MINI REVIEW

Mechanisms of hepatitis C virus-related insulin resistance

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Summary  The hepatitis C virus (HCV) infection has been shown to have direct and/or indirect effects on glucose metabolism, leading to insulin resistance and, in predisposed individuals, type 2 diabetes. This is supported by several experimental, clinical and epidemiological data. The detailed molecular events leading to insulin resistance in HCV-infected patients are unclear. HCV infects primarily the liver and, to a very minor extent, mononuclear cells. Direct interactions between HCV products and the hepatocyte insulin signaling pathway have been reported by several authors. However, recent evidence supports the existence of a significant extrahepatic component of HCV-induced insulin resistance. Thus, the molecular pathogenesis of glucose metabolism disturbances observed in hepatitis C is much more complex than expected. The clinical management of such condition remains empirical.

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Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic progressive liver disease, including its long-term sequelae, i.e. cirrhosis and hepatocellular carcinoma [1]. Although some pathological features, such as steatosis, suggest a direct effect of HCV on cellular constituents, most liver damage is considered as being mediated by the host immune response [2]. This results in mononuclear infiltration of the liver parenchyma, leading to hepatocyte necrosis and progressive deposition of fibrotic tissue, in a typical wound-healing process [3]. However, the pathophysiology of hepatitis C must consider also some additional, clinically significant interactions between HCV and the host cell machinery, which may impact on HCV-related morbidity and mortality. It is well established that HCV perturbs the glucose metabolism, leading to insulin resistance and, in predisposed individuals, type 2 diabetes [4]. These alterations may lead to all common sequelae of diabetes, and, in addition, to accelerated fibrogenesis, increased incidence of hepatocellular carcinoma and impaired response to treatment with interferon alpha (IFN-α) [4]. Thus, the knowledge of the molecular pathways involved in the pathogenesis of insulin resistance in HCV infection is of paramount importance to determine its appropriate management.

Insulin signaling

The binding of insulin to its receptor in the plasma membrane of the cell triggers a complex cascade of downstream
signaling events (Fig. 1) [5]. The insulin receptor (IR) is a heterodimeric complex consisting of two α-subunits and two β-subunits with tyrosine kinase activity. Insulin binding to the α-subunits determines the β-subunits to transphosphorylate each other, increasing their kinase activity. Several IR substrates (IRS) have been described, but only IRS-1 and IRS-2 are relevant to hepatocyte signaling. IRSs contain several tyrosine residues that can be phosphorylated by the IR tyrosine kinase. Once phosphorylated, IRSs function as docking molecules for proteins containing an src homology region 2 (SH2) domain, such as the phosphatidylinositol 3-kinase (PI3K), activating them. PI3K is a heterodimer, consisting of a p110 catalytic subunit and a regulatory subunit: once activated, it phosphorylates the phosphoinositides at their 3-position to produce phosphoinositide 3-phosphates (PIPs). These molecules firstly activate the PI-dependent kinase 1 (PDK1) and then recruit the serine/threonine kinase Akt (also known as protein kinase B, PKB) to the plasma membrane. In order to be activated, Akt must be phosphorylated twice: first, by the mammalian target of rapamycin (mTOR) complex 2 at the serine 473, and then by the activated PDK1 at its threonine 308.

The protein kinase Akt/PKB is key effector of insulin action. In the liver, Akt prevents the transcription factor FoxO1 from entering the nucleus and activating genes that control gluconeogenesis, like the phosphoenoxyruvate carboxykinase and the glucose 6-phosphatase. In addition, Akt inactivates the glycogen synthase kinase 3 and allows for the persisting localization of glucose transporters at the plasma membrane, leading to increased glycogen synthesis and glucose uptake.

The lipogenic effects of insulin proceed also through the activation of the PI3K/Akt pathway. Insulin-stimulated lipogenesis is mediated by the transcription factor sterol regulatory element–binding protein (SREBP)-1c, although the full details of this are still unclear. The SREBPs family consists of three transcription factors: SREBP-1c is the dominant isoform in liver and adipose tissue, where it activates the fatty acid synthesis. Its regulation is complex and occurs at the transcriptional and posttranslational levels. Insulin seems to activate the transcription factor, liver X receptor (LXR), which is known to bind to the SREBP-1c promoter, thus activating its transcription. However, recent evidence suggests that also the mTOR complex 1 is required for the insulin-stimulated induction of SREBP-1c [6]. Akt activates mTOR by phosphorylating and inhibiting TSC2, an mTOR inhibitor. The mTOR is an important target of activated Akt, and it is not only involved in control of lipogenesis, but also in protein synthesis regulation.

Finally, another major pathway activated by insulin involves the mitogen-activated protein kinase (MAPK). This pathway is triggered by the IR-mediated tyrosine phosphorylation of IRS proteins, Gab1, and Shc, and is independent of PI3K. The MAPK pathway is associated with the cell growth effects of insulin.

Several factors may modulate/suppress insulin signaling, and their activation may lead to insulin resistance: protein tyrosine phosphatases (PTP) Shp1 and Shp2 may dephosphorylate IRSs [7], lipid phosphatases such as SH2-containing phosphoinositide 5-phosphatase 2 (SHIP2) and the phosphatase and tensin homolog (PTEN) dephosphorylate PIPs [8], while the suppressors of cytokine signaling (SOCS) promote IRSs degradation [9]. Some kinases, including the mTOR substrate p70 ribosomal S6 kinase (p70S6K), the protein kinase C (PKC) and the c-Jun N-terminal kinases (JNK), induce insulin resistance through phosphorylation of serine residues of IRSs, which inactivates them [10].

HCV infection causes insulin resistance

Several epidemiological, clinical and experimental observations have provided convincing evidence that HCV plays a direct role in altering glucose metabolism, leading to insulin
resistance and diabetes. Cross-sectional studies, comparing the prevalence of diabetes in HCV-infected patients with that of a comparator group, have shown that chronic hepatitis C patients have diabetes more often than patients with chronic liver diseases of other causes [11]. This observation has been confirmed by general population-based and longitudinal studies [12,13]. Since the odds of developing diabetes mostly concern patients presenting with other risk factors of diabetes [14], one can conclude that HCV infection increases the rate of developing diabetes in predisposed individuals. The association between HCV infection and glucose metabolism abnormalities is confirmed also when assessing insulin resistance in patients without overt diabetes [15,16]: in chronic hepatitis C, insulin resistance—measured as the homeostasis model assessment of insulin resistance, or HOMA-IR score—is increased at early stages of liver diseases, i.e. even in patients without liver fibrosis [15], and is, on average, significantly higher than that found in patients with chronic hepatitis B, matched for body mass index (BMI), age and stage of fibrosis [16]. Finally, eradication of HCV by antivirals results into an amelioration of insulin resistance and decreased incidence of diabetes after the end of therapy [17—20].

Mechanisms of HCV-induced insulin resistance

Experimental data suggest a direct interference of HCV with the insulin signaling pathway. In an early study, fresh liver samples obtained from 42 nonobese, non-diabetic chronic hepatitis C patients and 10 non-HCV-infected subjects, matched for age and BMI, were incubated ex vivo with insulin and assessed as to the integrity of the insulin signaling pathway [21]. This work found a reduced tyrosine phosphorylation (hence decreased activation) of IRS-1, reduced association of IRS-1 with its downstream effector PI3K but increased expression of the IRS-1 protein in HCV-infected livers [21], suggesting a postreceptorial interaction between HCV products and the insulin signaling pathway. However, in another study, the immunohistochemical detection of IRS-1 in HCV-infected livers shows a reduced membrane staining [17], which was restored upon successful therapy with antivirals. Thus, whether IRS-1 is increased or reduced in the liver of chronic hepatitis C patients remains to be clarified. In experimental models, based on the expression of the sole HCV core protein, an increased proteasome-mediated degradation of IRS-1, mediated by the activation of members of the SOCS family, was reported [22,23]. One of these studies postulated also HCV genotype-specific mechanisms of impairment of the insulin signaling transduction [23]. Here, a downregulation of peroxisome proliferator-activated receptor-γ (PPAR-γ) and an upregulation of SOCS-7 were observed upon expression of the HCV genotype 3 core protein, whereas the core protein of genotype 1 activated mTOR. Interestingly, subsequent work suggested that PPAR-γ may directly control SOCS-7 level in cells expressing the HCV genotype 3 core protein [24]. It was also shown that the activation of SOCS family members may be a mechanism common to all major HCV genotypes [25], including genotype 1, since the variant originally associated with the in vitro mTOR activation is rather infrequent among known isolates [25]. In addition, SOCS activation in human livers (both at the mRNA and at the protein level) has been reported in several studies, with the level of activation being correlated, in some cases, with metabolic alterations, including obesity [26] and hepatic insulin resistance [27]. The activation of SOCS family members—namely SOCS-1, SOCS-3 and SOCS-7—is not the only mechanism suggested to account for HCV-induced, hepatic insulin resistance. The integrity of the insulin signaling may also depend on the proteasome activator 28gamma (PA28γ) [28]. Transgenic mice expressing the HCV core under the control of a liver-specific promoter are insulin resistant [29]. In this model, both tyrosine phosphorylation of IRS-1 and IRS-2 expression are decreased [28]. However, these defects are totally reversible when the PA28γ is deleted, suggesting that the HCV core protein suppresses insulin signaling through a PA28γ-dependent pathway [28]. The involvement of PA28γ is worthy of note, because this activator plays a role also in the development of steatosis and HCC [30].

Other molecular mechanisms triggered by HCV may include an increased endoplasmic reticulum (ER) stress [31] and the activation of the c-Jun N-terminal kinase [32]. The work by Bernsmeier et al. [31] stemmed from the observation [33] that the protein phosphatase 2A (PP2A) is upregulated in the liver of chronic hepatitis C patients. In vitro, HCV and hepatitis B virus lead to the overexpression of PP2A by inducing ER stress [34]. Using cells allowing the regulated overexpression of all HCV proteins, it was shown that PP2A inhibits insulin signaling by dephosphorylation of Akt at the level of the serine 473 [31]. Impaired insulin signaling was confirmed in transgenic mice and human livers, although these authors could not establish a correlation between the intraportal level of PP2A and insulin resistance [31].

In another study, the HCV core protein alone or in the presence of other viral proteins increased the phosphorylation of IRS-1 at the level of its serine residue 312 [32], an effect that was abolished by the inhibition of the JNK signaling pathway. The latter also restored the hepatocyte glucose uptake reduced by the HCV core expression. Thus, JNK may contribute to HCV-induced insulin resistance, and recent data in chronic hepatitis C seem to confirm these in vitro observations [35].

HCV may also induce insulin resistance by triggering the production of pro-inflammatory cytokines. In the aforementioned transgenic mouse model, the liver specific expression of the HCV core protein leads to insulin resistance via a PA28γ-dependent pathway, as shown by the knockout model [28]. However, also the administration of a TNF-α inhibitor was capable of restoring the insulin sensitivity, and in particular to revert the serine phosphorylation of IRS-1 [29]. In hepatitis C, circulating TNF-α levels are increased [36—38]. However, correlations between TNF-α (and/or other inflammatory cytokines and/or adipokines) and insulin resistance has been difficult to establish and data are scanty [39]. In a nicely controlled study [40], serum levels of TNF-α and IL-6 were measured in 154 HCV-infected nondiabetic males and compared to 75 matched uninfected controls. No correlation was found with insulin resistance. Circulating levels of the adiponectin leptin and adiponectin were independently associated with insulin resistance, but not with the presence of HCV. Thus, it was concluded that HCV-associated insulin
resistance does not seem to be mediated by adipokines or pro-inflammatory cytokines.

Interestingly, recent evidence obtained by combining the use of euglycemic hyperinsulinemic clamp, infusion of labeled tracers and indirect calorimetry, shows that HCV infection is associated with a substantial peripheral component of the total insulin resistance [27,41]. Since HCV infects primarily the liver, these results raise the issue of the cross-talk between the liver and extrahepatic tissues involved in the glucose homeostasis, i.e. the striated muscle or the adipose tissue. Currently, most—if not all—of the extrahepatic insulin resistance seems to originate from muscles, since the adipose tissue retain the sensitivity to insulin when lipolysis is measured at the time of the euglycemic hyperinsulinemic clamp [41]. The circulating mediators of these effects are currently unknown.

Has our knowledge on the mechanisms of HCV-induced insulin resistance modified patients’ management?

Insulin resistance and diabetes have significant clinical consequences on chronic hepatitis C: (i) accelerated fibrogenesis [15,16,42—45], (ii) increased incidence of hepatocellular carcinoma [46,47] and (iii) reduced virological response to IFN-α-based therapy [19,45,48,49]. Thus, to unravel the mechanisms of insulin resistance induced by HCV is of paramount clinical importance. However, all approaches attempted so far have been empirical, i.e. using lifestyle measures and insulin sensitizers already in use in non-HCV-related insulin resistance and diabetes. This is partly due to the lack of SOCS inhibitors for clinical use, and to the fact that there is little, inconclusive evidence supporting the use of etanercept and/or infliximab (two TNF-α inhibitors) for correcting metabolic disturbances [50—53]. A single study proposing lifestyle changes was able to reduce insulin resistance and liver fibrosis in a small cohort of chronic hepatitis C patients [54]. The effects of such measures on the response to antiviral therapy were not assessed. The data reported in four independent studies using different schedules containing pioglitazone added to the pegylated IFN-α/ribavirin combination [55—58] fell short of demonstrating the efficacy of this insulin sensitizer on the virological response rate, although insulin resistance was reduced. The only study that used metformin has shown only a marginal, insignificant increase of the sustained virological response rate, together with an amelioration of the metabolic parameters [59].

In conclusion, HCV seems to induce insulin resistance via direct and indirect mechanisms. The knowledge of the molecular interactions underlying these effects is still very poor, and does not allow implementing mechanistic approaches to patients’ management.

Conflict of interest statement

The author confirms the absence of conflicts of interest related to the work presented here.

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