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Ⅳ. 研究成果の刊行物・別刷

EXPERT REVIEWS

Simeprevir for the treatment of chronic hepatitis C genotype 1 infection

Expert Rev. Anti Infect. Ther. 12(8), 909–917 (2014)

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Simeprevir is a second-wave hepatitis C virus NS3/4A protease inhibitor that was designed to optimize its antiviral activity, safety, drug-drug interactions, and pharmacokinetic profile. When used to treat patients with hepatitis C virus genotype 1, simeprevir is coadministered with peginterferon and ribavirin for 12 weeks, followed by double therapy with Peg-IFN and ribavirin for an additional 12 or 36 weeks. Phase III studies achieved a sustained virologic response in 80–90% of treatment-naïve patients (International Phase III studies QUEST-1/2: 80/81%; Japanese Phase III trial CONCERTO-1: 89%). Unlike with the first protease inhibitors, telaprevir or boceprevir, used in triple therapy, when using simeprevir the frequency of clinically problematic adverse events such as anemia, rash, and digestive symptoms is almost comparable to that of double therapy. The advent of simeprevir has enabled interferon therapy, which started as monotherapy in early 1990s, to reach its maximum efficacy and arrive at what can be considered its final form at least in genotype 1b.

Keywords: direct acting antiviral • hepatitis C virus • peginterferon • protease inhibitor • Simeprevir

An estimated 130–150 million people worldwide are infected with the hepatitis C virus (HCV) [1]. The majority of these individuals are infected as adults through blood exposure, and although 20–30% develop only a transient infection, the other 70–80% develop a persistent infection. Spontaneous clearance of the virus after the development of a persistent infection is rare (~0.2% per year), and infections lasting 20–30 years can lead to decompensated liver cirrhosis or liver cancer. In the USA and Japan, these diseases are the leading indications for liver transplantation because of end-stage liver disease. Therefore, it is important to completely eradicate the virus to defer the development of liver disease during the persistent infection stage.

Interferon monotherapy was introduced as an antiviral therapy for hepatitis C in the early 1990s, and it has made achieving a sustained virologic response (SVR; defined as the inability to detect HCV RNA at 24 weeks after completing treatment) possible in roughly 1 of 3 or 4 cases. However, it was not effective for patients with HCV genotype 1, particularly those with a high viral load, and the SVR rate was found to be approximately 5–10%, which led to these cases being called 'difficult-to-treat hepatitis C' (Figure 1). In the 2000s, two-drug combination therapy with peginterferon (Peg-IFN) and ribavirin (RBV) became the standard antiviral therapy for chronic HCV infection. With this treatment, the SVR rate for genotype 1 patients after 48 or 72 weeks of treatment became 40–50% and the rate for genotype 2 patients after 24 weeks of treatment became approximately 80% [2]. The release of the protease inhibitor telaprevir (TVR), and also boceprevir (BOC), as a treatment for genotype 1 patients in 2011 ushered in the era of triple therapy, drastically shrinking the treatment duration to 24 weeks at least for some naive patients and relapsers and improving the SVR rate to roughly 80% [3]. Although TVR and BOC had problematic aspects including complex drug interactions and strong side effects such as anemia and skin manifestations, simeprevir (SMV), a second-wave protease inhibitor with very limited side effects, was approved at the end of 2013 [4]. This paper reviews the use of triple-drug therapy with SMV for the treatment of patients with HCV genotype 1.

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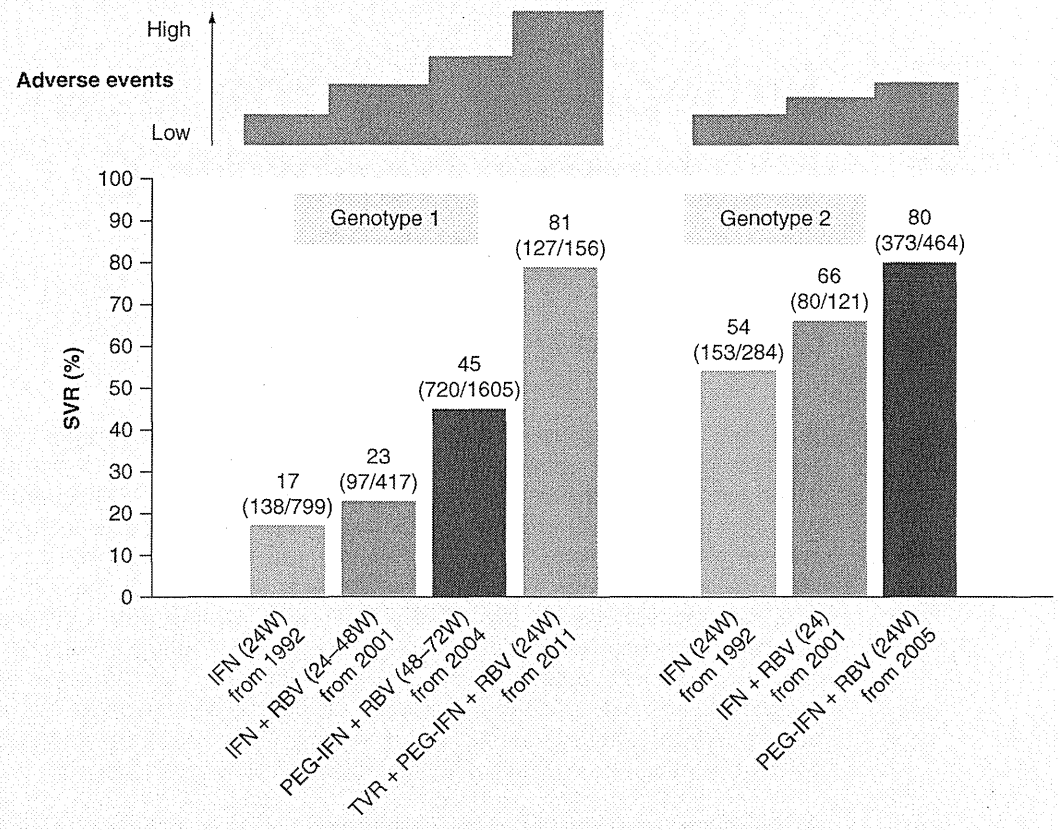

Figure 1. Changes to interferon treatment before the advent of simeprevir. Sustained virologic response rates are from therapies using postmarket IFNs conducted at Osaka University and associated facilities. Advancements in IFN therapy have led to improvements in the sustained virologic response rates for patients with hepatitis C genotype 1 and genotype 2, but the frequency of side effects has also increased. In particular, decreased compliance with long-term treatment and the increased side effects associated with additional dosage of telaprevir are problematic for patients with genotype 1.
IFN: Interferon; Peg-IFN: Peginterferon; RBV: Ribavirin; SVR: Sustained virologic response; TVR: Telaprevir.

History of NS3/4A protease inhibitor development

IFN and RBV have long been used as the primary antiviral drugs to treat hepatitis C [2] as both drugs are nonselective HCV inhibitors. IFN is a cytokine prototype discovered in the 1950s, and RBV is an early nucleoside analog antiviral drug synthesized in 1970. Although HCV was discovered in 1989, its discovery in itself did not lead directly to the development of new drugs for treating hepatitis C infection. The main reason for this was that although the genetic structure of the virus had been characterized, no efficient assay system for HCV replication was developed quickly – it took 10 years after the discovery of the virus for a replicon that could reproduce HCV proliferation *in vitro* to be created in 1999 [5]. Another important step that laid the groundwork for research on new drugs for treating HCV infection was that the structures of HCV proteases, which are essential to HCV replication, were elucidated in 1996 [6]. HCV NS3 protease was shown to undergo inhibition by the N-terminal substrate products. This made it possible to design functional molecules to target the active sites of the proteases. In the 2000s, the conformation of the substrate peptides predicted from the structures of proteases was altered to a nonpeptide form, and after the antivirus activity of small molecules that could be taken orally was screened using replicons, a new era of clinical development of drugs with direct antiviral activity against HCV (direct-acting antivirals) began.

One of these HCV-selective antiviral drug prototypes was BILN 2061 (Figure 2). The BILN 2061 molecule is a noncovalent protease inhibitor with a macrocyclic structure resembling a lid placed from above on the active site of the NS3/4A HCV protease [7]. BILN 2061 was the first drug to inhibit HCV replication in humans, but subsequent studies on monkeys revealed cardiotoxicity at high doses [8], and thus its development was suspended. Another molecule developed slightly after BILN 2061 was VX-950, which is a covalent protease inhibitor with a linear structure that binds to the bottom part of the groove of the active site of the NS3/4A HCV protease. VX-950 immediately gained attention because of its strong HCV inhibitory effect both *in vitro* and *in vivo*, and following clinical trials, it was released for clinical use as TVR in 2011. The release of TVR improved the SVR rate for hepatitis C genotype 1 after 24 weeks of combined treatment with Peg-IFN + RBV to 73%

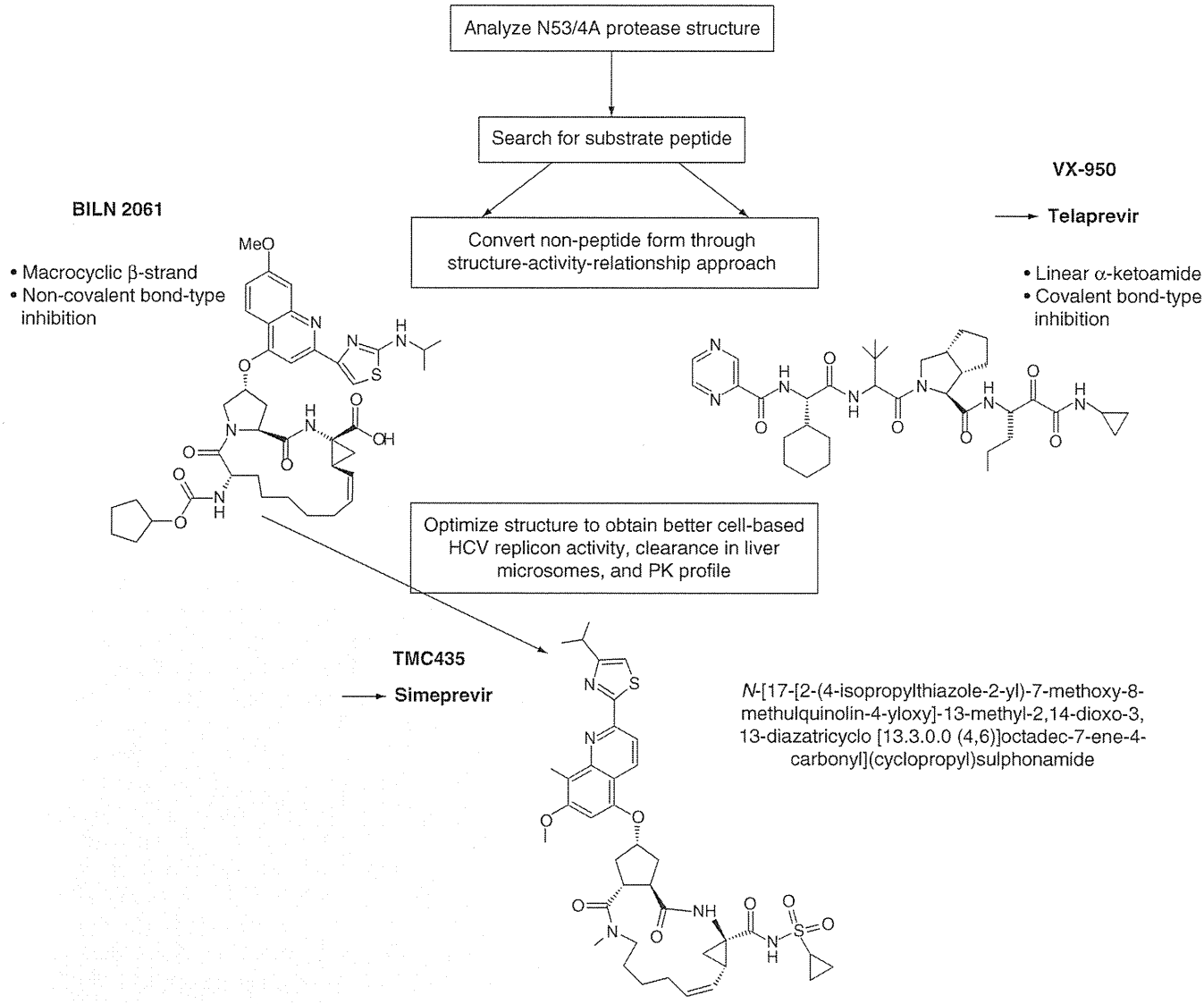

Figure 2. Structure of protease inhibitors and development of simeprevir.
HCV: Hepatitis C virus; PK: Pharmacokinetic.

(in a Japanese Phase III development study). Although TVR is certainly a first-in-class drug, particular caution must be taken with its use because of its complex interactions with many other drugs and its unique side effect profile that includes anemia, skin reactions and kidney damage [3,9].

Although development of the macrocyclic molecule BILN 2016 was suspended, several other drugs are being developed based on its structure with the conformation changed to avoid toxicity. One of these is SMV (code name, TMC435), and other similar drugs currently in clinical development include faldaprevir and vaniprevir. To differentiate them from the first drugs TVR and BOC (the latter not approved in Japan), they are second-wave protease inhibitors. SMV is based on a lead compound developed from BILN 2061 and was selected after performing tests including an enzyme inhibitory activity test using recombinant proteins, an inhibitory effect on replication test using a replicon, a clearance test using human liver microsomes, a permeability test using Caco-2 cells and a pharmacokinetic test in rats [10]. Although SMV was approved for clinical use after TVR, its molecular structure has been carefully optimized as described above, making it highly superior with respect to its safety, drug interactions and pharmacokinetic profile.

Clinical development of SMV

Preclinical studies

Protease inhibitors are classified as linear or macrocyclic based on their molecular structure. TVR and BOC, which have already been approved in Europe and the USA, possess linear keto-amide structure, whereas SMV possesses macrocyclic structure. SMV has a molecular weight of 750 Da and can be taken orally. This drug is a strong inhibitor of the NS3/4A protease

that originates in HCV genotypes 1a and 1b (the enzyme inhibition constant [Ki] of SMV against NS3/4A proteases is 0.5 nmol/l in genotype 1a [H77 strain] and 1.4 nmol/l in genotype 1b [Con1 strain]) and strongly suppresses replication in genotype 1b replicon cells (EC_{50} = 8.1–25.2 nM) and genotype 1a replicon cells (EC_{50} = 28.4 nM) [11]. In combination with other drugs in a replicon system, it has synergistic antiviral effects with IFNs and NS5B polymerase inhibitors, as well as additive effects with RBV. Studies on the drug distribution among organs in rats showed high accumulation in the liver (liver-to-plasma ratio, 32:1–65:1). The plasma concentration 8 h after administration reached the same EC_{99} value measured in the replicon, and the concentration remained at EC_{50} even after 24 h, indicating that a once-daily dose of the drug could yield sufficiently long-lasting effects. It is known that the mutations to the NS3 domain that confer resistance to SMV differ from those that confer resistance to TVR. Specifically, the antiviral effect of SMV is not affected by substitutions of amino acids 36, 54 and 170, which confer resistance to TVR, whereas substitutions of amino acids 43, 80, 155, 156 and 168 are known to confer resistance to SMV [12]. Mutations at position 155 and 156 also confer resistance to TVR, but the others are not related to TVR resistance.

Phase I/IIa studies

A randomized double-blind placebo-controlled trial in 49 healthy adults and a nonplacebo-controlled open-label study in six patients with hepatitis C (TMC435350-C101 study) were Phase I studies of a single administration of TMC435 conducted outside Japan [13]. Healthy adults were administered a single dose from 50 to 600 mg in an ascending dose study and continuous doses of 100 (once daily), 200 (once daily), 200 (twice daily) and 400 mg (once daily) over 5 days. The increase in blood concentration after the start of administration was greater than the increase in dose. Some subjects experienced issues such as headache and photosensitivity while taking the drug, but no serious adverse events were observed. Patients with hepatitis C (4 genotype 1a, 2 genotype 1b) were administered a 200 mg dose once daily for 5 days. Three days after the start of treatment, blood levels of HCV RNA fell by a median magnitude of 3.46 $\log_{10}$ IU/ml (1.6–3.8) and ultimately decreased by at least 3 $\log_{10}$ IU/ml in all six subjects. Although no viral breakthrough was observed in the 8 days after starting administration, new protease domain variants were detected in all six subjects after starting treatment. The 24 h area under the curve after 5 days of treatment was approximately threefold higher than that of healthy subjects, suggesting that the pathology of hepatitis C may influence elimination of the drug.

A study where TMC435, Peg-IFNα-2a and RBV were administered to patients with hepatitis C, genotype 1 was conducted outside Japan as a Phase II proof-of-concept study (OPERA-1 study [TMC435-C201 study]). Considerable mean reductions in HCV RNA levels were observed after 4 weeks of triple therapy; specifically, 3.64 $\log_{10}$ IU/ml in the placebo group, 4.74 $\log_{10}$ IU/ml in the 25 mg TMC435 group, 5.52 $\log_{10}$ IU/ml in the 75 mg group and 5.44 $\log_{10}$ IU/ml in the 200 mg group [14]. To assess the efficacy of treatment in patients with other genotypes, single doses of TMC435 (200 mg once daily) were administered for 7 days to 37 treatment-naive patients with HCV genotypes 2–6 (TMC435-C202 study) [15,16]. Although this considerably reduced the viral load in subjects with genotypes 4–6, it only reduced HCV RNA by roughly half in subjects with genotype 2 and did not reduce the viral load in any subject with genotype 3.

Phase IIb studies

The international PILLAR study (TMC435-C205 study) and the Japanese DRAGON study (TMC435-C215) began as Phase IIb studies in 2009 at around the same time (Tables 1 & 2) [17,18]. In the DRAGON study, 92 IFN therapy-naive patients with chronic hepatitis C genotype 1 infection and a high viral load were randomly assigned to a group that received triple therapy with SMV + Peg-IFNα-2a + RBV for 12 weeks followed by Peg-IFNα-2a + RBV (600–1000 mg depending on body weight) for 12 weeks, a group that received triple therapy with SMV + Peg-IFNα-2a + RBV for 24 weeks, or a control group that received Peg-IFNα-2a + RBV for 48 weeks [18]. The 12-week and 24-week groups were both subdivided into 50 mg and 100 mg SMV dose groups, which are lower doses than used in the PILLAR study, which is discussed in a later section. The DRAGON study protocol called for response-guided therapy (RGT). Total Peg-IFN + RBV duration would be 24 weeks if HCV RNA was <1.4 $\log_{10}$ IU/ml at week 4 and undetectable at week 12, 16 and 20, and if this criteria were not met total Peg-IFN + RBV duration was 48 weeks. As a result, only one patient was treated for 48 weeks. The SVR rates in the SMV groups (listed by 12/24 week subgroups) were 78/77% in the 50 mg/day groups and 77/92% in the 100 mg/day groups, indicating that treatment with SMV was significantly more efficacious than treatment with the Peg-IFNα-2a + RBV (46%). Although adverse events such as decreased hemoglobin levels and drug eruptions occurred, most events were grade 1 or 2, and the frequency of events did not differ between the TMC435 and control groups. Transient hyperbilirubinemia after 1–2 weeks of treatment was a side effect observed in the SMV groups, but it was not found to be associated with any other clinical symptoms or laboratory test parameters such as aspartate aminotransferase or alanine aminotransferase levels. These results suggest that in Japanese treatment-naive patients infected with HCV genotype 1 with high viral load, triple therapy with SMV is superior to double therapy with respect to its SVR rate with a comparable safety and tolerability.

The PILLAR study, an international study of IFN treatment-naive patients with HCV genotype 1, had a similar treatment design to the DRAGON study but used higher doses of SMV (75 and 150 mg) and RBV (1000–1200 mg depending on body weight) [17]. The SVR rates of the SMV groups in

Table 1. Summary of international Phase IIb/III studies.

	PILLAR	ASPIRE	QUEST-1	QUEST-2	PROMISE
Phase	IIb	IIb	III	III	III
Patient population	Naïve	Experienced (relapsers/partial responders/null responders)	Naïve	Naïve	Experienced (relapsers)
Study design	5 arms	7 arms	2 arms	2 arms	2 arms
Simeprevir dose (mg)	75/150	100/150	150	150	150
Patient number	386	462	394	391	393
Genotype 1b (%)	55	57	44	58	58
Cirrhosis (%)	Excluded	Included (18)	Included (12)	Included (8)	Included (15)
Peg-IFN	α2a	α2a	α2a	α2a/α2b	α2a
Treatment duration (weeks)	24 (RGT)	48	24 (RGT)	24 (RGT)	24 (RGT)
Met RGT criteria (%)	79–86	NA	85	91	93
SVR† (SMV arms) (%)	75–86	38–59 (null responders) 48–86 (partial responders) 77–89 (relapsers)	80	81	79
(Control PR48 arm) (%)	65	19 (null responders) 9 (partial responders) 37 (relapsers)	50	50	37
Ref.	[17]	[19]	[20]	[21]	[22]

†SVR shown is determined at 24 weeks for PILLAR and ASPIRE and at 12 weeks for QUEST-1/2 and PROMISE after the end of treatment.
Peg-IFN: Peginterferon; RGT: Response-guided treatment; SMV: Simeprevir; SVR: Sustained virologic response.

the PILLAR study (listed by 12/24 week subgroups) were 82/75% in the 75 mg/day groups and 81/86% in the 150 mg/day groups, indicating that treatment with SMV was more effective than treatment with the Peg-IFNα-2a + RBV (65%). According to RGT criteria, 79–86% of SMV-treated patients completed treatment by week 24; 85–96% of these subsequently achieved SVR. No clear relationship between the dosing period of SMV and the SVR rate was observed. The SVR rate was lower for genotype 1a than for genotype 1b in the 75 mg dose group, but did not differ between genotypes in the 150 mg dose group.

In SMV/Peg-IFN/RBV triple therapy, the effect of prior treatment is currently the most important factor influencing the SVR. Outside Japan, a second Phase IIb study was conducted in patients who had previously received Peg-IFN/RBV therapy (ASPIRE study [TMC435-C206 study]) [19]. The dosing period for each regimen was set to 48 weeks. The control group received Peg-IFNα-2a + RBV for 48 weeks, whereas the experimental groups received Peg-IFNα-2a + RBV for 48 weeks along with 100 mg/day or 150 mg/day of SMV for the first 12 weeks, the first 24 weeks or the entire 48 weeks of the study. The SVR rates of the SMV groups (listed by 12/24/48 week subgroups) were 70/66/61% in the 100 mg/day groups and 67/72/80% in the 150 mg/day groups, respectively, significantly higher than the 23% of the control group; the SVR rate did not differ with respect to the SMV dosing period. However, the effect of prior treatment strongly influenced the SVR rate, which (for 100 mg/150 mg doses) was 85/85% for relapsers, 57/75% for partial responders (≥2 log reduction in HCV RNA at week 12 of previous treatment) and 46%/51% for null responders (<2 log reduction in HCV RNA at week 12 of previous treatment). The SVR rate did not differ between genotypes 1a and 1b in prior relapsers, but was lower for genotype 1a in prior partial responders and prior null responders. Transient hyperbilirubinemia and rash were observed more frequently in the SMV group than in the control group. However, the incidence of clinically problematic major adverse events did not differ between the treatment groups. In the ASPIRE study, unlike the PILLAR study, 18% of subjects had liver cirrhosis, but no particularly problematic adverse events were observed in subjects with advanced fibrosis.

Phase III studies

Following the success of the Phase II studies, Phase III studies of triple therapy with SMV + Peg-IFN + RBV were initiated. Outside Japan, the QUEST-1 and QUEST-2 studies were conducted in treatment-naive patients, [20,21], and the PROMISE study was conducted in prior relapsers [22]. In Japan, the CONCERTO-1 study [23] was conducted in treatment-naive patients, the CONCERTO-2 and CONCERTO-3 studies [24] were conducted in treatment-experienced patients and the CONCERTO-4 study [25] was conducted separately and used Peg-IFNα-2b. Studies outside Japan included subjects with liver cirrhosis, whereas Japanese studies did not. In addition,

Table 2. Summary of Japanese Phase IIb/III studies.

	DRAGON	CONCERTO-1	CONCERTO-2	CONCERTO-3	CONCERTO-4
Phase	IIb	III	III	III	III
Patient population	Naïve	Naïve	Experienced (nonresponders†)	Experienced (relapsers)	All
Study design	5 arms	2 arms	2 arms	1 arm	3 arms
SMV dose (mg)	50/100	100	100	100	100
Patient number	92	183	106	49	79
Genotype 1b (%)	100	98	97	98	99
Cirrhosis	Excluded	Excluded	Excluded	Excluded	Excluded
Peg-IFN	α2a	α2a	α2a	α2a	α2b
Treatment duration (weeks)	24 (RGT)	24 (RGT)	24 (RGT)	24 (RGT)	24 (RGT) for naïve/relapsers 48 for nonresponders
Met RGT criteria (%)	83–90	92	74–81	96	92 for naïve 97 for relapsers
SVR‡ (SMV arms) (%)	77–92	89	36–51	90	92 for naïve 39 for nonresponders 97 for relapsers
(Control PR48 arm) (%)	46	57	NA	NA	NA
Ref.	[18]	[23]	[24]	[24]	[25]

†Nonresponder means partial responder plus null responder.
‡SVR shown is determined at 24 weeks after the end of treatment.
Peg-IFN: Peginterferon; RGT: Response-guided treatment; SVR: Sustained virologic response; SMV: Simeprevir.

about half of the subjects in studies outside Japan were genotype 1a, whereas most subjects in Japanese studies were genotype 1b.

The QUEST-1 (n = 394) and QUEST-2 (n = 391) studies compared the treatment efficacy of SMV (150 mg/day, 12 weeks) + Peg-IFN + RBV in patients with hepatitis C genotype 1 infection (including those with compensated cirrhosis) with the control treatment of Peg-IFN + RBV. The SVR rate for triple therapy with SMV + Peg-IFN + RBV was 80–81% overall and 82–90% for genotype 1b. By *IL28B* genotype (rs12979860), the SVR rate was 94–96% for the CC allele, 76–80% for the CT allele and 58–65% for the TT allele. By the degree of fibrosis (METAVIR score), the SVR rate was 83–85% for F0-2, 67–78% for F3 and 58–65% for F4.

The CONCERTO-1 study conducted in Japan compared the treatment efficacy of SMV (100 mg/day, 12 weeks) + Peg-IFNα-2a + RBV in 183 treatment-naive patients with hepatitis C genotype 1 infection with the control treatment of Peg-IFNα-2a + RBV. While the SVR rate of control arm was 57% (34/60), that for triple therapy with SMV/Peg-IFNα-2a/RBV was 89% (109/123) overall, 87% (20/23) for subjects aged ≤45 years, 90% (70/78) for those 44–64 years and 86% (19/22) for those ≥65 years (but ≤70), indicating no difference with respect to age. By *IL28B* genotype (rs8099917), the SVR rate was 94% (77/82) for the major, favorable, allele TT and 78% (32/41) for the minor alleles TG/GG. Although the rate was significantly higher for the TT allele, the efficacy of treatment for the TG/GG alleles was also relatively high. The incidence of serious adverse events was lower in the study group than in the control group (3.3 vs 10%). Although a transient increase in bilirubin levels was observed in subjects soon after treatment began in the study group, but not in the placebo group, this increase was not accompanied by an increase in transaminase and subsided in all cases as treatment continued. In the CONCERTO-4 study, a clinical trial of SMV-based triple therapy using Peg-IFNα-2b, the SVR rate for treatment-naive subjects was 92% (44/49), almost the same as that achieved with SMV-based triple therapy using Peg-IFNα-2a (CONCERTO-1 study).

The CONCERTO-2 study was conducted in 106 prior nonresponders infected with the hepatitis C genotype 1. Subjects were administered Peg-IFNα-2a + RBV for 24 weeks along with SMV (100 mg/day) for the first 12 weeks or the full 24 weeks. The SVR rate was 51% (27/53) and 36% (19/53), respectively. The SVR rate was 50% (7/14) for the TT allele and 42% (39/92) for the TG/GG alleles, indicating no clear relationship between the IL28B genotype (rs8099917) and therapeutic efficacy. The CONCERTO-3 study was conducted in 49 prior relapsers infected with the hepatitis C genotype 1. Subjects were administered Peg-IFNα-2a + RBV for 24 weeks along with SMV (100 mg/day) for the first 12 weeks as with CONCERTO-1. The SVR rate was 90%

(32/35) overall and was high for all IL28B genotypes (rs8099917) at 91% (32/35) for the TT allele and 86% (12/14) for TG/GG alleles. In the CONCERTO-4 study, a clinical trial of SMV-based triple therapy using Peg-IFNα-2b, the SVR rate was 97% (28/29) for prior relapsers and 38% (10/26) for nonresponders, almost the same as the rates achieved with SMV-based triple therapy using Peg-IFNα-2a (CONCERTO-2, 3 studies).

The four CONCERTO studies examined genetic mutations of the NS3 protease domain in 87 cases of treatment failure defined by viral breakthrough, virologic stopping due to insufficient antiviral efficacy, HCV RNA-positive status at completion of treatment or relapse following completion of treatment. Mutations conferring SMV resistance were detected in approximately 90% of treatment failure cases, and almost all were due to substitutions of amino acid 168, with approximately 90% involving D168V (single D168V substitutions and multiple substitutions including D168V). It should be noted here that, in total, 98% of subjects in these CONCERTO studies had genotype 1b, while the non-Japanese PILLAR and ASPIRE studies similarly reported the involvement of D168 in almost all SMV-resistant mutations in genotype 1b. A study of NS3 polymorphism before treatment showed that the main polymorphs were S122G (16–27%), S122T (3–13%) and Q80L (8–10%); it should be noted that, in Japanese studies, almost all patients are genotype 1b. However, susceptibility to SMV did not differ between these polymorphs in an *in vitro* system, and no differences were observed in the efficacy for the S112G polymorph observed in the CONCERTO studies. Moreover, almost no D168 polymorphism was present before treatment. The therapeutic efficacy of triple therapy with SMV is known to be slightly lower in genotype 1a patients than in genotype 1b patients due to the Q80K mutation seen in a proportion of genotype 1a patients; of note proportion of Q80K differs substantially within genotype 1a patients ranging from 5 to 10% depending on region [20,22]. Indeed, in the QUEST-1 study, the patients with genotype 1a and Q80K mutation had a similar efficacy in the triple therapy arm compared with control arm. It is also known that in genotype 1a patients, in contrast with genotype 1b patients, R155K is the most common resistance mutation that appears with treatment failure.

Conclusion

Triple therapy with SMV + Peg-IFN + RBV yields a high SVR rate (≥80%) after a standard 24-week treatment period in patients with hepatitis C genotype 1 infection. High percentage of subjects who met RGT criteria and the high SVR rates in these subjects support the fixed treatment duration recommended in the USPI based on Phase III trial data. The side effect profile of this treatment, in contrast with that of TVR, is comparable to the side effect profile of double therapy with Peg-IFN + RBV, although it should also be noted that SMV still has several side effects such as hyperbilirubinemia and photosensitivity. In addition, the therapeutic efficacy depends on factors such as responsiveness to IFN (*IL28B* genotype or responsiveness to prior treatment) and the stage of liver fibrosis, but efficacy remains relatively high for patients with minor *IL28B* genotypes, prior nonresponders and patients with advanced liver disease. The incidence of side effects is also comparable to that of double therapy with Peg-IFN + RBV, making it an excellent treatment and an easy choice for a large number of patients.

Expert commentary

IFN therapy for hepatitis C was started in the 1990s, but the therapeutic efficacy for genotype 1 patients with high viral load was soon discovered to be low, leading to such cases being called 'difficult-to-treat hepatitis C.' In the 2000s, Peg-IFN + RBV became the standard treatment, but genotype 1 cases required a longer treatment period (48–72 weeks) than genotype 2 cases, and the therapeutic efficacy was only 40–50%. Today, the addition of SMV to therapy has shortened the treatment period for patients with chronic hepatitis C genotype 1 to 24 weeks, and superior, or equal at least, treatment results can now be expected from Peg-IFN + RBV therapy in patients with genotype 2. It should also be noted that the addition of SMV has not yet been reported to increase the incidence of clinically important adverse events. The advent of triple therapy with SMV has led to IFN therapy, which has improved the method of treating difficult-to-treat cases of hepatitis C since its development in the 1990s, to what can be considered nearly its final form. Both SMV + Peg-IFN + RBV and sofosbuvir (SOF) + Peg-IFN + RBV therapies have been approved in the USA for genotype 1 cases, [26], but the SOF triple therapy regimen is not being developed in Japan and thus SMV + Peg-IFN + RBV is the first-line treatment for genotype 1 cases.

Hepatitis C treatments that are planned for release include not only other types of triple therapy with IFN, but also treatments such as daclatasvir (DCV) + asunaprevir (ASV) and SOF + ledipasvir (LDV) that omit IFN. [27,28]. Antiviral therapy for hepatitis C must be started as soon as possible to control the progression of liver disease and the development of liver cancer, particularly in aged patient populations like that of Japan. At the same time, secondary options must also be considered in advance because treatment failure occurs in 10–20% of cases. In the era of direct-acting antiviralss, it is important to avoid creating resistant viruses to the greatest possible extent. It is generally believed that treatments that include IFN present a high barrier to the emergence of resistant viruses. In patients who developed virologic failure to SMV + Peg-IFN + RBV, mutations can develop, more specifically, D168V for genotype 1b and R155K for genotype 1a. Although these two resistance mutations are shared by the second-wave protease inhibitors, their susceptibility to SOF + LDV will likely be maintained. The fact that such secondary options are available is yet another reason why SMV + Peg-IFN + RBV combination therapy can be considered an excellent treatment method.

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Key issues

- Simeprevir (SMV) is a second-wave hepatitis C virus NS3/4A protease inhibitor that was designed to optimize its antiviral activity, drug–drug interactions and pharmacokinetics.
- SMV is coadministered with peginterferon (Peg-IFN) and ribavirin (RBV) for 12 weeks, followed by double therapy with Peg-IFN and RBV for an additional 12 or 36 weeks for genotype 1 patients.
- SMV + Peg-IFN + RBV therapy yields a high sustained virologic response rate (≥80%) for treatment-naive patients.
- The efficacy of SMV + Peg-IFN + RBV therapy depends on factors such as responsiveness to IFN (IL28B genotypes for naive patients or responsiveness to prior treatment for experienced patients) and the stage of liver fibrosis.
- Therapeutic efficacy of triple therapy with SMV is slightly lower in genotype 1a patients than in genotype 1b patients due to the Q80K mutation seen in a proportion of genotype 1a patients.
- In patients who developed virological failure to SMV + Peg-IFN + RBV therapy, mutation can develop specifically D168V for genotype 1b and R155K for genotype 1a, which may have little influence to give the effect of the later therapy such as a combination of NS5A inhibitor and NS5B inhibitor.

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ダクラタスビル・アスナプレビル併用療法

肝胆膵69巻増刊号

II. 各論

作用機序と薬剤特性

竹 原 徹 郎[*1]

索引用語：ダクラタスビル，アスナプレビル，HCVライフサイクル，作用機序，耐性ウイルス

1 はじめに

DAA治療の時代は，2011年のテラプレビル(TVR)，2013年のシメプレビル(SMV)の登場により幕を開けた[1]．これらのプロテアーゼ阻害薬はペグインターフェロン(PEG-IFN)／リバビリン(RBV)と併用することによりIFN治療効果を最大限に引き出すIFN-based therapyとして開発された．2014年に登場したダクラタスビル(DCV)＋アスナプレビル(ASV)治療は初めてのIFN-free therapyであり，IFNの使用できない患者に対してウイルス排除の可能性を開き，またIFNに対して低反応性の患者にも高い有効性を発揮する．ASVはTVRやSMVと同じくプロテアーゼ阻害薬であるが，DCVは初めてのNS5A阻害薬である．この治療を可能にしたのはプロテアーゼ阻害薬と異なる作用機序の薬剤が開発されたことに負うところが多い[2]．

本稿では，DCVの作用機序と薬剤特性を中心に解説し，後半でASVのそれについて概説する．

2 ダクラタスビル

1. 開発の経緯

DCVはgenotype 1bレプリコンを用いて100万個以上の候補化合物がスクリーニングされ，得られたリード化合物を基に至適化された薬剤である[3]．一般にプロテアーゼ阻害薬がレプリコンに対してnMオーダーで抗ウイルス活性を示すのに比べ，DCVはpMオーダーで抑制効果を示し，極めて抗ウイルス活性が強い．genotype 1bに対して最もEC_{50}が低く，次に4a，1a，2aの順であるが，細胞障害活性はμMオーダーであり，いずれのgenotypeに対しても安全域が広く，広範なgenotypeに対して有効な薬剤である．対象的な化学構造を有し，NS5A replication complexに対する阻害活性を示す(図1)．genotype 1のC型肝炎ウイルス(HCV)感染患者に対するdose-escalation単回投与試験[3]では，1 mg投与から効果がある．10 mg，100 mg投与では，6～12時間から血清HCV-

Tetsuo TAKEHARA: Action mechanism and drug properties
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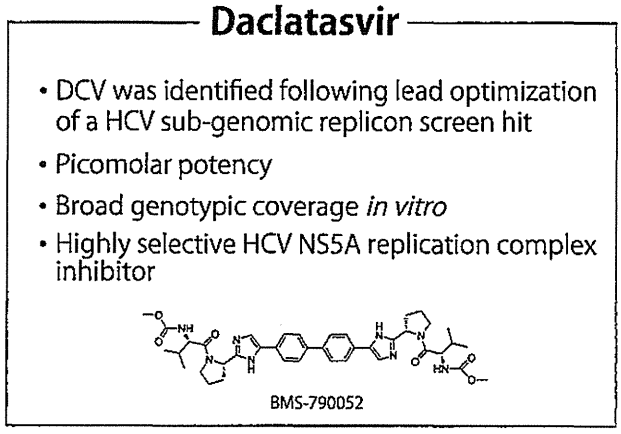

図1　ダクラタスビルの薬剤特性

RNAが低下し，24時間で最大の低下が得られ，72～144時間まで持続的な効果を発揮する．プロテアーゼ阻害薬に比して迅速なHCV-RNA抑制効果があり，効果が持続的なことが特徴である[4]．

2. 作用機序

プロテアーゼ阻害薬はHCVポリプロテインの非構造領域のプロセッシングを阻害し，ポリメラーゼ阻害薬はHCV-RNAの複製を阻害する．これらの薬剤のHCV増殖サイクルにおける阻害機序は明確であるといえる[5]．

一方，NS5Aは酵素活性がなく[6]，その阻害薬がどのようにして抗ウイルス効果を発揮するのかは明確ではなかった．近年，その機序が明らかになりつつあり，また逆に，NS5A阻害薬が登場したことがHCVのライフサイクルをより明瞭にしたともいえる．

HCVは細胞内でmembranous webで増殖する[7,8]．これはHCVに特異的な現象ではなく，一般にプラス鎖のRNAウイルスはER膜上で形成される多小胞体内で増殖することが明らかになりつつある[9]．HCV-RNAから一つの読み枠で読まれたポリプロテインはER膜上に整列し，切断される．ER膜は湾入することによりERの周辺で多小胞体を形成し，この膜構造の中でRNAが複製され，ウイルス粒子の成熟が行われる．NS5AはN末端側から両親媒性のαヘリックス領域，ドメイン1，ドメイン2，ドメイン3からなり，ドメイン1とドメイン2の移行部にリン酸化部位が存在する．NS5Aは両親媒性領域でER膜に結合している．NS5Aのドメイン1はER膜上でダイマーを形成し，それにより形成されるRNA-binding grooveがHCV-RNAの複製に関与している．ドメイン2～3は構造が確定されていない領域であるが，サイクロフィリンAやPI4KⅢαなどの宿主分子と相互作用することが知られている．ドメイン1のダイマー形成に関してはback-to-backとclam-likeの2つのモデルがあるが，いずれの場合もL31，Y93はドメイン1のRNA-binding grooveとは対側のER膜に近い側に存在し，NS5A阻害薬はこの底部に結合する．DCVが対象形をしているのはこのようなダイマー構造を標的にするからである．NS5A阻害薬はNS5Aのダイマー形成，安定性，リン酸化には影響しないが，NS5AのERへの正常な配列を阻害する可能性が指摘されている．NS5A阻害薬の投与によりHCVの増殖の場である，membranous webが破壊されることが報告されており，このことがNS5A阻害薬が極めて低濃度で有効で，さらにその抗ウイルス作用が迅速であることに関与している可能性が示唆されている[10]．

3. 薬剤耐性

DCVも他のDAA製剤と同様に単独投与では耐性ウイルスが出現し，持続的な抗ウイルス効果を達成できないことが知られている．genotype 1aおよびgenotype 1bのHCV感染者に対する14日間の連続投与試験の結果では，抗ウイルス効果はgenotype 1b＞genotype 1aでレプリコンのデータと一致していたが[11]，いずれの場合も持続投与によ

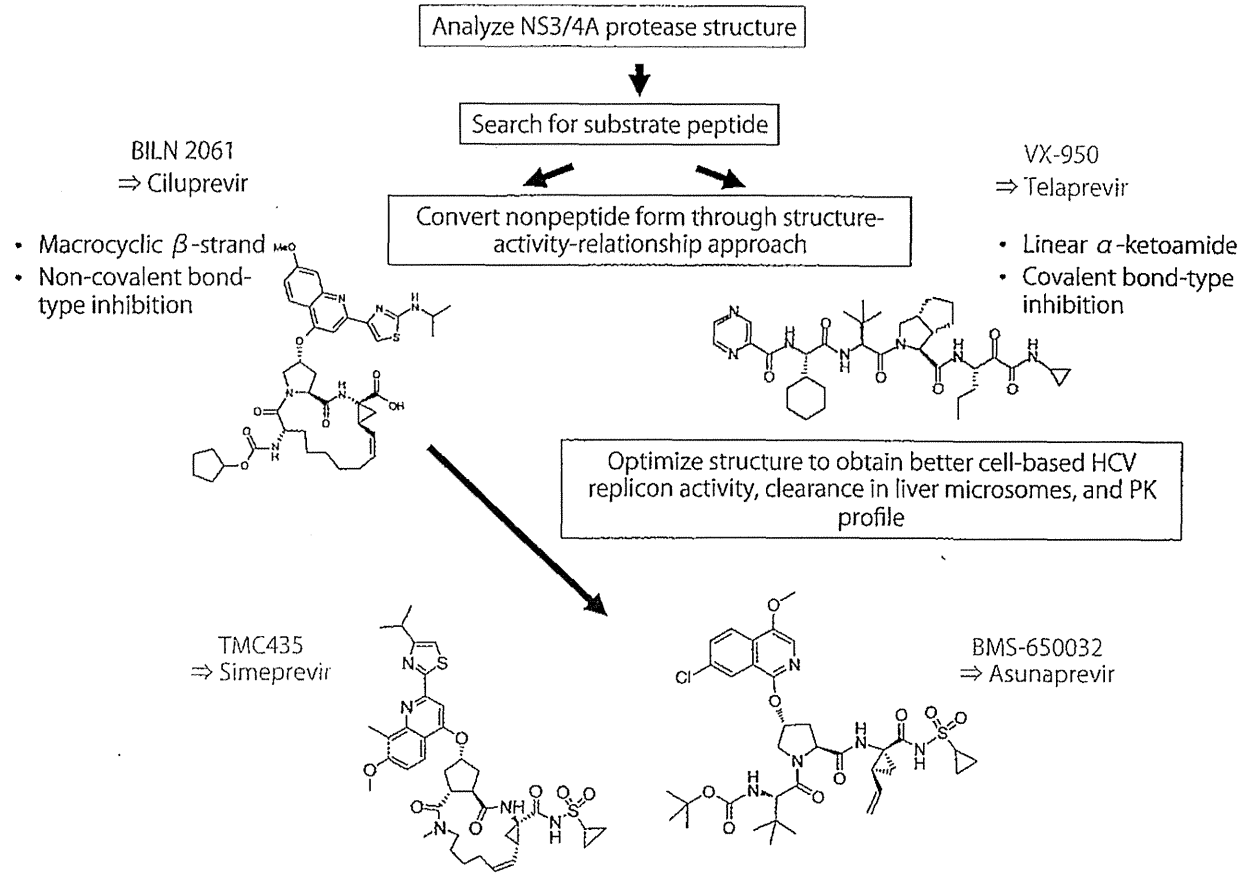

図2　第2世代プロテアーゼ阻害薬の開発

り耐性ウイルスが出現している[12]．耐性変異の生じるアミノ酸部位のプロファイルは*in vitro*のデータと一致しており，genotype 1aではM28，Q30，L31，Y93，genotype 1bではL31，Y93であった．これらの耐性変異ウイルスはreplication fitnessが高く，患者血清中に長期に残存することが示されている[13]．

3　アスナプレビル

ASVはSMVと同じく第2世代に属するプロテアーゼ阻害薬であり，多くの説明は要しないかもしれない[14]．プロテアーゼ阻害薬は，初期に大環状型の構造を有するBILN 2061（Ciluprevir）と直鎖状の構造を有するVX-950（TVR）が開発されたが，前者は大動物で毒性が出現し，その後の臨床開発が断念された．SMV，ASVはいずれもBILN 2061を基本骨格に，構造改変が行われ，最適化が行われた薬剤である（図2）[1]．ASVはSMVと比較的類似した構造を有しており，NS3/4Aプロテアーゼに対する結合様式モデルから，その結合にはSMVと同様にD168，R155，Q80が重要であることが示されている[15]．genotype 1aとgenotype 1bのHCV患者に対するASVの単回投与試験の結果では，genotype 1bの患者のほうがgenotype 1aの患者に比し，血清HCV-RNAの低下が強い傾向がある．これはQ80変異に関係しており，genotype 1aで観察されるQ80Kを有する患者ではHCV-RNAの低下が少ないためである．このような抗ウイルス効果に関連するQ80変異の関与もSMVと類似している．

4 おわりに

DAAの開発はNS3/4Aプロテアーゼ阻害薬が先行したが，ここにきてNS5A阻害薬が開発され，NS5B阻害薬も本邦では来年には臨床に登場する予定である．このような異なる作用機序を持つDAAの開発は，それらを組み合わせることによりIFNを使用しない治療法の開発を可能にした．IFNやRBVなどの非選択的抗ウイルス薬と異なり，DAAはその宿命として薬剤耐性の問題を抱えており，できるだけSVR率の高いレジメンを作ることによりこのことが克服されなければならない．DCV＋ASVはgenotype 1に対して，世界に先駆けて承認されたIFN-free therapyであり，今後の展開が期待される．

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ORIGINAL ARTICLE

Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects

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Keywords
BAMBI – IMT – NASH/NAFLD – TGF-β1

Abbreviations
BAMBI, bone morphogenic protein and activin membrane-bound inhibitor; CHE, choline esterase; FBG, fasting blood glucose; GGT, γ-glutamyl transpeptidase; Hb, haemoglobin; HSC, hepatic stellate cell; IFG, impaired fasting glucose; IMT, intima media thickness; Mets, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NGSP, National Glycohemoglobin Standardization Program; sBP, systolic blood pressure; T-Chol, total cholesterol; TGF-β1, transforming growth factor-β1; TG, triglyceride; TLR4, toll-like receptor 4; TNF-α, tumour necrosis factor-α; TβRII, TGF- receptor type II.

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Abstract

Background & Aims: Fetuin-A (α2HS-glycoprotein), a liver secretory glycoprotein, is known as a transforming growth factor (TGF)-β1 signalling inhibitor. Serum fetuin-A concentration is associated with nonalcoholic fatty liver disease (NAFLD) and cardiovascular disease. However, the usefulness of serum fetuin-A as a predictive fibrosis biomarker in NAFLD patients remains unclear. In this study, we investigated the relationship between circulating fetuin-A levels and fibrosis-related markers [platelet count, NAFLD fibrosis score and carotid intima media thickness (IMT)] in subjects with NAFLD. *Methods:* A total of 295 subjects (male, 164; female, 131) who received medical health check-ups were enrolled in this study. NAFLD was diagnosed using abdominal ultrasonography. Serum fetuin-A was measured by ELISA. IMT was assessed using a high-resolution ultrasound scanner. Using recombinant human fetuin-A, we investigated the effects of fetuin-A on hepatic stellate cells, which play a pivotal role in the process of hepatic fibrosis. *Results:* Serum fetuin-A concentration was significantly correlated with platelet count ($R = 0.19$, $P < 0.01$), NAFLD fibrosis score ($R = -0.25$, $P < 0.01$) and mean IMT ($R = -0.22$, $P < 0.01$). Multivariate analyses revealed that the fetuin-A concentration is a significant and independent determinant of platelet count, NAFLD fibrosis score and mean IMT. Recombinant fetuin-A suppressed TGF-β1 signalling and fibrosis-related gene expression and increased the expression of TGF-β1 pseudoreceptor bone morphogenic protein and activin membrane-bound inhibitor (BAMBI). *Conclusions:* Serum fetuin-A level is associated with liver/vessel fibrosis-related markers in NAFLD patients. Circulating fetuin-A could be a useful serum biomarker for predicting liver and vascular fibrosis progression in NAFLD patients.

Nonalcoholic fatty liver disease (NAFLD) is among the most common causes of chronic liver disease in the world and is a growing medical problem in industrialized countries (1). A wide spectrum of hepatic histological changes has been observed in NAFLD, ranging from simple steatosis, which is generally nonprogressive, to nonalcoholic steatohepatitis (NASH). A proportion of patients with NASH develop cirrhosis and hepatocellular carcinoma (2). About 30% of the general population has NAFLD and up to 5% of this population has NASH (3–5). To evaluate liver disease progression in NAFLD patients, liver biopsy remains the gold standard for diagnosing NASH and grading the severity of liver damage

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(6, 7). However, invasive liver biopsy is not suitable as a large-scale diagnostic test, and this, in turn, restricts therapeutic interventions (8).

NAFLD is a hepatic manifestation of the metabolic syndrome (Mets) and appears to be an almost indispensable prerequisite for Mets development, including development of type 2 diabetes mellitus (DM) and cardiovascular disease (CVD) (9–11). A recent systematic review indicated that NAFLD patients are prone to CVD, independent of Mets (12). In addition, many studies have demonstrated that the presence of NAFLD is an independent risk factor for progression of atherosclerotic disease (13–15). Atherosclerotic disease reportedly plays an important role in the natural course of NAFLD (16). Therefore, there is an urgent need to develop and validate a reproducible and noninvasive test that can accurately grade the severity of liver and vascular disease progression in NAFLD patients.

Recent investigations demonstrated that circulating secreted factors, such as adiponectin, leptin, tumour necrosis factor-α (TNF-α) and fetuin-A significantly affect pathophysiological progression in NAFLD (17–19). Fetuin-A (α2HS-glycoprotein) is a liver glycoprotein secreted into the circulation at high concentrations (20). Fetuin-A is an endogenous inhibitor of insulin receptor tyrosine kinase in the liver and skeletal muscle (21). In mice, a lack of fetuin-A enhances glucose clearance and insulin sensitivity (22). Moreover, Pal *et al.* recently reported that fetuin-A acts as an endogenous ligand for toll-like receptor 4 (TLR4) and enhances both insulin resistance and inflammation (23). These findings suggest that fetuin-A may worsen the course of NAFLD by increasing insulin resistance.

Transforming growth factor-β1 (TGF-β1) is a major pro-fibrogenic growth factor, and enhanced TGF-β1 signalling promotes fibrotic changes in many organs and tissues, including the liver and arteries (24, 25). Fetuin-A is also known to inhibit TGF-β1 signalling (26, 27). The disulphide-looped sequence in the *N*-terminal cystatin domain of fetuin-A shares homology with the extracellular domain of TGF-β receptor type II (TβRII), and this disulphide-looped peptide from fetuin-A binds to TGF-β1 (26). Indeed, fetuin-A knockout mice exhibit worsened organ fibrosis through enhanced TGF-β1 signalling (28, 29). Therefore, the anti-TGF-β1 signalling effects of fetuin-A could prevent organ fibrotic changes, including changes in the liver and vasculature. Thus, fetuin-A seems to promote (e.g. promote insulin resistance) and inhibit (e.g. attenuate organ fibrotic changes) NAFLD progression. However, the significance of serum fetuin-A in liver fibrosis and atherogenic changes in NAFLD subjects remains unknown.

The aim of this study was to elucidate the role of fetuin-A in the progression of liver and vascular fibrosis. To address this issue, we investigated the relationship between the serum fetuin-A level and serum fibrosis markers (platelet count, NAFLD fibrosis score) in NAFLD subjects. We also evaluated the relationship between the serum fetuin-A level and intima media thickness (IMT) and investigated the effects of fetuin-A on hepatic stellate cells (HSCs), which play a pivotal role in the progression of hepatic fibrosis.

Methods

Study subjects

Among 343 Japanese adult subjects (205 males, 138 females) who underwent a health check-up at aMs New Otani Clinic (Osaka, Japan) from 2008 to 2009, 295 subjects (164 males, 131 females) were initially recruited into this study. Exclusion criteria included a history of hepatic disease, such as chronic hepatitis C or concurrent active hepatitis B (seropositive for hepatitis B surface antigen), autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, haemochromatosis, α1-antitrypsin deficiency, Wilson's disease, or hepatic injury caused by substance abuse, as well as a current or past history of consumption of >20 g of alcohol daily. The diagnosis of fatty liver was based on the results of abdominal ultrasound performed by trained technicians, after exclusion of competing aetiologies of steatogenic liver disease. Fatty liver was defined as liver parenchyma with echogenicity higher than the kidney cortex, the presence of vascular blurring and deep attenuation of the ultrasound signal (30, 31). Of the 295 subjects recruited into this study, 275 (151 males, 124 females) were diagnosed with fatty liver by abdominal ultrasound, whereas 20 subjects (13 males, seven females) were diagnosed as not having fatty liver. Serum samples were collected from the subjects at the time of the health check-up and kept frozen at −80°C until used.

The protocol and informed consent were approved by the institutional review board of the Osaka University Graduate School of Medicine. Written informed consent was obtained from all subjects at the time of health check-up, and this study was conducted in accordance with the Helsinki Declaration.

Anthropometric and laboratory evaluation

Anthropometric variables (height and weight) were measured using a calibrated scale after requesting the patients to remove their shoes and any heavy clothing. Body mass index (BMI) was calculated as weight (kg) divided by the square of height in metres (m^2). Systolic blood pressure (sBP) values were measured in the sitting position to the nearest mm Hg. Venous blood samples were obtained in the morning after overnight fasting for 12 h. Laboratory evaluations for all patients included determination of platelet counts, haemoglobin (Hb), and measurement of serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), total bilirubin (T-Bil), choline esterase (CHE), creatinine, albumin,

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total cholesterol (T-Chol), triglyceride (TG), HDL-cholesterol (HDL-C), fasting blood glucose (FBG), iron and uric acid. All parameters were measured using standard techniques. Impaired fasting glucose (IFG) was defined as a FBG of 110–125 mg/dl. The presence of DM was defined as FBG ≥126 mg/dl, HbA1c (NGSP) ≥6.5% or treatment with antidiabetic drugs. The NAFLD fibrosis score was calculated for each of the subjects as previously reported [1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/DM (yes = 1, no = 0) + 0.99 × AST/ALT − 0.013 × platelet count (×10^9/L) − 0.66 × albumin (g/dl)] (32).

IMT measurements

A B-mode examination of the carotid artery was performed using an ultrasound scanner (SSA-660A, Xario; Toshiba Medical Systems Corporation, Tochigi, Japan). The maximum carotid IMT (max IMT) and mean IMT of the common carotid artery were measured on both the right and left sides in the areas of the common carotid artery, bulbus and internal carotid artery (but not the external carotid artery) in the supine position with a 7.5-MHz transducer. The mean IMT was the average thickness of the max IMT at two adjacent points (33).

Enzyme-linked immunosorbent assay (ELISA) for fetuin-A, fucosylated haptoglobin and adiponectin

Fetuin-A levels were determined using a competitive ELISA system, which was established using polyclonal antihuman fetuin antibodies as described previously (34). Briefly, a human fetuin polyclonal antibody was coated as the solid phase (96-well plate), and diluted serum samples were then added to the ELISA plate.

Fucosylated haptoglobin (Fuc-Hpt) levels were measured using our lectin antibody ELISA developed as described previously (35). Briefly, the Fab fragment of antihuman haptoglobin IgG (Dako, Carpinteria, CA) was coated onto the bottom of the wells of a 96-well ELISA plate because IgG has a fucosylated oligosaccharide in its Fc portion. Diluted serum samples were then added to the ELISA plate. To detect Fuc-Hpt, biotinylated *Aleuria aurantia* lectin at a 1/1000 dilution was placed into each well. Adiponectin was measured using a sandwich ELISA system according to the manufacturer's protocol (Otsuka Pharmaceutical Co., Tokushima, Japan) (36). Each assay was performed in duplicate using diluted serum samples.

In vitro assay

The human HSC line LX-2 was kindly donated by Scott Friedman (Mount Sinai School of Medicine) (37). Cells were maintained at 37°C under 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal bovine serum (FBS). LX-2 cells (1.5 × 10^5/well) were seeded in 24-well plates, rested for 24 h, then incubated in serum-free DMEM for 48 h. Cells were stimulated with fetuin-A (15 μM) (Sigma-Aldrich, St-Louis, MO, USA), TGF-β1 (5 ng/ml) (PeproTech EC Ltd., Rocky Hill, CT) or TGF-β1 (5 ng/ml) + fetuin-A (15 μM) dissolved in serum-free DMEM. After 6 h of stimulation, total RNA was extracted from the cells with QIAshredder and an RNeasy Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany) and then transcribed into complementary DNA using a ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan). Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was performed using the THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan) with specific primers on a LightCycler according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN, USA). The primers used were TGF-β1 (QT00000728), collagen Iα1 (QT00037793), bone morphogenic protein and activin membrane-bound inhibitor (BAMBI; QT00091329) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; QT01192646) (Qiagen). mRNA expression levels were normalized to GAPDH mRNA expression and expressed in arbitrary units.

Immunoblot analysis was performed to investigate the phosphorylation of Smad3 (p-Smad3) in LX-2 cells. LX-2 cells (5 × 10^5/well) were seeded in 6-well plates, rested for 24 h, then incubated in serum-free DMEM for 24 h. Cells were stimulated with TGF-β1 (5 ng/ml) with or without fetuin-A (15 μM) for 30 min. Immunoblotting was performed as described previously using rabbit anti-p-Smad3 antibody, anti-Smad3 antibody (Cell Signaling Technology, Beverly, MA, USA) or rabbit anti-GAPDH antibody (Trevigen, Gaithersburg, MD, USA) (38).

Statistical analysis

Statistical analyses were conducted using JMP Pro 10.0 software (SAS Institute Inc., Cary, NC, USA). Continuous variables were expressed as the mean ± standard deviation (SD). Qualitative data were represented as numbers, with percentages indicated in parentheses. Kruskal–Wallis and Wilcoxon tests were used to assess any significant differences in continuous clinical or serological characteristics between groups. Chi-square tests were used for categorical factors. As TG, HDL-C, AST, ALT, GGT and FBG did not show a Gaussian distribution, these parameters were common log transformed before analysis. Spearman's correlation coefficient was used to estimate the association between serum fetuin-A and several factors of interest. The prediction performance of the serum fetuin-A level for increased mean IMT (≥1 mm) was assessed by analyzing receiver operating characteristic (ROC) curves. The probabilities of true positive (sensitivity) and true negative (specificity) assessments, the positive predictive value (PPV) and the negative predictive value (NPV) were determined for selected cut-off values, and the area under the receiver