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Original Contribution

Silybin combined with phosphatidylcholine and vitamin E in patients with nonalcoholic fatty liver disease: A randomized controlled trial

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ABSTRACT

The only currently recommended treatment for nonalcoholic fatty liver disease (NAFLD) is lifestyle modification. Preliminary studies of silybin showed beneficial effects on liver function. Realsil (RA) comprises the silybin phytosome complex (silybin plus phosphatidylcholine) coformulated with vitamin E. We report on a multicenter, phase III, double-blind clinical trial to assess RA in patients with histologically documented NAFLD. Patients were randomized 1:1 to RA or placebo (P) orally twice daily for 12 months. Prespecified primary outcomes were improvement over time in clinical condition, normalization of liver enzyme plasma levels, and improvement of ultrasonographic liver steatosis, homeostatic model assessment (HOMA), and quality of life. Secondary outcomes were improvement in liver histologic score and/or decrease in NAFLD score without worsening of fibrosis and plasma changes in cytokines, ferritin, and liver fibrosis markers. We treated 179 patients with NAFLD; 36 were also HCV positive. Forty-one patients were prematurely withdrawn and 138 patients analyzed per protocol (69 per group). Baseline patient characteristics were generally well balanced between groups, except for steatosis, portal infiltration, and fibrosis. Adverse events (AEs) were generally transient and included diarrhea, dysgeusia, and pruritus; no serious AEs were recorded. Patients receiving RA but not P showed significant improvements in liver enzyme plasma levels, HOMA, and liver histology. Body mass index normalized in 15% of RA patients (2.1% with P). HCV-positive patients in the RA but not the P group showed improvements in fibrogenesis markers. This is the first study to systematically assess silybin in NAFLD patients. Treatment with RA but not P for 12 months was associated with

Abbreviations: AE, adverse event; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; HCV, hepatitis C virus; HOMA, homeostatic model assessment; IL, interleukin; MMP, matrix metalloproteinase; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; P, placebo; PP, per protocol; SF-36, short form 36; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; γGT, γ-glutamyltranspeptidase.

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improvement in liver enzymes, insulin resistance, and liver histology, without increases in body weight. These findings warrant further investigation.

Nonalcoholic fatty liver disease (NAFLD) is an important emerging liver damage epidemic. When characterized by only steatosis, NAFLD is associated with increased cardiovascular risk and worsens the course of hepatitis C; when represented by nonalcoholic steatohepatitis (NASH), NAFLD can evolve into liver fibrosis and cirrhosis [1,2]. Currently there is no effective therapy for NAFLD, except for lifestyle modification [3]. Both dysregulated glucose metabolism and oxidative stress in hepatocytes are key pathogenic factors in the onset of steatosis and progression of NASH, its inflammatory and potentially evolutive form. Studies have shown that insulin-sensitizing agents and antioxidants may improve clinical and histologic features of NAFLD, although data remain inconclusive [4,5].

Silybin is a potent antioxidant representing about 50–70% of the silymarin extract of *Silybum marianum* (milk thistle). Silybin modulates inflammation and fibrogenesis and interferes with intrahepatic glycolysis and gluconeogenesis [6,7]. Silybin treatment attenuated liver damage and diabetes in animal models of NASH and type 1 diabetes mellitus [8–11] and showed benefits in patients with poorly controlled type 2 diabetes with or without alcoholic liver cirrhosis [12,13].

As with other flavolignans, limitations of silybin use include low water solubility, low bioavailability, and poor intestinal absorption [14]. Derivatives of silybin with improved solubility may surmount these pharmacologic limitations [15,16]. The silybin phytosome complex (silybin plus phosphatidylcholine) has been coformulated with vitamin E (Realsil (RA); Istituto Biochimico Italiano, Lorenzini S.p.a., Italy). Pharmacokinetic analyses indicated that silybin phytosome bioavailability is much higher than silymarin bioavailability [17,18].

Given the potentially beneficial effects of silybin on liver function, and the need for effective therapies for liver steatosis, we carried out a placebo-controlled, double-blind, phase III, randomized clinical trial to assess RA in NAFLD patients.

Experimental procedures

Trial design

The trial was conducted at 11 Italian and 2 Romanian centers. Protocol and patient consent forms were designed by the coordinating center and reviewed by all participating centers. The protocol conformed to ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board at each participating center. All patients gave informed written consent. The trial was registered with the European Clinical Trials Database (EudraCT, Ref. 2005-000860-24). No significant changes were made to the protocol or prespecified outcomes after trial commencement.

Inclusion criteria were histologically documented liver steatosis or steatohepatitis diagnosed within 12 months, age 18–65, persistent increase in ≥1 plasma aminotransferase (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT)) and/or γ-glutamyltranspeptidase (γGT) within 6 months, and negative pregnancy test (females). Hepatitis C virus (HCV)-positive patients (confirmed by HCV RNA assay) + NAFLD with prior HCV treatment failure could enroll. Even if both pathogenesis and treatment of HCV-related chronic hepatitis were very different from NAFLD/NASH, in this study we aimed to verify the effect of RA on the metabolic component of fatty liver also in HCV-positive patients.

Exclusion criteria were liver cirrhosis, presence of other major diseases including type 1 diabetes, hepatitis B or D infection, genotype 3 HCV infection, any treatment for liver disease or HCV infection within 6 months, participation in other trials, hypersensitivity to treatment, daily ethanol consumption ≥20 g, and substance abuse. Drinking habits were assessed by questionnaires on lifetime drinking history and self-administered AUDIT C test [19,20]. Patients with normal values of one liver enzyme at baseline (T_0) were not excluded.

Patients were allocated (1:1) to receive active treatment (RA; active components: silybin 94 mg, phosphatidylcholine 194 mg, vitamin E acetate 50% (α-tocopherol 30 mg) 89.28 mg) or placebo (P; extrawhite saccharine replacing active components) orally twice daily for 12 consecutive months, on a consecutive basis within each site according to a centrally randomized list generated by block allocation. The block allocation sequence was based on numbers randomly generated by a computer program and randomization was not stratified. Investigators recorded patients' randomization numbers on the case report form. Patients and investigators were blinded to treatment until trial completion; treatment assignment code was sealed in the randomization envelopes.

Other treatments were disallowed. Patients received recommendations for lifestyle modification and an individually designed diet, plus dietary recommendations including reduction of simple carbohydrates (glucose, sucrose, fructose), fatty dressings (butter, mayonnaise), fatty cheeses, and sausages and increases in vegetable proteins and fiber.

Patients with <70% treatment compliance or who missed a visit were withdrawn.

Clinical and laboratory assessments

Clinical history, physical examination, and liver biopsy results were recorded at baseline; histology was defined by NAFLD activity score (NAS) [21]. NAS uses a standardized grading system for steatosis (0 to 3), lobular inflammation (0 to 3), and hepatocellular ballooning (0 to 2), higher scores indicating increased severity. Monthly check-ups assessed compliance, adverse events (AEs), and drug delivery; patients had to record their drug consumption daily. AST, ALT, and γGT plasma levels were determined at baseline and every 3 months. The following assessments were completed at baseline, 6 months (T_6), and 12 months (T_{12}): body mass index (BMI; overweight, BMI ≥25 and <30 kg/m^2; obese, BMI ≥30 kg/m^2); abdominal circumference and waist-to-hip ratio; abdominal ultrasonography (severity graded on semiquantitative scale: 0, absent; 1, mild; 2, moderate; and 3, severe) [22]; quality of life (QoL; 36-item short-form health survey (SF-36)) [23]; routine biochemistry tests (blood urea nitrogen, creatinine, bilirubin, total protein, alkaline phosphatase, uric acid, blood cell count, and prothrombin activity); HCV RNA levels (HCV-positive patients); plasma levels of glucose, triglycerides, cholesterol, ferritin, and insulin; and homeostatic model assessment (HOMA) [24]. Cytokines (tumor necrosis factor α (TNF-α), interleukin 10 (IL-10), transforming growth factor β (TGF-β)) and liver fibrosis markers (hyaluronic acid, matrix metalloproteinase 2 (MMP-2), procollagens I and III, tissue inhibitor of metalloproteinases 1 and 2 (TIMP-1 and -2)) were assessed at baseline and at T_{12} [25,26]. A second liver biopsy was performed at T_{12} if allowed by the Institutional Review Board and the patient had resigned the informed consent form.

Fasting blood samples were collected and plasma for cytokine level assessment was stored at −80 °C. Cytokines and markers of liver fibrosis were assessed by the coordinating center using enzyme-linked immunosorbent assay kits (IL-10, TNF-α, MMP-2, TGF-β, R&D Systems, Minneapolis, MN, USA; hyaluronic acid, Echelon