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Dual Therapy With the Nonstructural Protein 5A Inhibitor, Daclatasvir, and the Nonstructural Protein 3 Protease Inhibitor, Asunaprevir, in Hepatitis C Virus Genotype 1b–Infected Null Responders

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Patients with chronic hepatitis C virus (HCV) infection and previous null response to pegylated interferon (Peg-IFN) and ribavirin (RBV) have limited therapeutic options. HCV genotype 1 is the most common worldwide and the most difficult to treat; genotype 1b is the most common subtype of genotype 1 outside North America. The enhanced antiviral activity achieved by combining two direct-acting antiviral (DAA) agents may improve clinical outcomes. This open-label, phase IIa study included 10 patients with chronic HCV genotype 1b infection and previous null response (<2 log_{10} reduction in HCV RNA after 12 weeks) to Peg-IFN and RBV. Patients received dual DAA treatment for 24 weeks with the nonstructural protein 5A replication complex inhibitor, daclatasvir (60 mg once-daily), and the nonstructural protein 3 protease inhibitor, asunaprevir (initially 600 mg twice-daily, then subsequently reduced to 200 mg twice-daily). The primary efficacy endpoint was the proportion of patients with sustained virologic response (SVR) at 12 weeks post-treatment (SVR_{12}). Nine patients completed 24 weeks of treatment; 1 patient discontinued treatment after 2 weeks. In the 9 patients who completed the full course of treatment, HCV RNA was undetectable at week 8 and remained undetectable through the end of treatment; all 9 patients achieved SVR_{12} and SVR_{24}. HCV RNA also remained undetectable post-treatment in the patient who discontinued after 2 weeks. There was no viral breakthrough. Diarrhea and headache, generally mild, were the most common adverse events; transaminase elevations were reported in 3 patients, but did not result in discontinuation. *Conclusions*: Dual therapy with daclatasvir and asunaprevir, without Peg-IFN and RBV, can achieve high SVR rates in difficult-to-treat patients with HCV genotype 1b infection and previous null response to Peg-IFN and RBV. (HEPATOLOGY 2012;55:742-748)

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Chronic hepatitis C virus (HCV) infection affects approximately 180 million individuals worldwide and is a common cause of chronic liver disease and hepatocellular carcinoma (HCC) in Japan, the United States, and many European countries.[1,2] Among the six major HCV genotypes, genotype 1 is the most common and the most difficult to treat, and its two main subtypes may differentially influence therapeutic outcomes.[3,4] Genotype 1b is the most prevalent worldwide and predominates in Japan and China, whereas genotype 1a is most common in the United States; subtype prevalence in Europe is similar.[5-7]

Abbreviations: ALT, alanine aminotransferase; cEVR, complete early virology response: undetectable HCV RNA at week 12; DAA, direct-acting antiviral; EOTR, end-of-treatment response: undetectable HCV RNA at week 24; eRVR, extended rapid virologic response: undetectable HCV RNA at weeks 4 and 12; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IL28B, *interleukin-28B; INR, international normalized ratio; LLQ, lower limit of quantitation; NS3, nonstructural protein 3; NS5A, nonstructural protein 5A; Peg-IFN-α, pegylated interferon alpha; PCR, polymerase chain reaction; RBV, ribavirin; RVR, rapid virologic response: undetectable HCV RNA at week 4; SNP, single-nucleotide polymorphism; SVR, sustained virologic response: undetectable HCV RNA post-treatment; SVR_{12}, sustained virologic response 12 weeks post-treatment; SVR_{24}, sustained virologic response 24 weeks post-treatment; ULN, upper limit of normal.*

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Treatment of chronic HCV infection with pegylated interferon alpha (Peg-IFN-α) and ribavirin (RBV) elicits a sustained virologic response (SVR) in 40%-50% of treatment-naïve patients with genotype 1 infections; SVR rates in this population increase to 66% or 75% when boceprevir or telaprevir, respectively, is added to the regimen.[8-12] Response rates are influenced by viral load and genotype and by patient demographics, disease history, and genetics.[10] Peg-IFN/RBV retreatment of patients with previous nonresponse to Peg-IFN/RBV is frequently unsuccessful, with SVR rates of only 6%-9%.[13,14] Null responders are the subset of nonresponders who have responded most poorly to Peg-IFN/RBV, and their urgent need for more potent therapies has prompted the evaluation of regimens containing direct-acting antivirals (DAAs). SVR rates of 27% (genotype 1a) and 37% (genotype 1b) were achieved in null responders with a regimen combining telaprevir with Peg-IFN/RBV in a study of nonresponders.[15] These results suggest that DAA-containing regimens can benefit this population, but greater antiviral potency is needed to increase response rates further.

Combinations of two DAAs may overcome IFN nonresponsiveness in null responders by increasing antiviral activity and reducing the risk of developing resistance-associated variants.[16] In HCV-infected human hepatocyte chimeric mice, dual DAA treatment eradicated HCV without resistance, whereas resistance emerged rapidly with single DAA treatment.[17] In a clinical study that included null responders, marked antiviral effects were observed after 13 days of dual DAA treatment, supporting the evaluation of longer term dual DAA therapy reported in this study.[18] Daclatasvir (BMS-790052) is a first-in-class, highly selective nonstructural protein 5A (NS5A) replication complex inhibitor with picomolar potency and broad genotypic coverage; asunaprevir (BMS-650032) is a nonstructural protein 3 (NS3) protease inhibitor active against HCV genotypes 1a and 1b.[19,20] Daclatasvir and asunaprevir are associated with different resistance-associated variants, consistent with their different molecular targets, and showed no meaningful pharmacokinetic interactions in healthy volunteers.[20-22]

In a 24-week study of null responders in the United States, daclatasvir and asunaprevir demonstrated potent antiviral effects, both as a dual DAA regimen and in a quadruple regimen that included Peg-IFN/RBV.[23] Overall, 36% of dual-therapy recipients achieved SVR, including both of the 2 patients with genotype 1b infection. However, patients with genotype 1a experienced frequent viral breakthrough with the dual regimen and only 2 of 9 achieved SVR, suggesting subtype-associated differences in resistance barrier and response. We present the results of an open-label trial evaluating dual therapy with daclatasvir and asunaprevir in Japanese patients with chronic HCV genotype 1b infection and previous null response to Peg-IFN/RBV.

Patients and Methods

Study Design. This open-label, phase IIa study (clinicaltrials.gov identifier NCT01051414) evaluated the antiviral activity and safety of daclatasvir combined with asunaprevir in patients with HCV genotype 1 infection and previous null response to treatment with Peg-IFN/RBV, defined as <2 $\log_{10}$ reduction of HCV RNA after 12 weeks of therapy. This sentinel cohort provided safety data for review by an independent study safety committee before the enrollment of additional cohorts that will be described in a subsequent report. Written informed consent was obtained from all patients. The study was approved by institutional review boards at each site and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and local regulatory requirements.

Patients. Patients eligible for enrollment in the sentinel cohort included men and women 20-75 years in age (women of childbearing potential were required to use adequate contraception) with chronic HCV genotype 1 infection for at least 6 months (all enrolled patients were genotype 1b because of the high prevalence of this subtype in Japan) and HCV RNA $\geq 10^5$ IU/mL. Eligible patients met criteria defining null responders and had no evidence of cirrhosis documented by laparoscopy, imaging, or liver biopsy within 2 years.

Patients were excluded if they had a history of HCC, coinfection with hepatitis B virus or human immunodeficiency virus, other chronic liver disease, or

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evidence of hepatic decompensation. Patients were also excluded if they had other severe or unstable conditions or evidence of organ dysfunction in excess of that consistent with the age of the patient, were unable to tolerate oral medication or had conditions that could affect the absorption of study drug, or were exposed to any investigational drug within 4 weeks of study participation or had any previous exposure to inhibitors of NS5A or NS3 protease. Laboratory findings that excluded participation were the following: alanine aminotransferase (ALT) >5× the upper limit of normal (ULN); total bilirubin ≥2 mg/dL; direct bilirubin >1.5× ULN; international normalized ratio (INR) ≥1.7; albumin ≤3.5 g/dL; hemoglobin <9.0 g/dL; white blood cells <1,500/mm^3; absolute neutrophil count <750/mm^3; platelets <50,000/mm^3; or creatinine >1.8× ULN.

Prohibited concomitant medications included inducers or inhibitors of cytochrome P450/3A4, non-study medications with anti-HCV activity, any prescription medication or herbal product not prescribed for a specific condition, liver-protection drugs, proton pump inhibitors, and erythropoiesis-stimulating agents. H_2 receptor antagonists were permitted, but administered ≥10 hours before or ≥2 hours after daclatasvir; other acid-modifying agents had to be taken ≥2 hours before or after daclatasvir.

Study Drug Dosing. All patients received oral combination therapy with daclatasvir and asunaprevir from the beginning of the study. Daclatasvir was dosed as two 30-mg tablets once-daily. Asunaprevir was initially dosed as three 200-mg tablets twice-daily; subsequently, the dose of asunaprevir was reduced to 200 mg twice-daily after reports of hepatic enzyme elevations in a clinical study of asunaprevir and Peg-IFN/RBV.[24]

Treatment was continued to week 24 for patients with HCV RNA below the assay lower limit of quantitation (LLQ; 15 IU/mL) on or after week 2; treatment was discontinued for patients with <2 $\log_{10}$ IU/mL decrease of HCV RNA from baseline or on or after week 2. For patients with viral rebound on or after week 2, or HCV RNA above LLQ on or after week 4, treatment was discontinued or weight-based Peg-IFN-RBV therapy was added for up to 48 additional weeks at the investigator's discretion, based on expected tolerance of Peg-IFN-RBV. Viral rebound was defined as an increase ≥1 $\log_{10}$ IU/mL from nadir at more than one time point or HCV RNA ≥15 IU/mL after declining to below that level.

Safety and Efficacy Assessments. Assessments, including HCV RNA, physical examination, vital signs, adverse events, laboratory tests, and review of concomitant medications, were conducted at screening, on study days 1 (baseline) through 7 and days 9, 11, and 14, at weeks 3, 4, 6, 8, 10, 12, 16, 20, and 24, and at post-treatment weeks 4, 8, 12, and 24. Twelve-lead electrocardiograms were recorded at all visits, except those at weeks 3 and 6. Additional pretreatment assessments included HCV genotype and host interleukin-28B (*IL28B*) genotype.

Serum HCV RNA levels were determined at a central laboratory using the Roche COBAS TaqMan HCV Auto assay (LLQ = 15 IU/mL; Roche Diagnostics KK, Tokyo, Japan). HCV genotype and subtype were determined at the central laboratory by polymerase chain reaction (PCR) amplification and sequencing. *IL28B* genotype was determined by PCR amplification and sequencing of the rs12979860 single-nucleotide polymorphism (SNP).

Outcome Measures. The primary efficacy endpoint was the proportion of patients with undetectable HCV RNA at 12 weeks post-treatment (SVR_{12}). Secondary endpoints included the proportions of patients with rapid virologic response (RVR; defined as undetectable HCV RNA at week 4), extended RVR (eRVR; undetectable HCV RNA at weeks 4 and 12), complete early virologic response (cEVR; undetectable HCV RNA at week 12), end-of-treatment response (EOTR; undetectable HCV RNA at week 24), and SVR at 24 weeks post-treatment (SVR_{24}).

The possible presence of HCV-resistance polymorphisms was analyzed using stored specimens. Resistance testing was performed on all samples at baseline and on samples indicative of virologic failure, defined as either (1) <2 $\log_{10}$ HCV RNA decrease from baseline at week 2, (2) virologic rebound (HCV RNA detectable after previously undetectable or ≥1 $\log_{10}$ increase from nadir), or (3) detectable HCV RNA at weeks 4 or 12 or at the end of therapy. Resistance analysis methodology included isolation of HCV RNA, PCR amplification, and population sequencing of HCV NS3 protease and NS5A domains.

Statistical Analysis. Categorical variables were summarized using counts and percents; continuous variables were summarized with univariate statistics.

Results

Patient Characteristics and Disposition. Twelve patients were screened; 2 patients failed to meet entry criteria (for HCC and elevated direct bilirubin, respectively), and 10 were enrolled and treated. Enrolled patients were generally older (median, 62 years); 6

Table 1. Baseline Demographic and Disease Characteristics

Parameter	Value
N	10
Age, median years (range)	62 (52-70)
Male sex, n (%)	4 (40)
Japanese race, n (%)	10 (100)
Host *IL28B* genotype,* n (%)	
CC	2 (20)
CT	8 (80)
HCV genotype 1b, n (%)	10 (100)
HCV RNA, mean $\log_{10}$ IU/mL (SD)	6.8 (0.61)
ALT, mean U/L (SD)	60.6 (32.9)
Platelets $\times 10^9$ cells/mL, median (min, max)	150.5 (84.0, 166.0)
Total bilirubin, median mg/dL (min, max)	0.8 (0.6, 1.2)
Albumin, median g/dL (min, max)	3.9 (3.1, 4.2)
INR, median (min, max)	1.0 (1.0, 1.1)

*SNP rs12979860.

Abbreviation: *IL28B*, interleukin-28B; HCV, hepatitis C virus; SD, standard deviation; ALT, alanine aminotransferase; min, minimum; max, maximum; INR, international normalized ratio; SNP, single-nucleotide polymorphism.

were female and all were Japanese (Table 1). All enrolled patients were infected with genotype 1b, reflecting the predominance of this subtype in Japan, although the study protocol did not exclude patients with HCV genotype 1a.[6] Two patients were *IL28B* genotype CC (SNP rs12979860) and 8 were CT. Nine patients completed 24 weeks of therapy; 1 patient discontinued at week 2 because of a grade 4 total bilirubin elevation (see below). Among the 9 patients treated for 24 weeks, asunaprevir was dosed at 600 mg twice-daily for 12-21 weeks before the dose was reduced to 200 mg twice-daily (Fig. 1).

Virologic Response. Serum HCV RNA levels decreased rapidly in all patients (Fig. 2); mean reductions from baseline were 4.4 $\log_{10}$ IU/mL at week 1,

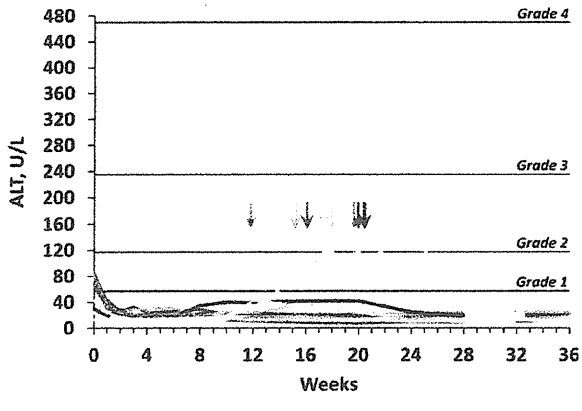

Fig. 1. ALT levels: individual patients. Serum ALT levels for the 9 patients who completed 24 weeks of treatment; the patient who discontinued at week 2 is not presented. Shaded area indicates the treatment period; arrows indicate the points at which the dose of asunaprevir was reduced from 600 to 200 mg twice-daily. Arrow and line colors are the same for each patient.

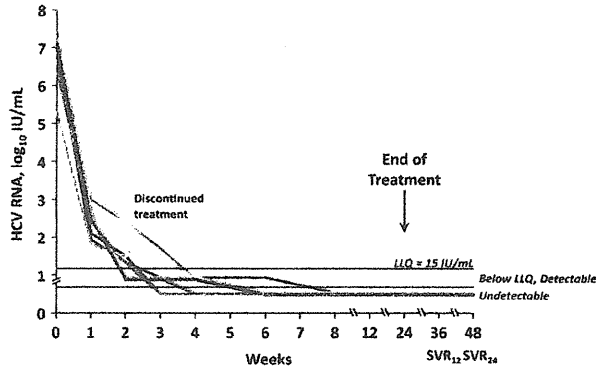

Fig. 2. HCV RNA levels: individual patients. Individual patient plasma HCV RNA levels during 24 weeks of treatment and through 24 weeks post-treatment (week 48) are shown. LLQ = 15 IU/mL.

5.3 $\log_{10}$ IU/mL at week 2, and 5.8 $\log_{10}$ IU/mL from week 4 through the end of treatment. At week 4, HCV RNA was undetectable (RVR) in 4 of 10 (40%) patients and below the assay LLQ in 9 of 10 (90%; Fig. 3). No patients qualified for discontinuation or addition of pegIFN/RBV. At week 8, HCV RNA was undetectable in 9 of 10 patients (all who remained on treatment) and remained undetectable through the end of treatment and follow-up. SVR_{12}, the primary endpoint, and SVR_{24} were achieved by 90% of patients, including all 9 who completed 24 weeks of therapy. The patient who discontinued treatment at week 2 had low-level HCV RNA at discontinuation (1.8 $\log_{10}$ IU/mL), but HCV RNA was undetectable at follow-up visits 2, 3, 4, 13, and 24 weeks after discontinuation.

Viral Breakthrough and Relapse. There was no viral breakthrough during treatment or relapse of HCV RNA post-treatment. Analysis of baseline samples revealed variants reported to confer minimal to low

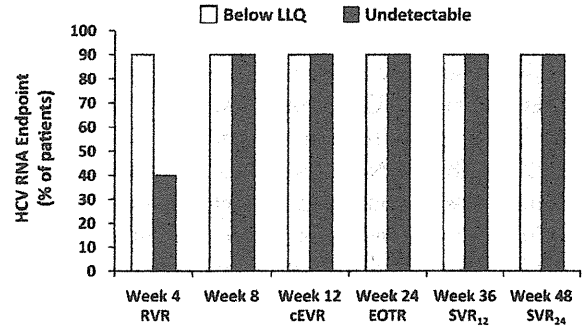

Fig. 3. HCV RNA endpoints. Categorical HCV RNA endpoints are indicated for the 10 study patients. One patient discontinued at week 2 and was counted as a treatment failure at the time points shown. However, HCV RNA was undetectable in this patient at 2, 3, 13, and 24 weeks post-treatment.

Table 2. On-Treatment Adverse Events Occurring in ≥2 Patients

Event	Patients, n (%)
Diarrhea	7 (70)
Headache	4 (40)
ALT increased	3 (30)
AST increased	3 (30)
Lymphopenia	2 (20)
Abdominal discomfort	2 (20)
Malaise	2 (20)
Pyrexia	2 (20)
Nasopharyngitis	2 (20)
Lipase increased	2 (20)
Back pain	2 (20)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

levels of resistance to daclatasvir.[22] NS5A substitutions L28M and L31M were detected in 1 patient each, and Y93H was detected in 2 other patients. NS3 protease substitutions reported to confer resistance to telaprevir, boceprevir, and TMC-435 were detected[25]; T54S was identified in 1 patient, and Q80L was identified in 3. In 1 patient, both NS3 protease substitutions (T54S and Q80L) and an NS5A substitution (Y93H) were detected. There was no consistent association between detection of these variants and virologic outcomes.

Safety. The most frequently reported adverse events were diarrhea and headache, all of which were mild (grade 1) (Table 2). The patient who discontinued (see below) experienced multiple grade 3 or 4 adverse events and laboratory abnormalities on treatment. In the other 9 patients, there were no grade 3 or 4 transaminase elevations or other grade 3 or 4 events, no clinically relevant changes in electrocardiogram parameters, and no lymphopenia of any severity. Two transient grade 1 ALT elevations were reported, and 1 grade 2 elevation that began at week 16 and persisted until the end of treatment, after which it normalized within 2 weeks (Fig. 1). There were no notable differences in ALT before and after asunaprevir dose reduction.

There were two serious adverse events. A 54-year-old male was hospitalized with grade 3 pyrexia and persistent diarrhea 11 days after initiating study treatment. Loxoprofen was initiated, and body temperature normalized and diarrhea improved after 4 days. The patient remained on study treatment. The second event concerned a 60-year-old woman with a history of ulcerative colitis who discontinued study treatment after 2 weeks because of a grade 4 bilirubin elevation with multiple complicating features. Five days before discontinuation, she presented with infectious gastroenteritis and was treated with cefotiam and was subsequently hospitalized with fever, vomiting, and diarrhea. Meropenem, human serum albumin, and furosemide were initiated. At discontinuation of study drugs, laboratory findings included total bilirubin of 7.7 mg/dL and grade 3 lymphopenia and serum phosphorus reduction; transaminases and alkaline phosphatase were within normal ranges. In the week after discontinuation, white cell and eosinophil counts became elevated; total bilirubin improved and transaminases remained normal. Two weeks after discontinuation, grade 4 ALT and aspartate-aminotransferase elevations and a grade 3 lipase elevation were reported. Six weeks after discontinuation, bilirubin and transaminase elevations were resolved and lipase improved to within 2× ULN.

Discussion

This study assessed combination oral DAA therapy in a difficult-to-treat population with multiple adverse prognostic features, including HCV genotype 1b infection, primarily *IL28B* CT genotype, generally older age, and null response to previous Peg-IFN/RBV therapy.[10,13,14] These patients represent a group with a significant need for new therapeutic options.

A DAA-only therapeutic strategy may be particularly appropriate for null responders, who have previously shown only marginal response to Peg-IFN/RBV.[13,14] The combination of two highly potent DAAs cleared detectable virus rapidly in this study; HCV RNA was undetectable by week 8 in all 9 patients treated for 24 weeks. This outcome compares favorably with those observed when null responders received a combination of Peg-IFN/RBV and a single NS3 protease inhibitor, telaprevir or TMC435.[15,26] In these studies, HCV RNA remained detectable in 36% to approximately 50% of patients after 12 weeks.

HCV RNA remained undetectable 12 (SVR_{12}) and 24 weeks (SVR_{24}) post-treatment in all patients who completed treatment. This contrasts with the poor results obtained with Peg-IFN/RBV retreatment and the reported 37% SVR rate of genotype 1b null responders who received Peg-IFN/RBV and telaprevir.[10,13-15] Additional follow-up of patients from this study will assess whether SVR_{24} is predictive of long-lasting viral clearance with this dual DAA therapy, as it is with Peg-IFN/RBV. It is interesting that HCV RNA was persistently undetectable post-treatment in the patient who discontinued after only 2 weeks of treatment. With early discontinuation data from only this single case, at present, the result must be considered an anomaly. The factors that contributed to viral

clearance are uncertain, although the patient's *IL28B* CC genotype suggests increased sensitivity to endogenous interferon[27]; the possible influence of concurrent acute gastroenteritis or other complicating factors is unknown. However, coupled with the attainment of SVR_{12} in all other patients, this outcome suggests that required duration of therapy, which is currently predicated on data from Peg-IFN-based regimens, may need reassessment for DAA-only regimens, and, possibly, that certain patient populations can be treated for very short durations.

The high SVR rate is consistent with limited data from a related U.S.-based study, in which 2 of 2 null responders with HCV genotype 1b and who were treated with daclatasvir and asunaprevir achieved SVR_{24}.[23] However, only 2 of 9 patients with genotype 1a achieved SVR_{24} with the dual DAA regimen, compared with 9 of 10 patients who received both DAAs and Peg-IFN/RBV. These differences suggest that viral genotype can influence responses to DAA regimens that do not include Peg-IFN/RBV, and outcomes can be optimized with individualized therapy that considers viral genotype, among other factors. Because of the high SVR rate, the potential influence of other baseline and on-treatment parameters could not be assessed, other than to observe that unfavorable predictors of Peg-IFN/RBV response, such as older age and *IL28B* CT genotype,[27,28] had no measureable impact on outcomes.

There was no viral breakthrough on treatment. In view of the rapid emergence of resistance in some studies of short-term DAA monotherapy,[29,30] these findings support the concept that dual DAA therapy reduces the risk of viral breakthrough, in addition to increasing antiviral activity. Resistance analyses revealed that before treatment, some patients carried NS5A and NS3 polymorphisms predicted to reduce sensitivity to daclatasvir and some HCV protease inhibitors, respectively.[22,25] There was no clear relationship between the presence of these polymorphisms and minor interpatient differences in the rate of early virologic response; however, further study in larger patient cohorts will help determine whether baseline polymorphisms can influence virologic response with this regimen.

The adverse event profile of the dual DAA regimen compares favorably with the more frequent and severe events reported with Peg-IFN/RBV, although patient numbers in this study were limited. The mild diarrhea experienced by several patients has been reported previously with asunaprevir and is common with other drugs of this class.[15,18,24] Though a role for daclatasvir and/or asunaprevir in the two serious adverse events could not be ruled out and the investigator considered these events drug related, multiple confounding factors existed. The case of pyrexia was consistent with a viral infection and resolved with treatment. In the case of hyperbilirubinemia that led to discontinuation, the time course of laboratory abnormalities and related events suggests a link to the use of cefotiam and meropenem for treatment of infectious gastroenteritis. Both of these agents have been associated with vomiting, diarrhea, and hyperbilirubinemia.[31,32]

The asunaprevir dose was reduced during treatment because of transaminase elevations observed with 600 mg twice-daily in a concurrent study.[24] In this sentinel cohort, viral suppression was maintained in all patients after dose reduction, and no grade 3 or 4 transaminase elevations occurred during treatment at either dose of asunaprevir. One patient experienced grade 2 transaminase elevations that began at week 16 and persisted during treatment, despite asunaprevir dose reduction at week 19. Although these elevations were not severe, their rapid normalization post-treatment suggests a possible relationship to study treatment. None of the 9 patients treated for 24 weeks experienced transaminase elevations post-treatment. Although grade 4 transaminase elevations occurred 2 weeks post-treatment in the patient who discontinued, the timing of these events and multiple other complications suggest that they were not related directly to study treatment.

In conclusion, the combination of daclatasvir and asunaprevir achieved a high rate of SVR_{24} in patients with HCV genotype 1b infections and previous null response to Peg-IFN/RBV. These results support the concept that HCV infection can be cured with two DAAs without Peg-IFN/RBV, even in difficult-to-treat populations that lack robust IFN responsiveness. Further research will assess the benefits of DAA combinations in larger, more diverse patient populations.

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5 石川県の肝癌撲滅計画

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肝癌撲滅には背景にある肝炎ウイルスに対する治療導入が重要である。石川県では肝炎ウイルス検診初年度より協議会を設立し，陽性者をフォローアップしてきた。インターフェロン療法導入率向上を目指してさまざまな施策を講じ，導入率は30%を越えるようになった。2010年度よりかかりつけ医と専門医の連携を強化した「石川県肝炎診療連携」を新たに開始して専門医受診勧奨，抗ウイルス療法導入を図ることにより肝癌撲滅を目指している。

はじめに

2009年度人口動態統計では肝癌による死亡者数は男性で第4位，女性では第6位であり，年間3万人を越えている。肝癌の多くはウイルス性慢性肝疾患を背景に発生しており，肝癌撲滅には肝炎ウイルス感染者を早期に発見し，早期に治療することが重要である。国は2002年度より5年間で肝炎ウイルス検診を行い，肝炎ウイルス感染者の発見に努めたが，検診受診率は決して高くなく，また医療機関を受診しても適切な観察，治療導入すなわち抗ウイルス療法が行われてきたとは言い難い。本稿では肝炎ウイルス検診開始当初より石川県で取り組んできた肝炎ウイルス症例への対策について述べる。

1 肝炎ウイルス検診の方針

2002年肝炎ウイルス検診会誌当初より，石川県では肝炎協議会を設置し，県健康福祉部・医師会・保健所・検査センター・学術経験者が一体となって協力した検診体制を確立した。地域により専門医療機関の過不足があるため，精密検査は特に指定医療機関とはせず，かかりつけ医でも可とした。このため検診精度の向上と経過観察の重要性を考え，以下7つの項目を検診事業の柱とした。

1. 検診陽性者への行政の関与することの通知と同意
2. 精密検査の全県での統一
3. 住民，担当医用の診断手引きの作成
4. 精密検査での画像検査の義務付け
5. 全症例を対象とした事例検討会
6. 前年度陽性者に対する事後調査
7. 保健師などを対象とした研修会の開催

このなかで石川県として独自性の高いと考えているものは検診陽性者を行政が継続フォローするために必要な1，6および担当医の肝炎への理解を深めた5である。毎年検診陽性者の医療機関受診・治療状況を把握することと，担当のかかりつけ医が正しく診断，治療導入することへの意識が高まるようこれら事業を継続した。

2 肝炎ウイルス検診の状況

石川県では5年間の肝炎ウイルス検診受診率は36.6～41.5%と全国平均[1]と比べると10%ほど受診率がよかったが半数には満たない。検診陽性者の精密検査受診状況は男性67.6%，女性75.0%，年齢では若年（65歳未満）66.1%，高齢（65歳以上）74.7%であった。性年齢でわけると若年男性53.4%，若年女性71.9%，高齢男性74.0%，高齢女性74.0%と若年男性で精密検査の受診率が低いことが明らかであり，仕事等で忙しく受診機会をつくりにくい状況がうかがえる。図1に性・年齢・医療圏別での精検受診状況を示す。検診自体の受診率は能登地方および南加賀で低い傾向にあった。しかし能登地方はウイルスキャリアと判明すると医療機関をきちんと受診する傾向にある。一方，南加賀ではウイルスキャリアと判明しても医療機関への受診率が悪い。能登地方ではキャリアの発掘が重要であり，南加賀ではキャリアの発掘と受診勧奨の両面が必要なことがう

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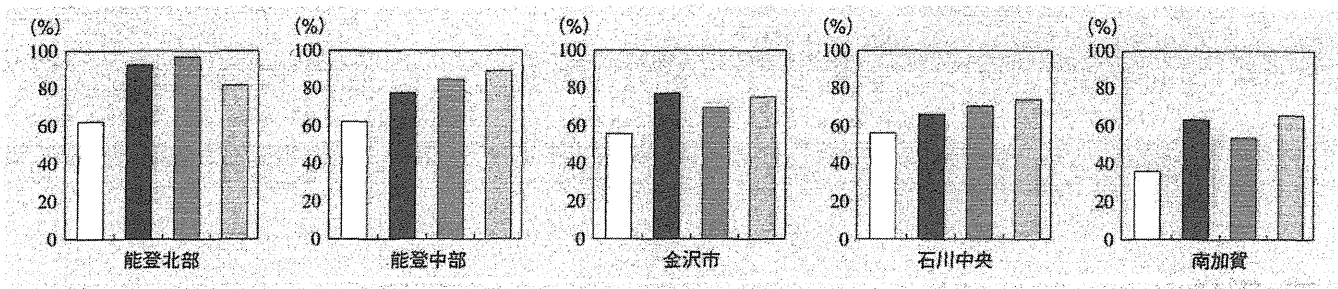

図1●検診陽性者の精密検査受診状況
□：男性・65歳未満 ■：男性・65歳以上 ■：女性・65歳未満 ▨：女性・65歳以上

表1●石川県精検未受診者のその後の状況

	検診初年度精検未受診	翌年以降医療機関受診	IFN療法/受診者
能登北部	18（14.8%）	12（66.7%）	3（25.0%）
能登中部	32（17.5%）	17（53.1%）	2（11.8%）
石川中央	71（31.8%）	45（63.4%）	7（15.6%）
南加賀	88（40.6%）	52（59.1%）	10（19.2%）
金沢市	147（28.1%）	39（26.5%）	2（5.1%）
合計	356（28.1%）	165（46.3%）	24（14.5%）

かがえる。また医療機関受診の時間がとりにくい若年男性の受診率が悪いのは地域で共通しており，受診動機を促す啓蒙活動が必要である。

フォローアップ事業の有用性 3

前述したように石川県では保健師が面談，電話，手紙などの方法で検診陽性者の状況把握に努めている。継続して医療機関で経過観察されているのはC型肝炎では48.7～63.7%であった。一方，各市町で少なくともフォロー期間（2～7年）中に1度は医療機関を受診した症例はB型肝炎ウイルス陽性者で49～100%，C型肝炎ウイルス陽性者で80～100%であった。表1に初年度精密検査未受診者のその後の状況を示す。受診勧奨を行った結果未受診者のうち能登北部66.7%，能登中部53.1%，金沢市26.5%，石川中央63.4%，南加賀59.1%がその後に医療機関を受診し，さらに受診者のうち能登北部25.0%，能登中部11.8%，金沢市5.1%，石川中央15.6%，南加賀19.2%がインターフェロン（IFN）療法を行っていた。継続した状況把握，受診勧奨が適切な医療へと結びつくことが明らかとなった。

IFN治療状況 4

肝癌撲滅という目標に対してC型肝炎であればIFN療法によりウイルスが排除されることが一番である。年齢，合併症などにより全ての症例でIFN療法を行うのは困難であるが，検診症例のIFN療法の施行率が低いことが問題となっている。厚生労働省研究班の報告では当初3年間では13.8～18.2%であった[1]。石川県でも2002年131例中5例（3.8%），2003年164例中14例（8.5%）とIFN療法施行率は低かった。特に65歳以上の高齢者ではIFN施行率は2.6%と，65歳未満の9.6%に対して有意に低かった[2]。IFN導入率が高齢者を含めて低い理由を検討するために，石川県全下で内科標榜医療機関にアンケート調査を行った。設問「一度はIFN療法を患者に説明するか（複数回答可）」に肝臓専門医の約8割は条件を問わずIFN療法について説明するが，非専門医師は約5割しか条件を問わずにIFN療法を説明していなかった。また「IFN療法を行わない理由」としては高齢であることをあげる医師が多数を占めたが，「何歳までがIFN療法の適応と考えるか」という設問では専門医は70～75歳までを適応と考えているが，非専門医はおおむね70歳以下と考えており，IFN適応年齢を非専門医は低く考えがちであることも明らかとなった[2]。このような実態を踏まえ，一例ごとの事例検討会，IFN療法をテーマにした講習会などを繰り返し行い，2004年102例中24例

表2●全国および石川県の検診C型肝炎陽性者のIFN施行率

		初年度IFN療法施行率	
		精検受診者中	慢性肝炎中
全国[1]	2002年	13.8%	
	2003年	13.3%	
	2004年	18.2%	
	2005年		
	2006年		
石川県	2002年	3.0%	3.8%
	2003年	5.7%	8.5%
	2004年	14.7%	23.5%
	2005年	24.5%	35.3%
	2006年	23.7%	31.0%

表3●「肝炎診療連携」で把握された75歳以下検診C型肝炎陽性症例のIFN治療状況

	キャリア（n：13）＋慢性肝炎（n：75） n＝88
IFN過去にあり	28（著効 6例）
現在投与中	7
投与開始	7
IFN施行数（率）	42/88（48%）
合併症不可（IP，うつなど）	4
IFN可能症例施行数（率）	42/84（50%）
IFN検討中	8

（23.5%），2005年68例中24例（35.3%），2006年71例中22例（31.0%）と後半2年間はIFN療法施行率が30%を超えていた（表2）。

石川県肝炎診療連携

年々IFN施行率は上昇してきたが，さらに向上させるには専門医が関わることが重要である。石川県では精密検査を専門医が行った症例では144例中53名（36.8%）がすぐにIFN導入され，翌年以降にさらに26例でIFN療法が施行，計79例（54.9%）でIFN療法が導入されていた。一方，かかりつけ医で診られた41症例では計8例（19.5%）のIFN導入にとどまり，IFN療法施行率をあげるには専門医がその診断，治療方針決定に関わることが重要であった。2007年にでた厚生労働省の肝炎検査後診療体制のガイドラインでも「状態に変化がなくとも年一回の専門医療機関受診が望ましい」とされており，かかりつけ医から患者を年1回の専門医に受診勧奨する「石川県肝炎診療連携」を立案した。個人情報保護の問題をクリアし，行政の保持する検診データを拠点病院と専門医療機関で構成する肝炎診療連携協議会に移行するために，行政・各市町と協議の上，患者より「石川県肝炎診療連携」への参加，データ移行に関して再同意をとり，専門医療機関を受診，順次データ移管することとなった。非同意，または返答のなかった症例は引き続き行政でフォローアップをすることとした。

2,570人の肝炎ウイルス検診陽性者に同意書・調査票が送付され494人が同意，非同意が90人，専門医療機関受診し調査票が回収されたのは328人であった。HBs抗原陽性148人，HCV抗体陽性174人であった。HBs抗原陽性では無症候性キャリアと診断されたのが79例で，そのうち5例でALT31IU/L以上の異常値であったが，4例ではHBV-DNA低値の情報が付加されており，診断が妥当であることが確認された。また核酸アナログ使用率も14%とHBs抗原陽性で治療を必要とする従来の割合と合致しているデータと考えられた。HCV抗体陽性者のうち慢性肝炎またはキャリアと診断された症例の治療方針をみると専門医がIFN療法が望ましいとしたのは全体の33%であった。一方経過観察が選択された症例では，ALT値が低いか，超高齢者が多く含まれていた。今回の専門医受診を契機にIFN療法導入が7例あり，過去のIFN歴も踏まえて現在までにIFN療法が行われたのは75歳以下の検診症例で48%であった（表3）。

おわりに

肝癌撲滅には背景となるウイルス性肝疾患への適切な経過観察，治療の導入が重要である。県下の肝炎ウイルス検診症例を専門医受診勧奨とデータ管理により早期に適切な治療導入に図りたい。

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ORIGINAL ARTICLE

Effect of Mosapride Citrate on Gastric Emptying in Interferon-Induced Gastroparesis

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Abstract
Background and Objectives Gastroparesis, a gastrointestinal autonomic neuropathy, is a common adverse reaction in chronic hepatitis C (CHC) patients receiving interferon therapy. Current therapeutic options are limited. We evaluated the efficacy of mosapride for IFN-induced gastroparesis.
Methods Twenty-four consecutive CHC patients were randomly assigned to either the control group, which received pegylated interferon α-2b at 1.5 μg/kg/week and ribavirin at 600–1,000 mg/day, depending on body weight (PegIFN/RBV), or the mosapride group, which received PegIFN/RBV plus mosapride at 15 mg/person/day. The solid-phase gastric emptying half-times (T1/2) of the total, proximal, and distal stomach (scintigraphy) and digestive symptoms (questionnaire) were measured within one week before and four weeks after initiation of the assigned therapy. The test meal comprised a 200-g pancake containing Tc-99m diethylenetriamine pentaacetic acid.
Results In the control group, after PegIFN/RBV initiation, a significant increase was observed in the total T1/2 (before: 84.0 ± 22.1 min versus after: 100.8 ± 28.9 min, $P = 0.03$), the distal T1/2 (before: 95.3 ± 32.2 min versus after: 115.3 ± 41.4 min, $P = 0.03$), and digestive symptom score (before: 3.2 ± 1.4 versus after: 8.1 ± 4.8, $P = 0.02$); proximal T1/2 change was not significant. In the mosapride group, no significant delays were observed in the total, proximal, and distal T1/2 values; the change in symptom scores was not significant.
Conclusions Mosapride improved total and distal gastric motility in IFN-induced gastroparesis, and consequently relieved symptoms.

Keywords 5-HT_4 receptor agonist · Pegylated interferon · Gastroparesis · Chronic hepatitis C · Gastric emptying scintigraphy

Abbreviations

PegIFN	Pegylated interferon
RBV	Ribavirin
T1/2	Half-time of gastric emptying
HCV	Hepatitis C virus
CHC	Chronic hepatitis C

Introduction

Hepatitis C virus (HCV) is a global health problem, and the virus infects approximately 170 million people [1]. Persistent HCV infection often progresses to chronic hepatitis and liver cirrhosis, and may lead to hepatocellular carcinoma, which is the third leading cause of cancer mortality worldwide [2, 3]. A combination of pegylated interferon (PegIFN) and ribavirin (RBV) has been the standard of care for chronic hepatitis C (CHC) in recent years. Some inhibitors of HCV nonstructural protein 3/4A serine protease, for example telaprevir and boceprevir, have become

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available very recently [4–6]. However, any novel direct-acting antiviral agent should be given simultaneously with PegIFN and RBV, because of the extremely high incidence of viral breakthrough when used alone. Therefore, PegIFN remains a crucial drug for the treatment of chronic HCV infection.

Treatment with PegIFN can cause adverse reactions, including fever of 38°C or higher, influenza-like symptoms, thrombocytopenia, leukocytopenia, proteinuria, alopecia, and gastrointestinal symptoms; these are the major reasons for PegIFN dose reduction and therapy withdrawal. Dose reduction and withdrawal of treatment at an early stage of administration reduces the sustained virologic response [7]. One study reported that 58% of patients who undergo PegIFN and RBV therapy had some gastrointestinal symptoms, including constipation and loss of appetite [8]. These gastroparetic symptoms are recognized as "IFN-induced gastroparesis," and are believed to occur because of autonomic dysfunction in the gastrointestinal tract [9].

Previously, in the era of conventional, non-pegylated IFN monotherapy, we showed by scintigraphic test measurements and assessment of gastrointestinal symptoms by symptom score, that IFN causes a delay in gastric emptying. In addition, we also showed that cisapride, a prokinetic agent that enhances gastrointestinal motility by increasing acetylcholine release via 5-hydroxytryptoptamine (5-HT) receptors, corrected the gastric emptying delay and relieved digestive symptoms [10]. However, use of cisapride has been withdrawn in many countries because of the risk of QT prolongation and fatal arrhythmias, for example torsade-de-pointes and ventricular fibrillation. Mosapride is a selective 5-HT_4 receptor agonist, with fewer adverse reactions than cisapride, that accelerates gastric motility [11] and results in gastroparetic symptom relief [12].

A randomized controlled study was performed to determine the effect of mosapride on gastric emptying capability and gastrointestinal symptoms of CHC patients treated with PegIFN α-2b/RBV at our institution.

Materials and Methods

Design of the Study

The study was a prospective, randomized, controlled, open-label, parallel group design to evaluate the effect of mosapride on IFN-induced gastroparesis and related symptoms in patients with CHC during PegIFN α-2b/RBV therapy. Enrollment was conducted in the Departments of Hepatology and Nuclear Medicine of Osaka City University Hospital. Written informed consent was obtained from all patients. This study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Osaka City University Medical School (UMIN Clinical Trials Registry, UMIN000003890). The primary endpoint of the study was the incidence of interferon-induced gastric emptying delay without mosapride intervention. The focus of this analysis was to determine the different responses between the control and mosapride groups, in terms of secondary endpoints of the study, i.e. the gastric emptying half-time of the total stomach (total T1/2), proximal stomach (proximal T1/2), and distal stomach (distal T1/2), determined by scintigraphy, and digestive symptoms scored at the time of gastric emptying.

Participants and Randomization

The eligibility criteria included persistent elevation of serum transaminase levels for at least six months, presence of serum HCV RNA, absence of serum hepatitis B surface antigen and other likely causes of chronic liver disease, histopathological features of chronic hepatitis in liver biopsy, no evidence of hepatocellular carcinoma on ultrasound or computed tomography, absence of gastrointestinal disease, and no history of antiviral therapy or gastric surgery. Patients enrolled in this study were randomly assigned in a 1:1 ratio to either the control group or to the mosapride group. Patients in the control group received only baseline therapy; 1.5 μg/kg PegIFN α-2b (Pegintron; Schering-Plough, NJ, USA) by subcutaneous injection once weekly and the antiviral drug RBV (Rebetol; Schering-Plough) orally twice a day at a total daily dose of 600–1,000 mg, depending on body weight, for a period of 24–48 weeks. Patients in the mosapride group received the standard oral dose of mosapride citrate (Gasmotin; Dainippon Sumitomo Pharma, Tokyo, Japan) of 15 mg/person/day (5 mg, 3 times daily) for a period of four weeks, starting on the same day as initiation of baseline therapy. The study is reported according to the 2010 update of the Consolidated Standards of Reporting Trials guidelines.

Gastric Emptying Scintigraphy

The scintigraphic test was performed within one week before and four weeks after initiation of PegIFN α-2b/RBV. A solid nutrient meal, consisting of a 200-g pancake (51.6 g carbohydrate, 8.1 g protein, 5.7 g fat; 291 kcal) containing 37 MBq Tc-99m diethylenetriamine pentaacetic acid (DTPA; Fujifilm RI Pharma, Tokyo, Japan) was consumed with 100 mL water. All subjects ingested the pancake within 2 min. Immediately thereafter, the subjects were placed in a standing position and the radioactivity was recorded over the upper abdomen at 0, 5, 10, 20, 30, 60, 90, and 120 min by use of a gamma camera (Vertex Plus; Adac Laboratories, CA, USA). Data were corrected for radionuclide decay. The relevant anterior region containing the total stomach was

divided into two regions corresponding to the proximal and distal gastric areas. The line separating these two regions was positioned such that a right angle was formed with the longitudinal axis of the stomach (Fig. 1), and the radioactivity of this region was plotted. The T1/2, the time at which 50% of the peak radioactivity had exited the stomach, was calculated by computer analysis (normal range at our institute: total T1/2, 73 ± 15 min; proximal T1/2, 42 ± 15 min; distal T1/2, 84 ± 21 min).

Digestive Symptom Score

Digestive symptoms were assessed at the time of gastric emptying, as measured by scintigraphy, and were scored using a modification of the method described by Gatto et al. [13]. Anorexia, nausea, vomiting, abdominal distension, early satiety, heartburn, belching, and epigastric pain were scored as 0, not present; 1, mild (symptom could be ignored if the patient did not think about it); 2, moderate (symptom could not be ignored, but did not affect daily activities); and 3, severe (symptom affected daily activities). When the sum of scores in each category is calculated, the maximum possible total score is 24.

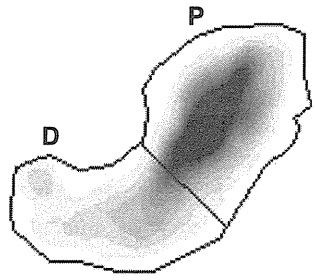

Fig. 1 Anterior image of the stomach obtained immediately after meal ingestion by a healthy subject. The image was outlined to define the total stomach region of interest, which was divided into halves corresponding to the proximal (*P*) and distal (*D*) gastric regions

Statistical Analysis

Data are expressed as mean ± SD. The significance of differences in the mean T1/2 values and symptom scores between one week before and four weeks after PegIFN α-2b/RBV initiation was assessed by use of the Wilcoxon signed rank-sum test. The significance of differences between mean patient characteristics at entry in the control and mosapride groups was assessed by use of the Mann–Whitney *U* test for continuous variables, and the χ^2 test for categorical variables. The correlation between total T1/2 and symptom score was tested by use of Spearman's rank correlation test. Study outcomes were analyzed on the basis of the intention-to-treat population. A *P* value of <0.05 was considered significant. Statistical analysis was performed using JMP 8.0.2 software (SAS Institute, NC, USA).

Results

Trial Enrolment

During the study period, 30 CHC patients were screened and 24 patients (80%; 11 men and 13 women; mean age 59.3 years) who met the eligibility criteria were enrolled (Fig. 2). Although six out of 24 patients enrolled in the

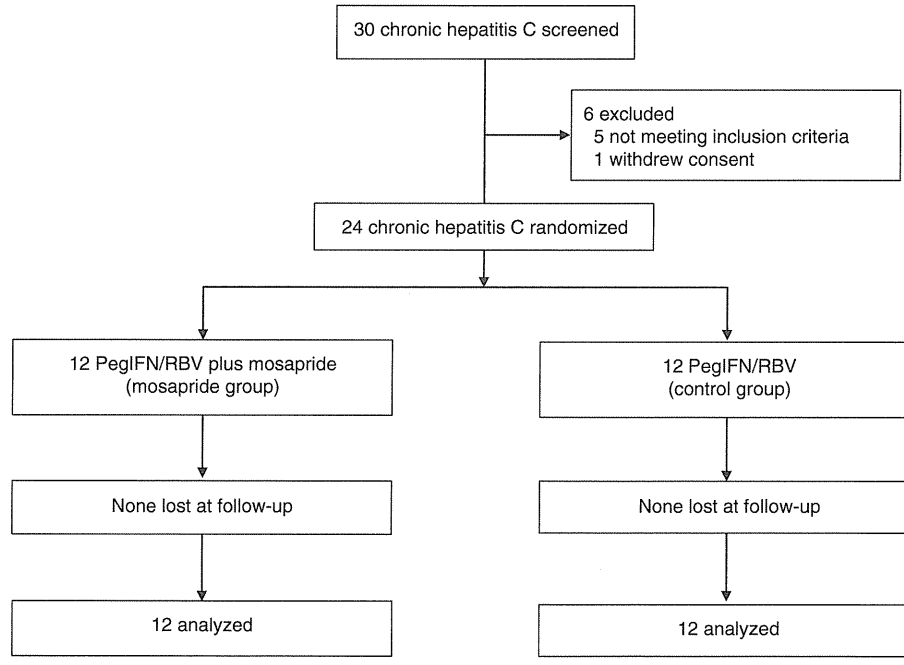

Fig. 2 Trial selection flow (mosapride group, pegylated interferon α-2b at 1.5 μg/kg/week and ribavirin at 600–1,000 mg/day, depending on body weight (PegIFN/RBV), plus mosapride citrate at 15 mg/person/day ($n = 12$); control group, PegIFN/RBV ($n = 12$, no mosapride)]

study underwent upper gastrointestinal endoscopy, these patients did not have gastrointestinal mucosal lesions such as peptic ulcers. Some other patients with digestive symptoms did not consent to an endoscopy. Twelve patients were assigned to the control group and received only the baseline therapy (PegIFN α-2b/RBV), and the other 12 patients were assigned to the mosapride group and received mosapride in addition to the baseline therapy. Six patients (20%) were excluded; reasons for exclusion included consent withdrawal (one patient), recent history of alcohol intake (two patients), recent interferon use (one patient), complicated evacuation disorder (one patient), and recent use of drugs affecting gastric motor performance (one patient). There was no difference in baseline characteristics between the two groups (Table 1). All of the 12 patients in the mosapride group continued to take mosapride for a period of four weeks. No adverse reactions to mosapride administration were observed in any patient.

Changes in T1/2 and Digestive Symptom Score After PegIFN α-2b/RBV Initiation in the Control Group

In comparison with before treatment, after PegIFN α-2b/RBV initiation, a significant increase was observed in the mean total T1/2 (before: 84.0 ± 22.1 min versus after: 100.8 ± 28.9 min, $P = 0.03$; Fig. 3a), mean distal T1/2 (before: 95.3 ± 32.2 min versus after: 115.3 ± 41.4 min, $P = 0.03$; Fig. 3c), and digestive symptom score (before: 3.2 ± 1.4 versus after: 8.1 ± 4.8, $P = 0.02$; Fig. 3d). The change in mean proximal T1/2 was not significant (before: 44.8 ± 14.7 min versus after: 59.3 ± 25.3 min, $P = 0.11$; Fig. 3b).

Changes in T1/2 and Digestive Symptom Score After PegIFN α-2b/RBV Initiation in the Mosapride Group

In comparison with before treatment, after initiation of treatment with PegIFN α-2b/RBV plus mosapride, non-significant increases were observed in the mean total T1/2 (before: 98.6 ± 23.7 min versus after: 106.3 ± 27.1 min, $P = 0.27$; Fig. 4a), mean proximal T1/2 (before: 47.3 ± 26.6 min versus after: 56.0 ± 21.3 min, $P = 0.64$, Fig. 4b), mean distal T1/2 (before: 103.1 ± 38.0 min versus after: 113.9 ± 35.5 min, $P = 0.06$; Fig. 4c), and digestive symptom score (before: 3.8 ± 2.4 versus after: 4.0 ± 1.9, $P = 0.77$; Fig. 4d).

Relationship Between T1/2 and Digestive Symptom Score Before PegIFN α-2b/RBV Initiation

The correlations between total, proximal, and distal gastric T1/2 and the sum of the digestive symptom scores were non-significant ($r = 0.04$, $P = 0.85$; $r = 0.18$, $P = 0.16$; and $r = 0.10$, $P = 0.45$, respectively). Among each gastric region and the eight specific symptoms, the correlation between the distal T1/2 and early satiety symptom score was significant ($r = 0.48$, $P = 0.02$; Fig. 5c). However,

Table 1 *Clinical characteristics of enrolled patients*

	Mosapride ($n = 12$)	Control ($n = 12$)
Male/female, n[a]	5/7	6/6
Age (years)[b]	57.3 ± 12.2	61.3 ± 4.2
HCV genotype (1/2), n[a]	8/4	7/5
AST (U/L)[b]	65.9 ± 43.9	52.0 ± 32.2
ALT (U/L)[b]	74.2 ± 47.5	69.4 ± 56.8
Total bilirubin (mg/dL)[b]	0.8 ± 0.4	0.9 ± 0.5
Serum albumin (g/dL)[b]	3.8 ± 0.3	4.0 ± 0.4
Platelet count ($\times 10^3/\mu L$)[b]	153 ± 43	155 ± 44
Fibrosis staging of the liver (F1/F2–3), n[a]	5/7	5/7
Total T1/2 (min)[b]	98.6 ± 23.7	84.0 ± 22.1
Proximal T1/2 (min)[b]	47.3 ± 26.6	44.8 ± 14.7
Distal T1/2 (min)[b]	103.1 ± 38.0	95.3 ± 32.2
Digestive symptom score[b]	3.8 ± 2.4	3.2 ± 1.4

All non-significant ($P \geq 0.05$) between groups mosapride and control via [a]χ^2 test and [b]Mann–Whitney U test. Data are mean ± SD values except where indicated

HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T1/2, half-time of gastric emptying

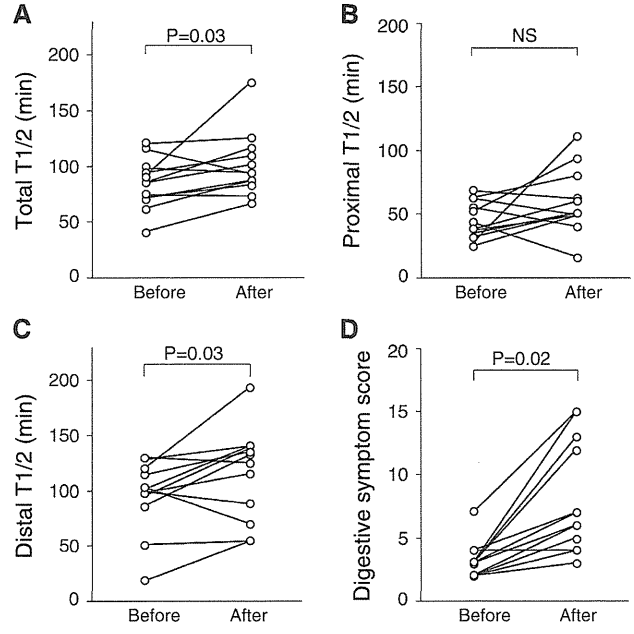

Fig. 3 Gastric emptying half-time (T1/2) and digestive symptom score in the control group: within one week before versus four weeks after pegylated interferon α-2b/ribavirin initiation ($n = 12$, no mosapride). *NS* non-significant (Wilcoxon signed rank-sum test)

the correlation between the total and proximal T1/2 and early satiety symptom score was non-significant ($r = 0.40$, $P = 0.05$; Fig. 5a; $r = -0.02$, $P = 0.92$; Fig. 5b).

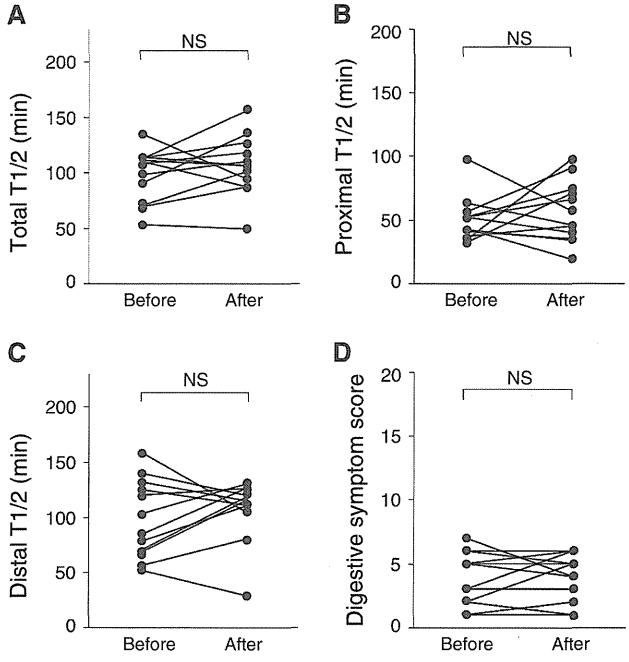

Fig. 4 Gastric emptying half-time (T1/2) and digestive symptom score in the mosapride group: within one week before versus four weeks after pegylated interferon α-2b/ribavirin plus mosapride initiation ($n = 12$, mosapride citrate at 15 mg/person/day). *NS* non-significant (Wilcoxon signed rank-sum test)

No early satiety symptom scores of 3 points were measured in either group.

Relationship Between T1/2 and Digestive Symptom Score After PegIFN α-2b/RBV Initiation

The correlations between total, proximal, and distal gastric T1/2 and the sum of the digestive symptom scores were not significant ($r = 0.23$, $P = 0.28$; $r = 0.30$, $P = 0.16$; and $r = 0.18$, $P = 0.41$, respectively). Among each gastric region and the eight specific symptoms, any correlations between T1/2 and symptom scores were not significant.

Discussion

PegIFN has been developed to prolong retention time in the blood, and thus enhance the antiviral effect. The occurrence of IFN-induced adverse reactions differs depending on whether or not IFN is pegylated; skin rash and cytopenia are more frequent with PegIFN treatment whereas mental and neurological symptoms are more frequent with non-PegIFN [14]. Comparison of subjects in this study with those in our previous study [10], as an ancillary analysis, reveals that subjective and objective gastroparetic adverse reactions were less pronounced in the PegIFN α-2b/RBV group ($n = 12$) than in the IFN α-2b group ($n = 12$) (data not shown). Therefore, gastrointestinal autonomic neuropathies, for example IFN-induced gastroparesis, might also

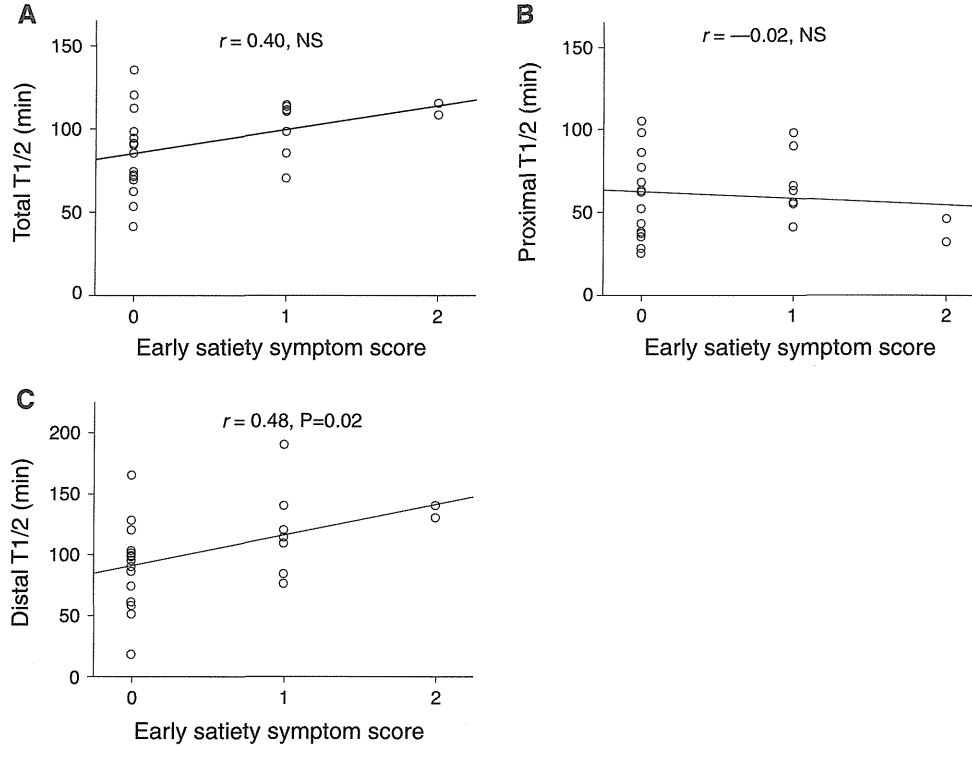

Fig. 5 Correlation between the gastric emptying half-time (T1/2) measurements and early satiety symptom score before pegylated interferon α-2b/ribavirin initiation ($n = 24$: groups control ($n = 12$) and mosapride ($n = 12$)) *NS* non-significant (Spearman's rank correlation test)

be an adverse reaction that is reduced by pegylation. Although adverse gastrointestinal reactions to RBV alone have not been thoroughly investigated [15], our results also suggest that RBV has little effect on gastroparesis.

The rate of gastric emptying is affected by factors such as regulatory peptides, neurotransmitters, and cytokines. Previously, non-pegylated IFN α-2b has been reported to stimulate corticotropin-releasing factor (CRF) release in the hypothalamus [16]. A new regulatory nociceptin/orphanin FQ (N/OFQ) and its receptor, which are expressed in the mammalian spinal cord and brain, constitute a neurotransmitter system that has been implicated in several types of non-opioid-related behavior, for example feeding [17, 18]. The N/OFQ peptide-receptor system activates the central inhibitory pathways that modulate gastrointestinal motility [19, 20]. This system is believed to inhibit gastric emptying through an integrated interaction between the orphaninergic system and endogenous CRF in which corticosterone plays a permissive role [21]. Although CRF may also function in the gastric emptying delay during PegIFN α-2b therapy, there is a difference in the pharmacokinetics between PegIFN and non-PegIFN. Because the blood concentration of PegIFN α-2b increased more slowly, delayed gastric motility was considered milder during PegIFN α-2b therapy than during non-PegIFN α-2b therapy. Gastric motility-related factors other than interferon, diabetes mellitus and hypothyroidism are known to cause delayed gastric emptying [22, 23]. However, our patients had no change in blood glucose levels or thyroid hormonal levels after PegIFN α-2b initiation (data not shown).

In this study, the mean total, proximal, and distal gastric T1/2 (min) after interferon therapy were 100.8 ± 28.9, 59.3 ± 25.3, and 115.3 ± 41.4, respectively, in the control group and 106.3 ± 27.1, 56.0 ± 21.3, and 113.9 ± 35.5, respectively, in the mosapride group. No significant differences were observed in the half-times between the two groups (data not shown). However, mosapride reduced the total and distal T1/2 in the mosapride group after PegIFN α-2b/RBV initiation (Fig. 4). 5-HT_4 receptors are found in structures located in the more distal regions of the stomach, for example the gastric antrum and corpus [24]. Thus, mosapride is not likely to affect gastric motion in the proximal region and seemed to accelerate total gastric motility primarily by promoting motility in the distal stomach. These data show that mean total, proximal, and distal T1/2 s before PegIFN α-2b/RBV were already longer than the normal range (84–99 vs. 73, 45–47 vs. 42, and 95–103 vs. 84 min, respectively; Figs. 3, 4). These findings are consistent with those in our previous study, which indicated that gastroparesis can occur in chronic hepatitis [25]. The mean total and distal T1/2 s after initiation of PegIFN α-2b/RBV were further prolonged. The delay in T1/2 s in these two regions after PegIFN α-2b/RBV initiation was improved in the mosapride group, although these T1/2 s were still longer than the normal range.

Evaluation of gastroparetic symptom intensity is mandatory when analyzing the relationship between visceral perception and potential pathophysiological factors. It has been reported that delayed gastric emptying of solids is associated with vomiting and postprandial fullness [26]. For CHC patients, this study indicated that total, proximal, and distal gastric motilities were not correlated with the sum of symptom scores. These results might be attributed to the fact that the sum of scores contains symptoms such as heartburn and epigastric pain that are not always indicative of gastric motility [13]. Our results indicate that gastric motility of the distal region was strongly associated with early satiety digestive symptoms in CHC patients not being treated with IFN (Fig. 5). However, IFN therapy caused no specific digestive symptoms (data not shown).

The acetaminophen method, breath test, ultrasonography, and radioisotope examination (gastric emptying scintigraphy) are known methods for determination of the gastric emptying time [27–30]. The acetaminophen method and breath test are indirect assessment methods, whereas ultrasonography is a direct method that lacks objectivity [31]. Therefore, radioisotope examination has now become the standard method worldwide. Three types of test meal are used for gastric emptying scintigraphy: solid, semisolid, and liquid. We have adopted solid meals that are as equivalent as possible to general meals, thereby ensuring high test reproducibility [32]. However, a weakness of this method is the difficulty of comparing results from independent centers because of methodological differences such as choice of test meal, radiolabel, data acquisition, and analysis.

In conclusion, our results show that PegIFN α-2b/RBV treatment in CHC patients causes adverse reactions, including a greater delay in gastric emptying, particularly of the distal stomach, and related symptoms. Mosapride promotes gastric motility of both the total and distal stomach, and relief of related symptoms. Mosapride administration could be used as supportive therapy for gastrointestinal adverse effects during PegIFN α-2b/RBV therapy.

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Conflict of interest The authors disclose no conflicts.

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Original Article

Changes in sequences of core region, interferon sensitivity-determining region and interferon and ribavirin resistance-determining region of hepatitis C virus genotype 1 during interferon-alpha and ribavirin therapy, and efficacy of retreatment

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Aim: Some regions associated with sensitivity to interferon-α and ribavirin have been identified in the hepatitis C virus (HCV) genome, including amino acid 70 in the core region (core a.a. 70), a.a. 2209–2248 (interferon sensitivity-determining region, ISDR) and a.a. 2334–2379 (interferon and ribavirin resistance-determining region, IRRDR).

Methods: We examined changes in the sequences of these regions in 25 patients with chronic HCV genotype 1 infection who had not had sustained virological response (SVR) to interferon-α and ribavirin for 24–48 weeks and subsequently received retreatment for 48–72 weeks.

Results: At baseline, the core a.a. 70 was mutant (resistant) type in seven patients. At the start of retreatment, the core a.a. 70 had changed from sensitive to resistant type in 2 patients, and SVR was not achieved by retreatment. The ISDR variations were resistant type (0–1 mutations) in 17 patients at baseline. After 2 weeks of treatment, amino acid change was found in two patients; in one, the substitutions returned to baseline status after treatment, and in the other, the substitution persisted. At the start of retreatment, ISDR sequences had changed from resistant to sensitive type in two patients and SVR was achieved and from sensitive to resistant type in three patients and SVR was not achieved. The IRRDR variations were resistant type (<6 mutations) in 19 patients at baseline and at the start of retreatment.

Conclusion: Sequences of the core region and ISDR sometimes change during anti-HCV therapy, potentially affecting the outcomes of retreatment.

Key words: core, hepatitis C virus, interferon, interferon and ribavirin resistance-determining region, interferon sensitivity-determining region

INTRODUCTION

HEPATITIS C VIRUS (HCV) affects approximately 170 million people worldwide and is a leading cause of liver cirrhosis and hepatocellular carcinoma.[1] Pegylated interferon-α (PEG IFN-α) and ribavirin have been the standard of care for chronic HCV infection in recent years, but the rate of sustained virological response (SVR) to combination therapy remains below 50% in patients with chronic hepatitis C genotype 1, the most prevalent type of HCV in North America and Europe.[2,3] Some inhibitors of HCV non-structural protein (NS)3/4A serine protease, such as telaprevir and boceprevir, have become available very recently.[4–6] However, any novel direct-acting antiviral agent should be given in combination with PEG IFN-α and ribavirin, because of the extremely high incidence of viral breakthrough when used alone.[7,8] Therefore, IFN-α remains a key drug for the treatment of chronic HCV infection.

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