Thérapie 2012 Septembre-Octobre; 67 (5): 423–427
DOI: 10.2515/therapie/2012058

Farnesoid X Receptor Targeting for Hepatitis C: Study Protocol for a Proof-of-concept Trial

Caroline Scholtes[1,2,3], Patrice André[1,2,3], Christian Trépo[4], Catherine Cornu[5,7,8], Laurent Remontet[6,7], René Ecochard[6,7], Theodora Bejan-Angoulvant[5,7,8] and François Gueyffier[5,7,8]

1 Hospices Civils de Lyon, Hôpital de la Croix Rousse, Laboratory of Virology, Lyon, France
2 Université de Lyon, Université Lyon 1, Lyon, France
3 Inserm U851, Lyon, France
4 Hospices Civils de Lyon, Hôpital de la Croix Rousse, Department of Hepatology and Gastroenterology, Lyon, France
5 Hospices Civils de Lyon, Hôpital Louis Pradel, Clinical Pharmacology Department, Lyon, France
6 Hospices Civils de Lyon, Biostatistic Service, Lyon, France
7 CNRS, UMR5558, Biometry and Evolutive Biology, Biostatistics-Health Team, Villeurbanne, France
8 Inserm CIC201, Lyon, France

Text received January 17th, 2012; accepted June 1st, 2012

Keywords:
clinical trial;
farnesoid X receptor;
hepatitis C;
proof of concept

Abstract – Objective. To test the modulation of farnesoid X receptor activity on the replication of hepatitis C virus in chronically infected patients. **Methods.** This is a proof-of-concept trial that was approved by the ex-French Agency for the Safety of Health Products (ex-Afssaps [currently ANSM]) and an ethics committee. It has started in January 2010. This one-arm, open-label study examines the safety and efficacy of the oral administration of guggulsterone. The main outcome measure will be the assessment of blood viral loads. **Results.** We planned to enrol 15 genotype 1-infected patients that failed a first-line therapy. **Conclusion.** We think guggulsterone might be an effective therapeutic option for HCV genotype 1 patients who do not respond well to first-line therapy.

Trial registration. ClinicalTrials NCT01492998

Mots clés :
essai clinique ;
récepteur nucléaire des acides biliaires ;
hépatite C ;
preuve de concept

Résumé – Ciblage du récepteur nucléaire des acides biliaires dans l'hépatite C : protocole d'un essai de validation de principe. Objectif. Évaluer la modulation de l'activité du récepteur nucléaire des acides biliaires appelé *Farnesoid X receptor* sur la réplication du virus de l'hépatite C chez les malades chroniques. **Méthodes.** Ceci est une étude de preuve de concept qui a été validée par l'ex-Agence française de sécurité sanitaire des produits de santé (Afssaps, devenue ANSM, Agence nationale de sécurité du médicament et des produits de santé) et par un comité de protection des personnes (CPP). Il a commencé en janvier 2010. Cet essai ouvert à un bras examine la sécurité et l'efficacité de l'administration orale de guggulstérone. Le critère de jugement principal sera l'évaluation de la charge virale plasmique. **Résultats.** Nous espérons inclure 15 patients de génotype 1 dont le traitement de première intention a échoué. **Conclusion.** Nous pensons que la guggulstérone pourrait être une option thérapeutique efficace pour les patients atteints d'hépatite C (génotype 1) qui ne répondent pas au traitement de première intention.

Numéro d'enregistrement. ClinicalTrials NCT01492998

1. Background

Hepatitis C is a global public health problem.[1] Current recommended therapy that combines ribavirin with interferon (IFN)-based treatment, has limited efficacy, poor tolerability, and is very expensive.[2] New treatment options that are more effective and less toxic are needed, especially for those who relapse or do not respond to current treatments. New therapies that combine protease inhibitors with the current regimen are currently under investigation.[3] One major obstacle in the fight against hepatitis C virus (HCV) infection is the unreliability of viral replication machinery. The virus can quickly mutate and resist compounds targeting viral enzymes. One example is monotherapy with Telaprevir, a protease inhibitor that was shown to select resistant viral populations within a few days

or weeks.[4] Virus tropism partly depends on cellular factors and metabolic pathways that are required to replicate the virus. An approach that targets host cofactors and prevents the propagation of viruses may be the ideal target for developing antiviral agents. This is because they have a lower rate of mutation than viral genomes, as long as there are no side effects. Host cofactors include viral entry receptors, host metabolism, and nuclear receptors and are necessary for virus propagation. These host factors are unlikely to be prone to mutations and have a higher genetic barrier for resistance than direct antiviral compounds such as protease or polymerase inhibitors.

HCV infection leads to a metabolic syndrome that is clinically evident. This syndrome is characterized by insulin resistance, liver steatosis and hypo-beta-lipoproteinemia.[5] Several different kinds of plasmatic HCV particles coexist in infected patients. Virions range in density from <1.06 g/mL to 1.30 g/mL for the canonical particles of flaviviruses. Lipo-viro-particles (LVPs) or low-density HCV virions are the result of an association of the virus with triacylglycerol-rich lipoproteins (TRLs).[6,7,8] The infectivity of viral particles is inversely correlated to their density.[9]

In its natural environment, HCV is in contact with high bile acid (BA) concentrations. Furthermore, serum BAs may be used as prognostic markers to predict the failure to reach a sustained virological response.[10] BAs, especially chenodeoxycholic acid (CDCA), regulate the replication of HCV with the farnesoid X receptor (FXR), a nuclear receptor.[11] The control of HCV replication by FXR relies on the metabolic status of the cells through numerous genes regulated by FXR (~50). These genes also regulate glucose and lipid metabolism. Guggulsterone (GGS), a vegetal sterol with FXR-antagonist properties, inhibits the replication of HCV RNA *in vitro*. Both GGS and interferon-alpha (IFN-α) inhibit HCV replication. FXR appears to be a host factor that may be targeted to control HCV replication.

GGS is the only FXR antagonist available to date. GGS has been used for centuries in Indian ayurvedic medicine and has already been tested in a clinical trial on dyslipidemia,[12] without causing major adverse events. It is possible to test the modulation of FXR activity on the replication of HCV in chronically infected patients.

We hypothesized that FXR inhibition by GGS is efficient in blocking *in vivo* HCV replication. Since the half-life of HCV viral particles in serum is very short (2-7 hours), we should be able to detect a rapid decrease in HCV viral load within one week of treatment.[13]

This study is a proof-of-concept trial that was approved by the ex-french Agency for the Safety of Health Products (ex-Afssaps currently ANSM) and an ethics committee. It has started in January 2010 and we planned to enrol 15 genotype 1 infected patients that failed a first-line therapy. This trial is funded by the french Directorate of Hospitalisation and Organisation of Care (DHOS), the french National Institute for Health and Medical Research (Inserm), and the french National Agency for AIDS and Viral Hepatitis Research (ANRS).

The aim of this study is to determine if GGS can reduce plasma viral load *in vivo* in patients who are chronically infected with HCV genotype 1. Our secondary objectives are to assess the impact of plasmatic BA concentrations on FXR inhibition by GGS and determine if GGS modifies the distribution of different viral particles.

2. Methods

2.1. Experimental design

This is a proof-of-concept, one arm, open-label, and monocentric trial (figure 1).

2.2. Objectives

2.2.1. Primary objective

Serum viral load is the main marker of viral replication. The primary objective is a $0.5\log_{10}$ decline of the viral load after one week of treatment by GGS. This decrease will be monitored by using an iterative measurement of the viral load.

2.2.2. Secondary objectives

- To assess the effect on the different viral particles.

The quantification of viral RNA in the different density fractions will be carried out. This will allow us to determine whether GGS inhibits the synthesis of different viral particles in the same way.

- To study the impact of BA concentrations on the response to FXR inhibition.

The evolution of BA concentrations will be evaluated after GGS administration and then compared with the modification of viral-load kinetics.

- To assess the safety of GGS administration.

2.3. Intervention

The study product Gugulipid® (Sabinsa Corporation) is a dietary supplement, made up of a standardized extract of *Commiphora mukul*. It contains 25 mg of Z-GGS and E-GGS and is commercialized by general nutrition centers.

Because there is a lack of data on the pharmacokinetics of GGS, we will use the same dose in our trial as the one in the reference trial.[12] The study drug GGS, will be given as two oral capsules of Gugulipid® (50 mg of GGS) three times daily during meals for one week.

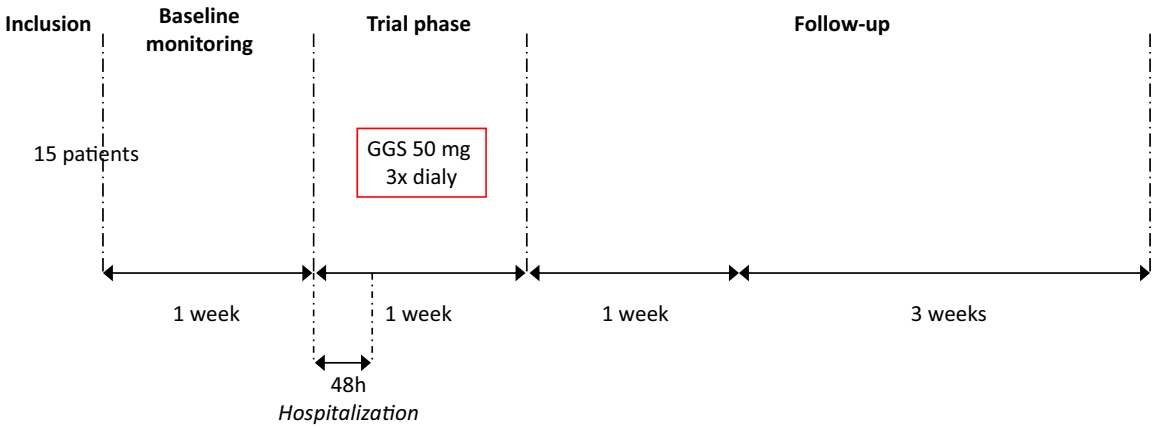

Fig. 1. Study diagram.

2.4. Outcomes

Our main outcome is the plasma viral load decrease under treatment. Each patient's viral load will be measured 13 times: five times before the trial (stability of the viral load), six times during the treatment phase, and two times after the end of treatment.

2.5. Study setting and participants

2.5.1. Inclusion/exclusion criteria

- Inclusion criteria were: adult men between 18 and 60 years old, with serologic evidence of chronic hepatitis C infection using an anti-HCV antibody test, a hepatitis C genotype 1 with positive viremia for over 6 months, a viral load above 10^5 UI/mL and a Metavir score <F4 by Fibroscan and Fibrotest at the pre-inclusion visit. Patients had to be non-responders to a previous treatment, without any antiviral treatment for two months. They must sign informed written consent.

2.5.2. Exclusion criteria

Exclusion criteria were:
- treatment with any anti-hepatitis C virus drug at the time of enrolment or within two months before the enrolment date;
- any pharmacological treatment catabolised by the P450 3A4 cytochrome;
- obesity defined as a body mass index (BMI) over 30;
- any immunosuppressive therapy;
- alcohol consumption over 20 g per day;
- other etiology of liver disease;
- dyslipidemia with specific treatment;
- cirrhosis or hepatocellular carcinoma;
- HIV- or HBV-associated infection;
- diabetes mellitus;
- chronic renal failure;
- heart failure;
- unwilling to use contraception during participation in the trial.

2.6. Assessments performed during trial visits

The study will last 10 weeks for each patient.

Patients will be included in the trial at the pre-inclusion visit (table I).

2.6.1. Clinical follow-up

Nine percent of patients in a previous trial on GGS developed mild rashes within 48 hours after the first intake. This hypersensitivity phenomenon resolved upon treatment discontinuation.[12] Patients included in our study will be hospitalized during the first 48 hours of GGS intake to insure a close surveillance during the period when skin rashes could appear. They will be followed up closely during the treatment phase.

Two additional visits including clinical assessment and blood tests will take place one week and one month after the completion of the trial.

2.6.2. Biological follow-up

- Viral load:

Viral load stability before GGS intake will be monitored one week before GGS administration.

Viral load will be measured in while fasting because it has been showed that viral load can be modified by feeding conditions.

- GGS pharmacokinetics:

Table I. Patient follow-up and assessments performed during trial visits.

	Inclusion Visit	Observation Week	Follow-up Visits						
			Hospitalisation						
		Day -7, -6, -5, -3	Day 0	Day 1	Day 2	Day 4	Day 6	Day 14	Day 35
Informed consent	√								
Treatment initiation			√						
Health exam (+ ECG on Day-6)	√		√	√	√	√	√	√	√
CBC, platelets, PT, CK	√		√		√		√	√	√
Fibrotest and Fibroscan	√								
HCV viral load	√	√	√	√	√	√	√	√	√
Genotyping	√								
Quantification of LVP	√	√	√				√		
Liver panel	√		√	√	√	√	√	√	√
Lipid profile	√		√		√		√	√	√
Fasting total plasma biliary salts	√	√	√	√	√	√	√		
GGS quantification in sera				√		√	√		

CBC=complete blood count; **CK**=creatine phosphokinases; **ECG**=electrocardiogram; **PT**=prothrombin time

Plasma drug levels will be monitored on days 1 (three times), 4, and 6.

- Other biological measurements (table I).

2.7. Statistical analysis

- Sample size

We plan to enrol 15 patients chronically infected with HCV-genotype 1 who failed to respond to prior pegylated-IFN plus ribavirin therapy. Assuming an expected 0.5 $\log_{10}$ decrease in viral load and a standard deviation in viral load of about 0.3 $\log_{10}$, a sample size of 15 patients will have the statistical power to detect a positive effect of GGS on viral load of more than 95% with an α-level of 0.05.

- Statistical analysis

A linear mixed model will be used for the main analysis because this type of model is well adapted to repeated measurement data.[14] The $\log_{10}$ of the viral load will be modelled[15] as a function of time and treatment. The following model will be adjusted:

$$Y_{ij}=\gamma_0+ u_{0i}+ \gamma_1\times intervention_{ij}\times time_{ij} +e_{ij}$$

with $Y_{ij}=\log_{10}(j^{th}$ measure of the viral load of the i^{th} individual),

$intervention_{ij}=0$ if the measurement is carried out before GGS intake, =1 otherwise.

The parameter γ_0 is the mean baseline whereas the random effect u_{0i} reflects the interindividual variability of the baseline.

The parameter of interest is γ_1 which is the mean decrease slope after GGS intake. A minimal decrease of 0.5 $\log_{10}$ of the viral load after 7 days will be considered as the main efficacy endpoint The corresponding test will be carried out on γ_1.

The non-linearity of the relationship and homoscedasticity of the residuals will be checked and the model will be adapted accordingly.

2.8. Consent

The informed consent document contains everything needed to ask for consent. The form clearly states that this is research, participation is voluntary, and that treatment with GGS may not be effective. Every effort has been made to inform participants of any known side effects. There are very few. All study participants will be followed closely. Their test results will be given to their primary care providers and them. The Clinical Investigation Centre of Lyon will monitor the study.

2.9. Ethical and organisational review

This study was approved by the ex-french Agency for the Safety of Health Products (ex-Afssaps, currently ANSM) and an ethics committee. No safety committee was set up, because we did not expect any risk of significant adverse events with the experimental drug. It was already marketed as a food supplement and this was not a potentially dangerous investigation.

3. Discussion

This is the first proof-of-concept study to determine if GGS, a potent FXR-antagonist *in vitro*, is efficient *in vivo* to inhibit HCV replication. A one-arm design will be used for this trial, because GGS is rapidly effective *in vitro* on HCV replication. We expect *in vivo* effects to be obvious after the first week of treatment. We do not think there will be long-term benefits for patients with this study.

Study participation was restricted to men to avoid gender variation because GGS has weak agonist properties on steroid receptors. An agonist property was shown on the pregnane X receptor PXR,[16] which induces the expression of Cyp3A4 cytochromes. Patients are required to avoid all pharmacological treatments that are metabolised by this cytochrome during the study.

This is the first clinical study to address this question. If GGS treatment can reduce HCV viral load, patients could benefit from a treatment combining GGS to the current IFN-ribavirin-based therapy.

We think GGS might be an effective therapeutic option for HCV genotype 1 patients that who do not respond well to first-line therapy.

Authors' contributions

Caroline Scholtes, Theodora Bejan-Angoulvant, Catherine Cornu, Patrice André, François Gueyffier were involved in drafting the manuscript. Caroline Scholtes, Theodora Bejan-Angoulvant, Patrice André were investigators in the study. Laurent Remonter, René Ecochard were in charge of the statistical analysis. All authors approved the final version to be published.

Acknowledgements

We thank Dr. Marie-Claude Gagnieu of the Department of Biochemistry, *Hospices Civils de Lyon* for help on GGS dosages. We are grateful to Kent Neal (supported by the French Cochrane Center) for proofreading the manuscript.
We also thank the Sabinsa Corporation, 70 Ethel Road West, Suite 6, Piscataway, NJ-08854, USA, and Sabinsa Corporation European Division, Sabinsa Europe GmbH,Monzastr. 4, D-63225, Langen, Germany for providing the study medication.

Funding. The study was funded by public grants from the french National Agency for AIDS and Viral Hepatitis Research (ANRS), the french Directorate of Hospitalisation and Organisation of Care (DHOS) and the french National Institute for Health and Medical Research (INSERM).

Competing interests. The authors have no competing interests to declare.

Abbreviations. Afssaps: french Agency for the Safety of Health Products (currently ANSM); BA: bile acid; CBC: complete blood count; CDCA: chenodeoxycholic acid; CK: creatinephosphokinases; DHOS : french Directorate of Hospitalisation and Organisation of Care; FXR: farnesoid X receptor; GGS: guggulsterone; HCV: hepatitis C virus; IFN: interferon; Inserm: french National Institute for Health and Medical Research; LVP: lipo-viro-particle; PT: prothrombin time; PXR: pregnane X receptor; TRL: triglyceride-rich lipoprotein.

References

1. Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat 1999; 6: 35-47
2. Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. Nature 2005; 436: 967-72
3. Rosen HR. Chronic Hepatitis C Infection. New Engl J Med 2011; 364: 2429-38
4. Sarrazin C, Kieffer TL, Bartels D, *et al.* Hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 2007; 132: 1767-77
5. Negro F, Sanyal AJ. Hepatitis C virus, steatosis and lipid abnormalities: clinical and pathogenic data. Liver Int 2009; 29 Suppl 2: 26-37
6. Andre P, Komurian-Pradel F, Deforges S, *et al.* Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. J Virol 2002; 76: 6919-28
7. Merz A, Long G, Hiet MS, *et al.* Biochemical and morphological properties of hepatitis C virus particles and determination of their lipidome. JBC 2011; 286: 3018-32
8. Nielsen SU, Bassendine MF, Burt AD, *et al.* Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients. J Virol 2006; 80: 2418-28
9. Bradley D, McCaustland K, Krawczynski K, *et al.* Hepatitis C virus: buoyant density of the factor VIII-derived isolate in sucrose. J Med Virol 1991; 34: 206-8
10. Jorquera F, Monte MJ, Guerra J, *et al.* Usefulness of combined measurement of serum bile acids and ferritin as additional prognostic markers to predict failure to reach sustained response to antiviral treatment in chronic hepatitis C. J Gastroenterol Hepatol 2005; 20: 547-54
11. Scholtes C, Diaz O, Icard V, *et al.* Enhancement of genotype 1 hepatitis C virus replication by bile acids through FXR. J Hepatol 2008; 48: 192-9
12. Szapary PO, Wolfe ML, Bloedon LT, *et al.* Guggulipid for the treatment of hypercholesterolemia: a randomized controlled trial. JAMA 2003; 290: 765-72
13. Neumann AU, Lam NP, Dahari H, *et al.* Hepatitis C viral dynamics *in vivo* and the antiviral efficacy of interferon-alpha therapy. Science 1998; 282: 103-7
14. Diggle PJ, Liang KY, Zeger SL. Analysis of longitudinal data. Oxford University Press. 1994
15. Pinheiro J, Bates D. Mixed-effects models in S and S-Plus. New York. Springer, 2000
16. Goodwin B, Gauthier KC, Umetani M, *et al.* Identification of bile acid precursors as endogenous ligands for the nuclear xenobiotic pregnane X receptor. Proc Natl Acad Sci U S A 2003; 100: 223-8

Correspondence and offprints: *François Gueyffier*, Clinical Investigation Centre (CIC), Louis Pradel Cardiologic Hospital, 28 avenue Doyen Lépine, 69677, Bron, France.
E-mail: francois.gueyffier@chu-lyon.fr