Silibinin modulates lipid homeostasis and inhibits nuclear factor kappa B activation in experimental nonalcoholic steatohepatitis

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Nonalcoholic steatohepatitis (NASH) is associated with increased liver-related mortality. Disturbances in hepatic lipid homeostasis trigger oxidative stress and inflammation (ie, lipotoxicity), leading to the progression of NASH. This study aimed at identifying whether silibinin may influence the molecular events of lipotoxicity in a mouse model of NASH. Eight-week-old db/db mice were fed a methionine-choline deficient (MCD) diet for 4 weeks and treated daily with silibinin (20 mg/kg intraperitoneally) or vehicle. Liver expression and enzyme activity of stearoyl-CoA desaturase-1 and acyl-CoA oxidase, and expression of liver fatty acid-binding protein were assessed. Hepatic levels of reactive oxygen species, thiobarbituric acid-reactive substances (TBARS), 3-nitrotyrosine (3-NT), inducible nitric oxide synthase (iNOS), and nuclear factor kappa B (NFkB) activities were also determined. Silibinin administration decreased serum alanine aminotransferase and improved liver steatosis, hepatocyte ballooning, and lobular inflammation in db/db mice fed an MCD diet. Gene expression and activity of stearoyl-CoA desaturase-1 were reduced in db/db mice fed an MCD diet compared with lean controls and were increased by silibinin; moreover, silibinin treatment induced the expression and activity of acyl-CoA oxidase and the expression of liver fatty acid-binding protein. Vehicle-treated animals displayed increased hepatic levels of reactive oxygen species and TBARS, 3-NT staining, and iNOS expression; silibinin treatment markedly decreased reactive oxygen species and TBARS and restored 3-NT and iNOS to the levels of control mice. db/db mice fed an MCD diet consistently had increased NFkB p65 and p50 binding activity; silibinin administration significantly decreased the activity of both subunits. Silibinin treatment counteracts the progression of liver injury by modulating lipid homeostasis and suppressing oxidative stress–mediated lipotoxicity and NFkB activation in experimental NASH. (Translational Research 2012;159:477–486)

Abbreviations: AOX = acyl-CoA oxidase; ALT = alanine aminotransferase; FFA = free fatty acid; iNOS = inducible nitric oxide synthase; L-FABP = liver-fatty acid binding protein; MCD = methionine-choline deficient; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; NFkB = nuclear factor kappa B; NO = nitric oxide; PCR = polymerase chain reaction; RNS = reactive nitrogen species; ROS = reactive oxygen species; SD = standard diet; SCD-1 = stearoyl-CoA desaturase 1; TBARS = thiobarbituric acid-reactive substances; 3-NT = 3-nitrotyrosine

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Conflicts of Interest: none.

Submitted for publication October 27, 2011; revision submitted December 5, 2011; accepted for publication December 6, 2011.

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1931-5244/$ - see front matter

doi:10.1016/j.trsl.2011.12.003

AT A GLANCE COMMENTARY

Salamone F, et al.

Background

NASH is associated with increased liver-related mortality. No pharmacologic treatment has been shown to be effective for NASH. Polyphenols can modulate the molecular pathways involved in liver steatogenesis and are able to counteract oxidative stress and inflammation.

Translational Significance

We demonstrated that silibinin exerts antisteatotic effects because of changes in liver expression of key enzymes involved in lipid homeostasis. Moreover, silibinin counteracts liver injury progression via antioxidant and anti-inflammatory activity. On the basis of these findings, there is a significant molecular rationale for the use of silibinin in patients with NASH.

Nonalcoholic fatty liver disease (NAFLD) is a chronic metabolic disorder with significant impact on all-cause mortality.[1] NAFLD is independently associated with the features of the metabolic syndrome,[2,3] having insulin resistance as a common metabolic determinant.[4] NAFLD includes a wide spectrum of histologic lesions ranging from nonalcoholic fatty liver to nonalcoholic steatohepatitis (NASH).[5] NASH is characterized by hepatocellular damage (ie, ballooning and inflammation) and may have a fibrogenic evolution leading to liver-related morbidity and mortality.[1] Patients with NASH have increased liver content of free fatty acids (FFAs) derived from adipose tissue lipolysis and hepatic de novo lipogenesis.[6] An imbalance in the production and scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS), mainly derived from mitochondrial FFA oxidation, can cause hepatocyte injury and may trigger the activation of inflammatory signaling (eg, nuclear factor kappa B [NFkB]) in the liver.[7,8]

Several pharmacologic treatments have been proposed for NASH, but currently available drugs have been reported to have limited efficacy and safety.[9] Experimental studies suggest that some natural polyphenols may be effective in counteracting oxidative stress and inflammation in NASH.[10] Silibinin is a polyphenolic compound contained in silymarin, a mixture of flavonolignans extracted for milk thistle (*Silybum marianum*) seeds, widely used as hepatoprotectant, although its molecular effects are not fully understood. Potent scavenging properties have been demonstrated in hepatic and non-hepatic cells;[11,12] several *in vivo* studies showed that silibinin may exert beneficial effects in different types of liver injury[13,14] and in diabetes and its complications.[15]

Previous clinical findings evidenced the efficacy of silibinin on insulin resistance and liver injury, assessed by surrogate markers, in patients with NASH;[16] the improvement of liver histology after silibinin treatment was recently reported in a multicenter randomized controlled trial.[17] However, the molecular mechanisms associated with the hepatoprotective activity of silibinin in NASH remain to be elucidated. The current study aimed at clarifying whether silibinin may favorably affect lipogenesis, oxidative stress, and NFkB activation in a mouse model of NASH. To this aim, we examined the effects of silibinin administration in db/db mice fed a methionine-choline deficient (MCD) diet, an experimental model combining the features of the metabolic syndrome with the histologic pattern of NASH.[18,19] db/db mice fed an MCD diet partially conserve the db/db phenotype, mainly increased visceral adiposity,[19] while developing hepatocellular injury and inflammation typical of the MCD diet.[18,19]

METHODS

Animals and treatments. Eight-week-old male BKS.Cg-m+/+ Leprdb/J (db/db) obese mice and 8-week-old male heterozygous db/m lean control mice were purchased from Charles River Laboratories (Calco, Italy). Animals were maintained in a temperature- and light-controlled facility and permitted ad libitum consumption of water; db/db mice were fed an MCD diet (ICN Biomedicals, Costa Mesa, Calif) for 4 weeks; db/m mice were fed an MCD diet supplemented with methionine and choline (ICN Biomedicals), that is, a standard diet (SD), for the same period. Mice were distributed in 3 groups: Group I included 8 db/m mice fed a control diet and treated with vehicle (db/m + SD); group II included 8 db/db mice fed an MCD diet and treated with vehicle (db/db + MCD); group III included 8 db/db mice fed an MCD diet and treated with silibinin (db/db + MCD + silibinin). Silibinin dihydrogen succinate (Indena, Milan, Italy) was dissolved in saline and administered daily intraperitoneally at a dosage of 20 mg/kg of body weight. This dosage has been showed to be safe both in healthy volunteers[20] and in patients with chronic hepatitis C.[21,22] Treatment was administered for a 4-week period; at the end of treatment, animals were sacrificed after an overnight fast. Blood and liver samples were processed and