Hepatitis C Virus Infection and Nonalcoholic Steatohepatitis

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Abstract: Nonalcoholic fatty-liver disease (NAFLD) is one of the most prevalent liver diseases in the Western hemisphere. The rising rates of obesity and diabetes mellitus correlate with the increasing incidence of NAFLD, which is the hepatic manifestation of metabolic syndrome. Hepatitis C virus infection is another common cause of liver disease worldwide. Up to 70% of patients with chronic hepatitis C (CHC) will have concomitant steatosis. The presence of NAFLD has been implicated as a cause of lower viral response rates in CHC patients who are treated with pegylated interferon and ribavirin. This review will focus on the factors that lead to NAFLD in the setting of hepatitis C virus infection, including viral and host factors—in particular, inflammatory mediators, cytokines, and lipid peroxidation. This paper will also discuss the implications of NAFLD and nonalcoholic steatohepatitis regarding fibrosis progression, risk of hepatocellular carcinoma, and limitations with antiviral therapy.

Keywords
Hepatitis C virus, nonalcoholic fatty-liver disease, nonalcoholic steatohepatitis, insulin resistance

Worldwide, chronic hepatitis C (CHC) affects up to 200 million individuals—3% of the world's population—and has an estimated progression to cirrhosis as high as 20%.[1] Africa and the Middle East have the highest occurrence of CHC (>3%), with Northern Europe and the United Kingdom having the lowest occurrence of CHC (1.1%).[2] Approximately 3 million individuals are affected in the United States, with the National Health and Nutrition Examination Survey (NHANES) reporting an estimated prevalence of 1.3%.[3] Of the 6 genotypes of hepatitis C virus (HCV), type 1 is the most common in the United States; unfortunately, type 1 HCV is also one of the more difficult genotypes in which to achieve sustained virologic response (SVR) with antiviral therapy.

The contribution of nonalcoholic fatty-liver disease (NAFLD) to the total burden of chronic liver disease is increasing due to the rapidly increasing prevalence of obesity. NAFLD affects up to 46% of the US population and is considered to be the most common chronic liver disease in North America.[4] Although the risk of developing cirrhosis over time is minimal, a subset of NAFLD patients with nonalcoholic

steatohepatitis (NASH) has a greater risk for cirrhosis and hepatocellular carcinoma (HCC). It is estimated that approximately 12% of NASH patients will progress to cirrhosis over an 8-year span.[5]

With the high prevalence rates of NAFLD and CHC, it is expected that these 2 disease states will occur together in a certain proportion of patients. Reported estimates of the prevalence of hepatic steatosis in conjunction with CHC range from 30% to 70%, which is significantly higher than the prevalence rates of each disease entity individually.[6,7] The exact mechanism for development of hepatic steatosis in the setting of HCV infection is not very well understood, but it appears that the association is dependent on both host and viral factors.[8] The interplay between CHC and steatosis has been shown to have implications in the response to antiviral therapy, rate of disease progression, and risk of developing HCC.[9-11] This review will focus on the complex interplay of CHC and NASH by describing the pathogenesis of the disease states, implications of concurrent diseases, and potential strategies to improve SVR rates.

Pathogenesis of Hepatic Steatosis

Mechanisms of Steatosis in Nonalcoholic Fatty-Liver Disease and Nonalcoholic Steatosis

A "multi-hit" hypothesis has been proposed for the pathogenesis of NAFLD, which incorporates the interplay of insulin resistance, oxidative stress, and an inflammatory cascade.[12,13] Insulin resistance, as a result of obesity, increases the activity of hormone-sensitive lipase, which enhances lipolysis in visceral adipose tissue, thus resulting in the release of free fatty acids (FFAs). FFAs are preferentially converted to triglycerides (TGs) within hepatocytes through either esterification or oxidization by hepatic mitochondria. TGs, in combination with apolipoproteins, are packaged and transported as very-low-density lipoprotein (VLDL). The oxidation of TGs and the increased FFA load can thus lead to hepatic steatosis and possible fibrosis through hepatic injury caused by the oxidized by-products.

De novo lipogenesis (DNL), referring to FFA synthesis in the liver, also plays a role in steatosis. As hyperinsulinemia leads to an increase in the expression of sterol regulatory element–binding protein (SREBP), FFA levels are increased through DNL, leading to accumulation of excess fat within the hepatocyte.[14] Further increases in DNL can occur due to overexpression of carbohydrate response element–binding protein secondary to hyperglycemia. The largest contributors to steatosis are increased FFA influx to the liver (60%) and DNL (26%).[15]

The term lipotoxicity has recently been invoked to describe the harmful effects of elevated FFA levels and ectopic fat accumulation that lead to organ dysfunction and cell death.[16] The storage capacity of tissues, including skeletal muscle and liver, is exceeded in obese patients due to the combination of high dietary fat intake and increased FFA production from insulin resistance. FFAs not only undergo limited oxidation but also produce reactive oxygen species (ROS) and activate other inflammatory cascades through more toxic pathways. The excess accumulation of FFAs places significant stress on the endoplasmic reticulum, which results in apoptosis through the accumulation of unfolded proteins.[17,18] Another mechanism by which saturated FFAs can induce mitochondrial dysfunction and oxidative stress is through direct lysosomal disruption.[19]

As FFA-induced hepatic steatosis develops, the interaction among inflammatory mediators, oxidative stress, and abnormal apoptotic mechanisms plays a critical role in the pathogenesis of NAFLD and NASH. Tumor necrosis factor-α (TNF-α) and adiponectin have been implicated in this pathogenesis. TNF-α levels have been shown to be elevated in obese patients, likely due to the infiltration of macrophages into the liver, resulting in increased fat deposition.[20] The proposed mechanism of TNF-α–induced hepatic injury indicates that inhibition of mitochondrial electron transport and release of ROS can stimulate lipid peroxidation.[21] Adiponectin, on the other hand, plays a protective role by increasing fatty acid oxidation and inhibiting hepatic gluconeogenesis, but low levels of adiponectin in mouse models were found to correlate with severity of hepatic inflammation.[22,23]

Mechanisms of Hepatitis C Virus–Induced Steatosis

The exact mechanism underlying HCV-induced steatosis is poorly understood, but the interaction of host and viral factors plays a major role in this process. Limited findings suggest that obesity plays a direct role in the development of steatosis in the setting of HCV infection. The contribution of obesity to steatosis appears to be greater in patients with genotype 1 HCV.[24] The distribution of fat may potentially play an important role, as visceral fat is a risk factor for insulin resistance and NAFLD. Adinolfi and colleagues reported that more than half of patients with central obesity had steatosis compared to only 43% of those with peripheral obesity.[24]

Insulin resistance is more prevalent among HCV-infected patients than noninfected patients.[25] Data from NHANES III show that, among patients greater than 40 years of age, CHC is associated with a higher prevalence of type 2 diabetes, ranging from 32% to 70%.[26,27] In a prospective case-cohort study of 1,084 adults, Mehta and colleagues determined that patients with CHC were twice as likely to develop diabetes compared to patients without CHC, irrespective of other risk factors.[28]

Genotype-specific mechanisms have been implicated in the development of insulin resistance and its complication

of hepatic steatosis. In one of the earliest animal models to assess this association, HCV genotype 1b core gene transgenic mice were shown to have insulin resistance in the absence of weight gain, revealing that HCV infection alone is a risk factor for insulin resistance.[28] The primary determinant of insulin resistance in these transgenic mice was the lack of inhibitory effect of insulin on hepatic gluconeogenesis.

The molecular mechanisms that underlie the induction of insulin resistance by HCV focus on insulin receptor substrates 1 and 2 (IRS-1/2), which are important in the insulin signaling pathway. Genotype 1 HCV infection downregulates IRS-1/2 through upregulation of TNF-α and suppressor of cytokine signaling–3 (SOC-3), leading to increased hepatic glucose output and worsening of hyperglycemia.[29] A study of transfected cells with genotype 2a HCV also linked SREBP upregulation to the phosphatidylinositol 3–kinase (PI3-K) pathway, which strengthens the argument for the interplay between HCV infection and insulin resistance.[30]

Host lipid metabolism has also been implicated in the development of steatosis caused by HCV infection. Perlemuter and coauthors demonstrated in a transgenic mouse model that overexpression of HCV core protein caused reduction in TG transfer protein activity, resulting in reduced secretion of VLDL and accumulation of TGs within hepatocytes.[31] SREBP upregulation has not only been demonstrated via the PI3-K pathway but also occurs via direct stimulation from HCV infection during the early viremic phase. SREBP stimulation, as demonstrated by Su and colleagues, directly correlated with a 2-fold increase in HCV replication levels in conjunction with increased FFA production.[32]

Mechanisms of Genotype-Specific Steatosis

Genotype-specific associations between steatosis and HCV infection have been confirmed in several studies, particularly among patients infected with genotype 3 HCV.[24,33-36] Genotype 3 HCV has been correlated with higher grades of steatosis independent of host insulin resistance or other related factors, including NAFLD.[35] The degree of steatosis in patients with genotype 3 HCV is directly related to HCV viral load, and patients who achieve SVR have been shown to have significant improvement in steatosis post-treatment.[24,37-39] The mechanism of steatosis has been suggested to occur through direct interference with TG secretion. Jackel-Cram and coauthors demonstrated that the genotype 3a HCV core protein leads to SREBP-1–mediated upregulation of fatty acid synthase, a key enzyme in lipid synthesis, when compared to genotype 1b HCV.[40] A follow-up study by Waris and colleagues confirmed the direct stimulation of SREBP by HCV core protein and NS4b proteins derived from genotype 3a HCV.[30]

Patients with genotype 1 HCV infection, on the other hand, are affected by metabolic risk factors, including visceral obesity. In patients with genotype 1 HCV, no correlation has been shown between the degree of steatosis and viral load; also, SVR has not been shown to improve viral-induced steatosis. Instead, studies have shown that activation of inflammatory pathways and underlying obesity and insulin resistance lead to the development of steatosis in genotype 1 HCV patients.[41-44] Using HCV core gene transgenic mice, Halse and coauthors demonstrated an elevated level of TNF-α, which was shown to induce insulin resistance through inhibition of IRS-1/2.[45] Genotype 1 HCV has been shown to inhibit microsomal TG transfer protein via HCV core protein interaction with apolipoprotein-A2 (apoA2), thus leading to hepatocellular steatosis.[46] NS5A, a nonstructural HCV protein, has also been implicated in the development of steatosis through interactions with apolipoprotein-A1 and apoA2, which lead to alterations in cholesterol transport within the hepatocyte.[47,48] Figure 1 summarizes the role of HCV in the development of hepatic steatosis.

Relationship of Nonalcoholic Steatohepatitis and Chronic Hepatitis C

The prevalence of concomitant NASH and CHC is estimated to be approximately 18%, but understanding of the impact of NASH on CHC patients and the associated risk factors is limited due to lack of prospective studies.[49] Younossi and coworkers demonstrated that risk factors including HCV genotype 3 and central obesity were independently associated with the presence of NASH and CHC infection.[49] However, the clinical significance of NASH in CHC patients has not been clearly characterized. Bedossa and coauthors performed a prospective study evaluating 296 liver biopsies in treatment-naïve CHC patients.[50] The prevalence of NASH in the study population was 9%, as determined by identifying pathologic criteria that are present in NASH but not in CHC: perisinusoidal fibrosis and ballooning hepatocytes. Specimens with concomitant CHC and NASH showed a greater degree of steatosis and more advanced fibrosis compared to controls. The study supported the idea that the development of NASH in the setting of CHC is a result of severe steatosis and is independent of the etiology of steatosis or HCV genotype.

Understanding the role of fatty-liver disease in CHC is important, as the interaction may play a significant part in the progression of liver disease. Several studies have demonstrated the relationship of NAFLD to fibrosis progression in the setting of CHC infection, which is suspected to be mediated by the combination of insulin resistance and steatosis. Adinolfi and colleagues confirmed that patients with higher grades of steatosis were more likely to have worsening hepatic disease progression and higher degrees of fibrosis.[51] Lo Iacono and coworkers further sup-

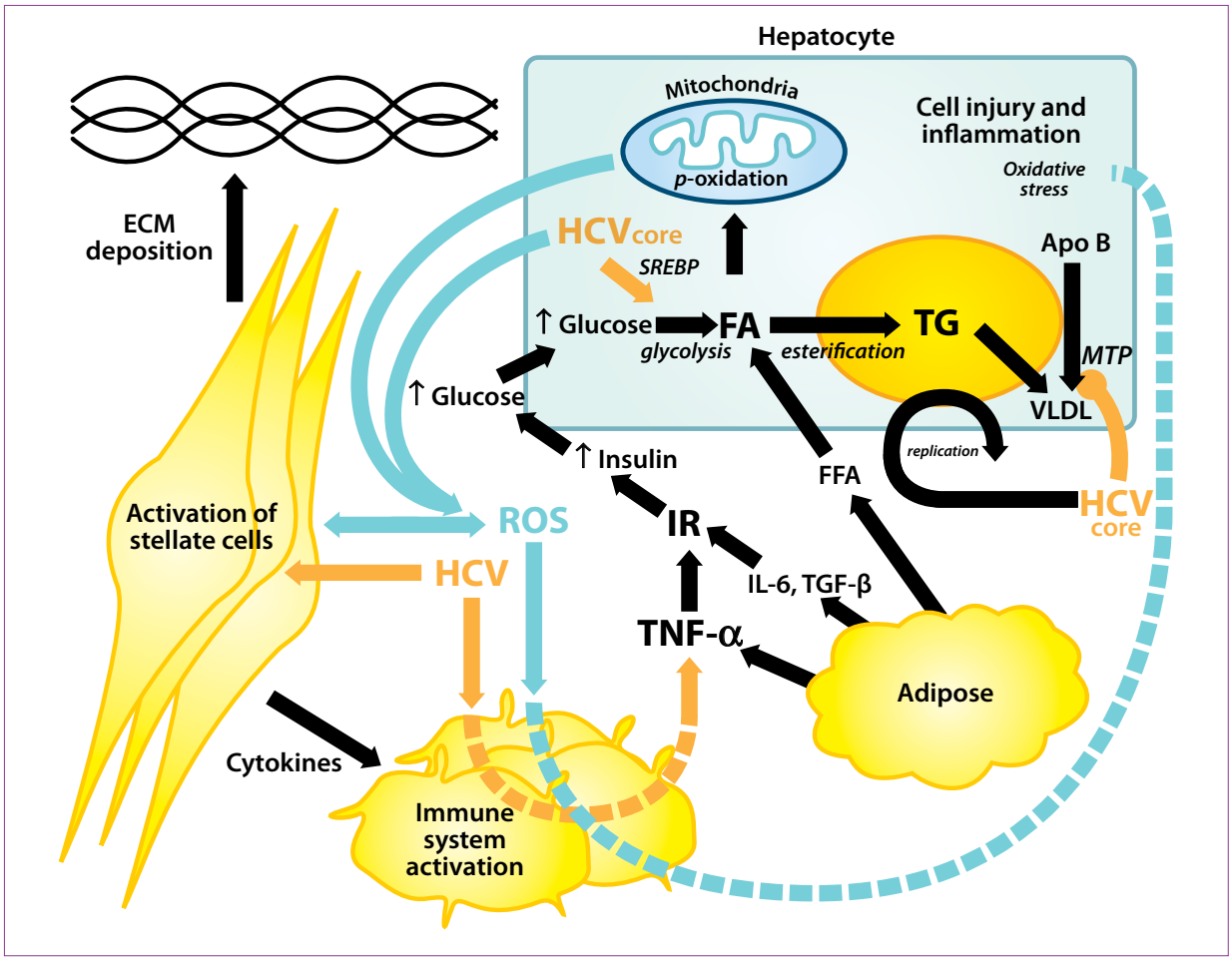

Figure 1. Summary of interactions of hepatitis C virus (HCV) on hepatic steatosis, insulin resistance (IR), oxidative stress, and hepatocellular injury. Arrows signify increases or activation; blunt ends signify inhibition.

Apo B=apolipoprotein B; ECM=extracellular matrix; FA=fatty acid; FFA=free fatty acid; HCV core=HCV core protein; IL-6=interleukin-6; MTP=microsomal triglyceride transfer protein; ROS=reactive oxygen species; SREBP=sterol regulatory element-binding protein; TG=triglyceride; TGF-β=transforming growth factor-β; TNF-α=tumor necrosis factor-α; VLDL=very-low-density lipoprotein.

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ported this finding by showing that steatosis and increased inflammatory activity were independently associated with advanced fibrosis.[52] Based on genotypic evaluation, studies have supported a higher predilection for fibrosis in patients with genotype 3 HCV infection, even though steatosis and fibrosis progression are evident in all genotypes.[53] Despite the genotype association, Sanyal and coauthors demonstrated that patients with concomitant CHC and features of NAFLD were more likely to have higher degrees of fibrosis compared to patients with CHC alone.[54]

The mechanistic role insulin resistance plays in fibrosis progression is not clearly understood. The pathogenesis is thought to mirror the process that occurs in NASH, with a heightened inflammatory response leading to hepatic stellate cell proliferation. In CHC patients with steatosis, there is an associated increase in production of cytokines, including TNF-α, along with an increase in cytochrome P450 2E1 levels, resulting in increased levels of ROS that ultimately are associated with fibrosis progression.[55] Hyperinsulinemia, which occurs secondary to insulin resistance, has been shown to directly stimulate hepatic stellate cells, leading to the deposition of extracellular matrix proteins and upregulation of cytokines, which lead to worsening fibrosis.[56] Leptin levels have also been implicated in the pathogenesis of fibrosis progression. Piche and coworkers demonstrated that elevated leptin

levels correlated with an increase in TNF-α expression, suggesting that the synergy potentially leads to leptin-induced hepatic fibrosis.[57]

Effect of Fatty-Liver Disease on Hepatocellular Carcinoma

A limited amount of data is available on the correlation between steatosis, CHC infection, and the development of HCC. There have been studies suggesting that CHC and NAFLD lead to an increased risk of developing HCC.[58] El-Serag and coauthors demonstrated that diabetes mellitus increased the risk of developing NAFLD and HCC independent of viral hepatitis and/or demographic characteristics.[59] It is reasonable to assume that patients with underlying CHC and steatosis are at an increased risk of developing HCC. A study by Ohata and coworkers that assessed a Japanese cohort of CHC patients found steatosis to be an independent risk factor for the development of HCC.[10] On multivariate analysis, this study concluded that hepatic steatosis, age, cirrhosis, and no prior interferon (IFN)-α treatment were all independent risk factors for HCC.

The pathogenic mechanisms linking steatosis and HCC are not clearly understood. Several studies have reported a possible role for oxidative stress and the ROS that are produced with steatosis and insulin resistance, which may predispose patients to carcinogenesis through cellular gene mutations.[60-62] Moriishi and colleagues supported this theory using *PA28Y* gene knockout mice.[63] This gene interacts with the HCV core protein and regulates insulin signaling, and lack of *PA28Y* in these mice halted the development of steatosis and subsequent HCC. However, the correlation between steatosis and HCC remains an area of contention, and further studies are needed to better clarify the pathogenesis linking steatosis to the risk of developing HCC. More data could potentially allow practitioners to modify the risk of HCC in patients with CHC by focusing on interventions in those with concomitant NAFLD.

Effect of Fatty-Liver Disease on Hepatitis C Virus Treatment

The concomitant presence of steatosis and CHC not only increases the likelihood of developing HCC but also impacts the response rate with IFN-α–based therapy for CHC. Data have suggested a reduction in SVR rates with combination IFN-α and ribavirin therapy in patients with CHC, especially those with steatosis greater than 30%.[64] Several studies have corroborated the significant role hepatic steatosis plays in reducing SVR rates, and these studies have also shown hepatic steatosis to be an independent risk factor for the limited response to antiviral therapy seen in CHC patients.[39,64,65] Poynard and coworkers found a statistically significant difference in SVR rates between patients with steatosis and patients without steatosis.[11] These results were confirmed in a similar multicenter randomized study in which treatment-naïve CHC patients with steatosis showed a reduction in viral clearance compared to patients without steatosis, irrespective of genotype.[65]

As hepatic steatosis and obesity are both independent risk factors for the reduction in viral clearance rates among CHC patients, one of the unifying factors at the root of these conditions is likely insulin resistance. Romero-Gomez and coauthors demonstrated that, in addition to causing hepatic steatosis and leading to fibrosis progression, insulin resistance also correlates with a reduction in SVR rates among CHC patients, especially patients with genotype 1 HCV.[66] Unfortunately, the specific mechanism involved in the interaction among insulin resistance, obesity, hepatic steatosis, and treatment response is not clearly understood. It has been theorized that, in obese patients with CHC, bioavailability of IFN-α is reduced in conjunction with a change in its pharmacokinetics. Giannini and coauthors proposed that steatosis in obese HCV-infected patients leads to an increased accumulation of lipid droplets within hepatocytes, and these droplets effectively act as a physical barrier between the virus and treatment drugs.[67] As obese patients are known to have poor lymphatic circulation, this effect can also limit the serum levels of pegylated IFN-α, leading to a reduction in SVR rates.[68]

Obesity can also potentially affect response rates through modifications in the IFN-α signaling pathway. Cellular signaling induced by IFN-α can be inhibited by HCV through the overexpression of SOC-3, especially in patients with genotype 1 HCV.[69] Obese patients are found to have increased messenger RNA expression of SOC-3, which inhibits insulin signaling and IFN-α expression, leading to lower SVR rates in treatment nonresponders. Oxidative stress could also potentially play a minor role in viral response rates due to its contribution to the development of hepatic steatosis. Such stress has been found to inhibit IFN-α–induced antiviral gene expression by blocking the janus kinase/signal transducer and activator of transcription pathway.[70]

Treatment Considerations in Patients with Concomitant Fatty-Liver Disease and Hepatitis C Virus Infection

Treatment strategies for NAFLD have focused on modification of risk factors—including obesity, diabetes mellitus, and hyperlipidemia—along with direct therapies such as insulin sensitizers, antioxidants, weight reduction, and cytoprotective agents.[71,72] The mainstay of therapy for fatty-liver disease has primarily been lifestyle modification, including exercise and weight reduction, but no definitive therapies have been

recommended and/or fully studied in patients with concomitant fatty-liver disease and HCV infection.

Weight Reduction in Patients with Fatty-Liver Disease and Hepatitis C Virus Infection

Weight reduction, typically achieved via diet and exercise, has been the mainstay of therapy for patients with fatty-liver disease. Caloric restriction, pharmacotherapy, exercise, and surgical weight loss have shown a modest improvement in steatosis in select patients.[72] Unfortunately, formal studies evaluating the benefit of weight reduction in patients with steatosis and HCV infection are lacking. Hickman and colleagues evaluated 19 patients with concomitant steatosis and HCV infection (10 of whom had paired liver biopsies) who underwent a 3-month exercise regimen.[73] The authors were able to demonstrate that weight loss in CHC patients may be associated with reduction in steatosis and improvement in fibrosis. Adherence to a strict, low-calorie diet for 3 months with a 10% weight reduction prior to CHC therapy has shown improvement in SVR rates among patients receiving combination IFN-α and ribavirin therapy.[74] Studies evaluating the benefit of weight loss in patients undergoing CHC therapy are still limited, but weight loss can be considered as an important adjunct to lifestyle modifications when treating CHC patients.

Statins and Hepatitis C Virus Infection

Hyperlipidemia is a common occurrence in patients with concomitant NAFLD and HCV infection. Lipids, especially cholesterol, have been implicated in the replication process of HCV, as it has been proposed that HCV enters hepatocytes via low-density lipoprotein (LDL) receptors.[75] One of the potent stimulators of LDL receptors is hydroxy-3-methylglutaryl-coenzyme A reductase, which is the main enzyme targeted by statins. Several in vitro studies have demonstrated that the use of statins reduces the ability of HCV to replicate.[76,77] A further study by Ikeda and coworkers demonstrated a statin-specific effect in terms of a drug's ability to maintain a 50% reduction in median inhibitory concentration, with fluvastatin showing the highest antiviral activity at the lowest dose, compared to other statins.[78] In a retrospective evaluation by Harrison and colleagues, data from the IDEAL trial were analyzed, and higher SVR rates were found in hyperlipidemic CHC patients who were receiving preemptive statin therapy.[79] Rao and coauthors demonstrated improvement in SVR rates in diabetic and nondiabetic patients who were treated with a combination of antiviral therapy and statins.[80] A recent prospective study evaluating the use of rosuvastatin in combination with IFN-α and ribavirin correlated the use of these drugs with improvement in SVR rates and resolution of steatosis and fibrosis.[81] Statins thus appear to be a viable option as an adjunct therapy in patients with steatosis and CHC, but more large, prospective, randomized studies need to be performed to evaluate the treatment response.

Pharmacotherapy for Fatty-Liver Disease and Hepatitis C Virus Infection

A reduction in oxidative stress, which is a key component in the pathogenesis of steatosis associated with HCV infection, is another ideal mechanism for possible therapeutics. Melhem and coauthors demonstrated a reduction in viral load and histologic improvement in CHC patients who received pretreatment antioxidant therapy.[82] However, the clinical significance of this study is limited due to the lack of a placebo control. The use of the antioxidant d-α-tocopherol has been shown to reduce the rate of fibrosis progression via inhibition of stellate cell activation, thereby limiting stellate cell–induced fibrogenesis in CHC patients.[83] Look and coworkers demonstrated a significant reduction in viral load when patients received combination therapy including vitamin E and IFN-α.[84] Even though early studies have shown some promise, further prospective trials involving antioxidant therapy have shown limited benefit in terms of SVR.

Several studies have shown that the presence of insulin resistance correlates with higher HCV viral loads and is associated with NASH.[27,85] The thiazolidinedione class of drugs, including rosiglitazone and pioglitazone, has demonstrated histopathologic benefit in patients with NASH, in part via improvement of underlying insulin resistance.[86] In a select group of Egyptian patients with genotype 4 HCV, pioglitazone showed improvement in insulin resistance and an increase in SVR rates.[87] However, this benefit has not been adequately demonstrated in patients with genotype 1 HCV.[88] Metformin, a biguanide agent that decreases hepatic gluconeogenesis, has been shown to reduce HCV-induced insulin resistance and improve SVR rates in female patients with genotype 1 HCV.[89] However, evidence regarding the use of metformin therapy is conflicting. Merat and coworkers demonstrated no significant improvement in SVR rates in CHC patients, irrespective of genotype status.[90] Newer data regarding combination therapy with direct-acting antiviral agents (telaprevir [Incivek, Vertex] or boceprevir [Victrelis, Merck]) plus IFN-α and ribavirin show improved SVR rates irrespective of negative predictive factors, including diabetes, with insulin resistance having limited impact on treatment outcomes.[91,92] With such a limited number of studies involving direct correlation of SVR with insulin resistance therapy, further evaluation with clinical trials is warranted to show clinical benefit.

Conclusion

CHC is one of the most common causes of liver disease in the Western world. Liver disease progression and risk of HCC are largely influenced by the presence of NAFLD and insulin resistance. The concomitant influence of NAFLD or NASH and insulin resistance, derived from both host and viral factors, has led to difficulty in curative therapy. Even though the prevalence of obesity is rising, therapeutic considerations for NASH or NAFLD are limited, which has led to difficulty in management of HCV-infected patients. Further studies are needed to evaluate the benefit of fatty-liver disease therapy in combination with HCV therapy.

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