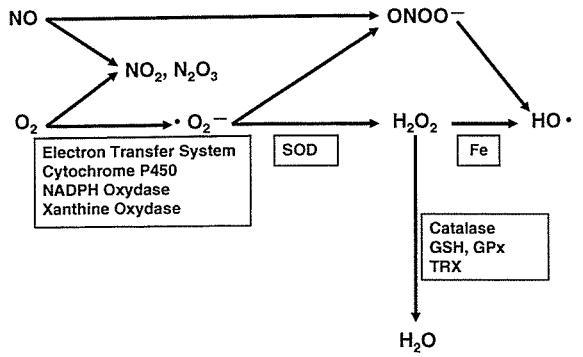

Fig. 2. Generation and scavenging of oxidative stress. SOD, super-oxide dismutase; GSH, reduced glutathione; GPx, glutathione peroxidase; TRX, thioredoxin.

and oxygen with GSH as an electron donor and reduces lipid peroxide to become neutralized. GSSG is converted back to GSH when glutathione reductase transfers an electron from NADPH to GSSG. Catalase in peroxisomes also decomposes H_2O_2 to water and oxygen. TRX is also a protein induced by oxidative stress, and is reduced via the S–S binding of the substrate protein by two SH groups in TRX and acts on the H_2O_2 elimination system via peroxiredoxins (Fig. 2).

ROS cause various forms of cellular damage. 4-Hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) are the peroxidation reaction products of lipids, and 8-hydroxydeoxyguanosine (8-OHdG) is the product of DNA base modification (Fig. 3). These products serve as oxidative stress markers.

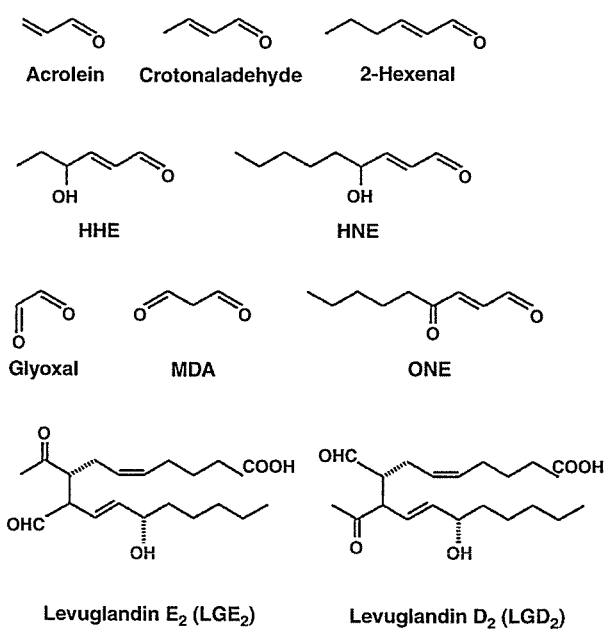

Fig. 3. Representative aldehyde species generated via lipid peroxidation reaction.

3. Viral infection and oxidative stress

3.1. ROS production associated with viral infection

Upon viral infection, ROS are produced by NADPH oxidase and xanthine oxidase in neutrophils and macrophages. In particular, NS3, one of the non-structural proteins of HCV, was reported to induce ROS production by NADPH oxidase in neutrophils [6]. Furthermore, in viral hepatitis, ROS are also produced in hepatocytes through the release of inflammatory cytokines such as TNF-α and IL-1β from inflammatory cells. Increased hepatic or serum 8-OHdG, HNE and MDA levels are observed in chronic hepatitis C, indicating an increase in ROS production [7–13]. Findings that indicate an increase in the activity of the ROS elimination system including decreased hepatic and blood GSH levels, an increased GSSG/GSH + GSSG ratio, and an increased serum TRX level have been reported [13–16]. The findings of markedly decreased HNE level following viral eradication with interferon [12] and decreased serum ALT and TRX levels following the administration of vitamin E, an antioxidant [17], also demonstrated that oxidative stress plays an important role in chronic hepatitis C.

3.2. Nitric oxide production associated with viral infection

In the presence of an inflammation, inducible nitric oxide synthase (iNOS) is induced in macrophages and hepatocytes by TNF-α and IFN-γ [18–20]. Other investigators reported that protein kinase (PKR) activated by double-stranded RNA formed during virus reproduction in turn activates the transcripts of NF-κB and IRF-1 to induce iNOS [21]. In the case of HCV, it was reported that its constituent proteins (E2 and non-structural (NS) protein 5A) inhibit PKR activity [22,23], but iNOS induction by viral RNA via PKR is also suspected. Indeed, iNOS synthesis correlates with intrahepatic viral load in chronic hepatitis C [24].

NO is generally synthesized as a non-specific defense reaction to infectious diseases; however, in viral infection, antiviral activity may be present or absent in various viral types [20]. NO is reported to exhibit no antiviral activity against a tick-borne encephalitis virus (TBE-V), flavivirus [25], and NO may also have no antiviral activity against HCV. On the contrary, NO causes cellular damage upon its reaction to O_2 or simultaneously produced O_2^- (reactive nitrogen species, RNS). Upon reaction to O_2^-, in particular, NO acts as a strong oxidant with the generation of peroxynitrous acid ($ONOO^-$), and $ONOO^-$ also produces nitrotyrosine through the nitration of aromatic amino acid residues in the presence of a transition metal. Nitrotyrosine accumulation was observed in correlation to inflammation severity in chronic hepatitis C tissue [26]; suggesting that the production of both NO and ROS increased. ROS and RNS are produced as defense factors for biological viral clearance, but these factors also have cytotoxic effects that

are assumed to contribute to the exacerbation of the disease state.

4. Oxidative stress caused by viral protein

The HCV genome comprises the genes of four structural proteins and six non-structural proteins (Fig. 4), and it has been reported that at least two viral proteins cause oxidative stress in cells. The core protein, a structural protein, was found to have various actions, including the induction of oxidative stress and accumulation of lipids, in experimental studies using cultured cells and transgenic mice [2,27]. Experiments using mice transgenic for the core gene showed an increased ROS production, an increased intrahepatic catalase activity, a decreased intrahepatic GSH level and a decreased GSH/GSH – GSSG ratio indicating an antioxidation effect inhibition, although there was no increase in serum ALT level nor a histological finding of hepatitis. Increased levels of intrahepatic peroxide lipids in the core gene transgenic mice with aging as compared with those in the control mice also indicate increased oxidative stress. As a mechanism underlying oxidative stress induction by the core protein, mitochondrial damage is considered. Morphological abnormalities of the mitochondria were observed in the core gene transgenic mouse liver [2], and an increased ROS production caused by damage of the mitochondrial electron transport system was noted in core-protein-expressing cells [27]. Mitochondrial DNA, which has no protective proteins such as histone, is susceptible to damage by ROS [28,29]. Mitochondrial DNA in the core gene transgenic mice showed damage as early as 3-months old. This mitochondrial damage disrupts the synthesis of proteins constituting the electron transport system complex and could also increase oxidative stress caused by damage of the electron transport system.

A study using a cell culture system demonstrated that nonstructural protein 5A (NS5A) also causes oxidative stress. NS5A induces endoplasmic reticulum calcium release via endoplasmic reticulum stress, and this leads to an increased ROS production in the mitochondria [4]. Although the effect of NS5A has not been confirmed yet by other study groups, HCV has the direct action of increasing intracellular ROS production via its proteins, separate from oxidative stress induction by inflammation caused by viral infection. A report that oxidative stress was also observed in HCV carriers with a normal ALT level [13] indicates that it is caused by a direct oxidative stress induction without being mediating inflammatory reactions.

5. Relationship of HCV infection with insulin resistance

The relationship of HCV infection with insulin resistance and type 2 diabetes has been suggested epidemiologically [30–32]. Insulin resistance was also observed in core gene transgenic mice before the onset of hepatic steatosis [33]. A disrupted tyrosine phosphorylation of the insulin receptor substrate (IRS-1) was observed in the liver of these transgenic mice. The analysis of hepatic tissues in patients with chronic hepatitis C not complicated by diabetes showed that insulin receptor and IRS-1 expression levels are elevated in patients with HCV infection, whereas the tyrosine phosphorylation of IRS-1 induced by insulin is inhibited. An excessive oxidative stress may be another potential cause of this insulin resistance. Oxidative stress indirectly blocks the phosphorylation of tyrosine residues of insulin receptors and IRS-1 and inhibits insulin signaling [34].

These reported results thus indicate an insulin signaling disorder in the liver infected with HCV [35]. There has been no report to date directly proving that hepatic insulin signaling disorder in patients with HCV infection is attributable to oxidative stress. However, because diabetes, which is the state of having abnormally high blood sugar levels that cannot be self-regulated by individual organisms, also induces oxidative stress [34], the close relationship between insulin resistance or diabetes and oxidative stress as the cause and the

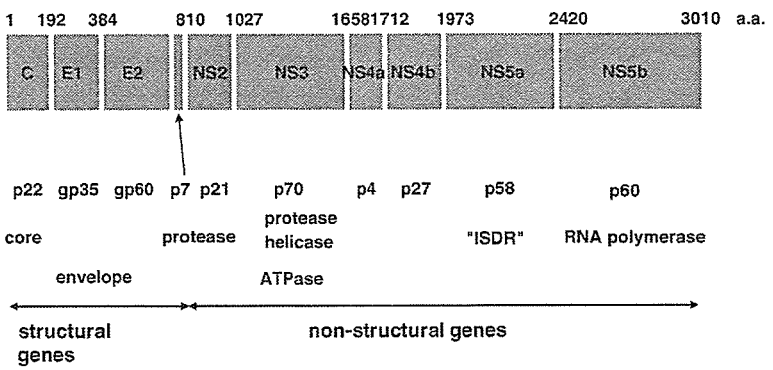

Fig. 4. Structure of hepatitis C virus genome. The genome of HCV consists of two parts, structural and non-structural regions. The former comprises the core and envelope regions, and the latter consists of NS2 to NS5a, which regions chiefly code enzymes necessary for viral replication. NS, non-structural; ISDR, interferon sensitivity-determining region.

result, respectively, is a very interesting issue to investigate in the future.

6. Relationship of HCV infection with hepatic steatosis

Hepatic steatosis is frequently observed in patients with HCV infection. The relationships of HCV infection with intrahepatic viral loads and core protein levels, different prevalence of hepatic steatosis by viral genotype [higher incidence for genotype 3a), and improved steatosis following viral eradication were reported [36–38]. It is presumed from these reports that HCV itself causes hepatic steatosis. A similar hepatic steatosis caused even by the core protein alone was observed in a study using an expression system in cultured cells and transgenic mice, and it was thus suggested that the core protein plays a significant role in hepatic steatosis as the direct action of HCV [39,40]. Hyperinsulinemia induced by insulin resistance mentioned above causes the overloading of the liver with fatty acids from fat cells, and mitochondrial damage inhibits the β-oxidation of fatty acids [41]. Furthermore, the core protein was reported to inhibit microsomal triglyceride transfer protein (MTP) activity that is required when neutral fat is released as very low-density liproproteins (VLDLs) [42]. All these actions could cause hepatic steatosis. In the liver of non-alcoholic steatohepatitis (NASH) patients, it was reported that β-oxidation in the mitochondria and peroxisomes or the metabolism of fatty acids by cytochrome P450 2E1 (CYP2E1) in microsomes is promoted under an excessive load of fatty acids, resulting in ROS production [43,44]. In HCV infection as well, intrahepatic fat accumulation possibly increases ROS production as in NASH. Because hepatic steatosis in chronic hepatitis C was reported to be a factor for disease progression [45–47], increased oxidative stress associated with hepatic steatosis is presumably involved in disease progression.

7. Iron and reactive oxygen

The iron content in the liver and spleen is high, and transition metals facilitate electron transfer and play an important role in the production of free radicals. Iron in combination with transferrin and ferritin is stable, but an unstable iron ion is freed when ferritin is decomposed by lysosomes [48]. ROS additionally promote iron release from ferritin [49]. A free iron ion catalyzes changes from relatively poor reactive O_2^- and H_2O_2 to a highly reactive $HO^\bullet$ (Fenton reaction) [50,51]. $HO^\bullet$ oxidizes membrane phospholipids, which compose cells and intracellular organelles, and iron forms radicals from produced peroxide lipids, thereby enhancing lipid peroxidation. Iron site-specifically combines with DNA and promotes DNA damage caused by ROS. Iron also increases ROS production by CYP2E1 [52]. A report that an enhanced peroxidation of intrahepatic lipids is attenuated by exsanguination in hemochromatosis also supports the involvement of iron in oxidative stress [53].

An excessively high iron content in the liver was observed in chronic hepatitis C [8,54]. Other investigators reported that iron removal therapy by exsanguination of chronic hepatitis C patients significantly improves serum ALT level without affecting viral load [55–57]. Another study showed that hepatic impairment is exacerbated following the administration of iron to chimpanzees with chronic hepatitis C [58]. Furthermore, oxidative stress is decreased by the iron removal therapy for chronic hepatitis C using intrahepatic 8-OHdG level as an index [57]. The above-mentioned reports show the close relationships of chronic hepatitis C with iron metabolism and oxidative stress.

8. Interactions with alcohol

Alcohol metabolism plays an important role in ROS production. Mainly alcohol dehydrogenase (ADH) in the cytosol and CYP2E1 (microsomal ethanol-oxidizing system) in microsomes are responsible for alcohol metabolism in the liver. When alcohol dehydrogenase oxidizes ethanol to acetaldehyde, the reduction from NAD^+ to NADH simultaneously occurs. NADH accumulation causes stress on the mitochondrial electron transfer system, leading to an increased production of ROS [59]. NADH also inhibits xanthine dehydrogenase activity, and xanthine is thereby oxidized by xanthine oxidase with the production of ROS [60]. CYP2E1 is induced by chronic alcohol intake and ROS are produced when CYP2E1 oxidizes ethanol to acetaldehyde [52,61].

There is no significant difference in hepatic peroxide level between core gene transgenic mice at 3–6-months old and control transgenic mice, but hepatic peroxide level significantly increases following the administration of a low dose of alcohol in the core gene transgenic mice [2]. ROS production increases upon glutathione reduction in HepG2 cells, with the co-expression of the core protein and CYP2D1, the latter of which is induced by alcohol [62]. These findings show that the core protein and alcohol in combination increase oxidative stress. Indeed, it was reported that alcohol intake plays a role in promoting the progression of chronic hepatitis C [63,64] and that increased levels of oxidative stress markers such as HNE and lipid hydroperoxide also support these findings [65]. From the viewpoint of oxidative stress also, HCV infection and alcohol intake are both considered to promote hepatic impairment.

9. Hepatocarcinogenesis and oxidative stress

It has been demonstrated that oxidative stress plays a key role in carcinogenesis [66,67]. Animal experiments using hepatocarcinogenesis models with the administration of a chemical substance (diethyl-nitrosamine, peroxisome proliferators) and with the administration of a choline-deficient

amino acid diet also indicates the involvement of oxidative stress [68–72]. In Long Evans Cinnamon (LEC) rats, an animal model that spontaneously develops heritable hepatitis and HCC caused by an abnormal copper accumulation, a congenitally decreased glutathione peroxidase expression level was reported, and the close relationship between oxidative stress and hepatocarcinogenesis was indicated [73].

The epidemiological relationship between HCV infection and HCC is evident [74,75], but the mechanism underlying this relationship has not been fully elucidated yet. Among postulated hypotheses on the mechanism of HCV-associated hepatocarcinogenesis, that of the involvement of the viral protein, in particular, the core protein of HCV is attractive: HCC develops in core gene transgenic mice, and carcinogenesis starts with well-differentiated carcinoma with an excessively high fat content, similar to hepatocarcinogenesis in human chronic hepatitis C, and poorly differentiated carcinoma with a low fat content develops in the form of "nodules in nodules" [76]. Because oxidative stress is increased in the core gene transgenic mice as mentioned above, it is assumed that oxidative stress plays an important role in hepatocarcinogenesis in chronic hepatitis C. Because the development of HCC is also observed in transgenic mice carrying the full-length HCV protein gene, the non-structural protein may have an additive effect to the effect of the structural proteins including the core protein, contributing to hepatocarcinogenesis [77]. NS5A, which was also reported to induce ROS production [4], may also contribute to hepatocarcinogenesis, although ROS induction by NS5A is not unequivocally confirmed yet.

Mitochondrial DNA has no potent protective proteins such as histone and is near the electron transport system, the major ROS production site. Hence, it is 10 to 15 times more susceptible to mutation caused by ROS than nuclear DNA [28,29]. In an investigation of mitochondrial DNA mutation in the human normal liver, both cancerous and non-cancerous liver tissues in patients with HCC showed very high incidences of DNA mutations [78]; thus, a relationship between oxidative stress persistence and hepatocarcinogenesis is suggested.

In the core protein expression system in the hepatic tissue and cultured cells of core gene transgenic mice, the activation of transcription factor AP-1 via mitogen-activated protein (MAP) kinase was observed [79–83]. The activation of the transcription factors AP-1, NF-κB, and signal transducer and activator of transcription (STAT) 3 by NS5A were also reported [4,84]. The activation of these transcription factors may facilitate cell proliferation, contributing to tumorigenic transformation.

It was also reported that ROS facilitate apoptosis via c-Jun N-terminal kinase (JNK)/p38 MAP kinase or by directly attacking the mitochondria. Apoptosis is a protective mechanism of the host against viral infection and carcinogenesis. Some reports stated that the core protein facilitates apoptosis [85–88], whereas other reports stated that the core protein inhibits apoptosis [89–92]; thus, no fixed view has yet been established. If it indeed inhibits apoptosis, it is assumed that this inhibition proceeds by maintaining oxidative stress and that the core protein has a beneficial effect against carcinogenesis and persistent viral infection.

In HCV infection, viral proteins such as the core protein and, possibly, NS5A protein induce oxidative stress, intracellular signaling, and transcription factors, which are not reflected in blood ALT level, contributing to the progression of carcinogenesis. Carcinogenesis, however, is slow as is observed in humans and core gene transgenic mice, the latter of which developed HCC in the latter half of their life. Recently, Okanoue et al. reported a long-term follow-up study of subjects with persistent HCV infection who had persistently normal ALT levels (PNAL) [93]. In their study, serum thioredoxin levels were not elevated in those with PNAL compared to those with chronic hepatitis. This may apparently seem contradictory to the results of our above-mentioned animal model studies. However, we should realize that anti-oxidant system is also instrumental in the liver. In these relatively younger people with PNAL than those with CH [93], active anti-oxidant system may erase the apparent elevation of ROS. Such a phenomenon was described in a mouse model by Moriya et al. [2], in which ROS was apparently normal in young core gene transgenic mice with the activation of catalase and reduction of GSH. Clinically, the presence of inflammation is thought to facilitate the process of hepatocarcinogenesis.

10. Conclusions

A very close pathological relationship between oxidative stress and HCV infection is observed, as shown by the above overview of relevant publications and discussion. The causes of oxidative stress in HCV infection are considered to include various factors such as mitochondrial damage, endoplasmic reticulum stress, iron accumulation, and lipid accumulation in the liver. Various study results demonstrated that even only viral proteins, mainly the HCV core protein, cause oxidative stress. When inflammation via immunoreactions to viral infection is added to oxidative stress, ROS production is expected to further increase, leading to a state in which the anti-oxidation system cannot cope with. In this sense, inflammation in chronic hepatitis C is considered to be qualitatively different from inflammation observed in other types of hepatitis such as autoimmune hepatitis or hepatitis B [94] (Fig. 5). As a treatment of chronic hepatitis C, the eradication of the virus is ideal. If it is not possible, however, the control of factors that exacerbate oxidative stress, such as inflammation via immune reaction and alcohol, and the relief of oxidative stress by the iron removal therapy and the administration of an anti-oxidation agent are considered to delay the progression of chronic hepatitis.

The development of such new anti-oxidation agents is being awaited. In further studies on the development of new therapies for hepatitis C and control methods for hepatocarcinogenesis in the future, the importance of those focusing on oxidative stress is expected to markedly increase.

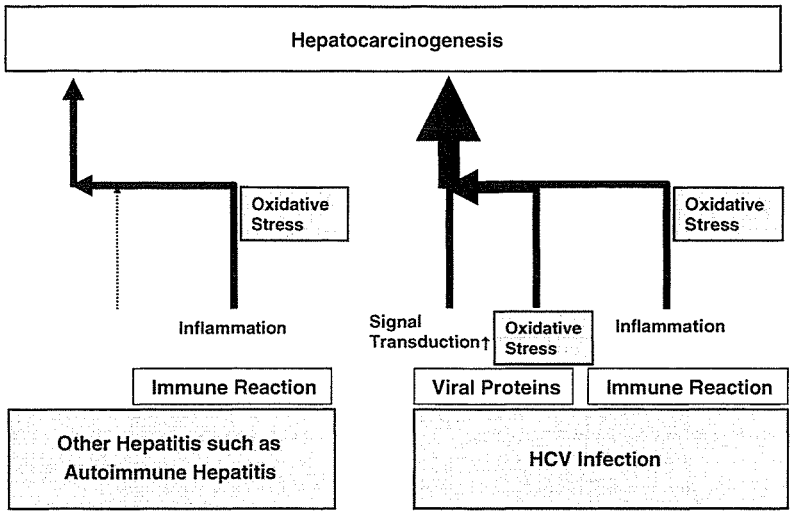

Fig. 5. Oxidative stress and hepatocarcinogenesis in various types of hepatitis (hypothesis). Oxidative stress is generated in all types of hepatitis via inflammation accompanied by continual cell death and regeneration. In HCV infection, HCV itself causes the production of oxidative stress in a synergy with inflammation. In this sense, the quality of "inflammation" in HCV infection may be different from that in other types of hepatitis. Additional impact of HCV proteins on the intracellular signal transduction would provoke the development of HCC. These may explain the conspicuous properties of HCC development.

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Journal of Gastroenterology

Editorial

Oxidative stress and apoptosis in hepatitis C: the core issue

Article on page 257
Hepatitis C virus core protein inhibits deoxycholic acid-mediated apoptosis despite generating mitochondrial reactive oxygen species
HARA Y, HINO K, OKUDA M, et al.

Extra- as well as intracellular stimuli elicit a wide range of responses, such as cell proliferation, differentiation, survival and apoptosis, via the regulation of intracellular signaling. Recent studies have revealed that stress-responsive signal transduction pathways are stringently regulated by the intracellular redox state.[1] The redox state of the cell is determined by the delicate balance between the levels of oxidizing and reducing equivalents, including reactive oxygen species (ROS) and endogenous antioxidants. The production of ROS, a representative of oxidative stress, fluctuates in response to alterations in both external and internal environments and, in turn, triggers specific signaling cascades, such as mitogen-activated protein kinases, which determine cell survival or death. Thus, ROS are profoundly involved in cell death or apoptosis.

In the liver, ROS are also key cytotoxic and signaling mediators in the pathophysiology of liver diseases, including viral hepatitis, in which hepatocytes and resident and infiltrating phagocytes can generate ROS. While ROS are able to cause cell death through massive lipid peroxidation, they also act to modulate signal transduction pathways by affecting redox-sensitive enzymes, transcription factors, and organelles, including mitochondria and endoplasmic reticulum. ROS, thus, directly regulate apoptotic and necrotic cell death.[2] In addition, ROS have indirect effects on the pathophysiology of cell death by supporting protease activity via inactivation of antiproteases.

In hepatitis C virus (HCV) infection, both ROS and apoptosis are closely involved in the process of progressive liver diseases from chronic hepatitis to cirrhosis and hepatocellular carcinoma (HCC).[3–7] ROS are assumed to play a major role in the pathogenesis of chronic hepatitis, which is characterized by continual cell death followed by regeneration. In this condition, the viral proteins of HCV, the core and nonstructural (NS) 5A proteins, have been shown to play a role in inducing ROS as well as in modulating apoptosis of hepatocytes.

The HCV genome comprises the genes of four structural proteins and six nonstructural proteins, and at least two of these viral proteins have been reported to cause oxidative stress in cells. The core protein, a structural protein, has been found to have various actions, including the induction of oxidative stress and the accumulation of lipids, in experimental studies using cultured cells and transgenic mice.[7,8] Experiments using mice transgenic for the core gene showed increased ROS production, increased intrahepatic catalase activity, a decreased intrahepatic glutathione (GSH) level, and a decreased GSH/GSH-GSSG (dimeric oxidized glutathione) ratio, indicating inhibition of antioxidation effects, although there was neither an increase in the serum alanine aminotransferase (ALT) level nor a histological finding of hepatitis.[7] Increased levels of intrahepatic peroxide lipids in the core gene transgenic mice with aging, compared with levels in control mice, also indicate an increase in oxidative stress. One possible mechanism underlying oxidative stress induction by the core protein is mitochondrial damage. Morphological abnormalities of the mitochondria have been observed in core gene transgenic mouse liver,[7] and increased ROS production caused by damage to the mitochondrial electron transport system has been noted in core protein-expressing cells.[8] Mitochondrial DNA, which has no protective proteins such as histone, is susceptible to damage by ROS. Mitochondrial DNA damage in the core gene transgenic mice appeared when they were as young as 3 months old. This mitochondrial damage disrupts the synthesis of proteins constituting the electron transport system complex and might also increase oxidative stress caused by damage to the electron transport system.

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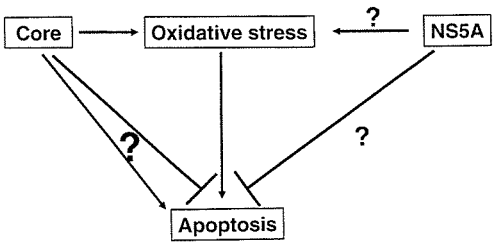

Fig. 1. Relationships among oxidative stress, apoptosis, and HCV proteins

A study using a cell culture system demonstrated that the NS5A protein also causes oxidative stress. NS5A induces the endoplasmic reticulum to release calcium by causing stress to the endoplasmic reticulum, and this release leads to increased ROS production in the mitochondria.[9] Although the effect of NS5A has not yet been confirmed by other study groups, HCV has the direct action of increasing intracellular ROS production via its proteins, separate from the induction of oxidative stress as a result of inflammation caused by viral infection (Fig. 1). A report that oxidative stress is also observed in HCV carriers with a normal ALT level[10] indicates that it is induced directly, without any mediating inflammatory reactions being necessary.

In contrast to the production of ROS by the core protein, which is now quite evident, the role of HCV core protein in apoptosis is rather controversial. Regarding the HCV-induced apoptotic mechanism, the HCV core protein may have a regulatory function in modulating apoptosis, either by enhancing or inhibiting it. In particular, the core protein exhibits both proapoptotic and antiapoptotic actions, depending on experimental conditions and the type of cells used,[11–14] whereas both the NS3 and the NS5A proteins have antiapoptotic effects (Fig. 1).[15] Modulation of apoptosis may involve binding of the core protein to the intracellular signal transducing portion of death receptors such as TNF-α, Fas, or lymphotoxin-αβ. Thus, HCV proteins may modulate hepatocyte apoptosis by indirect rather than by direct mechanisms. The real role of the core protein in the apoptotic process is, thus, not defined yet.

In the current issue of *Journal of Gastroenterology*, Hara et al.[16] tried to elucidate this core issue of HCV pathogenesis by separating the two properties of the HCV core protein with cultured cells, Huh-7 and HeLa. They confirmed that the core protein induced ROS, which was followed by activation of the scavenging system and insults to the cellular DNA, as shown previously.[6] In the study by Hara et al.,[16] the core protein inhibited the proapoptotic action of deoxycholic acid (DCA), which is known to cause both ROS production and apoptosis. Thus, the core protein seems to act to oppose the proapoptotic function of ROS, which ROS are also induced by the core protein itself. Such apparently opposing actions of the core protein, the production of ROS and the inhibition of apoptosis, might well explain the mode of hepatocarcinogenesis in HCV infection: hepatocytes with ROS-induced DNA damage may evade apoptosis by another effect of the core protein that inhibits apoptosis. Such a mechanism, similar to one previously postulated by other researchers,[3] in which both ROS production and mitogen-activated protein kinase activation are ascribed to the core protein, may clarify how cells with DNA damage can survive and develop into buds of HCC. Regrettably, the current study was done using DCA as an agent to induce both apoptosis and ROS, making it difficult to interpret the authentic role of the core protein in the execution of such biological functions. As noted above, the effect of the core protein in apoptosis varies depending on the system used. Therefore, further studies using different systems may be necessary to bring a conclusion to the core issue of HCV-induced pathogenesis associated with the multipotential HCV core protein.

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RAPID COMMUNICATION

Risk factors for retinopathy associated with interferon α-2b and ribavirin combination therapy in patients with chronic hepatitis C

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Abstract

AIM: To elucidate the frequency and risk factors for retinopathy in patients with chronic hepatitis C who are treated by interferon-ribavirin combination therapy.

METHODS: We prospectively analyzed 73 patients with histologically confirmed chronic hepatitis C, who underwent combination therapy for 24 wk. Optic fundi were examined before, and 2, 4, 12 and 24 wk after the start of combination therapy.

RESULTS: Fourteen patients (19%) developed retinopathy, which was initially diagnosed by the appearance of a cotton wool spot in 12 patients. Retinal hemorrhage was observed in 5 patients. No patient complained of visual disturbance. Retinopathy disappeared in 9 patients (64%) despite the continuation of combination therapy. However, retinopathy persisted in 5 patients with retinal hemorrhage. A comparison of the clinical background between the groups with and without retinopathy showed no significant differences in age, gender, viral genotype, RNA level, white blood cell count, platelet count, prothrombin time, complications by diabetes mellitus or hypertension, or pretreatment arteriosclerotic changes in the optic fundi. However, multiple logistic regression analysis revealed that complication by hypertension was observed with a high frequency in the group with retinopathy (P = 0.004, OR = 245.918, 95% CI = 5.6-10786.2).

CONCLUSION: Retinopathy associated with combination therapy of interferon α-2b and ribavirin tends to develop in patients with hypertension.

Key words: Retinopathy; Ribavirin; Chronic hepatitis C; Interferon

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INTRODUCTION

Chronic hepatitis C, which affects more than 170 million people in the world[1], may eventually lead to cirrhosis and/or hepatocellular carcinoma. The main treatment for this intractable disease is interferon administration. Published guidelines recommend interferon-ribavirin combination therapy as a first-line treatment[2]. Interferon is also used in the treatment of other viral and neoplastic diseases.

Various adverse effects have been reported due to use of interferon[3]. An influenza-like syndrome, characterized by fever, chills, myalgias, arthralgias, and headache, is the most common adverse effect. Toxicities of the central nervous, hematopoietic, gastrointestinal, urinary, cardiovascular, musculoskeletal and endocrine systems have also been described. However, ocular toxicity was not reported before the use of interferon for chronic hepatitis[3].

After the introduction of interferon for the treatment of hepatitis, retinal complications have been reported. Hayakawa *et al* showed that 17 of 43 patients developed retinopathy during interferon monotherapy. They also showed that the prevalence of retinopathy was higher in patients with diabetes[4]. Subsequently, several papers have shown that a substantial proportion of patients undergoing interferon monotherapy develop retinopathy[5-7]. However, the prevalence of retinopathy is variable, which is

presumably attributed to the difference in the treatment regimen and/or background of patients.

As mentioned above, interferon-ribavirin combination therapy has become the standard treatment for chronic hepatitis C. Results from recent studies have suggested that the prevalence of retinopathy associated with combination therapy may be higher than that associated with interferon monotherapy, which should be further investigated[8-10].

In spite of the high prevalence, risk factors for interferon-associated retinopathy are still unclear. Diabetes mellitus and the patients' age were reported to be possible risk factors for retinopathy associated with interferon monotherapy[4]. In interferon-ribavirin combination therapy, diabetes, hypertension[8], and response to treatment[10] were considered possible risk factors. However, the results are not conclusive because of the small number of patients examined.

The aim of the present study is to elucidate the prevalence and risk factors for retinopathy associated with interferon-ribavirin combination therapy.

MATERIALS AND METHODS

Patients

Seventy-three consecutive patients with histologically confirmed chronic hepatit is C (47 males and 26 females; median age, 53.4 years; ranges 26-73 years) were enrolled in this study from 2002 to 2004. The clinical backgrounds of the enrolled patients are shown in Table 1. All patients were treated with recombinant interferon α-2b (Intron A, Schering-Plough, Kenilworth, NJ, USA) and ribavirin (Rebetol; Schering-Plough, Kenilworth, NJ, USA) combination therapy. All the patients were treated daily with interferon α-2b at 6 MU for 2 wk followed by three times a wk treatment with interferon α-2b at 6 MU for 22 wk in combination with ribavirin. Ribavirin was given orally twice a day at a total daily dose of 600 mg for patients who weighed 60 kg or less and 800 mg for the remaining patients who weighed more than 60 kg for 24 wk.

All patients were assessed to determine the safety, tolerance, and efficacy of the treatment at the end of wk 1, 2, 4, and every 4 wk during the treatment. After the treatment was completed, patients were followed up on wk 4, 8, 12, and 24. The primary end point was indicated by a sustained loss of detectable HCV-RNA at 24 wk after the treatment.

Methods

Optic fundi were examined before, and 2, 4, 12 and 24 wk after the start of combination therapy. Ophthalmological examinations were carried out before the start of treatment and 2, 4, 12 and 24 wk after the start of treatment until the completion of treatment or until the retinopathy disappeared. Fundus photographs were taken for documentation and comparison when retinal abnormalities were detected.

Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committees of our institutions.

Table 1 Clinical findings of patients treated with interferon-ribavirin combination therapy

	Total	Retinopathy (+)	Retinopathy (-)
Patients			
Number	73	14	59
Age (yr)	53.4 ± 10.9	56.3 ± 10.5	52.8±38.6
Gender (M/F)	47/26	10/4	37/22
Hypertension (Yes/No)[a]	15/58	5/8	10/49
Diabetes mellitus (Yes/No)	2/71	1/13	1/58
Peripheral blood count			
Platelet count ($\times 10^4$/mm^3)	15.3 ± 6.0	12.5 ± 10.5	15.9 ± 38.6
White blood cell ($\times 10^3$/mm^3)	46.9 ± 12.6	46.5 ± 13.0	48.6 ± 10.9
Hemoglobin (×g/dL)	14.0 ± 1.3	14.0 ± 1.0	14.0 ± 1.4
Prothrombin time (%)	90.2 ± 13.3	87.1 ± 13.3	90.8 ± 13.3
ALT (IU/L)	109.4 ± 78.2	104.1 ± 41.0	110.4 ± 83.6
Viral factors			
Genotype (type 1/type 2)[2]	45/26	33/24	12/2
Viral load (kcopies/mL)	592.3 ± 271.2	505.6 ± 309.1	607.5 ± 271.2
Pretreatment/ Arteriosclerotic changes in optic fundi (Yes/No)	12/61	7/7	5/54
Response to therapy (SVR/non-SVR)	38/35	5/9	33/26

[1] Data are expressed as mean ± SD.
[2] Genotype could not be determined in 2 patients.
[a] $P = 0.004$

RESULTS

Before the start of the combinaiton therapy, one patient had scars from laser coagulation of a previous interferon-associated retinopathy and another patient had retinal central vein occlusion. Arteriosclerotic changes of the optic fundi were observed in 12 patients.

After the start of interferon-ribavirin combination therapy, 14 out of 73 patients (19%) developed retinopathy. The clinical profiles and laboratory data of the patients with and without retinopathy are shown in Table 1.

We compared the characteristics of patients who developed retinopathy and those who did not. The two groups showed no statistical differences in age, gender, subtype of virus, RNA level, white blood cell count, platelet count, prothrombin time before treatment or prevalence of pretreatment fundic arteriosclerotic changes. The patients with retinopathy were more frequently complicated by hypertension ($P = 0.004$) (Table 1).

Logistic regression analysis of factors affecting retinopathy was also carried out. Hypertension was found to be a factor for predicting retinopathy (Table 2).

Table 3 shows the optic fundi findings of the 14 patients with retinopathy. Retinopathy was initially diagnosed by the appearance of a cotton wool spot in 12 patients. In three of the 12 patients, retinal hemorrhage was also observed simultaneously or sequentially. Two of the 14 patients who developed retinopathy were diagnosed by retinal hemorrhage without a cotton wool spot. No patient complained of the visual disturbance.

Table 2 Logistic regression analysis of factors associated with retinopathy

Factor	P	Odds ratio	95% confidence interval
Sex	0.68	1.699	0.1-21.0
Age	0.203	1.099	1.0-1.3
Genotype	0.776	1.621	0.1-45.5
Levels of HCV RNA	0.114	1.006	0.99-1.0
Hypertension	0.004	246.32	5.5-10977.8
Diabetes mellitus	0.211	0.122	0.1-3.3
Abnormal findings in pretreatment optic fundi	0.904	1.192	0.1-20.3
Platelet	0.059	1.391	1.0-1.9
Prothrombin time	0.747	0.982	0.9-1.1
ALT	0.992	1	0.98-1.0
WBC	0.964	1.027	0.4-2.9
Response to therapy (SVR or non-SVR)	0.123	0.016	0.0-3.1

Table 3 Patients with retinopathy associated with interferon-ribavirin combination therapy

No	Age	Sex	Underlying disease		Optic fundi before treatment		Optic fundi after treatment	
			Hypertension	Diabetes mellitus	H	S	Cotton wool spot	Retinal hemorrhage
1	38	M	+	+	0	0	4 wk-	4 wk-
2	52	M	+	-	1	0	4-12 wk	-
3	40	M	-	-	0	0	6-36 wk	-
4	62	F	-	-	0	0	4-36 wk	-
5	61	M	+	-	0	0	12 wk-	-
6	58	M	-	-	1	1	12 wk-	-
7	73	M	-	-	2	2	4-28 wk	-
8	65	F	+	-	0	0	24-36 wk	-
9	59	F	+	-	2	2	2 wk-	4-24 wk
10	40	M	-	-	0	0	4-20 wk	-
11	62	F	-	-	1	2	2 wk-	4 wk-
12	65	M	-	-	1	1	2-24 wk	-
13	40	M	-	-	0	0	-	8-16 wk
14	40	M	-	-	0	0	-	2-4 wk

Retinopathy disappeared in 9 of the 14 patients despite the continuation of combination therapy. However, it continued in three patients with retinal hemorrhage and two without retinal hemorrhage.

Ocular manifestations other than retinopathy (e.g., ocular pain, a mild watery eye and conjunctivitis) were not observed in any patients.

DISCUSSION

Interferon associated retinopathy was first recognized in 1990 when Ikebe and associates reported a 39-year-old patient who developed retinal hemorrhages and cotton wool spots following intravenous administration of interferon[11].

The exact mechanism of interferon-induced-retinopathy is not known but is presumably related to the disturbance in retinal microcirculation[12]. Therefore, preexisting arteriosclerosis that affects microcirculation may promote interferon-induced retinopathy.

Our study shows that hypertension is a more frequent complication in patients with interferon-induced-retinopathy. Chronic hypertension is associated with the thickening of the walls of the arteries and small arterioles[13]. Therefore, systemic hypertension predisposes patients to interferon-induced-retinopathy. The fact that hypertensive retinopathy induces the formation of flame-shaped hemorrhages and white cotton wool spots, which are also seen in interferon-induced-retinopathy, implies that systemic hypertension and interferon-induced-retinopathy may be related each other.

Statistical analysis did not indicate pretreatment optic fundic changes or diabetes as predictive factors of retinopathy. This may be attributed to the following reasons: (1) pretreatment changes in the optic fundi as a predictive factor are included in hypertension; and (2) the number of patients with diabetes is too small. Regardless of these reasons, systemic hypertension is an important risk factor for interferon-related retinopathy.

The frequencies of interferon-induced retinopathy associated with interferon monotherapy and interferon-ribavirin combination therapy are reported to be 24%-58%[1-7,14,15] and 16%-64%[8-10,16], respectively. The frequency in the present study (20%) was lower than that in previous reports. Furthermore, the ocular side effects of ribavirin, which include a mild watery eye and conjunctivitis, were not seen in this study. Therefore, the frequency of induced retinopathy associated with combination therapy may be considered as high as that associated with interferon monotherapy.

Retinopathy developed by 12 wk in most (13/14, 93%) of the patients after the start of combination therapy and disappeared in majority (10/14, 71%) of the patients during the 4-8 wk period, in which the patients were receiving the treatment. This suggests that treatment can be continued despite the development of retinopathy in many patients. However, two patients who developed cotton wool spots early in the therapy (2 wk) therafter suffered from retinal hemorrhage in a prolonged manner. Therefore, patients who develop cotton wool spots early in the therapy should be carefully monitored. However, as reported in previous studies[4,8,17], most of the patients with retinopathy in this study were asymptomatic. Therefore, combination therapy may be continued in most patients.

The fact that retinopathy occurred more frequently in patients with hypertension, suggests that these patients should be carefully monitored. With periodic examination of the optic fundi, major bleeding that causes visual symptoms may be prevented or detected at an early stage. Therefore, patients who undergo interferon-ribavirin combination therapy, particularly those with hypertension, should undergo periodic examination of the optic fundi.

To conclude, retinopathy associated with combination therapy of interferon α-2b and ribavirin tends to develop in patients with hypertension.

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REVIEW ARTICLE

Kazuhiko Koike

Antiviral treatment of hepatitis C: present status and future prospects

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Abstract Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis. A substantial proportion of patients with chronic hepatitis C eventually develop hepatocellular carcinoma (HCC), which is one of the leading causes of death worldwide. Therefore, efficient antiviral treatments for HCV have long been needed. A recently developed combination therapy of pegylated interferon and ribavirin has dramatically improved the outcome of antiviral therapy for HCV infection. In genotype 1b HCV infection, 48 weeks of the combination therapy achieved eradication of the virus in 50% of patients, and in genotype 2 HCV infection, 24 weeks of the therapy resulted in viral eradication in 80%–90% of patients. By this eradication, an improvement in the hepatic fibrosis, an inhibition of HCC development, and an improvement in life expectancy were attained. Patients who did not respond to the combination therapy may be treated with long-term interferon monotherapy, which is not intended to eradicate HCV, but will lower the serum alanine aminotransferase (ALT) level. Thus, the treatment for HCV infection has progressed significantly, but therapies with new modalities, such as inhibitors of viral protease or RNA polymerase, are still being awaited.

Key words Hepatitis C · Interferon · Treatment

Introduction

Hepatitis viruses mainly infect the liver, causing hepatic diseases in humans. To date, five types of hepatitis virus, B, A, D, E, and C, have been found, in this order, and subjected to medical treatment. Hepatitis C virus (HCV) and hepatitis B virus (HBV) infections can develop into persistence, while hepatitis A virus and hepatitis E virus cause only transient infection. In Japan, chronic hepatitis caused by HCV infection currently poses the greatest problem because of the large number of patients affected and the high rate of patient mortality from complications, particularly hepatocellular carcinoma (HCC).[1]

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Chronic hepatitis C

It is estimated that there are approximately 170 million HCV carriers or patients with persistent HCV worldwide, and approximately 1.8 million patients in Japan. HCV infection occurs when blood contaminated with HCV enters the body directly. The infection routes include blood transfusion with HCV-contaminated blood products obtained a long time ago, sharing of needles among drug abusers, and the use of inappropriately disinfected acupuncture needles and tattoo needles, among others.[2] People undergoing folk remedies and hair-removal treatments should also be regarded as susceptible to HCV infection if these are invasive practices and nondisposable devices are used.

The problem with HCV infection resides in the very high rate of general HCV infections which are becoming chronic (approximately 70%). However, in the case of HCV infection via blood transfusion, the rate of reaching chronicity has been reported to reach 80%, probably because of a high virus load.

Virus markers of HCV infection required for the treatment of hepatitis

Some virus markers of HCV infection are available, as described below. Figure 1 shows a progress observation flowchart for anti-HCV antibody-positive patients obtained using these virus markers.

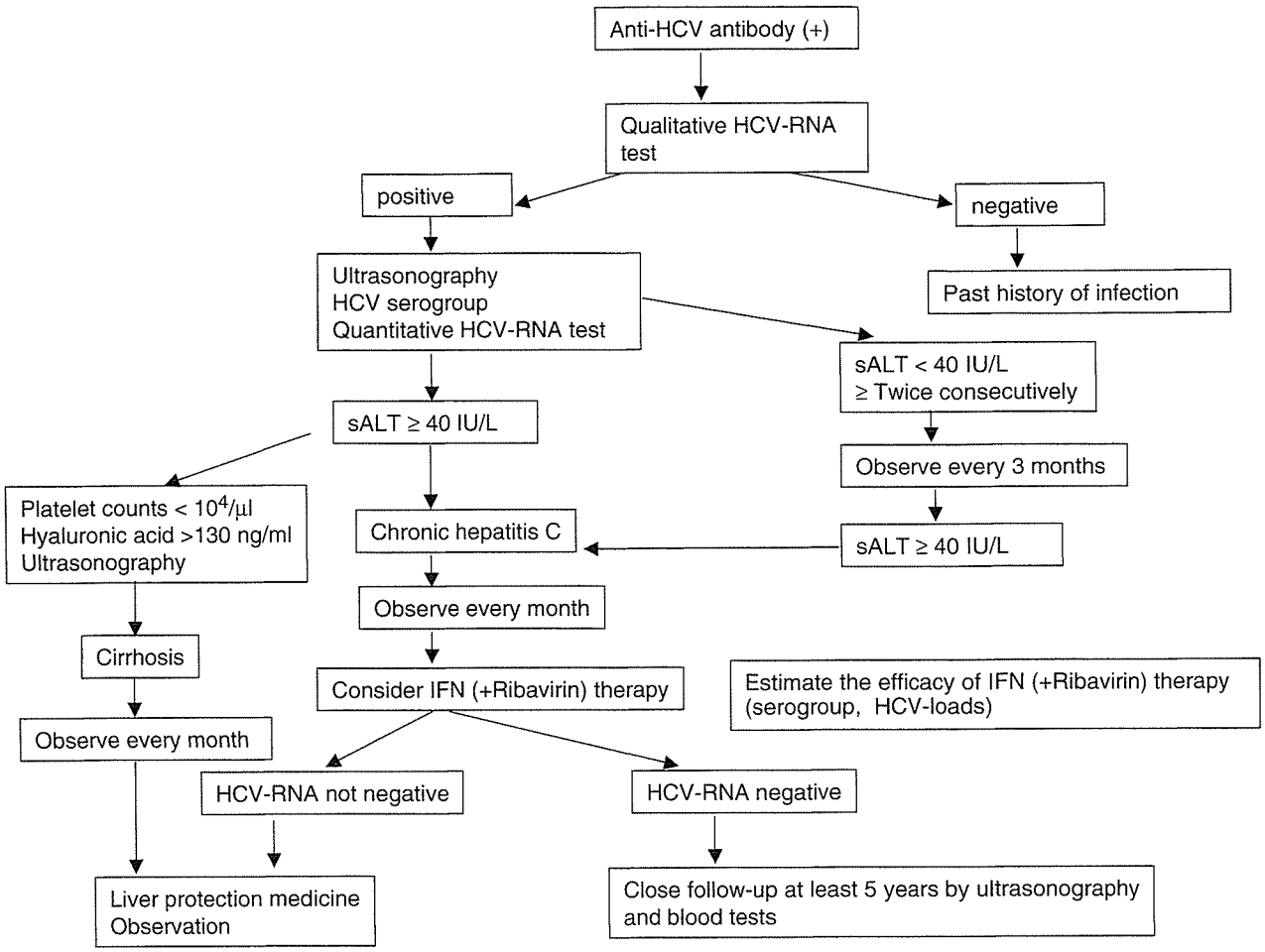

Fig. 1. Progress observation flow chart for anti-HCV antibody-positive patients. *HCV*, hepatitis C virus; *ALT*, alanine aminotransferase; *IFN*, interferon

Anti-HCV antibody

Anti-HCV antibody of low titer is frequently detected using sensitive HCV kits currently available in Japan. Patients with low anti-HCV antibody titers mostly have a history of remote HCV infection, while those with high titers generally have an ongoing infection. Hence, patients who test positive for anti-HCV antibodies are not necessarily infected with HCV at present. When the antibody titer is found to be low, a history of infection (i.e., currently cured) should be suspected. To verify this, a sensitive qualitative HCV–RNA measurement is required (reverse transcriptase–polymerase chain reaction (RT–PCR) method).

Meanwhile, it should be noted that during the early stage of HCV infection (2–3 months from the initial HCV exposure), patients do not test positive for anti-HCV antibody (window period).

HCV–RNA

To confirm the presence of HCV, we use an HCV–RNA assay by RT–PCR. There are two types of RT–PCR assay, a qualitative one and a quantitative one. However, the latter has a relatively low sensitivity. Therefore, the qualitative RT–PCR assay PCR is used to monitor the presence or absence of HCV, and hence the efficiency of an antiviral drug. For an estimation of the efficacy of antiviral treatment with interferon (IFN), a quantitative RT–PCR assay must be used.

Genotypes and serogroups of HCV

Many genotypes of HCV have been identified (i.e., there are HCV groups whose gene or genomic sequences differ to some extent). HCV genotypes are clinically important because the efficacy of IFN therapy varies depending on the HCV genotype. In Japan, the majority of HCV patients have HCV genotypes 1 or 2. Because the HCV genotype is determined on the basis of restriction fragment length polymorphism (RFLP) by PCR assay, the determination procedure is somewhat complicated. In order to determine the responsiveness of patients with chronic hepatitis C to IFN therapy easily (rapidly and accurately), serogroup (SG) identification by enzyme immunoassay is useful.[3] Patients

are classified as SG-1 (corresponding to HCV genotype 1) or SG-2 (corresponding to HCV genotype 2). Many patients classified as SG-1 are resistant to IFN, whereas many patients classified as SG-2 are generally responsive to IFN therapy.

Natural course of HCV infection

HCV patients commonly develop "acute hepatitis" 2 or 3 months after the initial exposure. However, many patients are unaware of this development because they have minor subjective symptoms and hardly exhibit jaundice. About 20% to 30% of patients exhibiting acute hepatitis recover spontaneously from the disease, but acute hepatitis develops into chronic hepatitis in the remaining 70% to 80% of patients (hepatitis persisting for more than 6 months is defined as chronic hepatitis). In general, these patients enter an "inactive phase" of hepatitis C, which persists for more than 10–15 years. The serum alanine aminotransferase (ALT) level, which indicates the extent of hepatocytic damage, is within the normal range during the inactive phase, but viral replication continues even during this period (Fig. 2).

Chronic hepatitis C frequently enters the "active phase" after an inactive phase of 10–15 years; however, this period varies greatly depending on the individual. In the active phase, the serum ALT level becomes approximately 2–3 times higher than the normal level. The problem with chronic hepatitis C is that it does not resolve spontaneously once it enters the active phase. If chronic hepatitis is left untreated, the risk of progression to cirrhosis increases without the patient realizing it. Thus, hepatitis C is characterized by its gradual but steady progression.[4]

With the progression to cirrhosis, there is an increasing risk of developing HCC. This risk has been reported to have an annual rate of 5% to 7%.[5] Ideally, HCV-infected patients should have the disease diagnosed during the inactive phase of chronic hepatitis so that, upon transition to the active phase, the patients can start receiving antiviral therapy for HCV.

Treatment of HCV infection

HCV infection is treated using mainly IFN preparations. These IFN preparations are outlined below in their order of development.

IFN monotherapy

IFN monotherapy was first introduced for the treatment of chronic hepatitis C. In Japan, the treatment of chronic hepatitis C generally starts with the daily administration of 6–10 million units of IFN for 2–4 weeks, followed by administration three times weekly for 6 months. In Europe and the USA, 3 million units of IFN are administered three times weekly from the start, and this is continued for a year. The efficacy of the therapy is evaluated after 6 months of IFN treatment. If an HCV-RNA test is negative by a qualitative RT-PCR assay at this time, it indicates that the patient obtained a sustained virological response (SVR) and is considered to be practically free of HCV.

IFN monotherapy had conventionally been used for non-A/non-B hepatitis from around 1985, prior to the discovery of HCV. A nationwide survey carried out by a research group supported by the former Ministry of Health and Welfare in 1995 showed that the overall SVR rate following IFN monotherapy for chronic hepatitis C (the administration of 6–10 million units per day) for 6 months was approximately 30%. SVR rates at facilities across Japan were nearly equal to this value. However, among patients with HCV genotype 1, who accounted for approximately 70% of all Japanese patients infected with HCV, and particularly those with a high viral load (defined as HCV-RNA >100 KIU/ml in Japan), a SVR was obtained in only 2% to 7% of cases; i.e., the efficacy of treatment by IFN

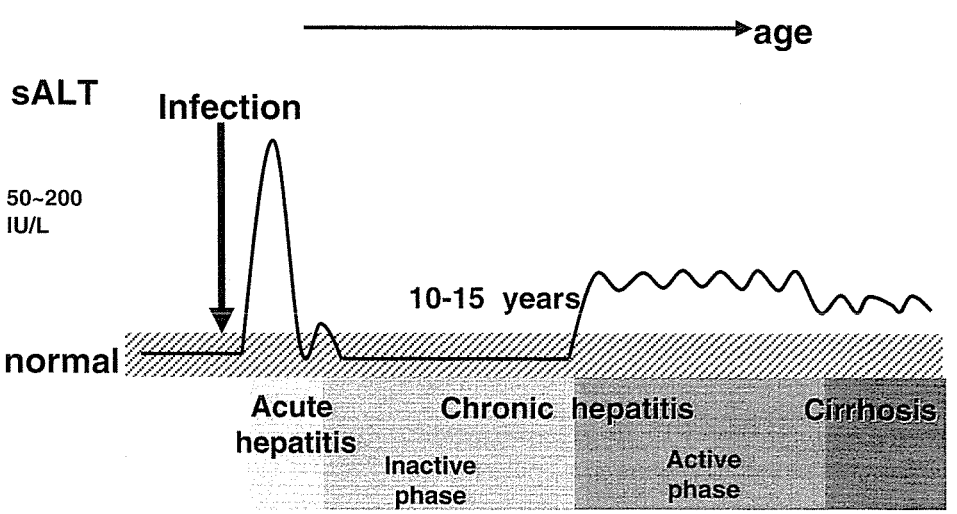

Fig. 2. Natural course of HCV-infected patients. Approximately 70% of acutely HCV-infected people develop persistent infection. After 10–15 years of the inactive phase, most chronic hepatitis C patients move into the active phase. One-third of chronic hepatitis C patients are assumed to develop cirrhosis. *sALT*, serum alanine aminotransferase

monotherapy was low. These patients with HCV genotype 1 at a high viral load have what is called "intractable hepatitis C."

IFN therapy in combination with ribavirin

IFN is also administered in combination with ribavirin, an antiviral drug. In Japan, the use of ribavirin was approved in December 2001. Ribavirin (600–800 mg daily, divided into two doses) is taken orally throughout the period of IFN injections. Ribavirin is a synthesized nucleic acid derivative and, when administered in combination with IFN, shows an increased antiviral activity.

In clinical studies of IFN therapy in combination with ribavirin conducted in Japan, a SVR rate of approximately 20% was obtained even in patients with HCV genotype 1 at a high viral load, i.e., "intractable hepatitis C," and who were less responsive to IFN monotherapy. Because patients on IFN monotherapy used as the control showed a SVR rate of only 2.3%, the concomitant use of ribavirin contributed to an approximately 10-fold increase in antiviral activity.[6]

The efficacy of IFN therapy in combination with ribavirin after its inclusion in the health insurance program is very similar to that found in a clinical study in Japan. However, the adverse effects of this combinational therapy have generally been more severe than those observed during the clinical study period. The drop-out rates of patients who could not complete the combinational therapy were as high as 15%–20%, and this led to a decrease in SVR rate calculated on intention-to-treat (ITT). In other words, the number of patients who dropped out of the treatment is added to the denominator. Adverse drug reactions that reduce the quality of life (QOL), such as hemolytic anemia, severe malaise, anorexia, and taste disorders, are frequently observed, particularly in many elderly patients. Indications for IFN therapy in combination with ribavirin should be considered carefully for patients aged 65 years or older.

Long-term interferon therapy

In cases of long-term IFN therapy, IFN is administered two or three times a week for a period of 2 years or more. The purpose of this therapy is not the eradication of HCV, but the normalization of serum ALT levels and eventually the suppression of HCC development. This is a promising therapy for patients who cannot be treated with ribavirin because of its adverse effects, or for those who were not able to continue with the combination therapy of IFN and ribavirin.

PEG–IFN therapy in combination with ribavirin

PEG–IFN is an interferon molecule covalently bonded to polyethylene glycol (PEG), which shows a sustained release. PEG–IFN characteristically requires subcutaneous administration only once weekly, as compared with the conventional type of IFN which requires administration three times a week. PEG–IFN therapy alone has a higher efficacy than the conventional IFN monotherapy, but it has been demonstrated that PEG–IFN therapy used in combination with ribavirin shows an even higher efficacy[7,8] (Fig. 3).

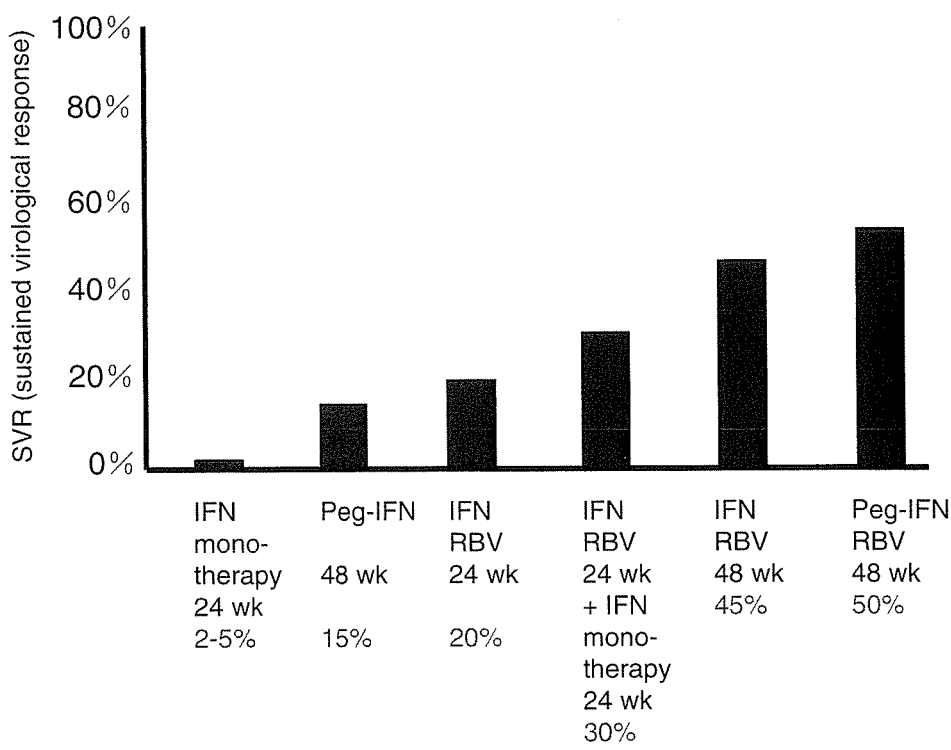

Fig. 3. Changes in anti-HCV therapy, including interferon for intractable (genotype 1b, high viral loads) chronic hepatitis C patients. After the introduction of IFN monotherapy for chronic hepatitis C, the efficacy of IFN therapy has gradually increased with the addition of ribavirin, the introduction of pegylated IFN, and an extension of the duration of therapy. *IFN*, interferon; *RBV*, ribavirin

PEG–IFN therapy in combination with ribavirin is expected to increase the SVR rate to approximately 50% even in patients infected with HCV genotype 1 at a high viral load, and to approximately 60% in all patients infected with HCV. The efficacy in those infected with genotype 2 HCV reaches 80%–90%. In Japan, treatment with PEG–IFN α-2a (Pegasys) alone was approved in December 2003. The combined use of PEG–IFN α-2b (PegIntron) and ribavirin (Rebetol) was also approved in December 2004. These treatments with PEG–IFN are generally administered for 48 consecutive weeks. Continuation of the treatments for 48 consecutive weeks is important, although it may be necessary to decrease the dose owing to adverse drug effects.

The adverse effects of PEG–IFN therapy in combination with ribavirin are almost the same as those of conventional IFN therapy. However, such adverse effects are generally minor (for example, fever), and the therapy requires administration only once per week, thereby improving the patient's QOL. Because there is the possibility of drug accumulation in the body and an associated exacerbation of adverse effects owing to the sustained-release formulation, very careful observation of patients is required. There have been reports of other problematic adverse effects of this combinational therapy compared with those of the conventional IFN preparation, e.g., decreased counts of leukocytes, and particularly of neutrophils. Some patients exhibit severe thrombocytopenia. It is mandatory to confirm neutrophil count immediately before every administration.

It is currently specified that PEG–IFN therapy used in combination with ribavirin is the best choice in the treatment of intractable hepatitis C of genotype 1 at a high viral load. This combinational therapy is thus administered first. It has recently been suggested that an extended administration period of 72 weeks for PEG–IFN therapy in combination with ribavirin proves effective in patients who are slow in showing a SVR.

Efficacy of antiviral therapy and its effect on patient prognosis

The following points have been reported in the literature: in patients in whom HCV was eradicated mainly by IFN monotherapy, hepatic fibrosis is improved,[9] the development of HCC is inhibited,[10] and life expectancy is also improved.[11] It has thus been indicated that if the eradication of HCV can be achieved, chronic hepatitis C prognosis is improved. It has also been reported that in patients in whom serum ALT level was normalized (even if this was transient), despite the failure to eradicate HCV (cases referred to as a biochemical response (BR)), the development of HCC was delayed in the short term. However, because no improvement in fibrosis was observed, it will probably be impossible in the long term to block the development of HCC. It has also been demonstrated that when curative treatment is carried out even after the development of HCC, subsequent IFN-based treatment could inhibit the recurrence of HCC.

Treatment of hepatitis C patients who do not respond sufficiently to IFN

Liver-protection therapy

Liver-protection therapy aims to delay the progression of chronic hepatitis by controlling inflammation in patients in whom HCV could not be eradicated. An ursodeoxycholic acid preparation (Urso) and a glycyrrhizin preparation (Stronger Neo Minophagen C) are used in combination as a liver-protection therapy. These drugs inhibit hepatic inflammation and decrease serum ALT level, but they do not decrease HCV load. It was reported that Stronger Neo Minophagen C delays the progression of chronic hepatitis and the onset of HCC.[12] The ursodeoxycholic acid preparation decreases serum ALT level, but its action of delaying the progression of chronic hepatitis has not yet been verified.

Hepatitis C generally progresses slowly and is less likely to aggravate rapidly, unlike hepatitis B, which may aggravate very rapidly, and progresses steadily. Liver-protection therapy, which retards the progression of the disease by controlling inflammation, can therefore be considered significant in hepatitis C. This therapy is applied mainly when it is impossible to use IFN due to its adverse effects, or when patients do not respond sufficiently to IFN therapy, including in combination with ribavirin. Liver-protection therapy is also administered as a temporary measure until a therapy in combination with IFN is started.

Phlebotomy

Iron deficiency leads to a decrease in serum ALT level, and its use as a therapy for chronic hepatitis C has begun to be appreciated. This is based on the observation that reactive oxygen species (ROS) production increases in hepatitis C patients, which leads to the development of liver disease and eventually HCC. Because intrahepatic iron plays an important role in ROS production (Fenton reaction), phlebotomy is designed to suppress ROS production by inducing intrahepatic iron deficiency. In fact, decreasing the serum ferritin level (an indicator of iron store) to 10ng/ml or lower leads to a significant decrease in serum ALT level.[13] This is a promising therapy for patients who do not respond sufficiently to IFN therapy, or who are unable to receive it and do not respond to the above-mentioned liver-protection therapy either.

Conclusions

An overview of the current status of research on the progression of chronic hepatitis C and the treatment methods available has been presented and discussed in terms of the effects and limits of these methods. The early detection of HCV infection makes it possible to apply antiviral therapy at the appropriate time. It is particularly worth noting that

it has become possible for antiviral therapies to eradicate viruses in a majority of HCV patients, and to suppress and control the progression of HCV infection (or acute hepatitis C) to chronic hepatitis and subsequently to HCC. However, the limits of the current IFN-based therapies have also become evident. Specific antiviral drugs targeting HCV enzymes (including viral proteases, helicase, and RNA polymerase) have recently been developed. The development of one antiviral drug has advanced to phase II clinical trials as of 2006.

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