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ORIGINAL PAPERS

Thalidomide with peginterferon alfa-2b and ribavirin in the treatment of non-responders genotype 1 chronic hepatitis C patients: proof of concept

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ABSTRACT

Background: fewer than half of patients infected with hepatitis C virus (HCV) achieve sustained viral clearance after peginterferon alfa/ribavirin (Peg-IFN/RBV) therapy.

Aims: thalidomide posses anti-inflammatory and immunomodulatory properties through inhibition of tumor necrosis factor and costimulatory effect on human CD8+ T cells.

Methods: we started a prospective, open label trial of retreatment of very-difficult-to-treat genotype 1 chronic hepatitis C patients (CHC) patients, who had failed to respond to the (Peg-IFN/RBV), with a triple therapy consisting in these same antivirals plus thalidomide 200 mg/day (the TRITAL study).

Results: none of the eleven patients fulfilling the inclusion criteria and included in the trial reached complete early virological response or sustained virological response. Viral load decline after 12 weeks of triple therapy thalidomide-based retreatment did not differ from viral dynamics during the first course. The triple therapy was well tolerated and only one patient developed mild bilateral neuropathy.

Conclusions: thalidomide addition to standard therapy is tolerated and did not increase the SVR rate in very-difficult-to-treat genotype 1 CHC patients. Different schedules are warranted to improve attempting retreatment of non responder CHC patients.

Key words: Hepatitis C. Host factors. Thalidomide. TNF-α. Clinical trial.

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INTRODUCTION

Chronic hepatitis C virus (HCV) infects approximately 170 million individuals worldwide and is a major cause of morbidity and mortality (1). Despite major improvements in therapy for HCV infection, approximately half of patients could become cured (2) and the other half do not achieve a sustained virological response (SVR) to treatment with peginterferon and ribavirin (Peg-IFN/RBV) (3), depending on baseline factors (4) and viral dynamics during the first weeks (5). These factors have led to an extensive search for novel therapies, especially for more difficult to treat groups as non-responders.

Up until now, treatment for this disease has consisted of therapies to stimulate the immune system and interfere in a non-specific manner with viral replication (6). IFN-α is known to exert a wide range of biological effects, including antiviral, antiproliferative, and immunomodulatory activities (7). The molecular mechanisms underlying failure of this approach of treatment are not well understood, but evidence indicates that both viral and host factors are involved (8). Several negative inflammatory cytokine, as suppressor of cytokine signaling (SOCS) family (9,10), interleukin (IL)-6, IL-1, IL-10 (11) and tumor necrosis factor (TNF)-α (12), that are elevated in HCV infection, are postulated to be an important primary mediator in reducing treatment efficacy by negatively regulating IFN-α signaling.

Unfortunately, attempts at retreatment in non-responders using the same therapies have yielded poor results, leaving such patients with few therapeutic options (13-15). Improving the re-treatment success rates of these nonresponsive patients remains a key challenge in hepatitis care. A number of new therapeutic approaches are being assessed (11,12). Most efforts to improve patients' outcomes have focused on antiviral therapy specifically targeted to HCV; these drugs are referred to as direct-acting antiviral agents (16,17). Although new targeted antivirals look very promising, emergence of antiviral resistance especially when used without

concomitant Peg-IFN/RBV, remain a great challenge (18). As a result, development of alternative strategies to improve IFN responsiveness remains a priority. Albeit, the limitation of the available data on that, targeting host factors may represent a potential novel approach that could improve response rates to treatment (19). Most of drugs examined in this approach are based on their immunomodulatory, anti-inflammatory or anti-oxidant properties.

Thalidomide is a glutaramide derivative that has been approved for the management of multiple myeloma (20) and leprosy (21). Thalidomide posses anti-inflammatory, immunomodulatory properties through decreasing TNF-α levels and enhancing CD8+ T-lymphocyte response (22,23). This observation led to studies of the potential value and antiviral activity of thalidomide in CHC. The use of thalidomide monotherapy was associated with normalization of serum alanine aminotransferase (ALT) and gamma-glutamyl-transpeptidase (GGT) levels (24). In addition, Caseiro reported in a series of six case reports that adding thalidomide to the standard of care therapy in retreatment of non -responder HCV patients, achieved SVR in all of them (25). The above clinical and laboratory observations, prompted the current pilot study in a generating hypothesis method (26), whose objective was to assess:

- The safety and antiviral efficacy of thalidomide with combination therapy in very-difficult-to-treat genotype 1 CHC patients who had previously failed to response to an adequate course of peg-IFN-based therapy (TRITAL study).
- Observation for HCV viral load changes, between the initial and the retreatment course by adding thalidomide to the Peg-IFN/RBV.

METHODS

Selection of patients

Patients with a documented history of non-response to combination treatment with adequate course of Peg-IFN/RBV were eligible for enrollment. Inclusion criteria included: 18 years of age or older, very-difficult-to-treat documented genotype 1 chronic HCV infection; HCV genotyping was detected using either a line probe assay or reverse hybridization (InnoLipa, Innogenetics, Genetics, Gent, Belgium), and a history of at least 3 months of full-dose combination antiviral therapy (with Peg-IFN/RBV) and failure to achieve an early virological response (EVR) defined as a 2-log10IU/mL decline in HCV RNA levels by week 12 of therapy or positive HCVRNA at week 24. Exclusion criteria included: decompensated liver disease, hepatocellular carcinoma, evidence of other forms of liver disease, human immunodeficiency virus (HIV) co-infection, active drug or alcohol abuse, any contraindication to Peg-IFN, RBV or thalidomide therapy, pathological electromyography, cardiac arrhythmias and pregnancy or refusal to use adequate contraception during therapy.

Study design

This study was conducted in accordance with the 1975 Declaration of Helsinki. Before inclusion of patients in this study, all patients were informed of its purpose, nature and duration, as well as the possible risks and benefits of this study intervention. All patients gave written, informed consent, and the study protocol and consent forms were approved by the institutional review board and registered in ClinicalTrials.Gov (#NCT00856804). The protocol of the trial was designed to be an "adaptive trial" protocol, a format that is particularly suited to pilot trials searching for dose safety and efficacy. The goal of an adaptive trial is to evaluate interim findings in order to gather the greatest amount of data while limiting drug exposure to the fewest patients.

Decisions on use of thalidomide and the used dosage were made by the principal investigator (MR-G). Thalidomide was provided as 200 mg tablets by Rasfer Int, Fargon Ibérica, S.A, Spain. The study was funded by Andalusia government (PI 07/09) and a non-restrictive grant from Schering-Plough, S.A with no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Analytical methods

After an overnight fast of 12 h, venous blood flow was drawn to determine serum levels of ALT, AST, □GT, glucose, triglyceride, high-density lipoprotein cholesterol, insulin, and C-peptide. Serum insulin and C-peptide were determined by an electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics, Indianapolis, IN). IR was determined by the HOMA method using the following equation: HOMA-IR = fasting insulin (mU/mL) × fasting plasma glucose (mmol/L)/22.5. Soluble tumor necrosis factor receptor II (sTNFRII) was measured using (R&D Systems, Minneapolis, Minn) at baseline and weeks 4, 8 and 12. Serum HCV RNA was assessed by using Cobas TaqMan real-time PCR (Roche Diagnostics, Palo Alto, CA; with a lower limit of detection of 15 IU/mL).

Treatment

Patients received triple therapy with thalidomide USP 200 mg/day in addition to Peg-IFN-α-2b (pegintron, Schering-Plough, Kenilworth, NJ, USA), given in weekly doses adjusted to body weight according to the manufacturer's instructions 1.5 mg/kg/week, plus weight-based RBV (Rebetol; Schering-Plough K.K.) at 1,000 or 1,200 mg daily in two divided doses. Patients with no or minimal change in their HCV-RNA titers (< 2 log10 drop at week 12 as compared with baseline), those whose viral loads dropped > 2 log10 at week 12 as compared with baseline and who still had positive HCV-RNA at week 24, were considered non-responder and therapy was discontinued.

Table I. Features of eleven chronic hepatitis C patients enrolled into the TRITAL study, before and after 12 weeks of therapy (PEG-IFN/ ribavirin + thalidomide)

#	Sex	Age	F score*	BMI	HOMA-IR	Baseline HCV-RNA (IU/ml)	Baseline TNF-α	Baseline ALT (U/L)	Baseline GGT (U/L)	Week 12 HCV-RNA (IU/ml)	Week 12 TNF-α (ng/ml)	Week 12 ALT (U/L)	Week 12 GGT (U/L)
1	M	46	F2	23	4.6	12100000	5.63	73	132	2630000	8.34	65	39
2	M	45	F0	28	4.3	2650000	9.87	47	59	15700	10.09	ND	28
3	F	53	F3	21	1.2	14000000	8.84	97	185	1640000	9.38	21	12
4	F	48	F4	24	2.6	7320000	11.3	118	27	54	12.4	69	29
5	F	62	F2	22	2.8	5470000	7.38	49	107	ND	14.7	ND	ND
6	M	47	F3	29	5.4	9760000	5.96	128	115	4790000	ND	73	75
7	M	43	F3	33	2.7	16800000	4.33	35	86	3360000	9.41	102	97
8	F	43	F4	24	2.6	561000	7.18	64	62	11900	10.3	21	55
9	M	38	F0	28	3.1	2140000	4.37	64	23	3620	5.57	15	10
10	F	58	F3	30	6.1	1540000	4.35	45	32	1060000	4.71	31	8
11	M	56	F4	24	2.6	3080000	7.31	20	42	127000	14.8	11	10

Assessment of efficacy

The primary efficacy parameter was SVR 24 weeks after the end of treatment. Secondary efficacy of parameters included rates of complete early virologic response (cEVR), defined as serum HCV RNA below the limits of detection after 12 weeks of combination therapy, comparison between viral dynamic during the first 12 weeks during both therapeutic regimens (previous Peg-IFN/RBV *vs*. thalidomide plus Peg-IFN/RBV), the rate of relapse, defined as patients with end-of-treatment response (ETR) but not reaching SVR, in addition, the rates of ALT normalization were assessed at multiple time points.

Safety assessments

The patients were monitored according to the schedule for weight, medication compliance and adverse events. Safety was assessed by means of clinical examinations and laboratory tests at week 4, 8, and 12. Standard 12-points EKG were done at baseline and after 12 weeks of therapy. Neurologic examination and standard neurophysiologic assessment was recorded at baseline and 12 weeks after starting treatment. Patients who developed clinical or neurophysiologic data of polyneuropathy were excluded immediately from the trial. Adverse events were graded as mild, moderate, severe or life threatening, and assessed by causality as probably related, possibly related, unlikely to be related or not related to the study medication. Side effects were assessed according to a standard protocol (ACTG toxicity grading scales) (27). Management of non-life-threatening adverse events was achieved by dose reduction of Peg-IFN-α-2b or RBV, or both in addition to thalidomide. Growth factors could be used at the discretion of the investigator.

Statistical analyses

All data were analyzed using SPSS 15.0 for Windows (Chicago, IL, USA). Comparisons between paired groups were with the Mann-Whitney U test, the Student *t*-test, Chi-square, or Fisher's exact test. Backward logistic regression was applied in the multivariate analysis.

RESULTS

Eleven patients were enrolled (Table I) between July 2007 and March 2008. The mean age of the patients was 51 ± 8 y, all the patients show the criteria of difficult to response. Five patients had advanced fibrosis (45.4%); liver fibrosis was assessed by image-based non-invasive methods transient elastography and fibroCT (28). Ten patients (90.9%) had high viral load and six patients (54.5%) had HOMA-IR > 4.

Two patients withdrew early from the study due to suffering from vasovagal syncope and delirium at the first and third week of therapy. These two patients were not included in the analysis.

Thalidomide and early HCV viral kinetics

In keeping with these difficult to treat patients' non-responder phenotype, the addition of thalidomide was not associated with improvement in viral decline at 12 week. Mean reductions in serum HCV RNA from baseline to the 12 week visit were -2.4 (from 6.66 ± 0.46 log10 IU/mL to 4.26 ± 1.6 log10 IU/mL) and -1.91 (6.57 ± 0.72 log10 IU/mL to 4.66 ± 1.62 log10 IU/mL) for the triple therapy with Peg-IFNα-2b /RBV and thalidomide and for Peg-IFN α-2b /RBV respectively (p = 0.81) (Fig. 1).

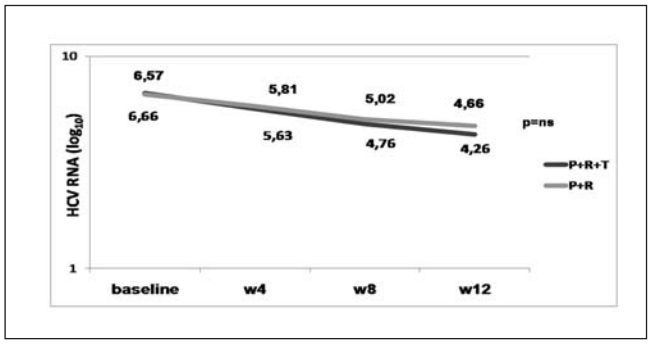

Fig. 1. Comparison of HCV RNA dynamics during the first 12 weeks between the first course of therapy (Peg-IFN-α/RBV) and second course of therapy (thalidomide plus Peg-IFN-α/RBV).

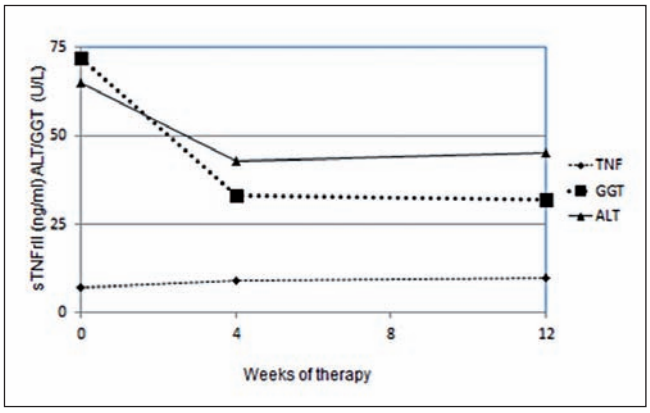

Fig. 2. Changes in ALT, GGT and sTNFRII levels during the first 12 weeks of therapy.

Treatment response

Two out of 11 patients (18%) achieved HCVRNA at week 12 lower than 125 IU/ml but none had cEVR. Both patients received therapy for 48 weeks and relapsed after peginterferon/ribavirin stopped. Thus, 9 patients were non-responders, 2 relapsers and none achieved SVR. Based on these discouraging results, we took the decision to terminate the study.

Biochemical response

Like in the first treatment with peginterferon plus ribavirin, a significant decrease of the mean levels of serum ALT and □GT were observed at week 12 compared to pretreatment levels (from mean value ± SD of 65 ± 35 IU/mL to 45 ± 34, paired-t-test; p = 0.3) (Fig. 2). Gammaglutamyltranspeptidase (GGT) showed a mean decrease (from 76 ± 53 IU/L to 36 ± 31 IU/L, paired-t-test, p = 0.047) (Fig. 2). Eight of the patients reached normalization of serum GGT levels. However, no significant change of sTNFRII levels was observed (Fig. 2).

Safety

Thalidomide was generally well tolerated; beyond the couple of patients who withdrew medications early due to vasovagal syncope and delirium at the first and third week of therapy; only one patient developed mild regressive bilateral peripheral neuropathy limited to digits, toes and ankles, the symptoms occurred two weeks after stopping treatment due to non-response but he was recovered one year after drug withdrawal.

DISCUSSION

Although advances in CHC therapy have improved SVR rates significantly in the past decade, non-responders continue to constitute a significant proportion of HCV-infected patients (3,4). Retreatment strategies aimed at viral eradication have included retreatment with the same or higher doses of the first Peg-IFN-α (29) used plus the same or higher doses of RBV, switching from one Peg-IFN-α to the other (30), increasing the duration of therapy (31), or using other IFN formulations (32,33). Unfortunately, recent studies have demonstrated SVR can be attained only in 6 to 8% of prior non-responders to Peg-IFN-α/RBV retreated for 48 weeks (34,35). Consequently, effective retreatment approaches for this population are urgently needed. Although, specifically targeted antiviral therapies for hepatitis C (STAT-C) have shown promising results in preclinical and early-stage clinical trials; the emergence of antiviral resistance especially when used without concomitant Peg-IFN-α/RBV, remains a great challenge (14,36). Ultimately, Peg-IFN-α/RBV are likely to remain the backbone of antiviral treatment for CHC for many years, even once new direct antivirals become available (14-16). As a result, improving the response to IFN-based therapy and exploring novel approaches to achieve major improvements in the treatment of non-responders remains a key challenge in hepatitis care. Targeting host cofactors of the HCV life cycle is attractive because it imposes a higher genetic barrier for resistance than direct antiviral compounds, as long as they have no serious side effects to patients (17).

Results from this pilot study show that addition of thalidomide (at the dosage of 200 mg/day) to standard therapy was generally well tolerated, associated with a significant decrease of ALT and GGT levels during a 12-wk course of treatment, without improvement of early viral kinetics. Ultimately, it has no positive impact on the final outcome (SVR). Based on these discouraging results, we took the decision to terminate the study.

The rationale of using thalidomide in non-responders lies on the premise that several intracellular factors are elucidated to be negatively involved in regulating the IFN-α signaling transduction, leading finally to IFN-α resistance. These negative factors include some members of the SOCS fami-

ly (7,8) and TNF-α (10). Theoretically, modulating the levels and/or the activity of these factors may help in establishing the IFN-α-induced antiviral activity on HCV replication. However, specific inhibitors of SOCS family members are either not available for *in vivo* administration or toxic (37). Alternatively, one may consider increasing IFN sensitivity by correcting the circulating and hepatic levels of TNF-α. TNF-α plays a major role in the host's immunomodulatory response (38). It has been implicated as a cofactor in liver injury associated with CHC (39) and in resistance to IFN-α therapy (40,41). The intrahepatic mRNA levels of TNF-α have been found to be significantly higher in non-responders than in the sustained responders (42). These findings call for an interesting study, has shown that etanercept, an anti-TNF agent, when used as an adjuvant to IFN/RBV therapy has beneficial effects with respect to virologic, biochemical, and possibly histologic endpoints in treatment-*naïve* CHC patients (43). However, up till now, still some caution has been suggested while administering anti-TNF-α therapy to CHC patients (44). Exploring another TNF-α modulators may be challenge. Several studies have demonstrated the anti-TNF-α activity of thalidomide *in vitro* (45) and *in vivo* (46). This anti-inflammatory effect is in addition with immunomodulatory properties by enhancing effect of T-lymphocyte response (20,21). Moreover, thalidomide was reported to accelerate the recovery from experimental cirrhosis in rats, probably mediated by TNF-α suppression effect in the liver (47). Ultimately, the few available clinical data generating also hypothesis supported the usefulness of thalidomide in the management of CHC. Milazzo et al. reported that he use of thalidomide monotherapy was associated with normalization of serum ALT and GGT levels (22). Also, in six case reports from Brazil, Caseiro reported that adding thalidomide to the standard of care therapy in retreatment of HCV patients, achieved SVR in all of them (23). The difference in the results of this trial and results of multiple case reports of Caseiro regarding the SVR may be multifactorial: firstly, all Caseiro's patients were nonresponsive to previous conventional IFN/RBV therapy and retreated with Peg-IFN-α/RBV. Data from the clinical trials of retreatment with Peg-IFN-α/RBV in patients who were nonresponsive to previous IFN-α/RBV, referred to that SVR rates ranged from 8 to 35% (48-50) and these rates were generally higher than the reported rates (6 to 8%) (28,32) in prior non-responders to Peg-IFN-α/RBV retreated by the same regimen for 48 weeks as in our trial. So, we can't exclude that the difference is related to difference in modality of treatment. Secondly, Caseiro didn't provide any data about the characteristic criteria of his patients or their HCV genotype. Indeed we enrolled very-difficult-to-treat documented genotype 1 patients. These factors are major determinant of the outcome of the second course of the therapy.

In our study, it remains to be unexplained that the use of thalidomide was not associated with significant changes in sTNFRII levels. In spite of the improvement of liver enzymes in our study, and that GGT levels seems to be a surrogate marker of TNF-α levels and inflammatory state (51). In contrast, Milazzo detected a reduced TNF-α production by stimulated PBMC *in vitro* (22). However, it worth mention that it has been recently reported that serum soluble TNF-α level rather than serum TNF-α level was a reliable indicator to reflect the activation of TNF-α system (52). Our study was not designed to assess histological improvement. Further studies might be needed to determine a potential beneficial effect.

This study has also demonstrated that thalidomide as an adjuvant to Peg-IFN-α/RBV is safe and not associated with serious toxic effects in HCV-infected patients. Only one patient developed mild peripheral sensory neuropathy. Thalidomide induces typically a sensory neuropathy, which reported to be partially reversible in 50% of cases (53). The mechanism of thalidomide-induced neuropathy is not completely understood; a dose-related correlation has been described. Cumulative dose of thalidomide higher than 20 gram (54) is the described dosage cut-off to be associated with increasing the risk of developing sensory neuropathy. This explanation may not be suited to our patient; as the patient received only a total dose of 18 g in spite of that the use of IFN-α *per se*, has been related to induce neuropathy in CHC patients. The pattern of IFN-induced neuropathy, which is commonly in the form of acute inflammatory demyelinating polyneuropathy (55) and polyradiculoneuropathy (56), ruled out this possibility in our patient who suffered from sensory neuropathy. Ultimately, thalidomide induced idiosyncratic neuropathy seem to be the more acceptable explanation.

Thus, although targeting host factors and modulating the negative intracellular factors may be a rational option in CHC patients not responding to current standard of care therapy, our approach was clearly inadequate to reach the stated goal. In conclusion, thalidomide could be discarded as possible therapy for CHC in combination with Peg-IFN/RBV.

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