Mechanisms and significance of liver steatosis in hepatitis C virus infection

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Abstract

The pathogenesis of liver damage associated with chronic hepatitis C virus (HCV) infection is thought to be largely immunomediated. However, some frequent histopathological features, such as steatosis, suggest a direct cytopathic effect of HCV. The direct responsibility of HCV in the pathogenesis of steatosis is shown by: (1) the association with HCV genotype 3 infection, suggesting that some viral sequences are involved in the intracellular accumulation of lipids; (2) the correlation between severity of steatosis and HCV replication levels; (3) association between response to treatment and disappearance of steatosis. Experimental studies have shown that the nucleocapsid protein of HCV (core protein) is capable and sufficient to induce lipid accumulation in hepatocytes. Moreover, the observation that chronic hepatitis C patients have reduced serum levels of ApoB suggests an interference with the very-low density lipoprotein (VLDL) assembly, although other mechanisms are possible. In patients with sustained virological response induced by antiviral therapy, such levels are normalized. Other observations suggest that the pathogenesis of steatosis in chronic hepatitis C is not solely due to HCV. The origin of the mild steatosis observed in most patients may be metabolic, since its severity correlates with body mass index and insulin resistance. Most studies have shown a correlation between presence and/or severity of steatosis and fibrosis stage, but it is unclear whether this effect is direct or mediated by the associated insulin resistance, increased susceptibility to apoptosis, or by inflammatory cytokines. Finally, steatosis negatively influences the rate of response to antiviral treatment, as confirmed by large clinical trials. Management of steatosis in chronic hepatitis C requires knowledge of its pathogenesis and may involve both life-style changes and pharmacological interventions, although the latter remain largely experimental.

INTRODUCTION

The hepatitis C virus (HCV) is a major cause of chronic liver disease with an estimated 170 million people infected worldwide. The spectrum of severity of the liver disease associated with HCV varies widely from non-specific, minimal inflammatory changes to cirrhosis and hepatocellular carcinoma[1]. The rate of progression of chronic hepatitis C is also variable, depending on many cofactors, mostly host-related, such as age, gender, alcohol consumption, overweightness and coinfections[2,3]. Steatosis, defined as an increased fat content of the liver, essentially accounted for by triglycerides, has been recognized as one of these factors capable of influencing both liver fibrosis progression and the rate of response to interferon-alpha-based therapy. Steatosis is a common lesion associated with many conditions affecting the liver, some of which, like overweightness and alcohol consumption, being very frequent. Interestingly, however, the proportion of chronic hepatitis C patients with steatosis is higher than one would predict by simple chance association, suggesting a direct role of HCV, at least in some cases, in the intrahepatic accumulation of triglycerides. This proportion is indeed so high that, in the pre-serology era, steatosis has been used as a diagnostic tool to identify patients with non-A, non-B hepatitis[4,5].

The proper appreciation of fatty liver associated with chronic hepatitis C is important due to its clinically significant consequences. The scope of this review is to discuss the pathogenetic, clinical and therapeutic aspects related to steatosis in chronic hepatitis C patients.

VIRAL STEATOSIS IN CHRONIC HEPATITIS C

The reported prevalence of steatosis in patients with chronic hepatitis C varies between 40% and 80%,
Some of the major mechanisms leading to steatosis in patients with chronic hepatitis C. HCV, especially (but not only) genotype 3a, has been shown to be directly involved in triglyceride accumulation by several mechanisms: activation of fatty acid neosynthesis via SREBP-1c (2) and RXRα (3), impaired degradation through down-regulation of CPT-1 (4) and PPARα (5), and inhibition and/or down-regulation of MTP (6). Another potential mechanism (not shown) is the cell damage induced by reactive oxygen species consequent to localization of the HCV core protein in the mitochondria. This may also trigger lipid peroxidation of microsomal membranes, leading to impaired VLDL secretion. In patients who are insulin resistant, the major mechanism leading to steatosis is the free fatty acid overflow from adipose tissue (1). Hyperglycemic/hyperinsulinemic states are also associated with activation of fatty acid neosynthesis via SREBP-1c (2) and impaired degradation via down-regulation of CPT-1 (4). It has to be added that HCV may contribute to insulin resistance by impairing the IRS-1 signaling in hepatocytes by multiple mechanisms. Not shown in the figure are also the interference with ApoB synthesis and the activation of MTP reported in insulin resistant states: the latter phenomenon may be partially counterbalanced by the strong inhibition of MTP observed in patients with predominantly viral steatosis.

Figure 1 Some of the major mechanisms leading to steatosis in patients with chronic hepatitis C. HCV, especially (but not only) genotype 3a, has been shown to be directly involved in triglyceride accumulation by several mechanisms: activation of fatty acid neosynthesis via SREBP-1c (2) and RXRα (3), impaired degradation through down-regulation of CPT-1 (4) and PPARα (5), and inhibition and/or down-regulation of MTP (6). Another potential mechanism (not shown) is the cell damage induced by reactive oxygen species consequent to localization of the HCV core protein in the mitochondria. This may also trigger lipid peroxidation of microsomal membranes, leading to impaired VLDL secretion. In patients who are insulin resistant, the major mechanism leading to steatosis is the free fatty acid overflow from adipose tissue (1). Hyperglycemic/hyperinsulinemic states are also associated with activation of fatty acid neosynthesis via SREBP-1c (2) and impaired degradation via down-regulation of CPT-1 (4). It has to be added that HCV may contribute to insulin resistance by impairing the IRS-1 signaling in hepatocytes by multiple mechanisms. Not shown in the figure are also the interference with ApoB synthesis and the activation of MTP reported in insulin resistant states: the latter phenomenon may be partially counterbalanced by the strong inhibition of MTP observed in patients with predominantly viral steatosis.

Historically, impaired secretion of lipids from the infected hepatocyte has been the first proposed mechanism of HCV-induced steatosis. In fact, serum levels of apolipoprotein B (ApoB) and cholesterol are reduced in chronic hepatitis C patients in whom steatosis responds to antiviral therapy[14,17], suggesting that HCV may interfere with the very-low density lipoprotein (VLDL) assembly and/or secretion. The disappearance of fatty liver in sustained virological responders to antiviral therapy correlates with normalization of ApoB and cholesterol levels[14,17]. Hypocholesterolemia in patients with chronic hepatitis C has been reported to be specifically associated with genotype 3[9]. Thus, clinical data suggest that HCV may interfere with VLDL secretion, a defect corrected by antiviral treatment.

In vitro studies and the transgenic mouse model have both suggested that the HCV core protein is sufficient to induce a lipid accumulation in hepatocytes[20-22]. This viral protein is localized on the surface of lipid droplets, and its over-expression seems to further stimulate the formation of lipid droplets. These models have predominantly used genotype 1-derived constructs, but recent work has reported similar results using other viral genotypes, including type 3a, which seems to be the most efficient in terms of fat accumulation[24]. In fact, although some degree of intra-hepatocyte fat deposition occurs with all viral genotypes, the genotype 3 core protein expression results in about 3-fold fat accumulation with respect to genotype 1[24], in agreement with the clinical evidence. Based on the experimental model of the transgenic mouse, the HCV core protein seems to inhibit the microsomal triglyceride transfer protein (MTP) activity[25]. Since this enzyme plays a key, rate-limiting role in VLDL assembly, the consequence of its inhibition is the accumulation of triglycerides, i.e. steatosis. A direct interaction between core protein and MTP is unlikely, as it would require the secretion of the viral protein into the endoplasmic reticulum lumen, which has not been reported. However, the MTP inhibition may still be indirect. Strangely enough, transgenic mice for the HCV core protein have normal ApoB levels in serum[26]. Were it not for this discrepancy, this mouse model may be an ideal candidate to study the HCV-related steatosis seen in chronic hepatitis C patients. Recent data in human liver are, however, in agreement with this proposed mechanism, since the intrahepatic levels of MTP mRNA were reduced in patients with chronic hepatitis C, especially those with steatosis and/or genotype 3[27]. According to another experimental model, the HCV core protein may accumulate in the mitochondria and
induce liver damage via the production of reactive oxygen species. These effects were prevented by a mitochondrial electron transport inhibitor. In HCV transgenic mice, increased intrahepatic lipid peroxidation products occurred in response to carbon tetrachloride. The subsequent production of ROS may result, among other effects, in the peroxidation of membrane lipids and structural proteins, such as those involved in the trafficking and secretion apparatuses. This would then block VLDL secretion, leading to steatosis. Furthermore, the intracellular accumulation of triglycerides would further contribute to the pathogenesis of steatosis by providing the fuel for continuing lipid peroxidation, in a typical free radical-driven amplification reaction.

HCV may also induce steatosis via ex novo synthesis of fatty acids. For example, HCV has been reported to upregulate the sterol regulatory element binding protein-1c (SREBP-1c) signaling pathway. SREBP-1c is a transcription factor leading to the up-regulation of enzymes involved in de novo lipogenesis, an event that can favor intracellular accumulation of triglycerides. Chimpanzees experimentally infected with HCV show an increased intrahepatic activity of enzymes involved in lipogenesis, such as ATP citrate lyase, which are regulated by SREBP-1c. The HCV core protein may additionally bind to and activate the DNA-binding domain of the retinoid receptor α (RXRα), a transcriptional regulator that controls many cellular functions, including cellular lipid synthesis. On the other hand, accumulation of fat in hepatocytes transiently expressing the HCV core protein seems to depend on the presence of exogenous lipids, which indirectly decreases the likelihood of a significant fatty acids neosynthesis activated by this viral protein. However, one cannot rule out that other viral proteins may activate the neosynthesis of fatty acids.

HCV may finally cause steatosis by impairing fatty acid oxidation. Transfection of hepatoma cells with the HCV core protein is followed by a reduced expression of peroxisome proliferators-activated receptor α (PPARα), a nuclear receptor regulating several genes responsible for fatty acid degradation. These same authors have also reported a down-regulation of mitochondrial carnitine palmitoyl transferase-1 (CPT-1), the rate-limiting enzyme of mitochondrial β-oxidation, which is the main catabolic pathway of fatty acids, and of the acyl CoA oxidase (AOX). A reduced expression of CPT-1 in the liver of chronic hepatitis C patients has been reported recently. However, the down-regulation of several genes, such as CPT-1 and AOX, is transcriptionally controlled by PPARα. Thus, the reported effects may be secondary to down-regulation of PPARα. PPARα mRNA is significantly reduced in the liver of patients infected with genotype 3 compared to genotype 1, and down-regulation of PPARα mRNA in chronic hepatitis C has been reported at least by another group. Overall, the data support the hypothesis that HCV core protein may modulate the expression of various lipid degradation-associated genes, possibly via the down-regulation of PPARα.

The search for the viral sequences responsible for the genotype-specific effects on triglyceride accumulation has been so far elusive. No single mutation has been identified as being responsible for steatosis, suggesting that more complex mutation clusters may be involved in virally-driven steatosis. The C-terminal signal sequence of the core protein appears particularly interesting. In this domain, the core protein of genotype 3a contains several unique mutations that, alone, or more likely in combination, may confer the steatogenic phenotype.

**STEATOSIS OF OTHER CAUSES OCCURRING IN HEPATITIS C**

The HCV-induced steatosis may co-exist with a fatty liver due to other causes. In chronic hepatitis C patients who do not drink alcohol and are infected with non-3a genotypes, the most frequent correlate of fatty liver is an increased body mass index. Not only being obese, but also being merely overweight (BMI higher than 25 but less than 30) is an independent risk factor for hepatic steatosis in patients infected with HCV. In genotype 1 infection, as observed in patients with non-alcoholic fatty liver disease (NAFLD), the fat distribution seems more important than total fat in determining steatosis. Visceral obesity, rather than merely increased BMI, seems to play a major role in the development of HCV-related steatosis. In initial studies, when genotype was taken into account, it soon became clear that there was no association between BMI and the prevalence and the severity of steatosis in genotype 3 infected patients whereas, among patients with non-3 genotype, steatosis correlated with BMI. These findings have been repeatedly confirmed and when patients with risk factors for NAFLD are excluded genotype 3 infection remains the single most important, independent predictor of steatosis.

This BMI-associated (or “metabolic”) steatosis is not or very little modified by successful antiviral therapy. However, a partial amelioration of steatosis is sometimes observed also in genotype non-3 infection following HCV eradication. This implies that the assignment of either of the two types of steatosis (viral and metabolic) to a specific viral genotype should not be so clear-cut. Careful analysis of experimental and clinical data indicates that also HCV genotypes non-3 may induce some degree of viral steatosis, whereas metabolic abnormalities may be associated with a fatty liver also in genotype 3-infected persons. Thus, it is likely that the two types of steatosis can coexist in at least some chronic hepatitis C patients, although, in genotype 3 infection steatosis will be primarily of viral origin and in genotype non-3 primarily metabolic.

The most likely cause of metabolic steatosis in chronic hepatitis C, therefore preceding its appearance, is the insulin resistance that accompanies overweightness. The mechanisms that underlie liver steatosis in the insulin resistant state are multiple. On the one hand, free fatty acid overflow from adipose tissue to the liver is a direct consequence of the failure to block lipoprotein lipase, resulting in increased uptake by peripheral tissues, including liver. Second, the deregulated hyperglycemic/hyperinsulinemic state stimulates the expression of a variety of enzymes involved in fatty acid neosynthesis, while at the same time inhibit the
mitochondrial β-oxidation. This imbalance between uptake, \textit{ex novo} synthesis and degradation results in excess triglyceride accumulation within hepatocytes. In case of HCV infection, some degree of synergism may occur at the level of the above metabolic pathways, such as CPT-1 inhibition. Moreover, insulin is a known down-regulator of ApoB synthesis. However, recent data support the notion that chronic insulin resistant state may stimulate MTP activity in order to increase hepatocyte VLDL output. If this is confirmed, two conflicting events would occur in insulin resistant chronic hepatitis C patients: MTP inhibition, directly or indirectly-mediated by HCV, and MTP stimulation, mediated by insulin. Presumably, these two mechanisms may not operate in the same subgroups of patients. In those with genotype 3, who have the lowest levels of insulin resistance (see below), the viral inhibition of MTP would predominate, with hypertriglyceridemia, low ApoB serum levels and “viral” steatosis as a final consequence. In patients with genotype non-3 infection, hypertriglyceridemia may occur, but the inability to counterbalance the stimulation of fatty acid neosynthesis and the inhibition of mitochondrial β-oxidation would still result in “metabolic” steatosis. Clearly, further work is warranted to dissect the role of each of these metabolic changes in the pathogenesis of steatosis in hepatitis C.

Insulin resistance is a hallmark of the metabolic syndrome and, as such, proceeds independently of HCV. However, there is some good evidence that HCV may play a significant role in influencing the level of insulin resistance, and this irrespectively of the stage of liver disease. The fact that HCV-infected cirrhotic patients may present with type 2 diabetes more frequently than patients with cirrhosis of other origin was first reported in 1994, and has been later confirmed by several cohort and case-control studies, also in special populations like patients having received transplantation. In particular, a retrospective analysis of 1117 patients with chronic viral hepatitis reported that diabetes was diagnosed in 21% of HCV but only in 12% of HBV-infected patients. By multivariate analysis, HCV infection and age resulted in independent predictors of diabetes. In the same study, when 594 diabetics were compared with 377 patients evaluated for thyroid disturbances, 4.2% of diabetic patients were found to be infected with HCV as compared with 1.6% of controls. In another study, conducted within the Third National Health and Nutrition Examination Survey (NANHES-III), a significant association with age was observed, i.e. chronic hepatitis C patients 40 years of age or older were more than three times more likely to have type 2 diabetes than those without HCV infection, . This raised the issue that diabetes may be due to the stage of liver disease rather than to the viral infection. However, Hui et al. have subsequently reported that 121 HCV-infected patients with stage 0 or 1 hepatic fibrosis had higher levels of HOMA scores compared with 137 healthy volunteers matched by sex, body mass index, and waist-to-hip ratio. This work also provided some evidence that the level of insulin resistance may be genotype-specific, since patients with genotype 3 had lower levels of HOMA scores than patients with genotype 1. Further evidence that HCV may provoke an insulin resistant state comes from some limited data on the correlation between the severity of insulin resistance and the HCV replication levels, and from the observation that insulin sensitivity may improve in patients who achieve HCV RNA clearance following antiviral therapy, while remaining unchanged in non-responders, despite a decrease in BMI. However, further, independent confirmation of these data is warranted.

Experimental data suggest a direct interference of HCV with the insulin signaling pathway via proteasomal degradation of the insulin receptor substrate-1 and -2. In addition, functional impairment of this signaling pathway may occur via increased levels of pro-inflammatory cytokines such as TNF-α or other post-receptor defects. It has been proposed that chronic hepatitis C patients with more severe liver disease may have an exaggerated intrahepatic TNF-α response, resulting in insulin resistance and a higher risk of developing diabetes. In patients with genotype 3a, HCV may alter the intrahepatic insulin signaling through a down-regulation of PPAR. Interestingly, although interference with the insulin signaling shows some HCV genotype-specificity, as discussed above, insulin resistance has been reported to occur in all HCV genotypes.

Thus, HCV may induce liver steatosis by interfering with lipid metabolism in hepatocytes, and, indirectly, by influencing the level of insulin resistance. As a consequence, the metabolic steatosis, although largely due to disorders independent of the viral infection, may at least partially be ameliorated by antiviral therapy. It has to be noted, however, that as many as 30% of patients with fatty liver who do not drink alcohol and are infected with genotypes other than 3a have normal BMI and HOMA score, suggesting that other causes of fatty liver exist in hepatitis C.

A relatively new field is represented by the metabolic impact of cytokines secreted by adipose tissue, i.e. the so-called adipokines. As an example, an association has been reported for serum levels of adiponectin and HCV-related steatosis. Adiponectin is a cytokine secreted by adipocytes with antilipogenic effects that may protect non-adipocyte tissues, such as liver, from fat accumulation. Chronic hepatitis C patients infected with genotype 3 have the lowest levels of adiponectin. Low circulating levels of adiponectin lead to increased serum free fatty acids, which are then taken up by hepatocytes. Further work is needed to fully appreciate the significance of adiponectin in steatosis associated with hepatitis C and more in general in the metabolic syndrome.

Apart from viral and metabolic factors, some specific host genetic polymorphisms may also play a role in the pathogenesis of steatosis. Hyperhomocysteinemia, by inducing endoplasmic reticulum (ER) stress, causes deregulation of the endogenous sterol response pathway via SREBP, leading to increased hepatic biosynthesis and uptake of cholesterol and triglycerides. This in turn leads to steatosis. It has also been suggested that hyperhomocysteinemia may increase oxidative stress by inhibiting the expression of several antioxidant enzymes, thus sensitizing hepatocytes to the cytotoxic effect of

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pro-oxidant agents. Interestingly, a close association has been reported between the severity of steatosis and homocysteine serum levels in chronic hepatitis C patients. The hyperhomocysteinemia-induced steatosis model may explain why only some, but not all, HCV-infected patients develop steatosis, and why only a minority of patients, e.g. those with higher homocysteine levels, accumulate a greater amount of fat in the liver. Hyperhomocysteinemia may result from a methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism. In that study, it was shown that the polymorphism of the MTHFR gene at position 677, which has a prevalence of 12%-15% for the TT genotype in the general population, was associated with both hyperhomocysteinemia and a greater degree of steatosis in chronic hepatitis C patients. It was estimated that the relative risk of developing more severe steatosis was six-fold higher for patients with the CT genotype and 20-fold higher for those with the TT genotype.

**IS THERE A ROLE FOR STEATOSIS IN HCV REPLICATION?**

HCV replication occurs entirely in the hepatocyte cytoplasm and, like many other plus-strand RNA viruses, proceeds in association with the endoplasmic reticulum membranes. Perturbation of host cell lipid and cholesterol metabolism can disrupt replication complexes by altering membranous structures where replication occurs. Thus, it is very likely that alterations brought about by HCV on lipid metabolism may affect its own replication.

The question that has been repeatedly raised is whether steatosis may play a role in the HCV life cycle, e.g. by stimulating HCV replication or facilitating cell-to-cell spread. Most models are currently highly speculative. For example, HCV is known for circulating bound to lipoproteins under the form of lipo-viro-particles (LPVs). Interestingly, binding LPVs to hepatocyte cell lines can be out competed by VLDL and LDL from noninfected controls and is blocked by anti-ApoB antibodies. Conversely, up-regulation of the LDL receptor increases their internalization. Thus, low levels of circulating VLDL, as induced by HCV genotype 3a and, in general, in all patients with viral steatosis, may facilitate cell-to-cell spread of LPVs. This fascinating hypothesis needs, however further confirmation.

Another interesting aspect of the interaction between HCV replication and lipids comes from the observation that HCV RNA replication in hepatoma cells can be disrupted by treatment with lovastatin, a drug that decreases the production of mevalonate by inhibiting 3-hydroxy-3-methylglutaryl CoA reductase. Mevalonate is a precursor of hydrophobic prenyl prosthetic groups, like the geranylgeranyl group, which are necessary to anchor various proteins to cell membranes. The inhibition of HCV RNA replication by lovastatin was overcome by the addition of geranylgeraniol, suggesting that HCV RNA replication requires one or more geranylgeranylated protein. This protein has been recently identified as the FBL2 protein (F-box and leucine-rich repeat-containing protein), which would form a stable complex with the non-structural protein 5A of HCV. Knockdown of FBL2 mRNA was paralleled by inhibition of HCV replication in vitro. It is interesting to add that several statins were recently shown to possess anti-HCV activity in vitro. Fluvastatin exhibited the strongest anti-HCV activity, atorvastatin and simvastatin showed moderate inhibitory effects, whereas lovastatin had weakest anti-HCV activity. Moreover, the anti-HCV activities of statins were reversed by the addition of both mevalonate and geranylgeraniol. If confirmed, these observations may have important consequences on the management of chronic hepatitis C.

Thus, the fatty acid content of the infected hepatocyte may be crucial to modulate the rate of viral replication. However, some caution is mandatory before hasty conclusions are drawn, based on these data. Fatty acids accumulated in the form of triglycerides as in the case of steatosis may not be available to replication complexes involving HCV, and their degree of saturation may also vary. It is known that, in patients with viral steatosis, the severity of the fatty liver correlates with HCV replication level: however, in these patients, replication precedes fatty accumulation, and not vice-versa, as shown by antiviral treatment data. Conversely, in patients with metabolic steatosis, in whom steatosis precedes viral infection and proceeds independently of it, the level of viral replication is not increased in parallel with the severity of fatty liver. Thus, further work is warranted to understand the relationship between steatosis and HCV life cycle, if any.

**STEATOSIS AS COFACTOR OF LIVER FIBROSIS PROGRESSION**

The vast majority of cross-sectional and longitudinal studies have repeatedly shown an association between steatosis and progressing liver fibrosis in HCV-infected patients. At present, it is unclear whether the two types of HCV-associated steatosis (viral and metabolic) have a similar or different impact on fibrosis progression, because the reported data are controversial. It is likely that the two forms of steatosis act in an additive way. However, data are not unequivocal, as some studies suggest that steatosis may accelerate fibrosis only in genotype 3-infected persons, whereas others support an association in patients infected with genotype 1. At least one study denies any association between steatosis and fibrosis. Interestingly, as the disease progresses to advanced cirrhosis, steatosis tends to disappear, a phenomenon already observed in NAFLD. Longitudinal studies are particularly important in emphasizing the role of steatosis in fibrosis progression. In a recent study on paired liver biopsies performed across a median interval of 61 mo in 135 untreated patients with chronic hepatitis C, steatosis was the only independent factor predictive of progression of fibrosis, and the probability of progression of fibrosis was significantly related to the percentage of hepatocytes with steatosis. The authors concluded by supporting the attitude of treating patients with mild
hepatitis and steatosis, regardless of HCV genotype.

The mechanisms by which HCV-related steatosis promotes liver fibrosis progression are far from being elucidated. Available data suggest that oxidative stress, pro-inflammatory cytokines, insulin resistance, and increased susceptibility to apoptosis may mediate the fibrogenic effect of steatosis. However, since virtually all studies are correlative, it is difficult to ascertain whether steatosis directly participates in the fibrogenic process, or whether it should be considered as an innocent by-product of another, directly fibrogenic mechanism.

The association between HCV and intrahepatic oxidative stress has been mentioned above\[26,27\]. It has been reported that in the presence of hepatic steatosis, oxidative stress is enhanced in HCV infection and may promote fibrogenesis, similarly to the second “hit” proposed in NAFLD\[34,71\]. In a recent study, Kitase et al\[70\] detected by immunohistochemistry some protein adducts with lipid peroxidation end-products in the liver of chronic hepatitis C patients. Interestingly, areas positive for 4-hydroxy-2-hexenal-protein adducts, a good marker for oxidative stress, were larger in steatotic livers compared to non-steatotic. Thus, the authors concluded that steatosis in chronic hepatitis C may amplify the oxidative stress-driven lipid peroxidation by providing the necessary fuel\[74\]. There is also some limited evidence that antioxidant therapies may ameliorate the necro-inflammatory activity in chronic hepatitis C\[75\], although long-term effects on the fibrosis progression rate have not been reported so far.

Pro-inflammatory cytokines may mediate fibrogenesis in the steatotic liver, although it is unclear whether steatosis would facilitate this process. A recent meta-analysis (the HCV MAID study)\[39\], which included individual patients data of 3068 patients with chronic hepatitis C from 10 centers in five countries, demonstrated that liver steatosis is associated with increased liver inflammatory activity and accelerates the progression of liver fibrosis. This observation has been reported by others\[39,76\].

Insulin resistance is fibrogenic in the liver. However, in most studies, the relative contribution of steatosis and insulin resistance to fibrosis has not been determined. The HCV MAID Study has suggested that steatosis and diabetes are both independent factors of fibrogenesis in patients with genotype 1 infection\[39\]. However, when insulin resistance, an earlier and more sensitive parameter of glucose metabolism dysfunction, is added to a logistic regression analysis, the association between steatosis and fibrosis disappears\[40\]. A body of epidemiologic work suggests that the presence of diabetes and insulin resistance per se are risk factors of severe fibrosis and more rapid fibrosis progression in chronic hepatitis C\[40,74,77\]. The connective tissue growth factor (CTGF) is over-expressed in the liver of patients with non-alcoholic steatohepatitis as well as of diabetic Zucker rats\[79\]. In addition, both CTGF mRNA and protein are significantly increased when hepatic stellate cells are incubated with either glucose or insulin\[78\]. Similar mechanisms may operate in chronic hepatitis C\[79\], where the level of intrahepatic expression of CTGF was correlated with serum levels of leptin and the scores of steatosis and fibrosis. Since hepatic stellate cells possess leptin receptors\[80\], it is intriguing to speculate that leptin may represent the link between steatosis, insulin resistance and fibrosis in chronic hepatitis C.

Finally, hepatocyte apoptosis is a well recognized condition associated with both necroinflammatory activity and fibrosis in NAFLD\[81\]. In chronic hepatitis C, in the presence of steatosis, increased apoptosis is associated with activation of stellate cells and increased stage of fibrosis\[82\]. In addition, steatosis is associated with decreased Bel-2 mRNA levels and an increase in the proapoptotic Bax/Bcl-2 ratio. It has also been reported that caspase activity, which controls apoptosis, is increased in both liver biopsy and sera from HCV patients\[83\] and is strictly correlated to the extension of steatosis. All these data seem to indicate a relationship between steatosis, apoptosis and development of fibrosis, although the fine pathogenetic mechanisms await clarification.

HCV-RELATED STEATOSIS AND HEPATOCELLULAR CARCINOMA

Experimental data from the transgenic mice model have shown that HCV may play a causative role in the development of steatosis and hepatocellular carcinoma (HCC)\[84,85\]. It has been speculated that, among the possible mechanisms, reactive oxygen species may play a major role in mutagenesis\[86,87\]. In addition, the HCV core protein may interact with RxR\[88\], a transcriptional regulator that controls many aspects of cell proliferation and differentiation. A recent study has reported steatosis to be an independent risk factor for the development of HCC in chronic hepatitis C patients\[89\]. The authors prospectively followed 161 patients with chronic HCV infection for up to 15 years and found that the presence of steatosis was significantly associated with the incidence of HCC by multivariate analysis. Recently, however, a retrospective study including a smaller number of chronic hepatitis C patients did not confirm that steatosis may be a risk factor for HCC development\[90\]. Thus, further prospective studies are needed to assess the role of HCV-associated steatosis in liver carcinogenesis.

HCV-RELATED STEATOSIS AND RESPONSE TO ANTIVIRAL TREATMENT

Steatosis has been recognized as a negative factor response to antiviral therapy for many years\[87\]. This observation has been repeatedly confirmed by data coming from large clinical trials\[91,92,93,94\]. The effect is significant for the steatosis seen among patients with non-3a genotype, hinting at the insulin resistance as the pathogenetic factor affecting responsiveness to interferon-alpha. This was confirmed by a recent study\[91\], where the sustained virological response rate was inversely correlated with the baseline HOMA score. Indirect evidence in favor of this negative association comes also from the reduced response to antivirals seen in African Americans, reportedly due to their high rate of visceral obesity and insulin resistance\[95\], and from the correlation between high levels of circulating TNF-α, as seen in insulin resistance, and poor response to interferon therapy\[96\].
The molecular reasons for the correlation existing between insulin resistance and interferon-alpha resistance are still unclear. Chronic hepatitis C patients who do not respond to interferon-alpha may have increased levels of suppressor of cytokine signalling 3 (SOCS-3) in the liver, a factor promoting the proteasomal degradation of IRS-1[91]. Interestingly, members of the SOCS family are negative regulators of STAT-1, a factor involved in the transduction of the interferon-alpha signaling[92]. Thus, we cannot exclude that HCV activates some members of the SOCS family as a mechanism to inhibit the interferon-alpha signaling, with the simultaneous impairment of the insulin signaling just being a collateral effect.

CONCLUSION

Steatosis is an established risk factor for disease progression in chronic hepatitis C. Furthermore, it has also been shown to impact significantly on the response to antiviral therapy. Thus, appropriate therapeutic strategies for HCV-related steatosis are required with the aim to improve both the natural history of chronic hepatitis C and its drug management. Ideally, it would be important to assess whether steatosis is mainly virally-induced (like in many cases of genotype 3 infection) or whether metabolic host factors play a predominant role, like in many patients infected with non-3 genotypes. In chronic hepatitis C patients with genotype 3, successful treatment is associated with disappearance or significant amelioration of fatty liver. Whether the efficacy of antivirals may be improved by specific interventions aimed at reducing the degree of steatosis remains to be proven. In patients with non-3 genotype infection, in whom insulin resistance may play a role as cofactor of disease and fatty infiltration of the liver, the first-line intervention is represented by those lifestyle modifications that may reduce body weight. In a recent study, a weight-reduction program followed by chronic hepatitis C patients was effective in reducing steatosis and improving liver biochemistry and fibrosis in the absence of effects on virological parameters and irrespectively of the infecting genotype[93]. One should be reminded that the diagnosis of viral and/or metabolic steatosis in any given patient should not be based solely on the infecting genotype, but should be corroborated by a whole set of clinical and laboratory parameters, including the assessment of the insulin resistance score. Finally, because alcohol, even at low doses, synergistically interacts with steatosis to promote fibrosis progression, it is mandatory to advise the patients to avoid its use. The additional role of drugs like metformin and thiazolidinediones, which improve insulin sensitivity, remains to be evaluated in carefully planned clinical trials. Of additional interest is the use of drugs that may inhibit the geranylgeranylation, a cellular enzymatic activity apparently essential for HCV replication. All these pharmacological strategies, however, remain experimental, for the time being, and should not be attempted in routine clinical practice until new data from controlled trials will be available.

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