavirin appears to markedly decrease the relapse rate (from ~50% after standard interferon or peginterferon monotherapy to 10-15% after combination therapy).[14] The mechanisms of action of ribavirin in mediating these effects are not well-defined.

For patients with genotype 1 HCV infection, the dose of ribavirin is based on body weight, being 1000 mg daily for individuals ≤75 kg and 1200 mg daily for those >75 kg. For patients with genotypes 2 and 3 HCV infection, a lower fixed dose of 800 mg daily is adequate to achieve high response rates and low relapse rates.[18] Higher doses of ribavirin have been evaluated indirectly in several large-scale studies[19] and directly in small pilot studies from Sweden and by the Liver Diseases Branch of the NIDDK.[20] Ribavirin is currently being tested as combination therapy with various DAAs either as interferon-containing or -free regimens.[21-26] These studies indicated that ribavirin appears to be an important component of the combination therapy to achieve optimal response.

C. The Future of HCV Therapy

As the future of HCV therapy is headed towards interferon-free regimens, various clinical trials are ongoing with oral combination antiviral therapy. Many of these DAA studies still utilize ribavirin as a component of the oral combination therapy. A recent phase 2a study exploring two DAAs plus ribavirin for 12 weeks has shown promise in treatment naive HCV genotype 1 patients with extended rapid virological response rates ranging from 79-89% and sustained virological response rates 12 weeks after the end of therapy from 93-95%.[27] Another study has evaluated an interferon-free oral combination DAA therapy utilizing the nucleotide polymerase inhibitor sofosbuvir plus ribavirin achieved a sustained virological response rate of 100% at 24 weeks after therapy.[23] However, in a phase 3 non-inferiority study evaluating HCV genotype 2 or 3 infection comparing sofosbuvir plus ribavirin or peginterferon plus ribavirin for 12 weeks, the response rates were nearly identical at 67%.[26] Thus, while the notion of an interferon-free DAA oral combination regimen appears achievable, the search for the ideal pan-genotypic oral combination regimen continues.

D. Chlorcyclizine HCl (CCZ)

Chlorcyclizine HCl is a first generation antihistamine of the phenylpiperazine class. Specifically, it functions as a competitive antagonist of histamine (H_1 and H_2) receptors. Chlorcyclizine HCl was originally granted marketing authorization by FDA under NDA 6-938 Di-Paralene Tablets marketed by Abbott Laboratories and NDA 6-947 Perazil Tablets marketed by Burroughs Wellcome Co. Clinical experience with chlorcyclizine HCl dates back to its initial introduction in 1949 as Di-Paralene and

Perazil. Di-Paralene was prescribed at an initial dose of 50 mg three times daily (150 mg per day) whereas Perazil was prescribed at a dose of 50 mg once or twice daily (50 or 100 mg per day).[28]

Chlorcyclizine HCl is currently marketed as a nonprescription drug product. Chlorcyclizine HCl is listed in 21 CFR 341.12 as being generally recognized as safe and effective for the use as an antihistamine. Chlorcyclizine HCl is currently available in various combination over-the-counter (OTC) formulations (e.g. NDC 58407-025-30) as an antihistamine drug for the indication of allergy symptoms. It is primarily used to treat allergy symptoms such as rhinitis, urticarial and pruritus, and may be used as an antiemetic. The Federal Drug Administration (FDA) listed the chlorcyclizine HCl final monograph in the Federal Register Notice (Volume 52, No. 163), dated August 24, 1987, as an antihistamine drug product for OTC human use.[29]

E. Chlorcyclizine HCl and HCV

Based on a cell-based HCV infection system, a high-throughput screen of chemical libraries with a collection of known pharmacological drugs (NPC Library) was performed. This screen identified a group of drugs in the class of anti-histamines that has potent anti-HCV activities *in vitro*. Further validation, characterization and structure-activity relation (SAR) analysis demonstrated that chlorcyclizine HCl has the most potent anti-HCV activities in various HCV assays. Further preclinical evaluation has identified the following:

1. Chlorcyclizine HCl Inhibits HCV Infection in a Dose-Dependent Manner

HCV harboring a luciferase reporter gene (HCV-Luc) was used to infect Huh7.5.1 cells in the presence of increasing concentrations of compounds. Viral infection and replication was measured by luciferase signal 48 hours after treatment. Cytotoxicity was evaluated in parallel with the ATP-based cell viability assay (ATPlite). Racemic, (*R*)- and (*S*)-chlorcyclizine HCl showed comparable potent EC_{50} values, low cytotoxicity and high selective indices (SI). (Figure 1 and Table below).

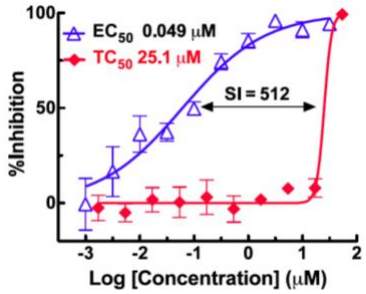
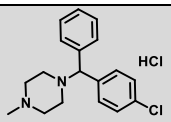
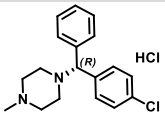
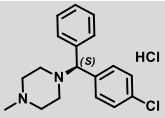


Figure 1. Chlorcyclizine HCl inhibits HCV infection in a dose-dependent manner, and exhibits low cell toxicity. The results shown are the means of three independent experiments ± SE.

ID	Chemical Structure	HCV-Luc EC_{50} (μM)	ATPlite TC_{50} (μM)	Selective Index
Racemic Chlorcyclizine HCl		0.049	25.1	512
(*R*)-Chlorcyclizine HCl		0.031	29.9	965
(*S*)-Chlorcyclizine HCl		0.054	27.1	502
Cyclosporin A	-	0.080	-	-
Doxorubicin	-	-	0.520	-

2. Chlorcyclizine HCl Dramatically Reduces HCV RNA Level in Cell Culture-derived HCV Assay

Cell culture-derived HCV (HCVcc) was used to infect Huh7.5.1 cells with or without the treatment of racemic, (*R*)- and (*S*)-chlorcyclizine HCl at 10 μM. 48 hours later, intracellular and extracellular viral RNA levels were evaluated by quantitative real-time PCR. This HCVcc system provides direct evidence of anti-HCV activity of the compounds. As shown in Figure 2 (below), the extracellular and intracellular viral RNA levels were dramatically reduced with the treatment of racemic, (*R*)- and (*S*)-chlorcyclizine HCl.

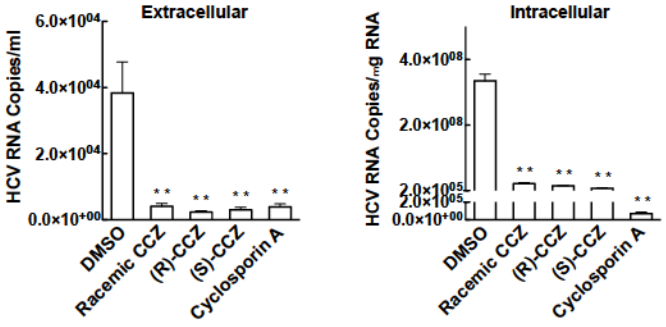


Figure 2. Chlorcyclizine HCl (CCZ) reduces HCV RNA level dramatically in HCVcc assay. The results shown are the means of three independent experiments ± SE. Asterisks (**P <0.0001) indicate statistically significant reduces of compound-treated results from the DMSO-treated results by Student's t test. Cyclosporin A was used as positive control.

3. The Antiviral Effect of Chlorcyclizine HCl is Highly Synergistic with Current Anti-HCV Drugs

As previously discussed, the combination of peginterferon, ribavirin and a DAA (telaprevir or boceprevir) is the new standard of care treatment for HCV genotype 1 infection. However, next generation DAAs (such as daclastavir and sofosbuvir) are

currently being investigated as potential therapies ± peginterferon and/or ribavirin in all HCV genotypic infections. Here we investigated the combination of (*S*)-chlorcyclizine HCl with different classes of anti-HCV drugs. HCV-Luc assay in parallel with the ATPlite assay (as described above) was performed in the presence of various concentrations of (*S*)-chlorcyclizine HCl in combination with various concentrations of each drug. As shown in the left panel of Figure 3 (below), the combination of (*S*)-chlorcyclizine HCl and each drug led to an greater HCV inhibitory effect than either of them alone in a dose-dependent manner without toxic effect on cell viability. In the right panel of Figure 3, three-dimensional surface plots were generated according to the Bliss independence model by using MacSynergy II program.[30] The surface represents theoretical additive plane, while peaks above the plane demonstrate synergy at the corresponding concentration combination and depressions below the plane shows antagonism. The antiviral effect of (*S*)-chlorcyclizine HCl is highly synergistic with ribavirin, interferon-α (IFN-α), telaprevir (NS3/4A inhibitor), daclatasvir (NS5A inhibitor), cyclosporin A, and 2'-C-methylcytidine (NS5B inhibitor), without significant cytotoxicity, supporting its use in combination therapy with these drugs.

The observed synergistic effects suggest that (*S*)-chlorcyclizine HCl inhibits HCV infection through a different mechanism from any one of these drugs. The mechanism of action of ribavirin and interferon-α is mediated through host antiviral response. Telaprevir is NS3/4A protease inhibitor and declatasvir inhibits HCV NS5A.[31,32] Cyclosporin A targets virus RNA replication and 2-C'-methylcytidine is a NS5B polymerase inhibitor.[32,33] The synergistic effect of (*S*)-chlorcyclizine HCl with these reagents suggests that its mechanism of action is novel and unique. This makes

chlorcyclizine HCl a more attractive agent for development with a possibly unique mechanism and lower probability of selecting resistant virus strains during treatment.

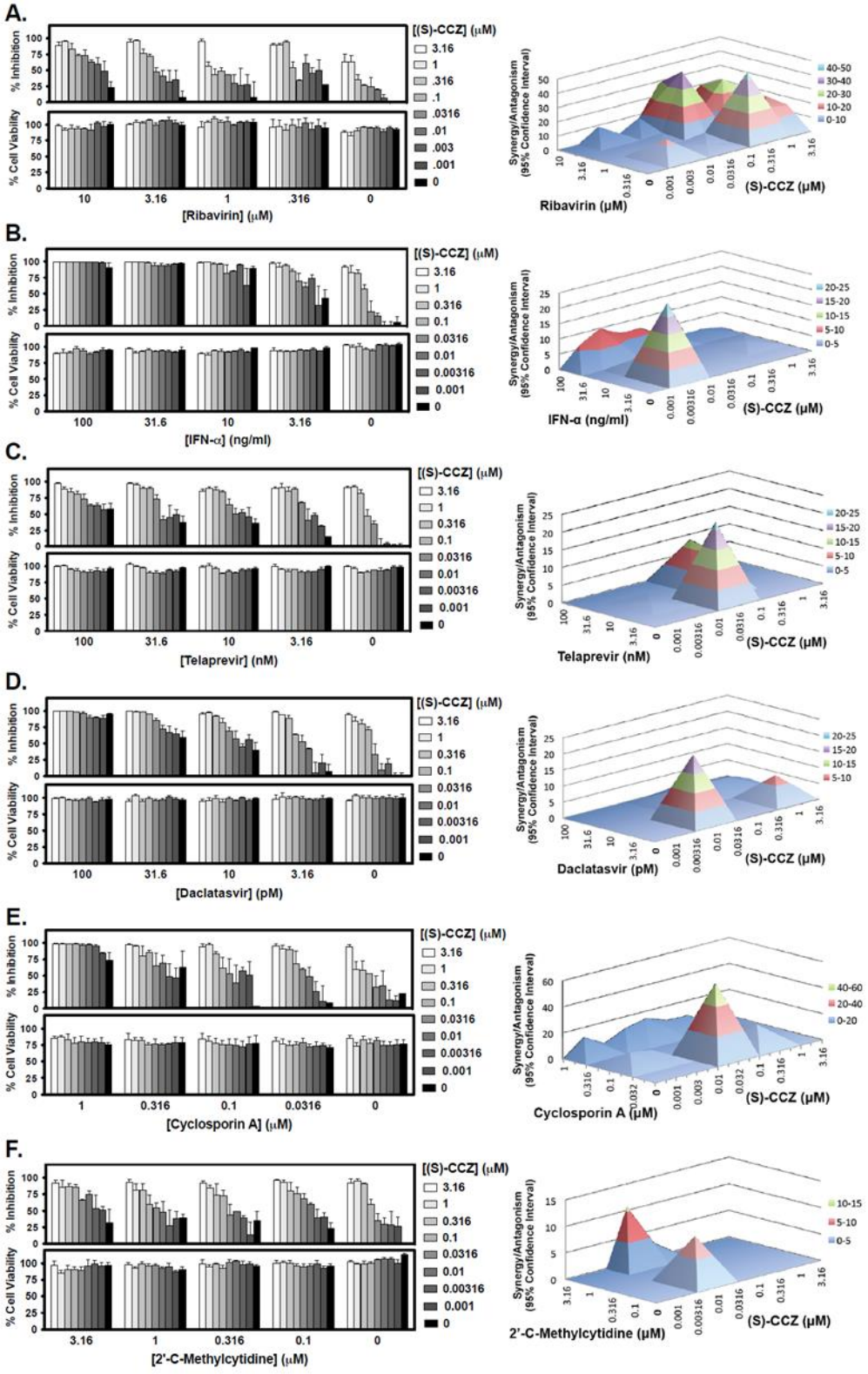


Figure 3. The antiviral effect of chlorcyclizine HCl (CCZ) is highly synergistic with (A) ribavirin, (B) IFN-α, (C) telaprevir, (D) daclatasvir, (E) cyclosporin A, and (F) 2'-C-methylcytidine. Left panel, normalized viral inhibition and cytotoxicity after treatment with various concentration of (*S*)- chlorcyclizine HCl in combination with various concentration of each drug. The results shown are the means of three independent experiments ± SE. Right panel, differential surface plots at the 95% confidence interval (generated by MacSynergy II). The three dimensional plots illustrate the differences between the observed effects and the theoretical additive effects at various concentrations of the two compounds. The colors indicate level of synergy or antagonism.

4. Chlorcyclizine HCl Inhibits HCV Genotype 1b and 2a Infection In Vivo

(*S*)-Chlorcyclizine HCl was tested in Alb-UPA/SCID chimeric mouse model infected with HCV genotype 1b and 2a respectively.[34,35] As shown in Figure 4 (below), the doses of 50 mg/kg and 10 mg/kg daily led to time-dependent reduction of HCV viral titer from pretreatment baseline in mice with genotype 1b and 2a infection (2-log and 1.5-log, respectively). Doses as low as 2 mg/kg daily also caused significant decreases of genotype 1b viral titer (approximately 1-log). A rebound of viral titer after stopping of treatment was observed in both genotype infections. However, HCV viral titers continued to decline during the treatment period without rebound, suggesting absence of emergence of drug-resistant virus. This antiviral profile is similar to that of mice treated with interferon-α.[36]

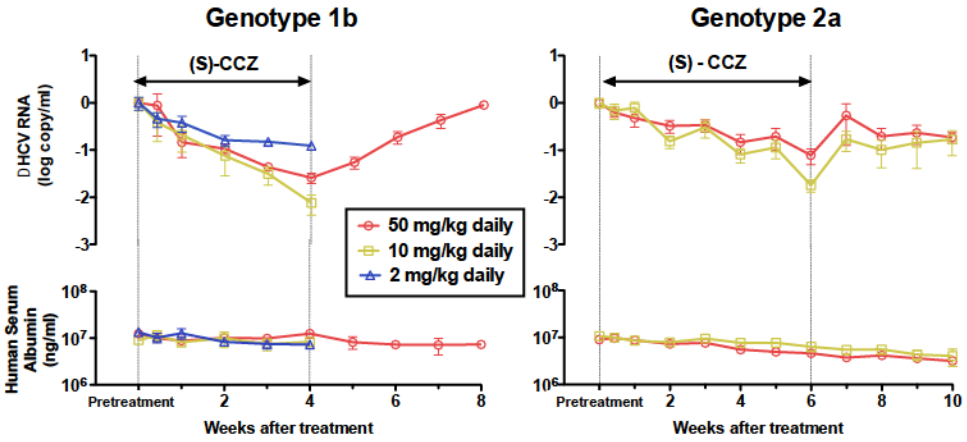


Fig Figure 4. Chlorcyclizine HCl (CCZ) inhibits HCV genotype 1b and 2a infection *in vivo*. Alb-UPA/SCID mice were engrafted with primary human hepatocytes and then infected with HCV serum samples of genotype 1b or 2a. The mice were monitored for serum HCV RNA and human albumin for 4-6 weeks before treatment. The serum HCV RNA levels were stable with little fluctuations during the weeks before infection, and the pretreatment HCV RNA values were determined by averaging HCV RNA levels of week -2, -1 and 0 before initiation of treatment. Left panel: changes in the genotype 1b HCV titers from pretreatment baseline over a period of 8 weeks with 4-week (*S*)-chlorcyclizine HCl treatment and 4-week of follow-up without treatment (only in the group received 50 mg/kg dose) in HCV-infected chimeric mice; Right panel: changes in the genotype 2a HCV titers from pretreatment baseline over a period of 10 weeks with 6-week (*S*)-chlorcyclizine HCl treatment and 4-week of follow-up without treatment (in both groups) in HCV-infected chimeric mice.

F. Summary

In both *in vitro* and animal *in vivo* studies, we have identified that chlorcyclizine HCl is effective against HCV for both genotype 1 and 2. In addition, chlorcyclizine HCl has shown to provide a synergistic effect in combination with ribavirin in *in vitro* experiments. We propose that chlorcyclizine HCl can be utilized as antiviral therapy in persons with chronic HCV infection and will result in a decrease in serum quantitative HCV RNA viral titers. The primary focus of this protocol is to evaluate the utility of chlorcyclizine HCl as a potential therapeutic modality and to assess the safety profile in patients chronically infected with HCV. We propose to treat patients with chlorcyclizine HCl with and without ribavirin for 28 days to evaluate for decline in quantitative HCV RNA during therapy. We will also evaluate safety, tolerability and viral kinetic responses during treatment.

Hypothesis and Aims

Hypothesis:

Therapy with chlorcyclizine HCl (CCZ) leads to a decrease in hepatitis C virus levels in serum and is safe in chronic hepatitis C patients.

Primary Endpoints:

1) Decline in serum HCV RNA viral titer during therapy as compared to baseline measured by PCR (qPCR).

2) The ability to tolerate the drug at the prescribed dose for the duration of therapy.

Secondary Endpoints:

1. A significant change in alanine aminotransferase (ALT) levels compared to baseline.
2. Quantification of chlorcyclizine HCl and its metabolites in serum during therapy.
3. Changes in symptom scale measurements during and after therapy.

Summary Of Investigational Agent

Chlorcyclizine HCl

For the purposes of this study, AHIST (chlorcyclizine hydrochloride) 25 mg tablets (Magna Pharmaceuticals, Inc, Louisville, KY) will be purchased for distribution by the NIH Clinical Center pharmacy.

Safety Pharmacology of Chlorcyclizine HCl

Chlorcyclizine HCl is a sedating antihistamine with clinical experience dating from its introduction in 1949.[37] *In vitro* pharmacology experiments have demonstrated it to be a low nanomolar antagonist of the histamine receptor.[38] The piperazine class of antihistamines including chlorcyclizine HCl also share anticholinergic, antiemetic, local anesthetic, and mild sedative properties presumably deriving from activity at muscarinic receptors and other targets.[39,40] Despite its polypharmacology, chlorcyclizine HCl retains a favorable side-effect profile:

> A mildly sedative antihistaminic agent with prolonged action and low incidence of toxic side effects. Chlorcyclizine HCl has slight anticholinergic and antispasmodic actions and enhances action of the epinephrine. It also has some local anesthetic action.[41]

Other antihistamine agents astemizole and terfenadine have been associated with rare reports of ventricular arrhythmias, but this is not believed to be a class effect.[42] The action of chlorcyclizine HCl on the QT interval or potassium channels in cardiac tissues has not been examined. In one preclinical study of cardiac repolarization with isolated, perfused, feline heart, chlorcyclizine HCl was the least potent of the antihistamines tested.[43] No reports of torsade de pointes have been associated with chlorcyclizine HCl.[44]

The absorption, distribution, metabolism and excretion of chlorcyclizine HCl in humans have not been characterized in detail. Chlorcyclizine HCl is metabolized in humans and other preclinical models primarily to norchlorcyclizine through oxidative *N*-demethylation and also a minor amount to chlorcyclizine *N*-oxide through *N*-oxidation which has been detected in urine in humans.[45,46] Both metabolic products lack

antihistamine activity.[45,46] It is expected that this is mediated by cytochrome P450 metabolism of chlorcyclizine HCl, though the specific metabolizing enzyme has not been identified to date. Chlorcyclizine HCl might therefore affect or be affected by concomitant administration of other cytochrome P450-interacting agents. Chlorcyclizine HCl may also have some potential to induce cytochrome P450 enzyme expression in studies in both rat[47] and dog[45] which may limit exposure to chlorcyclizine HCl. Furthermore, although described in an underpowered study, patients with chronic hepatitis C infections may have lower endogenous CYP3A4 and CYP2D6 activities than healthy volunteers which may increase exposure in this patient population if chlorcyclizine HCl metabolism is mediated by CYP3A4 or CYP2D6.[48] Thus, chlorcyclizine HCl exposure in the intended patient population might be greater or lower than the seasonal and perennial allergic rhinitis patient population.

The pharmacokinetics of oral chlorcyclizine HCl dosing is not known. The primary metabolite norchlorcyclizine preferentially distributes in preclinical models from plasma into certain tissues, especially to liver and spleen.[45] Norchlorcyclizine has a half-life of approximately 6 days in man with some amount detectable 20 days after the termination of dosing possibly reflecting this extensive tissue distribution.[49]

Human Experience with Chlorcyclizine HCl

Chlorcyclizine HCl is a sedating antihistamine[37] with clinical experience dating from its introduction in 1949 in two different products, Di-Paralene and Perazil. Di-Paralene prescribed initial dosing of 50 mg three times daily (150 mg per day) whereas Perazil labeling prescribed oral dosing of 50 mg tablets once or twice daily (50 or 100 mg

per day).[28] The OTC monograph for chlorcyclizine HCl 25 mg directs "1 tablet by mouth every 6-8 hours, not to exceed 3 tablets in 24 hours, or as directed by a doctor" (75 mg per day or as directed by a doctor).[29]

The effects of acute doses of chlorcyclizine HCl have been observed in humans, and are similar to other first-generation piperazine antihistamines. In general, adults have survived single doses of 2.5-5.0 grams of first generation antihistamines.[50] There is a case report of a 16 month old boy who consumed 900 mg; he experienced a prolonged convulsive state but eventually made a full recovery.[51] One patient suffering from an allergic reaction to penicillin was treated with 600 mg chlorcyclizine HCl over 12 hours and cumulatively 1300 mg over 3 days without side effect.[52]

The clinical profile of chlorcyclizine HCl seems consistent with other first generation antihistamines (excerpt taken from Pyrilamine, a first generation antihistamine):

> In acute poisoning with H_1 antagonists, their central excitatory effects constitute the greatest danger. The syndrome includes hallucinations, excitement, ataxia, incoordination, athetosis, and convulsions. Fixed, dilated pupils with a flushed face, together with sinus tachycardia, urinary retention, dry mouth, and fever, lend the syndrome a remarkable similarity to that of atropine poisoning. Terminally, there is deepening coma with cardiorespiratory collapse and death, usually within 2 to 18 hours.[53]

Single oral doses of 400 mg chlorcyclizine HCl have been administered without toxic effects to three patients.[52] One patient suffering from an allergic reaction to penicillin was treated with 600 mg chlorcyclizine HCl over 12 hours and cumulatively

1300 mg over 3 days without any side effects.[52] In other studies with single oral doses of 100 mg, drowsiness was the most common side effect, reported in 17 of 63 doses (this fact is further clarified in Brown 1950). Other mild side effects were reported in 21 of 63 doses (dryness of mouth, nausea, headache, light headedness, nervousness, difficulty in walking, vomiting, vertigo, palpitation, inability to concentrate, stimulation, and tingling of fingers).[54] By limiting single doses to 50 mg, patients are able to obtain allergy relief at cumulative doses equal to or greater than 100 mg per day without side effects, including 50 mg b.i.d. [54,55], 50 mg t.i.d.[49,54], 50 mg q.i.d.[54,55], though some drowsiness was also noticed at 50 mg q.i.d. in one study.[55]

The FDA has evaluated the safety of chlorcyclizine HCl in two ways, during the initial NDA process (NDA 6-947 Perazil Tablets marketed by Burroughs Wellcome Co. and NDA 6-938 Di-Paralene Tablets marketed by Abbott Laboratories) and in context of risk-benefit analysis through the DESI review process[56] for seasonal and perennial allergic rhinitis. This rhinitis patient population would be expected to a broad, heterogeneous patient population, and a wide safety margin would be expected for the use of this drug in any general population.

Chronic 4-week dosing of chlorcyclizine HCl has not been studied in a controlled clinical setting. The product is indicated for perennial allergic rhinitis and some chronic use is anticipated. In an early clinical study, a few patients took daily 50 mg doses for one month without significant side effects.[52] Moreover, patients experiencing side effects quickly recover after discontinuing treatment or reducing dosage. The OTC product label indicates “Uses: temporarily relieves these symptoms due to hay fever or other upper respiratory allergies.” It is assumed that “temporarily” in the indication refers to the

pharmacokinetics of the product and not to its potential chronic use. Cetirizine OTC products have very similar labels. Neither chlorcyclizine HCl nor cetirizine labels include any warning to stop use after an extended period, unlike Sudafed which has the warning "stop use and ask a doctor if … symptoms do not improve within 7 days or occur with a fever". Warnings in the chlorcyclizine HCl OTC product labeling include: "Do not take this product unless directed by a doctor. If you have; a breathing problem such as emphysema or chronic bronchitis, glaucoma, difficulty in urination due to enlargement of the prostate gland, ask a doctor before use or if you are taking sedatives or tranquilizers. If pregnant or breast-feeding, ask a health professional before use."

Toxicology of Chlorcyclizine HCl

The LD_{50} in mice is 137 mg/kg.[54] Kuntzman reported that the administration of a single 50 mg/kg oral dose of chlorcyclizine to a dog caused tremors and convulsions – the fate of that animal is not known, but data obtained for the 14 days following that single dose indicated recovery; other dogs were chronically dosed for up to 125 days with oral doses of at least 10 mg/kg without significant findings.[45,57]

Based on a detailed scientific review of preclinical and clinical data surrounding possible teratogenic effects of chlorcyclizine HCl and related compounds, the FDA found that the data does not support any special restriction on the use of chlorcyclizine HCl or a pregnancy warning[29] beyond the general precautionary labeling required on OTC products by § 201.57(f)(6). Chronic administration of chlorcyclizine HCl at high doses in rodents induces lipidosis and at similar 75 mg/kg doses also cause abortions in rat.[58,59] 50 mg/kg dosage during pregnancy in rats induced lipodosis in the fetus as well.[59] 60 mg/kg and 90 mg/kg doses during sensitive periods for palate development induced cleft-palate

and other malformations of rat fetal development.[60] Developmental toxicity has been noted with chlorcyclizine HCl and other antihistamines in rodents.[61,62]

Concomitant Use with Ribavirin

As discussed above, ribavirin appears to be an important component of combination therapy with various DAAs in HCV therapy and synergizes with chlorcyclizine HCl in anti-HCV in vitro assays. We therefore decided to use ribavirin in combination with chlorcyclizine HCl as an interferon-free combination therapy to compare with chlorcyclizine HCl monotherapy in this study. Because this is the first study utilizing the combination of the ribavirin and chlorcyclizine HCl for the therapy of chronic HCV infection, the side effects of combining the two drugs are not known. Results of *in vitro* studies of ribavirin using both human and rat liver microsome preparations indicated little or no cytochrome P450 enzyme-mediated metabolism, with minimal potential for P450 enzyme-based drug interactions[63] and its indicated drug interactions list only nucleoside analogues and azathioprine[64], and so the risk of an interaction with concomitant administration of chlorcyclizine is expected to be low.

Protocol

We propose to treat 50 patients with chronic hepatitis C infection (all genotypes) who are treatment naïve or relapsers to previous forms of interferon and ribavirin therapy with chlorcyclizine HCl with or without ribavirin for twenty-eight days monitoring HCV RNA levels as well as routine safety measures and liver function tests.

Study Population

Inclusion Criteria

(1) Adults, ages 18 and above;

(2) Chronic hepatitis C (HCV RNA in serum for more than 6 months);

(3) HCV RNA in serum at or above 10,000 IU/mL;

(4) **Treatment naïve** patients defined as individuals whom have never undergone any form of interferon and ribavirin therapy for chronic HCV infection or relapsers defined as reappearance of HCV RNA in serum after treatment (with any form of interferon and ribavirin therapy) was discontinued and an end-of-treatment response was achieved;

(5) No major contraindications to agents being used (chlorcyclizine HCl and ribavirin);

(6) Females of childbearing potential must have a negative serum or urine pregnancy test result (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours before the first dose of study drug;

(7) Women of childbearing potential and men, participants and partners, must use highly effective methods of birth control to minimize the risk of pregnancy and must follow instructions for birth control for the entire duration of the study including a minimum of 24 weeks after the last dose of ribavirin. Two forms of birth control are required from the time of screening throughout the duration of the on-treatment study period and for at least 24 weeks after the last dose of ribavirin. Examples of effective birth control include: condom with spermicide; diaphragm with spermicide; cervical cap with spermicide; female condom; intrauterine devices (IUDs); vasectomy in men;

Exclusion Criteria

(1) Liver or any other organ transplant (including hematopoietic stem cell transplants) other than cornea and hair;

(2) Current or known history of cancer (except in situ carcinoma of the cervix or adequately treated basal or squamous cell carcinoma of the skin) within 5 years prior to enrollment;

(3) Documented or suspected HCC, as evidenced by previously obtained imaging studies or liver biopsy (or on a screening imaging study/liver biopsy if this was performed);

(4) Evidence of decompensated liver disease including, but not limited to, bilirubin >4 mg/dL, albumin <3.0 gm/dL, prothrombin time >2 sec prolonged or a history or presence of ascites, bleeding varices, or hepatic encephalopathy. Patients with ALT levels >500 U/L will not be enrolled but may be followed until three determinations are below this level;

(5) Evidence of a medical condition contributing to chronic liver disease other than chronic HCV infection (such as, but not limited to: acute hepatitis C infection, hemochromatosis, autoimmune hepatitis, metabolic liver disease, alcoholic liver disease, toxin exposures);

(6) History of chronic hepatitis B virus (HBV) as documented by HBV serologies (eg, HBsAg-seropositive). Subjects with resolved HBV infection may participate (eg, HBsAb-seropositive with concurrent HBsAg-seronegative);

(7) Any prior exposure to direct-acting antiviral therapies for chronic HCV infection;

(8) History of HIV infection;

(9) History of hemoglobinopathies (eg. thalassemia major or sickle cell anemia), diagnoses associated with an increased baseline risk for anemia (eg, spherocytosis), hemolytic anemia, or diseases in which anemia would be medically problematic, or hemophilia;

(10) Confirmed, uncontrolled hypertension (any screening systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg should be excluded unless discussed with the central medical monitor);

(11) Any other medical and/or social reason, including active substance abuse as defined by DSM-IV, Diagnostic Criteria for Drug and Alcohol Abuse, which in the opinion of the investigator would make the candidate inappropriate for participation in this study;

(12) Significant systemic or major illnesses other than liver disease, including, but not limited to, clinically significant emphysema or chronic bronchitis, symptomatic benign prostatic hypertrophy, glaucoma, gastrointestinal motility related illnesses, congestive heart failure, renal failure (eGFR <50 mL/min), and active coronary artery disease;

(13) Significant prior history suggestive of cardiovascular instability, including but not limited to evidence of significant myocardial ischemia, unstable re-entry phenomena, other significant dysarrhythmias and/or uncontrolled hypertension;

(14) Inability to tolerate oral medication;

(15) For relapsers: exposure to interferon based therapy with ribavirin within 12 weeks prior to screening;

(16) Allergy or hypersensitivity to chlorcyclizine HCl or ribavirin;

(17) Any known contraindication to ribavirin, not otherwise specified;

(18) Inability to refrain from operating heavy machinery while on therapy;

(19) Breastfeeding women;

(20) Inability to understand or sign informed consent;

(21) Active use of chlorcyclizine HCl or another piperazine class antihistamine within 6 months of enrollment;

(22) Inability to abstain from piperazine class antihistamines during enrollment in the clinical trial period.

Initial Evaluation

Pre-treatment Phase: (screening visits)

Patients will be seen by the Liver Diseases Branch of NIDDK in the outpatient clinic of the Clinical Center, NIH, where they will initially undergo evaluation under the omnibus protocol 91-DK-0214 "Evaluation of Patients with Liver Disease." During this evaluation, patients will also undergo evaluation for assessment of eligibility:

(1) History and physical examination;

(2) Concomitant medication query (including dose and indication for each);

(3) A standard symptom questionnaire [which focuses on fatigue and abdominal pain], which provides information on categorical presence of symptoms as well as their severity, pattern and frequency (Appendix 1);

(4) Blood tests. These include complete blood count (CBC with differential and platelet count), prothrombin time (PT), partial thromboplastin time (PTT), sedimentation rate (ESR), reticulocyte count, plasma haptoglobin level, alanine

aminotransferase (ALT), aspartate aminotransferase (AST), direct and total serum bilirubin, albumin, total protein, lactate dehydrogenase (LDH), creatine phosphokinase (CK), sodium, chloride, bicarbonate, potassium, blood urea nitrogen, creatinine, glucose, magnesium, uric acid, calcium, phosphorus, cholesterol, triglycerides, iron and iron-binding capacity (transferrin), ferritin, immunoglobulin levels, thyroid stimulating hormone (TSH), antinuclear antibody (ANA), alpha fetoprotein (AFP), rheumatoid factor, cryoglobulins, hepatitis B surface antigen (HBsAg), antibody to HBsAg (Anti-HBs), antibody to hepatitis B core antigen (anti-HBc), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), hepatitis B virus DNA (HBV DNA), antibody to hepatitis d antigen (anti HDV), anti-HCV, hepatitis C virus RNA (HCV RNA), HCV genotype testing and anti-HAV. HBV DNA and HCV RNA will be tested by polymerase chain reaction. An additional 10 mL of whole blood will be collected and stored at -80 °C. Women of childbearing potential will have a pregnancy test;

(5) A routine urinalysis will be performed;

(6) An abdominal ultrasound will be performed;

(7) An electrocardiogram (EKG) in digital format will be performed.

Treatment

The treatment design of this study entails randomization of patients into one of two dosing groups (I and II) (Figure 1). Group I patients will receive chlorcyclizine HCl at a dose of 75 mg BID. Group II patients will receive chlorcyclizine HCl (75 mg BID) with ribavirin. For all genotypes, ribavirin will be dosed via a weight-based regimen of 1000 mg daily <75 kg and 1200 mg daily ≥75 kg. For patients receiving 1000 mg daily,

patients will take 2 (200 mg each) tablets in the AM and 3 (200 mg each) tablets in the PM. For patients receiving 1200 mg daily, patients will take 3 (200 mg each) tablets in the AM and 3 (200 mg each) tablets in the PM.

After the pre-treatment evaluation, patients will be admitted to the NIH Clinical Center for 48 hours, for induction of therapy, observation of side effects, administration of medication, and timed blood draws to facilitate analysis of virological response kinetics. Upon initiation of therapy, all patients will be administered a one-time dose of 150 mg (about 2mg/kg) of chlorcyclizine at time 0. From day 2 through 28, patients will take 75 mg of chlorcyclizine twice daily as prescribed. Approximately 10 patients receiving chlorcyclizine monotherapy will undergo a detailed pharmacokinetic (PK) study (measurement of chlorcyclizine and its metabolites in the blood). In patients undergoing PK analysis, sampling will begin upon initiation of therapy (Time= 0, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 18 h, 24 h and 48 h), while on therapy (days 7, 14, 21 and 28) and after the completion of therapy (post-therapy week 1 and 2). For viral kinetic analysis, all patients will have blood sampled at Time=0, 4h, 12h, 18h, 24h, 36h and 48h as outlined in Table 1.

It is possible that ribavirin co-administration may alter the pharmacokinetic parameters of chlorcyclizine, but there is no evidence in the literature that these two drugs interfere with each other's absorption and metabolism. We will be obtaining blood samples on all patients and therefore can measure the blood chlorcyclizine levels at certain time points after therapy to compare those receiving chlorcyclizine only and those receiving chlrocyclizine plus ribavirin.

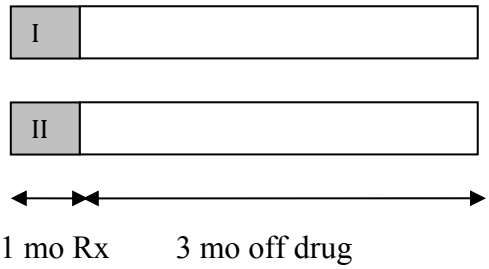
After induction of therapy, patients will be treated as outpatients and followed on a regular basis in the outpatient clinic at the NIH (See below and Table 1). Drug therapy compliance will be monitored through patient diaries. Therapy will be stopped for intolerance to chlorcyclizine HCl or ribavirin (which will be carefully defined). Patients will also be reminded to refrain from operating heavy machinery while on therapy.

At the 28-day point, patients will undergo a repeat outpatient evaluation. This evaluation will include all of the elements listed above under (Pre-treatment phase) which will include multiple blood tests, urine tests, symptom questionnaires, and virological testing.

After 28 days of therapy, patients will be monitored and followed off therapy for HCV without treatment at various intervals (Table 1) for the next 3 months. Patients may be offered standard therapies for disease flares that lead to clinically significant deterioration during the 3-month post-treatment monitoring period. Thereafter, patients will be followed in the usual fashion by the Liver Diseases Branch. If eligible, patients will be offered enrollment in other HCV studies offered by the Liver Diseases Branch.

Figure 1: Study Design

- 2 dosing groups:
  - I=Chlorcyclizine HCl
  - II=Chlorcyclizine HCl + ribavirin
- 1 mo Rx / 3 mo off drug



Randomization

After initial evaluation and determination of eligibility, patients will be asked to provide written informed consent. All patients who consent will then be randomized by a set of random numbers held in the Pharmaceutical Development Service (PDS) into either group I or group II. The randomized code will be blocked into groups of 5 so that equal numbers of patients will be randomized to each group of 25 patients have been enrolled. Randomization will be stratified by HCV genotype (HCV genotype 1 and non-HCV genotype 1).

Monitoring During Therapy

Patients will be seen, interviewed and have blood tests taken at regular intervals before, during and after therapy (Table 1). Patients will be seen and have blood drawn at least 3 times during the 6 months before treatment. An electrocardiogram will be performed prior to starting therapy and during therapy as described in Table 1. Patients will be admitted and have frequent blood samples during the first 48 hours of therapy (baseline, 4, 12, 18, 24, 36 and 48 hours), to assess the initial viral kinetics in response to

therapy. For patients undergoing intensive PK analysis, an additional tube of blood sampling will be obtained as previously described above. 48 hours after starting therapy, patients will be discharged and seen in the outpatient clinic for blood testing at 7, 14, 21 and 28 days. At each time point, an acceptable deviation would include +/-3 day. After stopping therapy, patients will be seen for blood testing at 1, 2, 4, 8, 16 and 24 weeks. At each time point, an acceptable deviation would include +/- 1 day. The timing of blood specimens, physical examination and other evaluations is provided in Table 1. Routine blood tests will include complete blood count and differential, serum chem-20 (ALT, AST, alkaline phosphatase, LDH, CPK, direct and total bilirubin, albumin, total protein, electrolytes, calcium phosphate, glucose, blood urea nitrogen, magnesium, creatinine and uric acid) and HCV RNA. Extended blood tests will also include prothrombin time, TSH and a pregnancy test. Serum to evaluate of chlorcyclizine HCl and its metabolites will also be drawn as shown in Table 1. When appropriate, blood will be drawn in pediatric tubes and the amount of blood that will be drawn from adult patients (i.e.: those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller over any eight week period. At specified outpatient visits during therapy (Table 1), patients will be asked about their symptoms, fill out a symptom questionnaire and will undergo assessment of vital signs and medication diaries. Potential neurological, cardiac, gastrointestinal, and psychiatric side effects associated with antihistamines will be carefully monitored at each visit (table 2). At extended outpatient visits, patients will undergo a brief history and physical examination.

HCV RNA will be tested at the NIH Clinical Center utilizing real time PCR (sensitivity ~15 IU per mL), COBAS Ampliprep/COBAS Taqman HCV Testing

Methodology which is an FDA approved assay intended for the use as an aid in the management of chronic HCV infection. Samples will be collected at baseline (at least 3 times) and at each sampling time point to enable resistance monitoring and analysis and viral kinetics. The latter will include determination of baseline determination of baseline isolate sequence analysis to enable identification and monitoring changes in viral genome sequences as a function of treatment duration. Analysis of both chlorcyclizine HCl and chlorcyclizine HCl + ribavirin will enable an assessment of background genomic variability. Any clinical evidence of breakthrough or rebound (defined as a greater than 1 log increase above an observed viral load nadir) will prompt a more in-depth viral genomic analysis. Because other unanticipated mechanisms of resistance could theoretically be operative; however, we would anticipate performing full genome sequencing to enable maximally unbiased assessment for candidate resistance mutations. We will also have the ability to engineer any identified candidate resistance mutation back into an HCV vector competent for in vitro replication, in order to confirm that the mutation is indeed causative of any suspected decreased sensitivity to chlorcyclizine HCl (CCZ) and/or ribavirin (RBV).

Table 1: Design of Study

Time	Therapy	Routine Visit	Extended Visit	Pharmacokinetic, Chlorcyclizine HCl and Metabolite Testing	HCV RNA Testing	Comments
-8 wk.	None	*			*	
-4 wk.	None	*			*	
Day 0-2 (Inpatient Stay)	Start Therapy with CCZ or CCZ+RBV with 150 mg CCZ, with days 2-28 with 75 mg BID.	*	*	*	*	Consent signed at or before Day 0, Symptom Questionnaire at Baseline, Baseline & 48 hr EKG. Perform PK studies.
Day 7	CCZ or CCZ+RBV	*		*	*	EKG, Symptom Questionnaire
Day 14	CCZ or CCZ+RBV	*	*	*	*	EKG, Symptom Questionnaire
Day 21	CCZ or CCZ+RBV	*		*	*	EKG, Symptom Questionnaire
Day 28	Stop Therapy	*	*	*	*	Symptom Questionnaire, EKG
Post-Therapy Follow-up						
Week 1	None	*		*	*	EKG, Symptom

						Questionnaire
Week 2	None	*		*	*	
Week 4	None	*			*	
Week 8	None	*			*	
Week 12	None	*	*	*	*	Symptom Questionnaire

Routine Visit: Review of diary, vital signs, review of symptoms, interim medical history, urinalysis, and routine blood tests for ALT, AST, alkaline phosphatase, direct and total bilirubin, albumin, total protein, BUN, creatinine, hematocrit, white blood cell count and differential, platelet count.

Extended Visit: Prothrombin time, TSH and physical examination. Women of childbearing age will have a pregnancy test.

Pharmacokinetic, Chlorcyclizine HCl and Metabolites Testing: 8 cc of blood will be collected in a red-top tube for PK testing at the designated time points. 10 cc of blood will be collected in a red-top SST tube to be tested for chlorcyclizine HCl and its metabolites.

HCV RNA Testing: Real-time quantitative polymerase chain reaction assay (qPCR) for viral titer with a sensitivity of 15 IU per mL.

Symptom Questionnaire: Will be administered on several occasions. Before and after 28 days of therapy and at the 6 month off-treatment follow-up visit.

Start Therapy: Includes blood drawn immediately before and then at 4, 12, 18, 24, 36 and 48 hours after the first dose of chlorcyclizine HCl or chlorcyclizine HCl+ribavirin for measurement of HCV RNA.

Assessment of Response

The primary therapeutic endpoint will be a decrease of HCV RNA viral titer as compared to baseline measurements in serum, as measured by quantitative PCR (qPCR). A secondary therapeutic endpoint will include a change in ALT levels compared to baseline measurements.

The primary safety endpoint will be ability to tolerate the drug at the prescribed dose for the full 4-week duration. Discontinuation of the medication will be considered failure to tolerate.

Secondary safety endpoints will include changes in specific values in symptom scales, in the safety questionnaire or worsening of liver disease and quantification of metabolites of chlorcyclizine HCl during therapy. Chlorcyclizine HCl has undergone various phase II, III & IV studies and has been used as an allergy medication with good safety and tolerability. Although we cannot be certain, we do not expect to have differing safety and tolerability issues in comparison to these studies.

Design of Trial/Statistical Considerations

This study is designed as a phase 1b proof-of-concept treatment trial to determine the ability of chlorcyclizine HCl to suppress HCV at tolerable doses. The effective dose is not known. This trial should show if HCV suppression is possible with chlorcyclizine HCl and also help in suggesting the optimal dose and duration for viral suppression and how well it is tolerated. Therefore assessment of virologic response and tolerance in each open label group will be the primary analysis. Secondarily the magnitudes of viral declines will be compared between the two groups with standard statistical methods to assess the combined effect of chlorcyclizine HCl and ribavirin. Normal fluctuation of

HCV RNA in serum over a short-term period (< 3 months) has been described to be approximately 0.5 log10.[65] In a prior study performed by our group, 28 days of ribavirin monotherapy resulted in a mean decrease of 0.55 log10 without significant difference between genotype 1 and non-genotype 1 infection.[66] Therefore, accounting for normal short-term fluctuation of HCV RNA in serum, and the effect of ribavirin therapy, to show at least a 0.5 log improvement while on therapy (comparing baseline with either chlorcyclizine HCl or chlorcyclizine HCl+ribavirin or comparing between groups) with an alpha of .05 and a power of .8, and accounting for a 10% dropout rate, the total needed for each group is 25 patients and a total for the study of 50 patients.

Hazards and Discomforts

The hazards associated with this study are the following.

(1) *The risks and discomforts of frequent phlebotomy*. To document stable levels of biochemical and serologic markers of chronic hepatitis and to monitor the effects and toxicities of the therapy, frequent blood sampling will be required. Patients will have between 15-20 venipunctures during their course of therapy. Each venipuncture will be for 15 to 70 mL of blood. The amount of blood that will be drawn from adult patients (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.

(2) *The risks and hazards of chlorcyclizine HCl therapy.* Chlorcyclizine HCl is a sedating antihistamine[37] with reported mild side effects including dryness of mouth, nausea, headache, light headedness, nervousness, difficulty in walking,

vomiting, vertigo, palpitation, inability to concentrate, stimulation, and tingling of fingers.[54] The extensive summary of chlorcyclizine HCl including risks and hazards of therapy are described earlier in this protocol in the section entitled "Summary of Investigational Agent" starting on page 17.

(3) *The hazards and discomforts of ancillary evaluations.* Patients with risk factors for coronary artery disease will undergo thallium stress testing. If one has been performed within one year prior to study enrollment, a repeat test will not be necessary. Exercise stress testing requires that the patient undergo treadmill exercise testing while being monitored for vital signs and EKG changes. The exercise itself takes 3 to 15 minutes and consists of graded exercise on a mechanical treadmill. The patient will have an intravenous line in place and EKG monitors. This is a standard means of evaluation of patients at high risk for coronary artery disease and is not a research procedure per se. Exercise stress testing has a small risk; deaths due to cardiac arrhythmias have been reported to occur once in every 10,000 patients studied. The cardiology service at the NIH Clinical Center is proficient in this methodology and perform it frequently as a part of evaluation of patients for both routine and research medical procedures.

(4) *The risks and hazards of ribavirin therapy***.** Treatment with ribavirin has been associated with few severe adverse side effects.[67-70] The following discussion of side effects is based largely upon our own experience in several pilots studies (90-DK-49, 91-DK-11 and 93-DK-140) and our randomized, double-blind controlled trials of ribavirin (91-DK-178 and 98-DK-003).[71-73]

a. **Hemolytic anemia.** Ribavirin results in dose dependent hemolysis of

varying degree in most patients.[73] In patients without hemolysis or bone marrow suppression, clinically significant anemia is not common. In large clinical trials of combination therapy using ribavirin, between 8-10% of patients required ribavirin dose modification because of anemia[67,6867,6867,6867,6866,6765,6665,6665,6664,65.63,64] In all cases, the hematocrit rose to pretreatment levels within a month of stopping therapy. Anemia can result in symptoms of fatigue, palpitations, dyspnea on exertion, headaches and general weakness. In the current study, patients will be monitored closely with blood counts every two to eight weeks. The initial dose of ribavirin will be 1000 to 1200 mg per day. Growth factors (epoetin alfa, darbopoeitin alfa) will not be started in this trial. Patients with pre-existing hemolysis or bone marrow compromise are more sensitive to the hemolysis induced by ribavirin.

b. **Lymphopenia.** Analysis of our controlled trial of ribavirin indicated that there was a 10% to 15% decrease in lymphocyte counts among the patients treated with ribavirin[72]. The lymphopenia has not been severe or associated with an increase in opportunistic infections. Lymphocyte counts return to pretreatment values when ribavirin is stopped.

c. **Gout.** Ribavirin is a purine analogue and is metabolized to uric acid. In some patients, uric acids increase during ribavirin therapy and these increased levels may trigger an acute attack of gout. Acute gout has been described in patients with a history of gout who receive ribavirin, and can be managed with typical therapy without stopping ribavirin, unless the

attack is severe and prolonged or does not respond to standard therapy.

d. **Cholelithiasis.** Both chronic hemolysis and chronic liver disease are associated with an increased risk of pigment gallstones. It is therefore possible that ribavirin could increase the risk of having gallstones, particularly in patients with pre-existing liver disease. Development of gallstones has been described in small numbers of patients receiving ribavirin for up to 48 weeks. Patients in this study undergo routine ultrasound of the abdomen before therapy and at the end of follow up.

e. **Pruritus.** Itching occurs in approximately 20% of patients receiving ribavirin for 24 weeks or more and is the reason for stopping therapy in some patients. The pruritus is usually mild, but can be severe in some patients, interfering with sleep. This side effect typically appears after several months of treatment and is improved by decreasing the dose. This side effect suggests that ribavirin has histamine-like effects.

f. **Nasal stuffiness, sinusitis and upper respiratory infections.** Respiratory symptoms are common among patients receiving ribavirin, being reported in as many as 20% of patients. These can include repeated upper respiratory infections, chronic cough, wheezing, sinusitis and ear ache. These side effects, like pruritus, tend to arise after several months of therapy and both may be related to histamine-like effects of ribavirin.

g. **Hepatic iron accumulation.** Studies of liver biopsies from before and after therapy with ribavirin have demonstrated an accumulation of iron with therapy. This is probably due to the chronic hemolysis induced by

ribavirin causing accumulation of macrophagic iron as well as increases in iron absorption caused by anemia. In most cases, the amount of iron appeared slight. In our controlled trial of combination therapy, quantitation of hepatic iron performed on liver tissue from before and after 48 weeks of therapy, the hepatic iron concentrations increased from a mean of *826 to 1857 □g per gram* of dry weight.[71] This degree of iron accumulation is not clinically significant. In other studies, we have performed follow up liver biopsies on four patients who developed stainable hepatic iron with ribavirin therapy and all four showed disappearance of iron staining; neither had been treated with phlebotomy. Thus, the increase in iron with therapy may gradually resolve spontaneously. In this study, quantitative analysis of iron concentration will be performed on liver biopsies done at the start of therapy. Patients with elevated levels of iron will undergo phlebotomy as would be recommended in standard practice.

h. **Fetal and reproductive effects.** The risks of ribavirin therapy to the fetus during pregnancy are not known.[74] Studies in animals indicate that ribavirin can cause fetal wasting and fetal abnormalities in all animal species studied. This effect is probably due to induction of severe and fatal hemolysis in the fetus. At doses equivalent to what is used in humans, ribavirin did not have teratogenic effects in rabbits and rats. Nevertheless, female patients of childbearing age will be strongly cautioned about the possible risk in pregnancy and will be counseled about practicing adequate

contraception for the duration of treatment and the subsequent six months. A pregnancy test will be done at the start of therapy in these individuals and repeated at each visit during treatment and for the next 6 months. Patients who are pregnant will not be entered into this study, and the drug will be discontinued immediately if pregnancy occurs. Ribavirin will also be discontinued in male patients should their partners become pregnant, as advised in the package insert for the medication. Male patients will also be cautioned and the potential for decrease in spermatogenesis will be discussed.

i. **Carcinogenesis.** *In vitro* assays have suggested that ribavirin has carcinogenic and mutagenic potential. However, in recent oncology studies in the mouse and rat, there was no evidence of carcinogenicity in either species. Nevertheless, the potential of carcinogenesis will be discussed in the consent form.

j. **Non-specific Symptoms.** Some patients in placebo-controlled trials of ribavirin complained of anxiety, irritability, fatigue and myalgias. These symptoms could not be clearly related to ribavirin and also occurred in some placebo recipients. The reasons for these complaints are not clear. However, many medications can cause such non-specific complaints and these will be mentioned in the consent form.

k. **Retinal Changes.** In rats given ribavirin for 12 to 24 months, retinal atrophy and degeneration were noted on ophthalmologic examination as well as microscopically. These findings led to a routine use of

ophthalmologic examinations at the beginning and end of therapy and in follow up in the U.S. multicenter trial of ribavirin for chronic hepatitis C. In that study there was no increase in retinal changes or cataracts in the treated patients compared to controls.

l. **Photosensitivity**. Ribavirin increases skin sensitivity to sunlight. This can lead to a range of problems from mild irritation with redness and pruritus to severe blistering sunburn. This risk extends to indirect sunlight, such as through a windshield or window. Patients are advised to use sunscreen and to cover up sun-exposed areas during treatment. This is of particular concern in fair-skinned individuals.

(5) *The risks of the combination of ribavirin and chlorcyclidine HCl.* As this the first study utilizing the combination of chlorcyclidine HCl and ribavirin for the therapy of chronic HCV infection, the side effects of combining the two drugs are not known. However, chlorcyclizine HCl is an FDA approved antihistamine therapy approved for temporary relief of allergy symptoms, and is also an over-the-counter drug. To the best of our knowledge, no reports have been described of significant side-effects with the combination of ribavirin and chlorcyclizine. Thus, we do not anticipate significant side effects aside from each drug's individual side effect profile mentioned above.

Adverse Events and Modifications of Dose of Chlorcyclizine HCl and Ribavirin

Patients will be monitored side effects. Discontinuation of chlorcyclizine HCl or ribavirin will be based upon the scoring of adverse events shown in table 2. The scoring of toxicity will be performed from the CTCAE Version 3.0 with modifications for

leukocytes, hemoglobin, hematocrit, platelets, prothrombin time, partial thromboplastin time, ALT, AST and bilirubin. These variables have been modified because the original version was designed by the National Cancer Institute (NCI) with use for cancer trials and not for clinical trials in liver disease. The modified version to be utilized for this clinical trial accounts for accepted variations of liver tests used in various other liver disease clinical trials by the Liver Diseases Branch. Factor that will lead to discontinuation of chlorcyclizine HCl with or without ribavirin include pregnancy, any grade 3 (except leukocyte and platelet categories) or any grade 4 adverse events or any adverse event, which, in the opinion of the investigator, places the patient at increased risk. Drug discontinuation may also be based on individual clinical presentations of each subject. For example, AST and ALT values will be evaluated in the context of each individual's baseline values. Rather than utilizing specific cutoff values, which may not be in the best interest of each subject, if the investigator identified a significant elevation in liver related laboratory tests which may jeopardize the patient's safety, the drug may be discontinued. If the female becomes pregnant, the patient's obstetrician will be provided with the standard of care guidelines to prevent HCV transmission. Chlorcyclizine HCl with or without ribavirin will not be restarted unless another cause for the abnormality or symptom is found.

Table 2. Scoring of toxicity for dose modifications. Scoring of toxicity from the CTCAE Version 3.0, with modifications for leukocytes, hemoglobin, hematocrit, platelets, prothrombin time, partial thromboplastin time, ALT, AST and bilirubin. Normal ranges for values at the NIH Clinical Center are used.

Scoring of toxicity for dose modification

		Grade				
Adverse Event	Name	1	2	3	4	5
Allergic reaction/ hypersensitivity (Including drug fever)	Allergic Reaction	Transient flushing or rash; drug fever <38 C (<100.4 F)	Rash; flushing; urticarial; dyspnea; drug fever >38 C (>100.4F F)	Symptomatic bronchospasm, with or without urticaria; parenteral medications(s) indicated; allergy-related edema/angioedema; hypotension	Anaphylaxis	Death
Anorexia	Anorexia	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); IV fluids, tube feedings or TPN indicated	Life threatening consequences	Death
Anxiety	Anxiety	Mild symptoms ; intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL; hospitalization not indicated	Life-threatening; hospitalization indicated	Death
Nausea	Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated <24 hrs.	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated >24 hrs.	Life threatening consequences	Death
Agitation	Agitation	Mild mood alteration	Moderate mood alteration	Severe agitation; hospitalization not indicated	Life-threatening consequences; urgent intervention indicated	Death

Fatigue (asthenia, lethargy, malaise)	Fatigue	Mild fatigue over baseline	Moderate or causing difficulty performing some ADL	Severe fatigue interfering with ADL	Disability	_____
Depressed level of consciousness	Depressed level of consciousness	Decreased level of alertness	Sedation; slow response to stimuli; limiting instrumental ADL	Difficult to arouse	Life-threatening consequences	Death
Somnolence (excessive sleepiness and drowsiness)	Somnolence	Mild but more than usual drowsiness or sleepiness	Moderate sedation; limiting instrumental ADL	Obtundation or stupor	Life-threatening consequences; urgent intervention indicated	Death
Tremor (uncontrolled shaking movement of the whole body or individual parts)	Tremor	Mild symptoms	Moderate symptoms; limiting instrumental ADL	Severe symptoms, limiting self care ADL	_____	_____
Dry Mouth	Dry Mouth	Symptomatic without significant alteration (e.g., dry or thick saliva)	Moderate symptoms; oral intake alterations (e.g., copious water, other lubricants, diet limited to purees and/or soft, moist foods)	Inability to adequately aliment orally; tube feeding or TPN indicated	_____	_____
Akathisia (uncomfortable feeling of inner restlessness and inability to stay still)	Akathisia	Mild restlessness or increased motor activity	Moderate restlessness or increased motor activity; limiting instrumental ADL	Severe restlessness or increased motor activity; limiting self care ADL	_____	_____
Concentration	Concentrati	Mild	Moderate	Severe impairment	_____	_____

Impairment	on impairment	inattention or decreased level of concentration	impairment in attention or decreased level of concentration; limiting instrumental ADL	in attention or decreased level of concentration; limiting self care ADL		
Dysesthesia (distortion of sensory perception, resulting in an abnormal & unpleasant sensation)	Dysesthesia	Mild sensory alteration	Moderate sensory alteration; limiting instrumental ADL	Severe sensory alteration; limiting self care ADL	_____	_____
Dizziness (lightheadedness, unsteadiness, giddiness, spinning or rocking)	Dizziness	Mild unsteadiness or sensation of movement	Moderate unsteadiness or sensation of movement; limiting instrumental ADL	Severe unsteadiness or sensation of movement; limiting self care ADL	_____	_____
Paresthesia (tingling, numbness, pressure, cold, warmth in the absence of stimulus)	Paresthesia	Mild symptoms	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL	_____	_____
Urinary retention	Urinary retention	Urinary, suprapubic or intermittent catheter placement not indicated; able to void with some residual	Placement of urinary, suprapubic or intermittent catheter placement indicated; medication indicated	Elective operative or radiologic intervention indicated; substantial loss or affected kidney function or mass to urinate	Life-threatening consequences; organ failure; urgent operative intervention indicated	Death
Diarrhea	Diarrhea	Increase of <4 stools per	Increase of 4-6 stools per day over	Increase of >6 stools per day over baseline;	Life threatening consequences	Death

		day over baseline; mild increase in ostomy output compared to baseline	baseline; IV fluids indicated <24 hrs.; moderate increase in ostomy output compared to baseline; not interfering with ADL	incontinence; IV fluids >24 hrs.; hospitalization; severe increase in ostomy output compared to baseline; interfering with ADL	(e.g. hemodynamic collapse)	
Distention/bloating, Abdominal	Distention	Asymptomatic	Symptomatic but not interfering with GI function	Symptomatic interfering with GI function	_____	_____
Vomiting	Vomiting	1 episode in 24 hrs.	2-5 episodes in 24 hrs.; IV fluids indicated <24 hrs.	>5 episodes in 24 hrs.; IV fluids, or TPN indicated >24 hrs.	Life threatening consequences	Death
Cardiac Conduction Disorder (pathological irregularities in the cardiac conduction system)	Cardiac conduction disorder	Mild symptoms; intervention not indicated	Moderate symptoms	Severe symptoms; intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Creatinine	Creatinine	>ULN-1.5X ULN	>1.5-2.0X ULN	>2.0-6.0X ULN	>4.0X ULN	Death
Glomerular Filtration Rate	GFR	<75-60% LLN	<60-40% LLN	<40% LLN, chronic dialysis not indicated	Chronic dialysis or renal transplantation indicated	Death
Glucose, serum-low (hypoglycemia)	Hypoglycemia	<LLN-55 mg/dL <LLN-3.0 mmol/L	<55-40 mg/dL <3.0-2.2 mmol/L	<40-30 mg/dL <2.2-1.7 mmol/L	<30 mg/dL <1.7 mmol/L	Death
Triglyceride, serum-high (hypertriglyceridemia)	Hypertriglyceridemia	>ULN-2.5X ULN	>2.5-5.0X ULN	>5.0-10X ULN	>10X ULN	Death
Pain-	Pain-	Mild pain	Moderate	Severe pain; pain or	Disabling	_____

Headache	Headache	not interfering with function	pain; pain or analgesics interfering with function, but not interfering with ADL	analgesics severely interfering with ADL		
Pain-Abdominal	Pain-Abdominal	Mild pain not interfering with function	Moderate pain; pain or analgesics interfering with function, but not interfering with ADL	Severe pain; pain or analgesics severely interfering with ADL	Disabling	_____
Infection-Upper Airway NOS	Infection-Upper Airway NOS	Mild	Moderate	Severe	Life-threatening; disabling	Death
Infection-Nasopharyngitis	Infection-Nasopharyngitis	Mild	Moderate	Severe	Life-threatening; disabling	Death
Leukocytes (total WBC)	Leukocytes	<2000/mm^3 <2.0X10^9/L	<1500-1000/mm^3 <1.5-1.0X10^9/L	<1000-500/mm^3 1.0-0.5X10^9/L	<500/mm^3 <0.5X10^9/L	Death
Hematocrit	Hematocrit	<34%	<30%	<28%	Life-threatening consequences; urgent intervention indicated	Death
Platelets	Platelets	<70,000/mm^3-60,000/mm^3 <70.0-60.0X10^9/L	<60,000-40,000/mm^3 <60.0-40.0X10^9/L	<40,000-25,000/mm^3 <40.0-25.0X10^9/L	<25,000/mm^3 <25.0X10^9/L	Death
INR (International Normalized Ratio of Prothrombin Time)	INR	>1-1.5XULN	>1.5-2XULN	>2XULN	_____	_____

PTT (Partial Thromboplastin Time)	PTT	>1-1.5 ULN	>1.5-2X ULN	>2X ULN	_____	_____
Bicarbonate, serum-low	Bicarbonate, serum-low	<LLN-18mmol/L	<18-15 mmol/L	<15-11 mmol/L	<11 mmol/L	Death
Acidosis (metabolic or respiratory)	Acidosis	pH<normal, but >7.3	_____	pH<7.3	pH<7.3 with life threatening consequences	Death
Alkaline Phosphatase (U/L)	Alkaline Phosphatase	>ULN-2.5X ULN	>2.5-5.0X ULN	>5.0-20.0X ULN	>20.0 X ULN	_____
Bilirubin (hyperbilirubinemia)	Bilirubin	>ULN-1.5X ULN	>1.5-3.0X ULN	>3.0-10.0X ULN	>10.0X ULN	_____
Albumin, serum-low (hypoalbuminemia)	Hypoalbuminemia	<LLN-3g/dL <LLN-30g/L	<3-2 g/dL <30-20 g/dL	<2 g/dL <20g/L	_____	Death
AST, SGOT (serum glutamine oxaloacetic transaminase)	AST	>3-5X patient's baseline values	>5-10X patient's baseline values	>10-20X patient's baseline values	>20X patient's baseline values	Death
ALT, SGPT (serum glutamine pyruvic transaminase)	ALT	>3-5X patient's baseline values	>5-10X patient's baseline values	>10-20X patient's baseline values	>20X patient's baseline values	Death

Data and Safety Monitoring

Data and safety will be monitored by the principal and associate investigators in this protocol. Data and safety are reviewed weekly in clinical research rounds by the Liver Diseases Branch, NIDDK. These rounds are separate from regular clinical rounds and consist of review of all study patients including flow sheets of major safety and efficacy measurements. The rational for not using an outside data and safety monitoring committee is that this is a small, single center study using a medication with defined side

effects. All measurements and tests are well established in clinical medicine. However, this study may be subject to audits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK monitoring plan. Audit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan. Yearly reports are made to the NIDDK/NIAMS IRB describing any issues with safety and efficacy

Adverse Events (definitions and classification)

Definitions

Adverse Event (AE): Any untoward medical occurrence in a human subject, including any abnormal sigh (for example, abnormal physical exam or laboratory findings), symptom, or disease, temporally associated with the subjects participation in research, whether or not considered related to the subject's participation in the research.

Serious Adverse Event (SAE): is any adverse event that:

1. Results in death,
2. Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
3. Results in inpatient hospitalization or prolongation of existing hospitalization
4. Results in a persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
5. Results in a congenital anomaly/birth defect, OR

6. Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Suspected adverse reaction (SAR): For the purposes of safety reporting, if evidence exists to suggest a causal relationship between the drug and the adverse event, it may be reported.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected.

Unanticipated problem (UP): Any incident, experience, or outcome that meets **all** of the following criteria:

1. **Unexpected** (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. **Related or possibly related** to participation in the research (**possibly related** means there is a **reasonable possibility** that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

Expected Adverse Events become unanticipated problems when they occur at a greater frequency or severity than was previously known.

Unanticipated problems that are not also an Adverse Event: Unanticipated problems that are not also AEs may involve greater risk of social or economic harm (to subjects or others) rather than physical/psychological harm.

Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol. A protocol deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety welfare or rights of subjects or others.

Assessment of Adverse Event Severity and Relationship to Treatment

Events will be collected, tracked and evaluated for expectedness, seriousness, severity and relatedness.

Investigators will "grade" the severity of all AEs using the CTCAE Version 3.0 as described earlier (Table 2).

The nomenclature for assessing the causal relationship between an AE and the study drug is listed in Table 3. The investigator / sponsor will determine the category that overall best "fits" the relationship between the AE and the study drug.

Table 3. AE or SAE Relatedness

Relatedness	Description

Unrelated	• No temporal association to study drug. • An alternate etiology has been established. • The event does not follow the known pattern of response to study drug. • The event does not reappear or worsen with re-challenge.
Probably not related / remotely related	• No temporal association to study drug. • Event could readily be produced by clinical state, environmental or other interventions. • The event does not follow the known pattern of response to study drug. • The event does not reappear or worsen with re-challenge.
Possibly related	• Reasonable temporal relationship to study drug. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study drug or as yet unknown pattern of response.
Probably related	• There is a reasonable temporal association with the study drug. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study drug. • The event decreases with de-challenge.
Definitely related	• There is a reasonable temporal relationship to the study drug. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study drug. • The event decreases with de-challenge and recurs with re-challenge.

Assessment of Adverse Event Outcome

The clinical investigator will follow every AE to a satisfactory outcome or stabilization of the event. This is particularly applicable to SAEs.

Actions taken in response to an AE and follow-up results (including lab results) will be recorded in the subject's medical record. When subjects are discontinued from the study

due to an AE, relevant clinical assessments and laboratory tests will be repeated as necessary until final resolution or stabilization occurs.

Waiver of Reporting to the IRB of Anticipated Adverse Events

Anticipated non-UP adverse events and serious adverse events will not be reported to the IRB at each occurrence unless they occur at a rate or severity greater than that known to occur in the natural history of chronic hepatitis C infection.[8,75] However, any episodes of acute hepatic decompensation or newly diagnosed hepatocellular carcinoma during the subjects participation in this trial will be reported to the IRB expeditiously. This waiver clarifies for the investigator(s) that these anticipated AEs and SAEs will be tracked, reported annually to the Sponsor and IRB, but do not need to be reported within the 14 day period as with the UPs in the section described below.

Required time frames for investigator expeditious reporting to the IRB and the Institute Clinical Director (CD):

The PI will report unanticipated problems, deaths, and protocol deviations **to the IRB Chair and the CD** according to the following schedule:

A. Serious Events

Serious unanticipated problems and protocol deviations will be reported to the IRB Chair and the CD, as soon as possible, preferably by phone or secure email and in writing not more than 7 days after the PI first learns of the event. Any other SAE unrelated to the research procedures associated with this protocol will not be reported expeditiously.

B. Non-Serious Events

All protocol unanticipated problems that are not serious will be reported to the IRB Chair and the CD, in writing, not more than 14 days after the PI first learns of the event.

Non-serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported. All other protocol deviations that are not serious will be reported to the IRB in writing, not more than 14 days after the PI first learns of the event.

C. Deaths

All deaths (whether or not they are unanticipated problems) will be reported to the CD and IRB as soon as possible preferably by phone or secure email and in writing not more than 7 days after the PI first learns of the event.

Investigator's reports to the IRB at the time of continuing review

A. All unanticipated problems will be reported.

B. All serious protocol deviations will be reported. Non-serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported. Other non-serious protocol deviations will be tracked, summarized and reported at the time of continuing review.

C. All adverse events thought to be possibly, probably, or definitely related to study drug will be summarized, tracked, and reported at the time of continuing review. Adverse events that arise after the Informed Consent is signed until 30 days after the last dose of study drug will be reported. Any adverse events with known relation to the natural history of the disease or to other pre-existing conditions will not be reported unless they occur at a rate or severity greater than that known to occur in the natural history of liver disease related to chronic HCV infection. Any accident, which results in an AE and is unrelated to the protocol, will not be reported.

Conflict of Interest

The Principal Investigator will assure that each associate investigator listed on the protocol title page receives a copy of the NIH Guide to preventing conflict of interest. Any potential conflicts will be reported to the IRB and the resolution of the conflict summarized.

Recruitment Strategy

We will advertise on the NIH web site and through local physicians and clinics for patients.

Recruitment of Women, Minority Individuals and Children

In recent trials of therapy for chronic hepatitis C performed by the Liver Diseases Branch, NIDDK, for the chronic hepatitis C (02-DK-0065), 35 of the 87 patients (40%) were women and 26 (30%) were minority individuals (17 African-Americans, 5 Hispanic-Americans and 3 Asian-Americans). Response rates to therapy have been lower in African American than other ethnic/racial groups in studies of interferon monotherapy,

but have been reported to be similar to other groups with interferon-ribavirin and interferon-ribavirin-direct acting antiviral combination therapy.

Hepatitis C is more common in men than women perhaps accounting for the higher proportion of males in most studies of hepatitis C. In the current study, we expect a slight under-representation of young women. The reason for this is the potential teratogenicity of ribavirin. We will not include women who intend to become pregnant during the 1-2 years of this study. In our recent study of alpha interferon in chronic hepatitis C, only 6 of the 87 patients enrolled were women below the age of 45 years and only 1 of the 6 expressed interest in having children in the future.

The epidemiology of hepatitis C in the United States indicates that this disease is more common among blacks and Hispanic whites than among non-Hispanic whites.[76] A major determining factor in the prevalence of hepatitis C is lower socio-economic class. To increase the representation of minority individuals in this trial, we will write to all hepatologists and gastoenterologists in the Washington DC area advertising this study.

This study will be limited to adults with chronic hepatitis C. The reasons for excluding children are that hepatitis C is uncommon in children and ribavirin has not been approved for use or adequately evaluated in children with hepatitis C.

Risk / Benefit Assessment

This study involves greater than minimal risk but offers the prospect of direct benefit to subjects and is likely to yield generalizable knowledge about hepatitis C.

Informed consent

The investigational nature and research objectives of the trial, the procedures and its attendant risks and discomforts, and alternative forms of therapy will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study. The principle investigator, an associate investigator, or the attending protocol investigator will lead this discussion. Informed consent will be obtained using forms approved by the Institutional Review Board (IRB) in accordance with good clinical practice for research involving human subjects. Each patient will be informed of the right of the patient to withdraw from the trial at any time without prejudice. After this explanation and before entering the trial, the patient will voluntarily sign and date an informed consent.

At any time during participation in the protocol that new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally in writing to all enrolled or prospective patient participants. Documentation will be provided to the IRB and if necessary, the informed consent amended to reflect relevant information.

Subjects will be provided with a copy of the informed consent that explains the purpose of the study, the medication(s) used in the study, procedures, and assessments. Subjects will also be provided with the telephone numbers of the investigator and qualified personnel who can assist with their questions and concerns.

Research Use, Storage and Disposition of Human Subject's Samples and Data

Patients will have serum stored from selected time points during this study. These specimens will be used for repeat virological testing and special tests as needed. Samples may be used to assess factors associated with response or non-response to therapy. These samples will be tested in the Liver Diseases Branch or the routine clinical and surgical pathology services of the Clinical Center. If residual samples are evaluated by outside collaborators in the future, this will be done so only after all identifying data have been removed from all samples. Samples will be numbered and a key to the number system will be stored and backed up by the principal investigator of this study. Research records and data as well as sera will be stored indefinitely in our locked offices and freezers and the medical record department. These materials will be protected and tracked by standard operating procedures in the medical record as well as a compulsive filing system in our locked offices and freezers. There will be redundant storage of clinical information in the medical record department and our offices. Computer files will be maintained on password-protected computers and servers. Serum samples will be processed and stored by the NIDDK core laboratory facility. These samples will be stored in locked freezers inside locked rooms. Access to these samples will require written approval from the Liver Diseases Branch chief, and will be recorded by the LDB and by the core laboratory. This should minimize the risk of loss or destruction of information and specimens. If that were to occur we would report it to the IRB. We do not plan to destroy this personal medical information or research subject sera after completion of the study because it may be critically important for physicians (here or elsewhere) to have access to this information when caring for these patients in the future.

Remuneration/Compensation

No compensation is offered to study participants.

Alternatives to Therapy

For patients infected with chronic hepatitis C, genotype 1, the FDA approved combination therapy with two direct-antiviral agents (boceprevir and telaprevir) in combination with pegylated interferon and ribavirin. The rate of sustained virological response with this triple therapy is 65-75%, which is significantly improved from the 40-50% with peginterferon and ribavirin. Thus, the recent guidelines issued by the American Association for the Study of Liver Disease state that the optimal regimen to treat previously untreated patients with chronic hepatitis C, genotype 1, is boceprevir or telaprevir in combination with peginterferon and ribavirin.[9]

However, not all patients with genotype 1 infection will be able or willing to tolerate the DAAs and/or peginterferon and their associated side effects, which can be significant. Others may not have access to the medications. Patients who are not interested in interferon-free regimens and interested in the standard of care treatment regimen may undergo treatment with a DAA-containing regimen, by a practitioner outside the NIH, in a clinical trial elsewhere or in studies at the Liver Diseases Branch if these become available. These options will be discussed clearly with the patients and will be documented in the patients' chart. If, following this discussion, a patient still elects to enroll in this study, knowing that he/she is receiving treatment that is not the current standard of care. Neither refusal to enroll in the current study nor enrollment in the current study will be an exclusion criterion in future studies. Patients who choose to

enroll in this study will be eligible for subsequently planned Liver Diseases Branch clinical trial utilizing interferon-free DAA-containing regimens. The subsequent trial is currently in the planning stages and is designed as definitive therapy for sustained virological response. Participation in the current study will not exclude patients from and in fact they will be offered the opportunity to participate in the planned DAA study.

For patients chronically infected with all other genotypes of HCV, the current standard of care therapy involves treatment with combination therapy of peginterferon and ribavirin for varying durations of therapy.[8] Patients who are not interested in interferon-free regimens and interested in the standard of care treatment may undergo therapy by a practitioner outside the NIH, in a clinical trial elsewhere or in other studies at the Liver Diseases Branch if these become available. These options will be discussed clearly with the patient and will be documented in the patients' chart. If, following this discussion, the patient still elects to enroll in this study, knowing that he/she is receiving treatment that his not the current standard of care. Neither refusal to enroll in the current study nor enrollment in the current study will not be an exclusion criterion in future studies.

Appendix 1

SYMPTOM SCALE

Name: _____ Date: (M/D/Yr) ____/____/____

Mark with an "x" the place on the lines below that best describes how you have felt during the past week.

None **Worst ever**

Drowsiness
|_____|

Nausea
|_____|

Dryness of Mouth
|_____|

Headaches
|_____|

Light Headedness
|_____|

Nervousness
|_____|

Inability to Concentrate
|_____|

Numbness or Tingling
|_____|

Difficulty Urinating
|_____|

In general, how do you feel overall?

Very Good **Awful**
|_____|

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