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FIELD OF VISION

Inhibition of apoptosis in the management of nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the developed world. The pathogenesis of NAFLD is multifactorial, involving obesity, insulin resistance, inflammation and oxidative stress. Accordingly, several treatments targeting these pathways have been evaluated in patients with NAFLD but have either shown limited efficacy or an unfavorable safety profile. On the other hand, increased hepatocyte apoptosis also appears to be implicated in the development and progression of NAFLD and recent pilot studies suggest that inhibition of apoptosis might represent a useful approach in this disease. However, several issues pertaining both to the efficacy and safety of this new class of agents remain unresolved and larger studies are required to clarify the role of this therapeutic modality in the management of NAFLD.

Key words: Apoptosis; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Fatty liver; Carcinogenesis; Cirrhosis; Caspase

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COMMENTARY ON HOT TOPICS

Nonalcoholic fatty liver disease (NAFLD) is a burgeoning health problem and is recognized as the main cause of chronic liver disease in the developed world[1,2]. It affects approximately 34%-46% of the general adult population in Western countries[3,4]. Moreover, the prevalence of NAFLD is substantially higher in obese and diabetic patients, reaching 70%[5-7]. Given the worldwide growing epidemics of obesity and type 2 diabetes mellitus, the prevalence of NAFLD is expected to rise further in the following years[1,5]. NAFLD covers a wide spectrum of histological abnormalities ranging from steatosis to the coexistence of steatosis with inflammation and a variable degree of fibrosis [nonalcoholic steatohepatitis (NASH)], to cirrhosis and even to hepatocellular carcinoma[1,8]. Patients with NAFLD, particularly those with NASH, have increased all-cause mortality compared with the general population, with cardiovascular disease and liver-related disease being the leading causes of death[9-11].

In light of the considerable prevalence of NAFLD and its associated increased mortality, there is a pressing need for identifying effective treatments for this disease. The pathogenesis of NAFLD is multifactorial, involving obesity, insulin resistance, inflammation, oxidative stress and increased hepatocyte apoptosis[8,12]. Accordingly, several treatments have been evaluated in this population, including lifestyle changes and pharmacological agents targeting the underlying pathogenetic mechanisms, including insulin-sensitizing, weight-reducing, antioxidant and antiinflammatory agents[1,12,13]. However, the evalu-

ated agents have either shown limited efficacy or have been associated with an unfavorable safety profile[1,12,13]. Accordingly, current therapeutic approaches propose lifestyle modifications including diet and exercise as first line treatment in patients with NAFLD[1]. However, diet and exercise are of limited efficacy and are characterized by low long-term adherence rates[1].

In this context, novel agents targeting hepatocyte apoptosis might represent a useful tool in the management of NAFLD. Apoptosis is a physiological, highly organized and genetically programmed form of cell death which contributes to body homeostasis by removing aged and damaged cells. Thus, apoptosis represents a major protective defense mechanism against a number of harmful factors including viral attacks and carcinogens[14]. However, aberrant hepatocyte apoptosis may induce hepatic injury and disease progression *via* up-regulation of inflammation and fibrosis[14-16]. Indeed, hepatocyte apoptosis is increased in NAFLD and correlates with the severity of inflammation and fibrosis[14-16]. Moreover, apoptosis is a main feature of NASH differentiating it from isolated steatosis and may also contribute to the progression from NASH to cirrhosis[16-18]. In experimental models, increased apoptosis appears to contribute to progression to hepatocellular carcinoma (HCC) independently from other carcinogens (*e.g.*, inflammation)[19]. It has been suggested that damaged hepatocytes become resistant to apoptotic death in more advanced NAFLD because of downregulation of proapoptotic molecules and upregulation of antiapoptotic mediators[19-21]. As a result, damaged cells escape apoptosis and high proliferation rates are observed leading to HCC[19-21].

Given the important role of apoptosis in NAFLD, a recently reported pilot study by Ratziu *et al*[22] evaluated the safety and efficacy of inhibition of hepatocyte apoptosis in this disease. This phase Ⅱ, randomized, double-blind, placebo-controlled, multicenter clinical trial evaluated GS-9450, an irreversible selective inhibitor of caspases 1, 8 and 9, in patients with NAFLD[22]. Caspases are intracellular proteolytic enzymes that are key effectors of the apoptotic process[14]. The study included 124 patients 18 to 75 years-old with biopsy-proven NASH and serum alanine aminotransferase (ALT) levels > 60 IU/L[22]. Exclusion criteria included histological findings of cirrhosis, HCC, platelets < 75 000/mm^3, neutrophils < 1500/mm^3, hemoglobin < 11.0 g/dL, creatinine clearance < 70 mL/min (estimated with the Cockcroft-Gault equation), weight lose > 4% within 8 wk before screening, daily alcohol consumption > 30 g in males and > 20 g in females, drug-induced fatty liver and liver damage attributed to other liver diseases (*e.g.*, viral hepatitis, autoimmune hepatitis and hemochromatosis). Patients with type 2 diabetes mellitus were eligible for inclusion in the study if they were not insulin-dependent, they were not under treatment with glitazones for at least 6 mo before screening, the onset of diabetes was within the last 10 years and there were no signs of peripheral diabetic neuropathy or gastroparesis. Patients were randomly assigned to receive GS-9450 1, 5, 10 or 40 mg or placebo once a day for 4 wk. All patients were required to follow a balanced lifestyle during the study. A follow-up of 4 wk followed the treatment period. The main efficacy endpoints were the change in serum ALT, aspartate aminotransferase (AST) and cytokeratin (CK)-18 fragment levels during the treatment period. CK-18 is a major cytoplasmic filament protein of the hepatocellular cytoskeleton that is cleaved mainly by caspase-3 during the apoptotic process leading to formation of CK-18 fragments[15]. Thus, CK-18 fragment levels reflect the extent of hepatocyte apoptosis[15].

Treatment with GS-9450 induced a significant, dose-dependent reduction in serum ALT levels whereas ALT levels did not change in the placebo group[22]. This reduction occurred as early as the third day of treatment. In the group that received 40 mg GS-9450, at week 4, only 2 patients (8%) were nonresponders (*i.e.*, had a decrease in ALT levels of < 10% relative to baseline) whereas 35% of patients showed normalization of ALT values. A dose-dependent reduction was also observed in serum AST levels in patients who received GS-9450. Among patients who received the highest GS-9450 dose, the percent of patients who had normal AST levels increased from 20% at baseline to 48% at the end of the treatment period. Serum CK-18 fragments decreased only in patients who were treated with 10 and 40 mg GS-9450 but this decrease did not differ from the change in the placebo group[22].

At 4 wk after treatment discontinuation, serum ALT levels returned to baseline levels in the groups that received 1, 5 and 10 mg GS-9450 but where lower than baseline in the 40 mg group[22]. This rebound effect was apparent from the first week of the follow-up period[22]. Serum AST levels increased within 1 wk of discontinuation of GS-9450 in all groups to modestly above baseline levels[22].

There was no change in markers of insulin resistance (serum glucose and insulin levels, homeostasis model of insulin resistance), serum γ-glutamyl transpeptidase levels, lipids or weight during treatment with GS-9450[22].

Regarding the safety of GS-9450, the majority of the adverse events recorded in patients treated with this agent were of mild to moderate severity and most were not attributed to GS-9450[22]. No serious adverse events were recorded during treatment with GS-9450. Moreover, there were no notable differences in the frequency of adverse events between the groups assigned GS-9450 and placebo[22].

Overall, the study of Ratziu *et al*[22] suggests that GS-9450 dose-dependently lowers serum ALT levels and is well-tolerated in patients with NAFLD. Previous studies evaluating this agent have also reported promising results. In a phase I clinical trial, GS-9450 was well-tolerated when administered to healthy individuals[23]. In a double-blind, placebo-controlled phase Ⅱa trial in patients with chronic hepatitis C, a disease also characterized by increased hepatocellular apoptosis, GS-9450 reduced serum

ALT levels[24]. Moreover, in a substudy of the latter trial, GS-9450 induced a moderate reduction in caspase-8 expression and a strong reduction in caspase-3 expression in peripheral T-lymphocytes[25].

Besides GS-9450, a wide range of pan-caspase inhibitors has been evaluated in pilot studies yielding encouraging results. IDN-6556, an irreversible, broad-spectrum caspase inhibitor, attenuated hepatocellular apoptosis and hepatic inflammation and fibrosis in animal models[26,27]. In humans, IDN-6556 was well-tolerated in a phase Ⅱ clinical trial by both normal volunteers and patients with elevated transaminase levels and lowered transaminase levels in the latter[28]. Moreover, in a phase I and Ⅱ clinical trial, IDN-6556 reduced aminotransferase levels in patients with chronic hepatitis C or NASH[29,30]. Another irreversible pan-caspase inhibitor, VX-166, reduced hepatocellular apoptosis, inflammation and fibrosis in experimental models but had a modest effect on ALT levels and markers of oxidative stress in animal models with established steatosis/steatohepatitis[31,32].

However, there are some concerns regarding the safety of GS-9450 and caspase inhibitors in general. There is a potential risk of carcinogenesis when apoptotic mechanisms are inhibited given the key role of apoptosis in protecting against tumor development[19,21]. The existing data regarding this possible association is meagre and controversial[21]. GS-9450 might theoretically be safer than pan-caspase inhibitors since it acts primarily on hepatocytes and blocks the activity of specific caspases. However, most information about the safety of caspase-inhibitors is from experimental models and therefore it is difficult to reach definite conclusions about their safety in humans[21]. The existing clinical studies are small and short in duration[22,24,28-30]; accordingly, larger and long-term studies are required to evaluate the carcinogenic potential, if any, of caspase inhibitors.

Another concern regarding the safety of caspase inhibitors is ALT overshoot, *i.e.,* elevation of ALT levels three times the baseline value after discontinuation of treatment, which could result in acute hepatic failure[29,30]. This adverse effect was observed in patients with chronic hepatitis C who were treated with the pan-caspase inhibitor IDN-6556 and could be due to massive apoptosis of hepatocytes, which escaped apoptosis during treatment, after the abrupt withdrawal of the drug[30]. In the study by Ratziu *et al*[22], although ALT values increased after discontinuation of GS-9450 and in some patients exceeded baseline levels, they did not reach three times the initial values. Therefore, GS-9450 might be safer than pan-caspase inhibitors but this has to be further evaluated in larger studies. It has been suggested that the risk of ALT overshoot might be reduced by the gradual instead of sudden removal of the caspase inhibitor but this remains to be evaluated in future studies[30]. On the other hand, the relapse of ALT levels after discontinuation of GS-9450 treatment suggests that long-term treatment will be necessary[22], limiting the clinical significance of ALT overshoot.

In addition to these safety concerns, there are some limitations regarding the evaluation of the efficacy of GS-9450 in the study by Ratziu *et al*[22]. In this study, the change in serum ALT and CK-18 fragment levels was used to assess the efficacy of GS-9450[22]. It is well established that both ALT and CK-18 fragment levels correlate with NAFLD severity[1,10,15,33]. However, more than 60% of patients with NAFLD have normal ALT levels, implying that normal ALT levels do not exclude the presence of the disease[1,4,33,34]. Moreover, the reduction in serum ALT levels correlates with the improvement in liver steatosis and inflammation but not fibrosis[1,35]. On the other hand, liver biopsy is the gold standard for the diagnosis, staging, monitoring and evaluation of drug response in NAFLD[1,3]. Given the short follow-up (4 wk), a second liver biopsy was not performed in the study by Ratziu *et al*[22]. Therefore, long-term studies that will evaluate the effects of GS-9450 on liver histology are needed before reaching definite conclusions on the efficacy of this agent.

In conclusion, despite its limitations, the pilot study by Ratziu *et al*[22] provides additional evidence that the inhibition of apoptosis might have a role in the management of NAFLD. Therefore, the efficacy and safety of this approach merits further evaluation in larger and longer-term studies. On the other hand, given that NAFLD has a multifactorial pathogenesis, a combination of agents targeting the multiple implicated mechanisms, including increased apoptosis, should be another focus of future studies. Finally, on the grounds of the strong genetic impact on NAFLD development and progression[36,37], investigating related genes and polymorphisms might allow the identification of patients who are at higher risk for progression of NAFLD and/or who might experience greater benefits from the different therapeutic approaches.

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