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Biochemical and histological effects of 26 weeks of glycyrrhizin treatment in chronic hepatitis C: A randomized phase II trial☆

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***Background/Aims*: Phase I/II studies of 4 weeks duration have confirmed the ALT lowering effect of glycyrrhizin in Western chronic hepatitis C patients. Our aim was to determine the dose frequency of glycyrrhizin required to maintain the ALT response beyond 4 weeks and evaluate its effect on liver histology and quality of life.**

***Methods*: HCV-RNA-positive patients with elevated ALT and marked fibrosis or necro-inflammation who were not eligible for interferon therapy were treated for 4 weeks with six infusions weekly of glycyrrhizin. Patients with an ALT response at week 4 were randomized to continue treatment for 22 weeks in three dose frequency groups: 6×, 3× or once weekly.**

***Results*: 72/121 (60%) patients were randomized. At the end of treatment the ALT response was maintained in 60%, 24% and 9% of patients in the 6×, 3×, and once weekly groups, respectively ($p < 0.001$). In ALT responders the necro-inflammation score improved non-significantly compared to ALT non-responders. Quality of life assessed by SF-36 increased in patients treated with the study drug, albeit unrelated to the occurrence of ALT response.**

***Conclusions*: ALT responses induced by 4 weeks glycyrrhizin therapy can be maintained in a subset of chronic hepatitis C patients receiving at least three injections weekly. The observed ALT response did not translate in a significant histological improvement after 6 months treatment.**

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1. Introduction

Chronic hepatitis C infection can be associated with progressive liver disease that may evolve insidiously to cirrhosis with an increased risk of hepatocellular carcinoma (HCC) and liver failure [1–3]. Antiviral treatment with (peg)interferon–ribavirin combination therapy has become successful in 50–85% of patients over the past decade [4,5]. For those not responding or those patients with absolute contraindications to (peg)interferon–ribavirin combination therapy, different treatment strategies have to be sought. These approaches might include viral suppressive or antifibrotic therapy. Studies on chronic hepatitis B and C have shown that persistent normalization of ALT is important in reducing the complications of chronic hepatitis, regardless of ongoing viral replication [6,7].

Glycyrrhizin, a natural compound extracted from the roots of *Glycyrrhiza glabra*, has in vitro antiviral effect against multiple viruses [8–12]. The underlying mechanism of its antiviral effect is not fully elucidated. Both inhibition and augmentation of T-cell cytotoxicity have been reported as well as stimulation of the endogenous production of interferon. It has been shown that glycyrrhizin alters cellular viral penetration and diminishes cell lysis through cell membrane stabilization [13–17]. In Japan glycyrrhizin has been used as a treatment for chronic hepatitis for more than 20 years. In a double-blind randomized placebo-controlled trial, Suzuki showed that in Japanese patients with chronic hepatitis the serum transaminases decreased during the treatment with glycyrrhizin given intravenously as Stronger Neo-Minophagen C (SNMC) [18]. After discontinuation of the medication the serum transaminases rebounded, but this could be prevented by maintenance therapy. In a retrospective study, Arase concluded that long-term usage of glycyrrhizin is effective in preventing HCC development when ALT normalizes during therapy in Japanese patients with chronic hepatitis C [19]. Besides pseudo-hyperaldosteronism, glycyrrhizin treatment has hardly ever been associated with side effects.

In contrast to Japan, the European experience with glycyrrhizin treatment has been limited. In two pilot studies of 4 weeks duration we confirmed the ALT lowering effect of glycyrrhizin in European patients even though there was no decrease in viremia [20,21]. We have now designed a study of 26-weeks treatment with glycyrrhizin to evaluate the dose and frequency required to maintain the initial ALT response beyond week 4. Furthermore, the effect of the 26-weeks treatment with glycyrrhizin on liver histology and quality of life was studied.

2. Patients and methods

2.1. Patients

Patients between 18 and 70 years of age were eligible if they met all inclusion criteria: serum antibodies against HCV; HCV-RNA-positive; serum alanine aminotransferase (ALT) levels at least twofold the upper limit of normal on two occasions in the 12 weeks before initiation of treatment; liver biopsy consistent with fibrosis stage 3–6 or necro-inflammation score 6–12 according to Ishak's score [22] and non-eligibility for interferon therapy (previous non-response or contraindications to interferon such as psychiatric co-morbidity or unwillingness to undergo interferon based treatment albeit fully informed about benefits and risks).

Patients were excluded if they had decompensated liver disease, hepatocellular carcinoma, other causes of liver disease, malignancy other than skin basocellular carcinoma in the previous 5 years; human immunodeficiency virus infection; immunosuppressive therapy; antiviral treatment in the preceding 3 months; pregnancy, breast-feeding; hypokalemia, hyperaldosteronism, myopathy, use of thiazide diuretics and liquorice addiction or if they were unwilling to use contraception for the whole study period including 3 months after treatment.

2.2. Study design

This randomized, open phase II clinical trial was conducted at tertiary care European centers. All patients provided written informed consent and the protocol was approved by each center's Institutional Ethics Committee. The Clinical Research Bureau of the University Medical Center Rotterdam coordinated the study and was responsible for verification of the inclusion criteria, randomization and data acquisition. An independent Contract Research Organization ensured that the study was conducted according to Good Clinical Practice.

All patients were treated with glycyrrhizin six times a week for the first 4 weeks. Glycyrrhizin was given as Stronger Neo-Minophagen C (SNMC, supplied by Minophagen Pharmaceutical Co. Ltd., Tokyo, Japan), consisting of 40 mg glycyrrhizin, 20 mg cysteine and 400 mg glycine in 20 ml physiological saline. Medication per visit consisted of 5 ampoules of 20 ml administered directly into a peripheral vein in a 3–5 min period through a 21 G butterfly needle or an indwelling 22 G plastic canula. All patients were treated as outpatients.

At week 4, patients with an ALT response, defined as a decrease of 50% or more of the baseline value or a serum ALT level $\leqslant 1.5 \times$ upper limit of normal, were randomized to one of the three study groups: glycyrrhizin administration six times per week, three times per week or once per week for an additional 22 weeks. Patients not tolerating the six times per week administration of the study drug were allowed to have the dose frequency reduced to a tolerable regimen.

Sample size was calculated using a simulation with ALT response at the end of treatment as primary outcome. Assuming that 70% of the included patients would continue beyond week 4 and ALT normalization of, respectively, 50%, 30% and 10% for the 6× weekly, 3× weekly and once weekly dose frequency group, at least 120 patients needed to be included to reach a power of 90% (trend test) at $\alpha = 0.05$ (two-sided).

2.3. Randomization

Randomization was done centrally at the coordination center directly after receipt of the week 4 patient data from the participating center. After verification of the week 4 ALT response, one of the 84 sealed opaque envelopes, prepared by the biostatistician according to a computer generated randomization list, containing the group alloca-