Safety of two different doses of simvastatin plus rifaximin in decompensated cirrhosis (LIVERHOPE-SAFETY): a randomised, double-blind, placebo-controlled, phase 2 trial

RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PHASE II TRIAL ASSESSING SAFETY OF TWO DIFFERENT DOSES OF SIMVASTATIN IN COMBINATION WITH RIFAXIMIN IN DECOMPENSATED CIRRHOSIS

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ABSTRACT

BACKGROUND: Statins have beneficial effects on the intrahepatic circulation and decrease portal hypertension, while rifaximin modulates gut microbiome and may prevent bacterial translocation in cirrhosis. Therefore, this drug combination may be of therapeutic benefit in decompensated cirrhosis. However, there is concern regarding safety of statins in patients with decompensated cirrhosis. We assessed the safety of two different doses of simvastatin, in combination with rifaximin, in patients with decompensated cirrhosis.

METHODS: We performed a multicenter, double-blind, randomized, placebo-controlled trial in patients with decompensated cirrhosis, Child-Pugh B and C, that were randomly assigned to receive simvastatin 40mg/day plus rifaximin 1,200mg/day(n=16) , simvastatin 20mg/day plus rifaximin 1,200mg/day (n=14) or placebo of both medications (n=14) for 12 weeks. Primary endpoint was development of liver or muscle toxicity, as defined by changes in liver transaminases (AST, ALT), alkaline phosphastase and creatine kinase (CK) levels. ITT and PP analysis were performed.

FINDINGS: Patients in the simvastatin 40mg/day group but not in the simvastatin 20mg/day group showed a significant increase in AST, ALT and CK levels during treatment, as compared to placebo (191 vs. 62IU/L, 96 vs 35 IU/L and 1160 vs. 152 IU/L for AST, ALT and CK, respectively; $p<0.001$, $p<0.001$ and $p=0.016$) . Moreover, 3 patients (19%) in the simvastatin 40mg/day group developed significant liver and muscle toxicity consistent with rhabdomyolysis.

INTERPRETATION: Treatment with simvastatin 40mg/day plus rifaximin in patients with decompensated cirrhosis appears to be associated with significant frequency of adverse events, particularly rhabdomyolysis. By contrast, the dose of simvastatin 20mg/day plus

rifaximin was not associated with an increased frequency of adverse events. We recommend simvastatin 20mg/day as the dose to be used in studies investigating the role of statins in patients with decompensated cirrhosis.

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BACKGROUND

There is accumulating evidence that statins have beneficial effects in cirrhosis[1,2]. This evidence mainly derives from retrospective cohort studies, some of which included large numbers of patients, and a few randomized clinical trials[3–6]. Results of the cohort studies consistently show that patients with cirrhosis that received treatment with statins to reduce cholesterol levels had lower risk of decompensation and death compared to patients that did not receive statins. The risk of development of hepatocellular carcinoma was also lower in one study. The favorable effects of statins were demonstrated with propensity score analyses and persisted after adjustment for the most important predictive variables. The primary endpoints of the few randomized controlled trials demonstrating the beneficial effects of statins were effects on portal hypertension[3,4,6] and survival[5]. Treatment with statins (simvastatin 40 mg/day) (in addition to standard treatment), was associated with significant reduction in portal pressure gradient compared to placebo[3,4]. Moreover, the most recent randomized, double-blind study, demonstrated that simvastatin 40 mg/day improved survival in patients with cirrhosis who recovered from a variceal bleeding[5]. The mechanism(s) by which statins exert their potential beneficial effects is not known but is thought to be related to an improvement in intrahepatic circulation through an increase in nitric oxide synthesis[7] and/or to anti-inflammatory properties[8]. In cirrhosis, there is enhanced systemic inflammation that increases as the disease progresses and is associated with poor prognosis[9]. Nonetheless, despite these positive findings, the most recent clinical guidelines still do not recommend the use of statins in clinical practice because there is only one positive randomized trial with hard endpoints; therefore results of further trials are awaited before statins can be advocated for use in clinical practice[10].

Rifaximin is a broad spectrum, poorly absorbed antibiotic that is effective in patients with cirrhosis to prevent recurrent hepatic encephalopathy[11]. Moreover, recent studies suggest that rifaximin could also be effective in preventing other portal-hypertension-related complications, yet conclusive evidence is lacking[12].The mechanisms by which rifaximin exerts its beneficial effects in patients with cirrhosis have not been completely elucidated, but may be related at least in part to modulation of gut microbiome and reduction in bacterial translocation, yet these effects have not been confirmed in all studies[13,14]. Therefore, considering the different mechanisms of action of statins and rifaximin, it would be of interest to explore the potential beneficial effects of this combined therapy in the prevention of progression of decompensated cirrhosis. However, the safety of this combination in patients with decompensated cirrhosis is of importance and has so far not been assessed. Rifaximin has been evaluated in many studies, from phase II to phase IV, including large numbers of patients with decompensated cirrhosis and no significant safety issues have been observed[15]. Rifaximin does not seem to increase the risk of infections by multidrug-resistant bacteria or have significant interactions with other drugs despite its potential effect on CYP3A4. Only drugs that inhibit p-glycoprotein and organic anion-trasnporting-polypeptides (OATPs), such as cyclosporine, have been shown to increase the systemic exposure to rifaximin. On the other hand, statins are thought to be safe in patients with chronic liver diseases without cirrhosis and in patients with compensated cirrhosis, but there is still very limited information about safety of statins in patients with decompensated cirrhosis owing to a possibly impaired metabolism of these drugs in the setting of liver failure. In the four prospective studies reported, the majority of patients included had either compensated or only mild decompensated cirrhosis. In the largest

RCT performed so far, two of the 69 patients treated with simvastatin 40 mg/day developed severe rhabdomyolysis, an incidence that was considered higher than expected[5].

On this background, the current study was designed to investigate the safety of two different doses of simvastatin (20 and 40 mg/day) associated with rifaximin (1,200 mg/day in both cases) compared to placebo in a double-blind manner, in a population of patients with decompensated cirrhosis. A group of patients treated only with rifaximin was not considered necessary given the extensive experience with the use of rifaximin in patients with decompensated cirrhosis and the lack of drug-related adverse events observed. This study is part of the LIVERHOPE project, which is aimed at assessing the efficacy of simvastatin in combination with rifaximin to prevent the progression of cirrhosis and development of acute-on-chronic liver failure (ACLF).

PATIENTS AND METHODS

Trial Design

The LIVERHOPE-SAFETY trial was a multicenter, randomized, double-blind, placebo-controlled trial that included patients with decompensated cirrhosis and moderate-to-severe liver failure from nine university hospitals in six European Countries (Italy, France, Holland, Germany, United Kingdom, and Spain). Candidate patients were randomly assigned to receive simvastatin 40mg/day in combination with rifaximin 1,200 mg/day (simv40+rif), simvastatin 20mg/day in combination with rifaximin 1,200 mg/day (simv20+rif) or matching placebo of both medications for three months. The study was

approved by regulatory Agencies of the six countries involved and by the Institutional Review Board of each participating center. Written informed consent was obtained from all patients. The study was monitored by the clinical trials units of the European Clinical Research Infrastructure Network (ECRIN, Paris, France) and the statistical analysis was performed by the Medical Statistics Core Facility of the Institut d'Investigacions Biomèdiques August Pi-Sunyer (IDIBAPS, Barcelona, Spain). The study was registered at European Union Clinical Trials Register (EudraCT Number 2016-004499-23) and at Clinicaltrials.gov (NCT 03150459).

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Participants

Patients with decompensated cirrhosis were considered eligible for the study if they met the following inclusion criteria: 1) age older than 18 yr; 2) cirrhosis defined by standard clinical and/or histological criteria; 3) Child-Pugh class B or C up to 12 points; and 4) written informed consent. Exclusion criteria were: serum bilirubin>5mg/dL; INR>2.5; serum creatinine≥2mg/dL; patients with ACLF (as defined by the CANONIC study)[16]; patients on the waiting list for liver transplantation; hepatocellular carcinoma beyond Milan criteria; gastrointestinal bleeding or active infection 15 days before study inclusion; current overt hepatic encephalopathy; antiviral treatment for HCV in the

previous six months; severe alcoholic hepatitis treated with corticosteroids; current alcohol consumption of more than three units per day; current use or contraindications to simvastatin or rifaximin or conditions that could increase the risk of adverse events related to these drugs; creatine kinase (CK) levels at inclusion of at least 50% above the upper normal limit (UNL); previous history or increased risk of intestinal obstruction; treatment with medications with potential interactions with simvastatin; infection with human immunodeficiency virus; extrahepatic diseases with poor short-term prognosis; extra hepatic tumors or hematologic disorders; women of child-bearing potential not using highly effective contraception; pregnancy or breastfeeding; and, psychiatric or social conditions precluding adequate understanding or compliance with the study.

Interventions

Patients were randomized to 3 groups to receive orally simvastatin (Alfasigma S.p.A., Bologna, Italy) at one of two different doses (40 mg/day or 20 mg/day) plus rifaximin 1,200 mg/day (Alfasigma S.p.A., Bologna, Italy) or matching placebo of both drugs. Patients from the first group received simvastatin 40 mg (2 tablets of 20mg) per day plus rifaximin 400mg every eight hours; patients from the second group received simvastatin 20mg (1 tablet of 20mg and 1 tablet of placebo of simvastatin) per day plus rifaximin 400mg every eight hours; and, patients from the third group received placebo of simvastatin (2 tablets per day) plus placebo of rifaximin (1 tablet every eight hours). The pharmaceutical formulation of rifaximin (rifaximin-EIR 400mg) used in the study was slightly different from the commercially available product, in that rifaximin is coated by

a gastro-resistant polymer that allows a higher bioavailability of the drug in the intestine compared to the commercial formulation.

Treatment was started after the baseline visit and was given for twelve weeks. Study visits were performed every two weeks; standard clinical and analytical data were collected and complications of cirrhosis and treatment-related adverse events were assessed and registered, if any. Compliance with the study medication was assessed at each visit by self-reporting and confirmed by counting the pills of simvastatin and rifaximin returned by the patients at the end of the treatment period. Subjects with a compliance of less than 70% of the total supplied study medication were excluded of the per protocol analysis. Study medication was permanently withdrawn if patients developed liver or muscle toxicity. In addition, treatment was also permanently withdrawn in patients who developed HE and met the criteria for treatment with rifaximin according to the EASL guidelines[17], and in those patients with severe treatment-related side effects, according to the judgement of the investigator. Complications of cirrhosis occurring during the study period were treated according to international guidelines[10].

Outcomes

Because this was a safety study, the primary endpoints were based on most common side effects related to statin therapy. No efficacy endpoints were addressed. The primary endpoint was the development of liver or muscle toxicity. Liver toxicity was evaluated by comparing increases from baseline in transaminases (AST, ALT) and alkaline phosphatase (AP) levels between the three groups during treatment; in addition, we assessed the percentage of patients who developed a three fold increase

in transaminases levels or a two fold increase in AP levels with respect to baseline. as a modification for patients with decompensated cirrhosis of the internationally accepted criteria for drug-induced liver injury case definition[18]. Muscle toxicity was evaluated by comparing changes in CK levels during treatment with respect to baseline and also by the percentage of patients who developed an increase in CK levels during treatment to a final value at least five times the UNL. If patients reached the primary endpoint of muscle toxicity the study medication was withdrawn; in the case of reaching the primary endpoint of liver toxicity, blood tests were repeated after two days, and if the increase in transaminases or AP persisted, the study medication was also withdrawn.

Secondary endpoints were: 1) development of muscle symptoms; 2) proportion of patients developing treatment-related adverse events in each study group; and 3) assessment of the relationship between muscle toxicity/symptoms and the rs4149056 polymorphism of the gene SLCO1B1; the existence of this polymorphism has been reported to be associated with an increased risk of muscle toxicity in patients treated with simvastatin[19].

Sample size

A sample size of 15 patients per arm was considered sufficient for this phase 2 exploratory trial. According to the binomial distribution, the study would have 80% probability to detect at least one muscle toxicity adverse event if the actual incidence in the population was >10%. However, for the sake of sensitivity in the dose-selection, the main outcome was focused on the detection of baseline-changes versus placebo of transaminases, AP and CK levels, which were expected to be more sensitive than the incidence of limiting adverse events. Due to the high uncertainty arisen from the lack of

previous data in this population and the exploratory nature of this trial no further numerical calculations were conducted.

Randomization, blinding and treatment allocation

Eligible subjects were randomized 1:1:1 to the different groups of the study. Randomization was stratified according to Child-Pugh class (B vs C) using the PROC PLAN of the SAS system and restricted using blocks multiple of 3 elements. A centralized computer-generated randomization was used through the electronic Case Report Form (eCRF). Unblinding was not expected until all subjects had completed the study and the database had been locked after data completion and verification. However, it was foreseen that a specific patient could be unblinded, if needed, as judged by the investigator in emergent cases with side effects possibly related to the study medication.

Main safety outcome measures in patients included in the study were monitored by an independent Data Safety Monitoring Board (DSMB), constituted by a group of experts. The study followed the regulatory recommendations regarding the functions and procedures of this committee[20]. The DSMB held a meeting after completion of the study of the first 10 patients. As a conclusion of this meeting, the DSMB provided a written report in which they recommended to stop the study medication in one of the study arms, without breaking the blinding procedure. The investigators decided to fully implement the advice of the DSMB and study treatment was discontinued in all patients allocated into one study arm, but continued in the other two arms.

Statistical methods

Categorical variables were summarized by counts and proportions and continuous variables by mean (standard deviation) as appropriate. Longitudinal continuous variables assessing the individual laboratory parameters measured to evaluate toxicity (AST, ALT, AP, and CK levels) were analysed using Mixed Models for Repeated Measurements including the Child-Pugh stratum and the baseline measurement. The Fischer's exact test was used to compare categorical variables.

Missing data for longitudinal variables was handled through the MMRM strategy which relies on the missing at random assumption. Categorical variables were handled using a worst-case imputation strategy and any treatment discontinuation due to relevant safety issues was considered as a failure. Since the objective of this exploratory trial was to assess and discard safety signals, no multiplicity adjusting strategy was planned. All analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC, USA) and the level of significance was established at the two-sided 5% level.

Role of funding source

The study funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author (P.G.) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Characteristics of study population

Recruitment period started July, 28th of 2017 and finished in January, 2nd, 2018. The follow-up period finished in March, 12th, 2018. The distribution of patients in the study is shown in figure 1. A total of 220 patients were evaluated and 163 of them were excluded because they met exclusion criteria or denied consent. Main causes of exclusion were current treatment with rifaximin or hepatocellular carcinoma. Seven of the remaining 57 patients were excluded because of screening failure. Therefore, 50 patients were randomly assigned to receive treatment with simvastatin 40mg/day plus rifaximin 1,200mg/day (simv40+rif group), simvastatin 20mg/day plus rifaximin 1,200mg/day (simv20+rif group) or placebo of both medications (placebo group). Six randomized patients were excluded: three of them because they did not meet the inclusion criteria, two patients because they did not initiate the study medication, and one because of a problem of supply of the study medication. Therefore, the full analysis set included a total of 44 patients (16 patients in the simv40+rif group, 14 patients in the simv20+rif, and 14 patients in the placebo group). The overall median follow-up period was of 84 days (IQR 36-84). The majority of patients were compliant with the study medication (mean compliance among the three treatment arms included in the study of more than 90% of fulfillment with rifaximin/ placebo and simvastatin/ placebo). Only four patients (three in the group of simvastatin 40mg/day plus rifaximin and one in the group of simvastatin 20mg/day plus rifaximin) were excluded of the per protocol analysis for lack of compliance with the study medication. Baseline demographical, clinical and analytical data were quite similar in the 3 groups, except for lower age in the simv20+rif group and slightly higher frequency of HE and variceal bleeding in the placebo group compared to the other two groups (table 1). As mentioned above, the study was prematurely stopped in one of the study arms that corresponded to the simv40+rif arm,

due to the recommendation of the DSMB based on safety issues. At the time this decision was made, all patients except one in this group had stopped treatment due to adverse events or already completed the treatment period .

Liver toxicity

Patients from the simv40+rif group showed a significant increase of AST and ALT levels compared to placebo (differences between the simv40+rif and placebo groups at the end of treatment 130 [54 to 205], and 61 [22 to 100] IU/L (mean[95%CI], p<0.001 and p=0.003, respectively). By contrast, no significant changes in AST and ALT levels were observed between the simv20+rif and placebo groups (differences between the simv20+rif and placebo groups -14 [-91 to 64] and -8 [-49 to 33] IU/L (mean[95%CI], p=0.73 and 0.70, respectively). Moreover, patients in simv40+rif group had significantly higher AST and ALT levels at the end of the treatment compared to those of patients from the simv20+rif group (differences between the simv40+rif and simv20+rif groups 143 [66 to 220] and 69 [29 to 109] IU/L, p<0.001 for both comparisons) (Table 2, Figure 2 and supplementary table 1). No significant changes in AP levels between groups were observed.

Three of the 16 (19%) patients in the simv40+rif group had an increase in AST or ALT levels of >3x the UNL. One patient had a marked increase in transaminases (peak values: ALT 696IU/L and AST 1,350IU/L) consistent with DILI related to simvastatin, and was associated with an increase in INR from 1.4 to 1.9. No other abnormalities were found and the patient did not develop complications of cirrhosis. Abnormal liver tests returned to baseline values two months after stopping the study medication. The other two

patients had moderate increases in ALT and AST levels (peak ALT: 141 and 147 IU/L; peak AST: 426 and 251 IU/L), without changes in other liver tests. Transaminase levels returned to baseline values 8 and 2 weeks after stopping the study medication. All three patients who developed increases in transaminases levels also had increases in CK levels indicative of muscle toxicity (see later). Only one patient in the simv20+rif group (8%) had an increase of serum ALT and AST levels of >3x the UNL at week 6 (peak ALT 206 IU/L, peak AST 143 IU/L), with no changes in other liver tests; ALT and AST levels returned to normal values without stopping the study medication. No further changes in AST/ALT levels were observed in this patient during the rest of the treatment period. No patient from the placebo group had an increase in AST/ALT of >3x during treatment.

Muscle toxicity

Patients from the simv40+rif group showed an increase of CK levels during treatment compared to placebo (mean [95%CI]:1009 [208 to 1809,] p=0.01). By contrast, no significant changes in CK levels were observed in the simv20+rif (4.2 [-804.5 to 813] IU/l, p=0.992) (Table 2 and Figure 3). Moreover, CK levels at the end of treatment were higher in the simv40+rif compared to levels in the simv20+rifgroup (1004 [192 to 1817] IU/L, p=0.016).

Three of the 16 patients (19%) from the simv40+rif group, but no patient from the simv20+rif or placebo groups, had an increase in CK levels to a final value >5x the UNL. Characteristics of these 3 patients and data related to the muscle toxic effect are reported in table 3. No patient developed renal failure in association with muscle toxicity. Out of all patients included in the simv40+rif group, one of the 12 (8%) Child-Pugh B patients and two of the 4 (50%) Child-Pugh C patients developed muscle toxicity.

The number of patients developing *de novo* muscle symptoms (cramps, aches, weakness) without meeting the criteria of muscle toxicity was not significantly different among groups (5, 6, and 3 patients in the simv40-rif, simv20+rif, and placebo groups, respectively).

In the whole population, the prevalence of C/T heterozygosis for the rs4149056 polymorphism of the gene SLCO1B1 was 30% (13 out of 44 patients). No patient was homozygote for the C/C polymorphism. The distribution of the C/T variant among the three treatment groups was similar (25, 36, and 29% in the simv40+rif, simv20+rif, and placebo groups, respectively). There was no relationship between both muscle toxicity or muscle symptoms and the C/T variant of the rs4149056 polymorphism of the gene SLCO1B1: 4 of 14 patients (19%) with muscle symptoms reported as adverse events during the study period had the SLCO1B1 rs4149056 polymorphism, versus 9 of 30 (29%) in the group of patients who did not have muscle symptoms (p=0.2). Among the three patients who had muscle toxicity presenting as rhabdomyolysis during the study period, two (67%) had the SLCO1B1 rs4149056 polymorphism, versus 11 of 41 patients (42%) who did not develop muscle toxicity (p=0.08).

Adverse events

Adverse events reported in all patients included in the study are shown in supplementary table 2. As expected for a study in patients with advanced cirrhosis, the number of patients with adverse events during follow-up was high. Overall, 36 of the 44 patients included (81.8%) reported a total of 107 adverse events. There was a clear trend for higher number of patients with adverse events and serious adverse events, and number of treatment-related serious adverse events in the group of patients treated

with simvastatin 40 mg/day compared to the other two groups. Remarkably, the number of patients who stopped treatment due to adverse events was significantly higher in simv40+rif group compared to the other two groups (56% vs 14% and 14%, respectively; p=0.017).

There were no cases of serious and unexpected adverse reactions reported during the study period.

DISCUSSION

In the LIVERHOPE-SAFETY trial we evaluated the safety of simvastatin 20mg/day or 40 mg/day, both combined with rifaximin 1,200 mg/day, compared to placebo of the two drugs for 12 weeks in patients with decompensated cirrhosis. Treatment with simvastatin 40 mg/day was associated with a significant increase in AST/ALT and CK levels, whereas no changes in these parameters were observed with simvastatin 20 mg/day or placebo. Moreover, patients treated with simvastatin 40 mg/day plus rifaximin had a high incidence of liver and muscle toxicity.

The safety of statins in patients with cirrhosis has been assessed in 4 randomized controlled trials aimed at investigating the efficacy of statins in reducing portal hypertension (3 trials) [3,4,6] or a combined endpoint of reducing variceal bleeding and mortality (one trial) [5]. No liver or muscle toxicity was observed in three of these studies. However, in these studies either the sample size was very small or treatment was given for only one month, which could have accounted for the absence of side effects[3,4,6]. The other study was a multicenter double-blind placebo-controlled randomized trial of 147 patients treated with simvastatin 40 mg/day or placebo followed

for up to 2 years[5]. In this study, 2 of the 69 patients (3%) treated with simvastatin developed clinically-relevant muscle toxicity that required treatment withdrawal. In the current study, 3 of the 16 (19%) patients (two Child C and one Child B) treated with simvastatin 40 mg/day plus rifaximin developed muscle toxicity associated with liver toxicity which required treatment discontinuation. Given that criteria for liver and muscle toxicity coincided in the three patients, it cannot be ruled out that the increase in AST and ALT was related, at least in part, to muscle necrosis. Therefore, the liver origin of increased AST/ALT levels cannot be established convincingly. Nevertheless, one of the patients developed a concomitant increase in INR, which suggests that liver toxicity in addition to muscle toxicity was present in this case. In sharp contrast with findings in the simvastatin 40mg/day plus rifaximin group, no case of muscle toxicity and only one case of transient mild liver toxicity, not requiring discontinuation of treatment, was observed in patients treated with simvastatin 20 mg/day plus rifaximin. These findings suggest that side effects related to simvastatin treatment in the setting of concurrent rifaximin therapy in patients with decompensated cirrhosis are dose-dependent and that 20 mg/day is safer than 40 mg/day. In this regard, it seems pertinent to mention that large studies in the general population without liver disease showed that simvastatin 80 mg/day dose was associated with high frequency of muscle toxicity, which led to the concept that the safe dose in the general population is 40mg/day[21].

The reason(s) for the higher frequency of side effects in the current trial compared to previous studies is unclear. A possible explanation is the greater severity of cirrhosis of patients included in the current study. In fact, all patients of the current study had decompensated cirrhosis and were Child B or C, whereas a significant proportion of the patients included in previous studies had compensated cirrhosis without previous of

complications of the disease, indicating lower disease severity. Compared to the study by Abraldes et al[5], patients included in the current study had higher frequency of ascites (39% vs 82% , respectively) and HE (3% vs 30%), higher MELD score (median 10 vs 14), and higher frequency of Child-Pugh C (14% vs 27%). Considering that simvastatin undergoes extensive biotransformation in the liver and is eliminated through the bile, severity of cirrhosis could affect exposure to the drug which may be responsible at least in part for the higher frequency of side effects observed[22]. Several mechanisms could theoretically lead to impaired biotransformation of simvastatin in the presence of advanced cirrhosis, including a reduction in the metabolic activity of CYP3A4[23], one of the key enzymes involved in simvastatin metabolism, and impaired transport of simvastatin to bile through MRP2, a canalicular membrane transporter which mediates transport of bilirubin conjugate to bile[22]. However, it should be mentioned that rifaximin treatment could theoretically induce CYP3A4 in *in vitro studies* and thus compensate for the reduced activity of CYP3A4 in cirrhosis; however this possible effect on CYP3A4 was not confirmed *in in vivo* studies[24,25]. In the current study, all three patients who had muscle and liver toxicity had increased baseline serum bilirubin levels (see table 3). Side effects were apparently not related to presence or frequency of polymorphisms of the gene SLCO1B1 encoding OATP1B1.

Another potential explanation for the high frequency of adverse events observed in the current study could be a toxic effect of rifaximin either by itself or by increasing the toxicity of simvastatin. The rifampin component of rifaximin could theoretically induce muscle toxicity through mitochondrial oxidative stress and perhaps act synergistically with statins. This potential mechanism was raised on the basis of a case reporting a chronological relationship between treatment with rifaximin and rhabomyolysis in a

patient with cirrhosis[26]. However, the possibility of muscle toxicity due to rifaximin seems remote due the following reasons: 1/ no cases of rhabomyolisis were reported in the pivotal trial of rifaximin for prevention of recurrent of HE and its long-term follow-up [15]; 2/ as of July 2019, only two cases of rhabdomyolisis possibly related to rifaximin have been collected in postmarketing safety databases of AlfaSigma (Bologna, Italy) over more than 10 years; 3/ rhabdomyolisis has been reported in the setting of advanced cirrhosis and is idiopathic in 25 to 58% of cases[27]; therefore, cause-effect relationship is difficult to establish; and 4/ no muscle toxicity was found in animal studies in rats and dogs treated with doses of rifaximin equivalent to those used in humans[28]. Nevertheless, since safety of the combination of simvastatin and rifaximin remains a relevant issue, the LIVERHOPE Efficacy Trial, a randomized double-blind trial comparing simvastatin 20 mg/day plus rifaximin vs placebo in the prevention of ACLF in patients with decompensated cirrhosis which has already started includes an assessment of safety at the time when the first 40 patients randomized to the treatment arm have reached at least one month of therapy (NCT03780673).

Other aspects of the study deserve a comment. First, it should be emphasized that liver safety monitoring and stopping rules for drug-induced liver injury in patients with decompensated cirrhosis enrolled in clinical trials remain a challenge because of potentially altered liver tests before initiation of treatment[29]. Therefore, potential liver toxicity was assessed by evaluating changes with respect to baseline. Furthermore, the role of simvastatin 40mg/day in development of liver toxicity is supported by the fact that causality assessment excluded other potential causes of liver damage and upon discontinuation of the study drug laboratory abnormalities returned to baseline values. Second, it is important to mention that although the dose of simvastatin given in the

randomized controlled studies of patients with cirrhosis published was of 40 mg/day, a recent large cohort study including more than 70,000 patients in which the effects of statins on survival of cirrhosis were evaluated, showed beneficial effects of simvastatin even at doses lower than 20 mg/day[30].

A possible limitation of this study is the restricted sample size. It is important to note that sample size was specifically calculated to investigate the safety of different doses of simvastatin in combination with rifaximin in patients with decompensated cirrhosis in the context of a phase II trial, and the design was correct for the primary endpoint of the study. However, possible signals of the efficacy of the combination of simvastatin plus rifaximin in patients with decompensated cirrhosis could have been identified with a larger sample size. Finally, we cannot rule out that the slight imbalance in some of the baseline characteristics among groups could have affected the results of the study, probably due to the small sample size. Nevertheless, the group with the highest imbalance was the placebo group, whereas the two groups treated with simvastatin 40 mg/day or 20 mg/day plus rifaximin were quite similar.

In conclusion, we conducted a multicentre, randomized, double-blind trial investigating the safety of two different doses of simvastatin, 40 mg/day or 20 mg/day, both combined with rifaximin 1,200 mg/day, for 12 weeks compared to placebo. We found that simvastatin 40 mg/day was associated with a high frequency of adverse events that required treatment discontinuation, specifically liver and muscle toxicity. By contrast, simvastatin 20 mg/day had a good safety profile similar to that of placebo. In studies investigating the efficacy of simvastatin in patients with decompensated cirrhosis, 20 mg/day should be preferred to the 40 mg/day dose.

RESEARCH IN CONTEXT

Evidence before this study

We systematically searched Pubmed database from inception to January 2019, for articles on the effects and safety of statins in patients with cirrhosis. Among 180 records, a total of 9 studies were identified, including four randomized controlled trials (RCTs) and five cohort studies. In 3 of the RCTs, statin therapy was associated with a decrease in portal pressure and no serious adverse events were observed. In the largest RCT reported to date, treatment with simvastatin 40mg/day was associated with a significant reduction in mortality compared to placebo, but with an unexpectedly high rate of rhabdomyolysis.

A search focused on rifaximin safety in liver cirrhosis was done for the same time period. Among 23 records, a total of 4 placebo-controlled clinical trials specifically designed to investigate rifaximin safety in patients with cirrhosis were identified. No increased frequency of serious adverse events associated with rifaximin therapy compared to placebo or control groups was reported.

Added value of this study

This is the first study investigating the safety of different doses of simvastatin, in combination with rifaximin, in a randomized, placebo-controlled trial in patients with advanced decompensated cirrhosis. Treatment with simvastatin at a dose of 40mg/day in combination with rifaximin resulted in an unexpectedly high rate of adverse events, particularly liver and muscle toxicity. By contrast, simvastatin 20mg/day plus rifaximin was safe and not associated with increased risk of adverse events compared to placebo.

Implications of all the available evidence

This RCT contributes to knowledge providing evidence based on a specifically designed study that simvastatin at 40 mg/day in combination with rifaximin in patients with advanced decompensated cirrhosis is associated with high rate of adverse events. This information is of high value for the design of future studies investigating the effects of simvastatin on disease progression and survival in decompensated cirrhosis. From a clinical perspective, this trial provides evidence in favour of using low doses of statins in patients with decompensated cirrhosis due to safety reasons. In the current setting of increasing prevalence of cirrhosis due to non-alcoholic fatty liver disease, which is frequently associated with dyslipidemia, this information can be of clinical value.

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CONTRIBUTORS

PG, EP, ML, and ES conceived the idea of the study, designed the protocol, and supervised study execution. LN, AA, DC, CJ, SP, OR, FU, KW, GZ, CA, PA, MB, UB, PC, FD, RM JT, VV, and MC identified candidates to the study, enrolled and treated patients, acquired data and collaborated in the design of the protocol. JP and JF provided administrative and monitoring support throughout the whole study period. GD and FT designed and performed the statistical analysis. EP drafted the manuscript. PG directly reviewed the manuscript. PK, JGA, and RA gave advice for the design and development of the study and contributed to the interpretation of the results and intellectual content of the manuscript. All authors provided critical revision of the manuscript for important intellectual content and approved the final draft of the manuscript for submission.

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TABLE LEGENDS:
Table 1.

Variables are mean and standard deviation (in brackets) or number and percentage (in brackets).

*Simv40+rif group: 3 Cryptogenic, 2 NASH, 2 Hepatitis C, 1 Autoimmune hepatitis; Simv20+rif group: 2 Hepatitis C, 2 Hepatitis B, 1NASH, 1 Autoimmune hepatitis, 1 Cryptogenic; Placebo group: 3 NASH, 3 Cryptogenic, 1 Hepatitis C.

Table 2.

Values are mean and 95% CI (in brackets).

*End of treatment values represent values at week 12 or last laboratory values available before study withdrawal due to side effects.

Table 3.

*Part of the duration of hospitalization was due to a concomitant spontaneous bacterial peritonitis.

FIGURE LEGENDS:

Figure 1.

*Other reasons for exclusion were: refusal to give informed consent (6 patients), inclusion in other clinical trials in the previous month (5 patients)

**Screening failures (7 patients) were due to patients meeting exclusion criteria after assessment for eligibility.

***Post-randomization failures were due to not meeting exclusion criteria (3 patients), not initiating the study medication (2 patients) and because of a problem with the

study medication supply (1 patient)

Figure 2.

0 level represents no changes with respect to placebo. Continuous line: simv40+ rif; discontinuous line: simv20+ rif. Values are mean and 95% CI

Figure 3.

0 level represents no changes with respect to placebo. Continuous line: simv40+ rif; discontinuous line: simv20+ rif. Values are mean and 95% CI