Review Article

Supplementing Conventional Treatment with Pycnogenol® May Improve Hepatitis C Virus–Associated Type 2 Diabetes: A Mini Review

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Abstract

Hepatitis C virus (HCV) infection and type 2 diabetes mellitus (T2DM) present a significant health burden, with increasing complications and mortality rates worldwide. Pycnogenol® (PYC), a natural product, possesses antidiabetic and antiviral properties that may improve HCV-associated T2DM. In this review, we present previously published data on the effectiveness of PYC against HCV replication and T2DM. We believe that supplementing conventional treatment with PYC may improve the current HCV therapy, attenuate HCV-associated T2DM, and reduce the risk of complications such as cirrhosis or hepatocellular carcinoma and cardiovascular disease.
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Introduction

Hepatitis C virus (HCV) affects about 3% of the world's population. A recent survey found that the number of people worldwide with HCV antibodies (anti-HCV) has increased from 122 million in 1990 to 184 million in 2005. Globally, 130–170 million people are chronically infected with HCV, leading to 54,000 deaths per year.[1] Chronic HCV infection can lead to cirrhosis and/or hepatocellular carcinoma (HCC).[2] In fact,

one of the major risk factors for the development of HCC in industrialized countries is HCV infection.

In the recent past, HCV therapy consisted of ribavirin (RBV) combined with either peg-interferon-alpha-2a (PEG-IFN-α-2a) or peg-interferon-alpha-2b (PEG-IFN-α-2b). Unfortunately, these treatments lead to a sustained virologic response (SVR) in less than 50% of individuals infected with HCV genotype 1 and less than 85% for those infected with genotypes 2, 3, 4, 5 and 6.[3,4] Moreover, severe side effects, such as flu-like symptoms, hemolytic anemia, autoimmune diseases and neuropsychiatric symptoms, are the frequent cause of treatment discontinuation.[5] In 2011, the nonstructural protein 3/4A (NS3/4A) protease inhibitors, telaprevir and boceprevir, were introduced. When used in combination with PEG-IFN-α and RBV, these agents increased the cure rate to approximately 70% for HCV genotype 1;[6–8] however, the triple-therapy regimens were reported to cause emergence of drug-resistant HCV variants and side effects.[6–8]

Recently, direct acting antiviral (DAA) regimens have improved HCV therapy by eliciting SVR in more than 90% of infected populations across all HCV genotypes.[9,10] In addition, a vaccine with substantial efficacy has been introduced and may reduce the incidence of new infections.[11] While the treatment paradigm for HCV infection has undergone substantial change since the discovery of HCV 27 years ago, the primary challenges remain the identification of all HCV carriers and worldwide accessibility to expensive drugs.

Type 2 diabetes mellitus

Globally, type 2 diabetes mellitus (T2DM) is one of the most serious common metabolic disorders. The prevalence of diabetes worldwide is estimated to rise from 2.8% in 2000 to 4.4% in 2030,[12] with the total number of people with diabetes estimated to increase from 382 million in 2013 to 592 million in 2035.[13] Most patients with T2DM will require multiple glucose-lowering medications to achieve glycemic goals.[14] However, the choice of subsequent drug therapy is not straightforward, given the diverse range of pharmacological agents (at least 12 drug classes) now available.[15] A recent meta-analysis of randomized-controlled trials suggested that combining metformin with other oral agents can improve glycemic outcomes, as compared to that of

metformin monotherapy.[16] However, the current management of patients with T2DM remains suboptimal.

Association between HCV and T2DM

HCV affects insulin resistance (IR) through several mechanisms. First, it impairs the insulin-signaling pathway. Researchers have found that insulin receptor substrate 1 (IRS-1) levels are significantly lower in Huh-7 cells transfected with HCV core proteins for genotypes 1b and 3a.[17] The HCV core protein for genotype 3a promotes IRS-1 degradation through down-regulation of peroxisome proliferator-activated receptor γ (PPARγ) and up-regulation of the suppressor of the cytokine signal 7 (SOCS-7) protein,[17] while the core protein for genotype 1b activates the mammalian target of rapamycin (mTOR).[17] HCV core proteins also inhibit activation of insulin receptor substrates 1 and 2 (IRS-1/2), which reduces protein kinase B (PKB) activity, lowers glucose transporter type 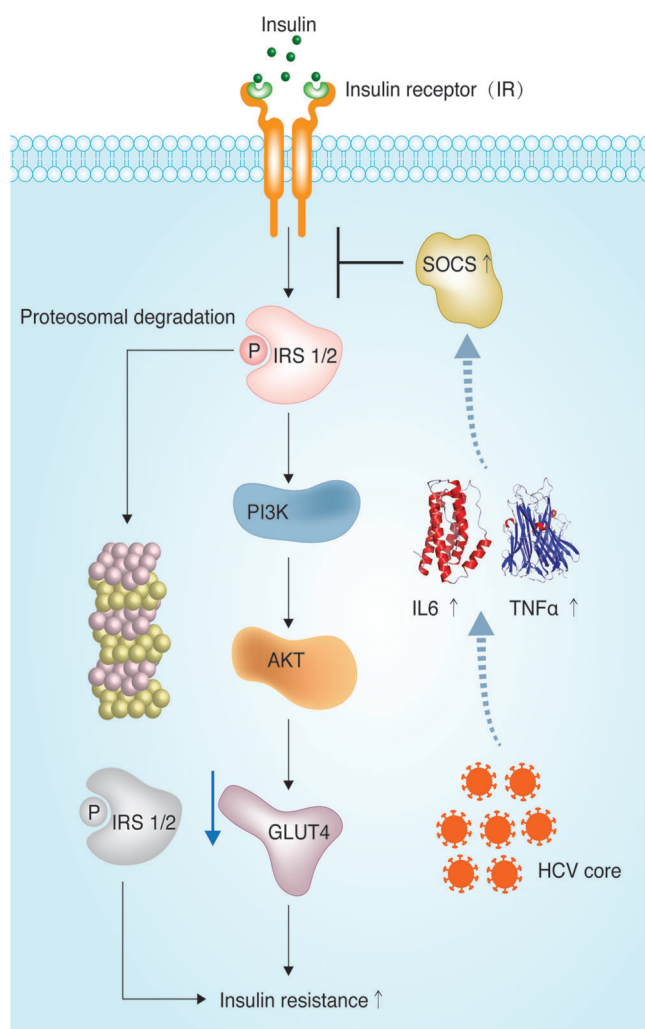4 (GLUT4) expression and increases gluconeogenic enzyme levels (Fig. 1). In addition, inhibition of hepatic IRS-1 tyrosine phosphorylation and phosphatidylinositol 3-kinase activation may disrupt insulin signaling and contribute to IR, which leads to the development of T2DM.[18,19]

HCV can also induce inflammation, which plays an important role in IR by increasing levels of interleukin 1 (IL-1), tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) to stimulate the expression of IKKβ, a protein kinase that induces IR by inhibitory phosphorylation of IRS-1.[20] In addition, reactive oxygen species (ROS) can activate nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB). Constitutive activation of NF-κB leads to increased expression of a variety of cytokines, including TNF-α and IL-6.[21]

Although several reports have shown that HCV increases the risk of T2DM,[22–24] the converse is also true. Individuals with T2DM are more prone to HCV infections.[25] For instance, a recent meta-analysis comparing patients with and with out T2DM clearly suggested that T2DM is associated with increased susceptibility to HCV infection (odds ratio = 3.50, 95% confidence interval: 2.54–4.82).[25]

Pycnogenol® (PYC) as adjunctive therapy for HCV–associated T2DM

Several studies have shown that HCV and T2DM induce oxidative stress.[26–28] Hyperglycemia increases oxidative stress through the overproduction of ROS,[29,30] and HCV induces oxidative stress via the HCV core, NS3, and NS5A proteins.[31] Several studies using various HCV cell culture systems have identified mitochondria as the source of ROS; thus, mitochondrial dysfunction is likely to be important in HCV-associated pathogenesis (Fig. 2).[32–34] More specifically, researchers believe that HCV gene expression elevates ROS levels through calcium signaling.[35,36] Calcium is taken up by the mitochondria, resulting in elevation of ROS and degradation of the inhibitory subunit (IκBα) of NF-κB by calcium/calpain.[35,37] ROS activates cellular tyrosine and serine/threonine kinases, which then activate NF-κB and signal transducer and activator of transcription 3 (STAT3).[35] Constitutive activation of NF-κB and STAT3 by HCV has been reported in acute and chronic liver disease.[35–37] In support of those studies, researchers have found that oxidative stress decreases to normal levels after viral eradication with PEG-IFN therapy.[38,39]

PYC (Horphag Research, Ltd., Geneva, Switzerland), an extract from the outer bark of the French maritime pine *Pinus pinaster* ssp. *Atlantica* (formerly known as *Pinus maritima* Aiton spp. *Atlantica des Villar*), is highly concentrated with flavonoids, with the primary constituents being procyanidins, taxifolin, ferulic acid, catechin, and caffeic acid.[40] PYC promotes a protective antioxidant state and anti-inflammatory action by up-regulating the scavenging systems,[41–45] and is generally recognized as safe for use as a supplement.[46]

PYC may inhibit HCV replication by interacting with viral proteins involved in oxidative stress, or through host-targeting by inducing endogenous lipid peroxidation that restricts HCV replication (Fig. 3).[47] We previously demonstrated PYC's direct antiviral effect on HCV replication *in vitro*, by using various HCV replicon systems, and *in vivo*, by using chimeric mice infected with genotype 1a.[45] We showed that combining PYC with RBV, interferon (IFN) and telaprevir can increase HCV antiviral activity, either synergistically or additively.[45] In addition, we found that PYC suppresses HCV replication in telaprevir-resistant replicon cells and may improve the

Fig. 1. Hepatitis C virus affects the insulin signaling cascade. Abbreviations: IRS-1, insulin receptor substrate 1; IRS-2, insulin receptor substrate 2; PI3K, phosphoinositide 3-kinase; SOCS, suppressor of cytokine signaling; GLUT4, glucose transporter type 4.
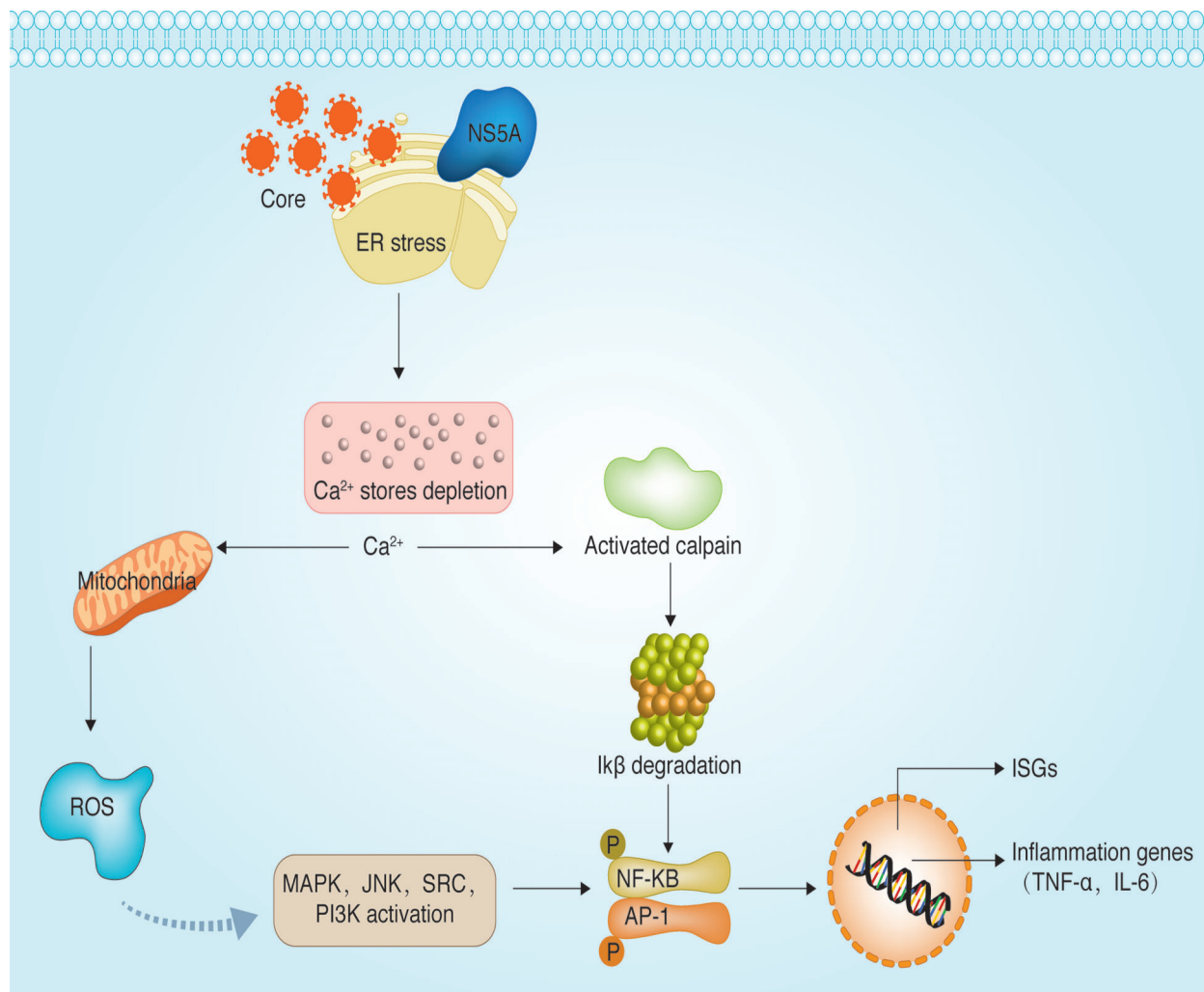
Fig. 2. Hepatitis C virus replication induces oxidative stress. Abbreviations: ROS, reactive oxygen species; NS5A, nonstructural protein 5A; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; PI3K, phosphoinositide 3-kinase; NF-κB, nuclear factor-kappa B; ISGs, interferon-stimulated genes.

response to protease inhibitors.[45] Deep sequencing of telaprevir-resistant replicon cells revealed the presence of V36A, T45V and A156T in the NS3 region that confers resistance to telaprevir treatment. The A156T mutation has previously been shown to confer resistance to simeprevir (TMC435; approved by the FDA in November 2013) *in vitro*.[48] We incubated the wild-type HCV and simeprevir-resistant replicon cells with either PYC or simeprevir. Results showed that PYC reduces HCV replication in both wild-type and resistant replicons.[45] In addition, we investigated the antioxidant capacity of PYC in HCV replicon cell lines and found strong scavenging activity against ROS.[45]

PYC may also improve blood glucose levels. In an animal model, treatment of streptozotocin-induced diabetic rats with PYC significantly reduced blood glucose concentrations and elevated antioxidant defense mechanisms and GLUT4 expression.[49,50] In addition, a previous study demonstrated that PYC treatment down-regulates high glucose–associated NF-κB nuclear translocation in renal tubular cells.[41] The effects were demonstrated clinically in a randomized, double-blind, placebo-controlled multicenter study that showed that the glucose-lowering effect was significantly greater for patients whose therapy was supplemented with PYC, as compared to that in patients supplemented with placebo ($p < 0.01$).[51] Glycosylated hemoglobin was assessed monthly and the values decreased continuously over the treatment period for both groups, with a more pronounced effect in the PYC group.[51]

Several mechanisms have been suggested for PYC's effect on blood glucose. Evidence from an *in vitro* study using 3T3-L1 adipocytes demonstrated that PYC enhances glucose uptake in a dose-dependent manner similar to insulin. In particular, PYC was shown to enhance GLUT4-mediated glucose uptake via phosphoinositide 3-kinase-dependent tyrosine kinase pathways involving PKB.[40] PYC also inhibits NF-κB and activator protein-1 (AP-1), and prevents the degradation of IκBα (Fig. 3).[52,53] More specifically, Choi and colleagues found that PYC inhibits the expression and secretion of TNF-α and IL-6, thereby reducing calcium uptake and suppressing NF-κB activation (Fig. 3).[54,50] Thus, PYC may improve T2DM therapy by enhancing GLUT4-mediated glucose uptake, inhibiting inflammatory genes such as IL6 and TNF-α, and also by inhibiting the SOCS pathways (Fig. 3).[50]
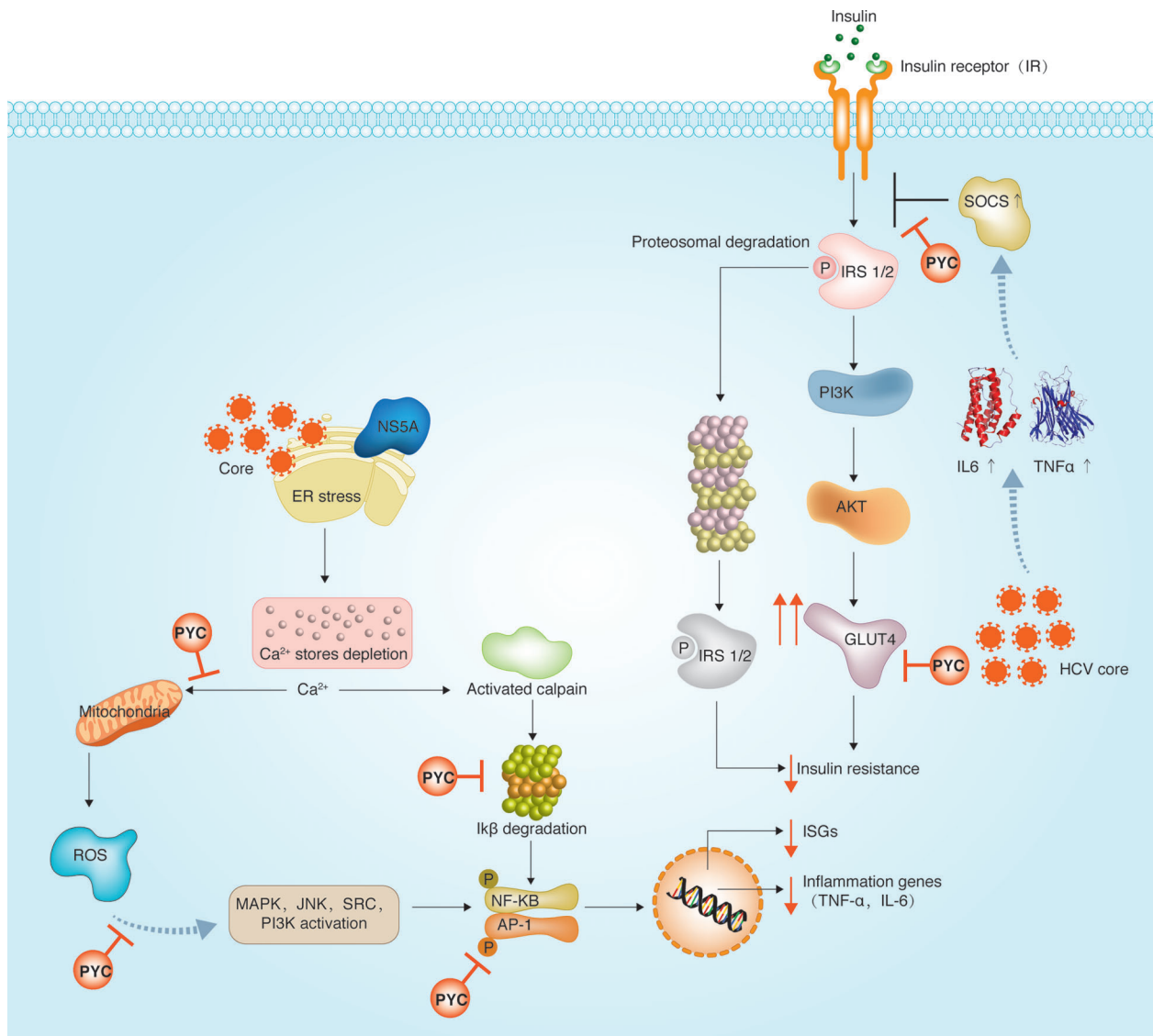
Fig. 3. Schematic model of possible actions of Pycnogenol® against hepatitis C virus-associated type 2 diabetes.

Conclusions

PYC's antidiabetic and antiviral properties make this natural remedy a potentially excellent adjunctive treatment for HCV-associated T2DM, possibly reducing medication burden and the risk of HCV complications such as cirrhosis or HCC. In addition, PYC's antioxidant and anti-inflammatory properties may attenuate HCV's effects on the insulin signaling pathway, reducing HCV-associated IR to improve T2DM therapy and help patients achieve and maintain normal blood glucose levels. For these reasons, it would be of interest to evaluate the usefulness of PYC *in vivo* and to conduct a clinical trial using PYC as adjunctive treatment for patients with HCV-associated T2DM.

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Conflict of interest

None

Author contributions

Review design and authorship (SE), collection of data (LW, SH, FJ), revision for important intellectual content (SE, KK, SB).

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