Direct-acting antiviral agents against hepatitis C virus and lipid metabolism

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Hepatitis C virus (HCV) infection induces steatosis and is accompanied by multiple metabolic alterations including hyperuricemia, reversible hypocholesterolemia and insulin resistance. Total cholesterol, low-density lipoprotein-cholesterol and triglyceride levels are increased by peginterferon and ribavirin combination therapy when a sustained virologic response (SVR) is achieved in patients with HCV. Steatosis is significantly more common in patients with HCV genotype 3 but interferon-free regimens are not always effective for treating HCV genotype 3 infections. HCV infection increases fatty acid synthase levels, resulting in the accumulation of fatty acids in hepatocytes. Of note, low-density lipoprotein receptor, scavenger receptor class B type I and Niemann-Pick C1-like 1 proteins are candidate receptors that may be involved in HCV. They are also required for the uptake of cholesterol from the external environment of hepatocytes. Among HCV-infected patients with or without human immunodeficiency virus infection, changes in serum lipid profiles are observed during interferon-free treatment and after the achievement of an SVR. It is evident that HCV affects cholesterol metabolism during interferon-free regimens. Although higher SVR rates were achieved with interferon-free treatment of HCV, special attention must also be paid to unexpected adverse events based on host metabolic changes including hyperlipidemia.

Key words: Cholesterol; Hepatitis C virus; Interferon-free; Lipid metabolism

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eradication of HCV, as well as lipid abnormalities, during treatment and after treatment. Further observations are needed to determine the long-term effects on lipid metabolism caused by HCV and by eradication of the virus.

Hepatitis C virus (HCV) encodes at least 10 viral proteins, which include structural (core, E1, E2 and p7) and non-structural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins[1]. HCV is a leading cause of cirrhosis and hepatocellular carcinoma in the United States and Japan. Eradication of HCV is important for preventing death due to these liver diseases.

Associations of HCV with host lipoproteins have been reported[2]. Hepatocytes take up low-density lipoproteins (LDLs) and very low-density lipoproteins through LDL receptors. Antibodies to the HCV envelope may disrupt the HCV lipid-containing envelope[3]. These antibodies could provide an efficient mode of viral entry into liver cells[4]. HCV core protein colocalizes with apolipoprotein AII at the surface of lipid droplets, suggesting a relationship between the expression of HCV core protein and cellular lipid metabolism[5]. HCV infection or core protein expression also increases the expression of sterol regulatory element binding protein 1c and its target, fatty acid synthase (FASN), which are both involved in lipid synthesis[5].

Although interferon-free regimens could result in higher sustained virologic response (SVR) rates, Endo et al[6] reported that serum cholesterol levels were significantly increased during combination treatment with the HCV NS5B inhibitor sofosbuvir and the HCV NS5A inhibitor ledipasvir, compared with those during interferon-included regimens[7]. Of interest, the authors also observed that regardless of the regimens, total cholesterol, LDL cholesterol and high-density lipoprotein (HDL) cholesterol levels increased post-treatment[6].

**ADVANCED LIVER FIBROSIS AND LIPID METABOLISM**

In most cells, the major source of new sterol is endogenous synthesis from acetyl-CoA[8]. HMG-CoA is formed from acetyl-CoA and acetoacetyl-CoA. There are at least 4 mechanisms for acquiring cholesterol: (1) de novo synthesis within the cells and uptake of unesterified or esterified cholesterol from the external environment via; (2) the LDL receptor (LDLR); (3) scavenger receptor class B type I (SR-BI); or (4) Niemann-Pick C1-like 1 protein (NPC1L1)[9]. HDL particles containing apoA-Ⅰ can be bound by SR-BI in hepatocytes and endocrine cells[10]. Interestingly, LDLR, SR-BI and NPC1L1 are candidate receptors that may be involved in HCV[11].

The total cholesterol pool in a human is 2.2 g/kg body weight. There is a continuous flow of cholesterol from the endoplasmic reticulum to the cell membrane and then from the plasma membrane to the liver and intestine[12]. In humans, the flux of cholesterol through the whole body is approximately 10 mg/d per kilogram body weight[13], although the half-life of plasma cholesterol is only a few days[14]. The serum lipids, total cholesterol, cholesteryl ester, LDL cholesterol and HDL cholesterol levels were significantly lower in HCV-related cirrhosis patients than in controls. HCV-related cirrhosis severely impairs liver lipid metabolism[14] (Figure 2). The serum total cholesterol level is an independent predictor of significant fibrosis[15]. When evaluating serum total cholesterol levels, liver function should also be checked (Figure 2).
LIPID METABOLISM DURING TREATMENT WITH INTERFERON-BASED REGIMENS AGAINST HCV

Total cholesterol, LDL cholesterol and triglyceride levels are increased by peginterferon and ribavirin combination therapy when an SVR is achieved in patients with HCV genotype 1\(^{[20]}\). HCV eradication is closely related to lipid metabolism in patients treated with interferon-based regimens. Lange et al\(^{[21]}\) examined the serum lipid profiles of 575 European HCV genotype 1-infected patients before, during and after treatment with peginterferon-\(\alpha\)-2a (180 \(\mu\)g/wk) and ribavirin (1000-1200 mg/d) for 48 wk. The authors found substantial pretreatment hypocholesterolemia with a nonresponse to interferon-\(\alpha\)-based therapy, and lower pretreatment cholesterol levels were an independent predictor of not attaining an SVR\(^{[21]}\). After treatment-induced HCV eradication, the median cholesterol levels increased above baseline. Kuo et al\(^{[22]}\) reported that chronic HCV infection is associated with hypocholesterolemia and hypotriglyceridemia, and these conditions can be reversed by successful antiviral therapy.

HCV GENOTYPE 3

Serfaty et al\(^{[23]}\) reported that hypobetalipoproteinemia is prevalent and associated with steatosis, especially in patients infected with HCV genotype 3. It has been reported that HCV, particularly genotype 3, is associated with steatosis. Poynard et al\(^{[24]}\) reported that an SVR, achieved with interferon-based regimens, is associated with a reduction in steatosis in HCV genotype 3 patients, as well as a correction of serum cholesterol levels at baseline. Steatosis is significantly more common in HCV genotype 3 patients than in those with other HCV genotypes, and in patients treated with peginterferon alpha-2a plus ribavirin, an SVR is associated with reduction of steatosis\(^{[25]}\). New treatments using HCV NS3/4A protease inhibitors have limited activity against HCV genotype 3\(^{[26]}\). HCV NS5B and HCV NS5A inhibitors have also performed poorly in HCV genotype 3 patients\(^{[26]}\).

LIPID METABOLISM DURING TREATMENT WITH INTERFERON-FREE REGIMENS AGAINST HCV

Endo et al\(^{[6]}\) studied 276 patients with chronic HCV genotype 1b infection who were treated with interferon-free regimens. Of these 276 patients, 141 were treated with the HCV NS5A inhibitor daclatasvir plus the HCV NS3/4A inhibitor asunaprevir for 24 wk\(^{[27,28]}\) and 135 were treated with sofosbuvir plus ledipasvir for 12 wk\(^{[6]}\).

In the daclatasvir plus asunaprevir-SVR group, the total cholesterol levels were significantly increased throughout the observation period, and the total cholesterol levels were significantly increased at 4 wk after treatment and 12 wk after treatment, compared with those at the end of treatment (EOT)\(^{[6]}\). Serum LDL cholesterol levels increased after the EOT. HDL cholesterol was significantly increased throughout the treatment period, but there were no significant changes in serum triglyceride levels\(^{[6]}\).

In the sofosbuvir plus ledipasvir-SVR group, the total cholesterol levels were markedly increased from the early stage of therapy and lasted until the EOT\(^{[6]}\). The total cholesterol levels were sharply decreased after the EOT (\(P < 0.001\)). Changes in the LDL cholesterol levels were quite similar to those found in the total cholesterol levels. After the EOT, the HDL cholesterol levels were decreased compared to those during therapy (\(P < 0.001\), but there were no significant changes in triglyceride levels\(^{[6]}\). Hashimoto et al\(^{[29]}\) also reported that the increase in cholesterol levels during treatment was much greater in the sofosbuvir plus ledipasvir-SVR group than in daclatasvir plus asunaprevir-SVR group. The authors also observed that a rapid increase in the serum LDL cholesterol concentration during the interferon-free treatment was associated with the type of regimen and decrease in the HCV core protein level. Morales et al\(^{[30]}\) also reported that there was a significant increase in the LDL and total cholesterol levels after treatment, compared to the pre- and post-treatment laboratory data from 52 patients receiving sofosbuvir-based regimens, but there was no change in body mass index between pre- and post-treatment. Among HIV/HCV coinfected patients, an increase in LDL cholesterol was observed after an SVR was achieved with interferon-free treatment\(^{[31]}\).

CONCLUSION

HCV infection induces steatosis and is accompanied by multiple metabolic alterations, such as hyperuricemia, reversible hypocholesterolemia, insulin resistance, arterial hypertension and visceral adipose tissue...
expansion. Eradication of HCV with interferon-free regimens increases total cholesterol levels. Because of the worsening nutritional status as an adverse event of interferon-based regimens, it is difficult to examine the effects of HCV on serum lipid profiles. It is evident that HCV affects cholesterol metabolism during interferon-free regimens because these regimens have no influence on the nutritional status of the host. The increase in cholesterol levels during treatment was much greater in the soybean burr plus ledipasvir-SVR group than in the daclatasvir plus asunaprevir-SVR group. Although higher SVR rates were achieved with interferon-free treatment of HCV, special attention must also be paid to unexpected adverse events based on host metabolic changes.

REFERENCES

1. Kanda T, Imazeki F, Yokosuka O. New antiviral therapies for chronic hepatitis C. *Hepatol Int* 2010; 4: 548-561 [PMID: 21063477 DOI: 10.1007/s12072-010-9193-3]

2. Prince AM, Huima-Byron T, Parker TS, Levine DM. Visualization of hepatitis C virions and putative defective interfering particles isolated from low-density lipoproteins. *J Viral Hepat* 1996; 3: 11-17 [PMID: 8736235 DOI: 10.1111/j.1365-2803.1996.tb00075.x]

3. Hikijikata M, Shimizu YK, Kato H, Iwamoto A, Shih JW, Alter HJ, Purcell RH, Yoshikura H. Equilibrium centrifugation studies of hepatitis C virus: evidence for circulating immune complexes. *J Virol* 1997; 61: 1953-1958 [PMID: 8383220]

4. Barba G, Harper F, Harada T, Kohara M, Goulionet S, Matsuura Y, Eder G, Schaff Z, Chapman MJ, Miyamura T, Bréchot C. Hepatitis C virus core protein shows a cytoplasmic localization and fatty liver and hepatic cirrhosis in transgenic mice. *Hepatol* 2001; 33: 175-177 [PMID: 11201275 DOI: 10.1002/hep.20161]

5. Bose SK, Kim H, Meyer K, Wolins N, Davidson NO, Ray R. Forkhead box transcription factor regulation and lipid accumulation by hepatitis C virus. *J Virol* 2014; 88: 4195-4203 [PMID: 24478438 DOI: 10.1128/JVI.02337-13]

6. Endo D, Sato H, Shimada N, Hikijikata M, Aizawa Y. Impact of interferon-free anti-virus therapy on lipid profiles in patients with chronic hepatitis C genotype Ib. *World J Gastroenterol* 2017; 23: 2355-2364 [PMID: 28428715 DOI: 10.3748/wjg.v23.i13.2355]

7. Kanda T, Ikeda M, Suzuki E, Ara M, Oku Y, Ogasawara S, Chiba T, Saito T, Haga Y, Takahashi K, Sasaki R, Wu S, Nakamoto S, Tawada A, Maruyama H, Imazeki F, Kato N, Yokosuka O. Real-World Experiences with the Combination Therapy with interferon-free antivirus therapy on lipid profiles in patients with chronic hepatitis C. *World J Gastroenterol* 2014; 20: 7318-7325 [PMID: 24854173 DOI: 10.3748/wjg.v20.i23.7318]

8. Kanda T, Nakamoto S, Miyamura T, Jiang X, Wu S, Yokosuka O. No correlation between PNPLA3 rs738049 genotype and fatty liver and hepatic cirrhosis in Japanese patients with HCV. *PLoS One* 2013; 8: e81312 [PMID: 24349054 DOI: 10.1371/journal.pone.0081312]

9. Miyanari Y, Itazawa K, Usuda N, Watsuki K, Hishiki T, Zayas M, Bartschlerager R, Wakiya T, Hikijikata M, Shimotohno K. The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol* 2007; 9: 1089-1097 [PMID: 17721513 DOI: 10.1038/ncb1631]

10. Spady DK, Dietschy JM. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. *J Lipid Res* 1983; 24: 303-315 [PMID: 6842066]

11. Dietschy JM, Turley SD. Thematic review series: brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J Lipid Res* 2004; 45: 1375-1397 [PMID: 15254070 DOI: 10.1194/jlr.R400004-JLR200]

12. Monazahian B, Böhme I, Bonk S, Koch A, Scholz C, Grethe S, Thomessen R. Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. *J Med Virol* 1999; 57: 223-229 [PMID: 10027971]

13. Scarselli E, Ansuini H, Cerino R, Roccasecca RM, Acali S, Filocamo G, Traboni C, Nicosia A, Cortese R, Vitelli A. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 2002; 21: 5017-5025 [PMID: 12356718 DOI: 10.1093/emboj/cdf529]

14. Sainz B Jr, Barretto N, Martin DN, Hiraga N, Imamura M, Hassain S, Marsh KA, Yu X, Chayama K, Alefswa FA, Uprichard SL. Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. *Nat Med* 2012; 18: 281-285 [PMID: 22231557 DOI: 10.1038/nm.2581]

15. Orth M, Bellotta S. Cholesterol: its regulation and role in central nervous system disorders. *Cholesterol* 2012; 2012: 292598 [PMID: 23191149 DOI: 10.1155/2012/292598]

16. Yue CC, Streba CT, Streba L, Rogoeanu I. Lipid serum profile in patients with viral liver cirrhosis. *Med Princ Pract* 2012; 21: 566-568 [PMID: 22722340 DOI: 10.1159/000339206]

17. Sud A, Hui JM, Farell GC, Bandara P, Rench JG, Fung C, Lin R, Samarasinghe D, Liddle C, McCaughan GW, George J. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. *Hepatology* 2004; 39: 1239-1247 [PMID: 15122752 DOI: 10.1002/hep.20207]

18. Tada S, Saito H, Ebinuma H, Ojoro K, Yamagishi Y, Kumagai N, Inagaki Y, Masuda T, Nishida J, Takahashi M, Nagata H, Hibi T. Treatment of hepatitis C virus with peg-interferon and ribavirin combination therapy significantly affects lipid metabolism. *Hepatology* 2009; 49: 195-199 [PMID: 19054155 DOI: 10.1001/1872-034X.2008.00430.x]

19. Lange CM, von Wagner M, Bojunga J, Berg T, Farrik H, Hassler A, Sarrazin C, Herrmann E, Zeuzem S. Serum lipids in European chronic HCV genotype 1 patients during and after treatment with pegylated interferon-α-2a and ribavirin. *Eur J Gastroenterol Hepatol* 2010; 22: 1303-1307 [PMID: 20729742 DOI: 10.1097/MEG.0b013e32836de92c]

20. Kuo YH, Chuang TW, Hung CH, Chen CH, Wang JH, Hu TH, Lu SN, Lee CM. reversal of hypolipidemia in chronic hepatitis C patients after successful antiviral therapy. *J Formos Med Assoc* 2011; 110: 363-371 [PMID: 21741004 DOI: 10.1016/S0929-6646(11)60054-5]

21. Serfaty I, Andreani T, Giral P, Carbonell N, Chazouillères O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. *J Hepatology* 2001; 34: 428-434 [PMID: 11322205 DOI: 10.1016/S0168-8278(00)00363-2]

22. Poynard T, Ratziu V, McHutchison J, Manns M, Goodman Z, Zeuzem S, Younossi Z, Albrecht J. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. *Hepatology* 2003; 38: 75-85 [PMID: 12892989 DOI: 10.1053/jhep.2003.05267]

23. Reddy KR, Govindarajan S, Marcellin P, Bernstein D, Dienstag JL, Bodenheimer H Jr, Rakela J, Messinger D, Schmidt G, Ackrill A, Hadziyannis SJ. Hepatic steatosis in chronic hepatitis C: baseline host and viral characteristics and influence on response to therapy with peginterferon alpha-2a plus ribavirin. *J Viral
Ampuero J, Romero-Gómez M, Reddy KR. Review article: HCV genotype 3 - the new treatment challenge. *Aliment Pharmacol Ther* 2014; 39: 686-698 [PMID: 24612116 DOI: 10.1111/apt.12646]

Kumada H, Suzuki Y, Ikeda K, Toyota J, Karino Y, Chayama K, Kawakami Y, Ido A, Yamamoto K, Takaguchi K, Izumi N, Koike K, Takehara T, Kawada N, Sata M, Miyagishi H, Eley T, McPhee F, Damokosh A, Ishikawa H, Hughes E. Daclatasvir plus asunaprevir for chronic HCV genotype 1b infection. *Hepatology* 2014; 59: 2083-2091 [PMID: 24604476 DOI: 10.1002/hep.27113]

Kanda T, Yasssi S, Nakamura M, Suzuki E, Arai M, Haga Y, Sasaki R, Wu S, Nakamoto S, Imazeki F, Yokosuka O. Daclatasvir plus Asunaprevir Treatment for Real-World HCV Genotype 1-Infected Patients in Japan. *Int J Med Sci* 2016; 13: 418-423 [PMID: 27279790 DOI: 10.7150/ijms.15519]

Hashimoto S, Yatsuhashi H, Abiru S, Yamasaki K, Komori A, Nagaoka S, Saeki A, Uchida S, Ikek S, Kugiyma Y, Nagata K, Nakamura M, Migita K, Nakao K. Rapid Increase in Serum Low-Density Lipoprotein Cholesterol Concentration during Hepatitis C Interferon-Free Treatment. *PLoS One* 2016; 11: e0163644 [PMID: 27680885 DOI: 10.1371/journal.pone.0163644]

Morales AL, Junga Z, Singla MB, Sjogren M, Torres D. Hepatitis C eradication with sofosbuvir leads to significant metabolic changes. *World J Hepatol* 2016; 8: 1557-1563 [PMID: 28050236 DOI: 10.4254/wjh.v8.i35.1557]

Townsend K, Meissner EG, Sidharthan S, Sampson M, Remaley AT, Tang L, Kohli A, Osinusi A, Masur H, Kottilil S. Interferon-Free Treatment of Hepatitis C Virus in HIV/Hepatitis C Virus-Coinfected Subjects Results in Increased Serum Low-Density Lipoprotein Concentration. *AIDS Res Hum Retroviruses* 2016; 32: 456-462 [PMID: 26559180 DOI: 10.1089/AID.2015.0170]

Lonardo A, Adinolfi LE, Restivo L, Ballestri S, Romagnoli D, Baldelli E, Nascimbeni F, Loria P. Pathogenesis and significance of hepatitis C virus steatosis: an update on survival strategy of a successful pathogen. *World J Gastroenterol* 2014; 20: 7089-7103 [PMID: 24966582 DOI: 10.3748/wjg.v20.i23.7089]

González-Reimers E, Quintero-Platt G, Rodríguez-Gaspar M, Alemán-Valls R, Pérez-Hernández O, Santolaria-Fernández F. Liver steatosis in hepatitis C patients. *World J Hepatol* 2015; 7: 1337-1346 [PMID: 26052379 DOI: 10.4254/wjh.v7.i10.1337]

Chang ML. Metabolic alterations and hepatitis C: From bench to bedside. *World J Gastroenterol* 2016; 22: 1461-1476 [PMID: 26819514 DOI: 10.3748/wjg.v22.i4.1461]
