Sildenafil is metabolized predominantly by the cytochrome P-450 3A4 hepatic microsomal isoenzyme. The mechanism of sildenafil-induced hepatotoxicity is unclear. In general, cholestasis can result from several mechanisms, including decreased fluidity of the sinusoidal plasma membrane, inhibition of ATP-dependent bile acid transporters, disruption of the cytoskeleton, and loss of canalicular integrity (4). It is often difficult to determine the main cause in a given case.

To our knowledge, we have documented the first reported case of severe liver toxicity ascribed to sildenafil. In our patient, sildenafil was not prescribed for a diagnosis of erectile dysfunction, but was obtained for 'recreational' use from a friend. Recently, this drug can be easily purchased from dealers or through the Internet. Patients who illicitly take sildenafil may not report a drug history, causing difficulty in the diagnosis of adverse events. Thus, the incidence of sildenafil-induced liver injury may have been underestimated; clinicians should be aware that sildenafil can cause severe liver damage.

In summary, we have described a case of sildenafil-induced severe cholestatic hepatotoxicity. Albeit rare, sildenafil is a possible cause of severe drug-induced liver toxicity.

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A Case of Rectal MALT Lymphoma Treated by Endoscopic Resection

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To the Editor: We report a case of rectal mucosa-associated lymphoid tissue (MALT) lymphoma that was treated successfully with endoscopic resection and empiric antibiotics. Extra gastric locations of MALT lymphomas include the skin, thyroid, lungs, urinary bladder, and salivary glands and rarely at other locations in the gastrointestinal tract including the rectum. The association of these with *Helicobacter pylori* is less well established.

A 65-year-old woman, in her usual state of health, had a screening colonoscopy, during which a 1.2-cm sessile rectal polyp—described as fleshy and different from a usual polyp—was resected at endoscopy with clear margins using a hot snare guillotine technique. On pathologic examination, the polyp was found to be a MALT lymphoma (**Table 1**, **Figure 1**). Upper endoscopy with biopsies and camplyobacter-like organism test showed chronic duodenitis and was negative for *H. pylori*. A liver function profile and a triphasic abdominal computerized tomography scan were unremarkable. She was started on empiric anti-*H. pylori* treatment (amoxicillin, lansoprazole, and clarithromycin). She remains asymptomatic and has returned for surveillance exams, four times over 4 years and has had no evidence of recurrence. The site of resection showed a clear scar on all occasions (**Figure 1**).

Mucosa-associated lymphoid tissue lymphoma, entered the literature in 1983, when Isaacson and Wright (1) described the first two cases. Most MALT lymphomas emerge from gastric lymphoid tissue looking remarkably like Peyer's patches—a paradoxical finding, as the stomach is generally devoid of lymphoid tissue (2). MALT lymphoma cells appeared to participate in immune responses and the stimulus eliciting this response remained unclear until Isaacson and co-workers described the association with *H. pylori* and later, its treatment (1–3). Today, 75% of gastric MALT lymphomas can be treated successfully by eradicating the *H. pylori*, relegating therapies such as chemo- or immunotherapy, radiation, or surgical resection to second- or third-line treatment options (4).

Gastric MALT lymphomas remain localized to the stomach for long periods. The *H. pylori* infection gives rise to a chronic inflammatory state in which a clone of neoplastic B cells evolves localized to the involved mucosa because of antigen-dependent growth. Subsequent mutations upregulate the production of NF-uB (1,5) and the B-cell line reproduces independently of *H. pylori* stimulation, rendering the disease no longer amenable to treatment with antibiotics

Table 1. Immunohistochemical characteristics of resected tumor

CD 3	Negative
CD 5	Negative
CD 20	Positive
CD23	Negative
CD43	Negative
BCL-1	Negative
BCL-2	Negative
Cytoplasmic Ig light chain	Suggestive, not diagnostic of i-excess

Ig, immunoglobulin.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Noninvasive laboratory tests proposed for predicting cirrhosis in patients with chronic hepatitis C are also useful in patients with non-alcoholic steatohepatitis

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Abstract

Background Several noninvasive tests have been proposed to predict cirrhosis in patients with chronic hepatitis C, but not in patients with non-alcoholic steatohepatitis (NASH). We assessed whether noninvasive laboratory tests designed to predict the risk of cirrhosis in patients with chronic hepatitis C virus (HCV) infection could be used in patients with NASH.

Methods The subjects were 50 patients with biopsy-proved NASH and 100 age- and sex-matched patients with HCV. Aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR), age-platelet (AP) index, AST-to-platelet ratio index (APRI), cirrhosis discriminant score (CDS), and the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) model were calculated.

Results The areas under the receiver-operating characteristic curves of the AAR, AP index, APRI, CDS, and HALT-C model for predicting cirrhosis were respectively 0.813, 0.877, 0.786, 0.949, and 0.908 in patients with NASH and 0.555, 0.652, 0.761, 0.782, and 0.782 in patients with HCV. A CDS cutoff value of less than 5 misclassified none of the 9 patients with NASH who had cirrhosis, while a value of more than 8 misclassified none of the 41 patients with NASH without cirrhosis. With the HALT-C model, a cutoff value of less than 0.6 classified non-cirrhotic NASH, while a cutoff value of 0.97 or higher classified cirrhotic NASH. The use of CDS and HALT-C model could avoid liver biopsy for predicting cirrhosis in 60 and 48% of the patients with NASH, respectively.

Conclusions Noninvasive laboratory tests designed to predict cirrhosis in patients with HCV are also useful in patients with NASH.

Keywords NASH · Noninvasive test · Cirrhosis discriminant score · HALT-C · Fibrosis

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become a common diagnosis in clinical practice owing to the increasing prevalence of obesity and type 2 diabetes mellitus in the general population worldwide [1]. The spectrum of NAFLD ranges widely from simple steatosis to non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis and liver failure [2, 3]. NASH is defined histologically as steatohepatitis similar to alcoholic steatohepatitis and is characterized by the presence of macrovesicular steatosis, mixed inflammatory cell infiltration, hepatocyte ballooning and necrosis, Mallory body formation, and perisinusoidal fibrosis [4, 5]. Adams et al. [6] followed 21 patients with cirrhotic-stage of NASH for a median duration of 6.8 years and reported that 62% had complications and 33% died of liver-related causes.

Liver biopsy is considered the gold standard for diagnosing chronic liver disease, grading inflammatory activity, and staging fibrosis. However, biopsy is not suitable for

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repeated evaluations because it is costly, invasive, and associated with a risk of major complications (0.3–0.5%), including death (0.03–0.1%) [7, 8]. Sampling error may lead to underestimation of underlying cirrhosis, especially when biopsy specimens are small or fragmented. Thus, an inexpensive, noninvasive, and accurate method for diagnosing cirrhosis is required [9–11].

Because of the risks and limitations of liver biopsy, cirrhosis is sometimes diagnosed on the basis of imaging studies, such as ultrasonography and computed tomography, in clinical practice. In addition, several laboratory tests, indices, and scores have been proposed for the noninvasive prediction of hepatic fibrosis in patients with chronic hepatitis C. Among these, the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR) [12, 13], age-platelet index (AP index) [14], AST-to platelet ratio index (APRI) [15], and cirrhosis discriminant score (CDS) [16] are based on routine laboratory variables and thus can be readily determined. A new model for predicting cirrhosis has recently been developed on the basis of data derived from the large Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) cohorts (HALT-C model) [17]. The HALT-C Trial was a randomized, controlled study designed to determine whether long-term therapy with pegylated interferon can reduce the risk of progression to end-stage liver diseases in patients with chronic hepatitis C.

The aim of this study was to determine whether the noninvasive tests designed to predict the risk of cirrhosis in patients with chronic hepatitis C virus (HCV) infection (such as AAR, AP index, APRI, CDS, and HALT-C model) could also be used in patients with NASH. First, the receiver-operating characteristic curve (ROC) of each test for predicting cirrhosis was constructed in patients with NASH and in age- and sex-matched patients with HCV. Second, we estimated the percentage of patients with NASH in whom liver biopsy for the diagnosis of cirrhosis could be avoided by using noninvasive predictive tests.

Materials and methods

Patients

We studied 50 patients with NASH who underwent liver biopsy at Osaka City University Hospital between 1998 and 2007. Data from an age- and sex-matched group of 100 patients with HCV were used as a control. The diagnosis of NASH was based on the following: (1) histological features of steatohepatitis, (2) an absence of clinically significant alcohol consumption (20 g/day), and (3) no other identifiable causes of liver diseases, including drug-induced hepatotoxicity, infection with hepatitis B and C viruses, autoimmune liver diseases, Wilson's disease, hemochromatosis, and α_1-antitrypsin deficiency. Informed written consent was obtained from each patient. The study was approved by the local ethics committee and was carried out according to the provisions of the Helsinki Declaration of 1975 (2000 revision).

Laboratory assessment

AST, ALT, alkaline phosphatase, total bilirubin, total cholesterol, triglycerides, plasma glucose, prothrombin time (international normalized ratio, INR), and platelet count were routinely determined by standard procedures within 4 weeks of the liver biopsy. Patients with a fasting plasma glucose level above 125 mg/dl were given a diagnosis of diabetes mellitus. Patients with a triglyceride level above 150 mg/dl and/or a total cholesterol level above 220 mg/dl were given a diagnosis of hyperlipidemia. Patients with a blood pressure above 140/90 mmHg were regarded to have hypertension. These routine laboratory values were used to calculate AAR, AP index, APRI, CDS, and HALT-C model as follows:

1. AAR = AST/ALT
2. AP index = the sum of the age score (age expressed in years: <30 = 0; 30–39 = 1; 40–49 = 2; 50–59 = 3; 60–69 = 4; ≥70 = 5) plus the platelet count score [platelet count ($\times 10^9$/l): ≥225 = 0; 200–224 = 1; 175–199 = 2; 150–174 = 3; 125–149 = 4; <125 = 5) (possible value 0–10)].
3. APRI = [AST/upper limit of normal)/platelet count ($\times 10^9$/l)] × 100.
4. CDS = the sum of the platelet count score [platelet count ($\times 10^9$/l): ≥340 = 0; 280–339 = 1; 220–279 = 2; 160–219 = 3; 100–159 = 4; 40–99 = 5; <40 = 6] plus the AST/ALT ratio score (>1.7 = 0; 1.2–1.7 = 1; 0.6–1.19 = 2; <0.6 = 3) plus the INR score (<1.1 = 0; 1.1–1.4 = 1; >1.4 = 2) (possible value 0–11).
5. HALT-C model = formula used to predict the probability of cirrhosis: exp (log odds)/(1+exp (log odds), where the log odds (predicting cirrhosis) = –5.56–0.0089 × platelet count ($\times 10^9$/l) + 1.26 × AST/ALT + 5.27 × INR.

Liver biopsy and histology

The liver tissues were obtained by ultrasound-guided biopsy using a 15-gauge Tru-cut needle (Hakko, Nagano, Japan). All specimens fulfilled the requirements for size as suggested by Janiec et al. [18]. Liver tissues were fixed in formalin immediately after biopsy and embedded in paraffin. Five-micrometer-thick sections were cut with a

microtome. One section of each biopsy specimen was stained with hematoxylin–eosin to assess hepatic steatosis and inflammatory activity; another section was stained with Azan–Mallory to evaluate hepatic fibrosis. Histological diagnosis was performed. Fibrosis was staged and inflammatory activity was graded according to the classification of Brunt et al. [19] in patients with NASH, and according to the classification of Desmet et al. [20] in patients with HCV.

Statistical analysis

Statistical analysis was performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC). The chi-square test or Wilcoxon rank sum test was used to compare the clinical characteristics of the patients between two groups. ROC curves were constructed for the AAR, AP index, APRI, CDS, and HALT-C model. To evaluate the diagnostic accuracies of the noninvasive predictive tests, the area under the ROC curve (AUROC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Individual AUROC values of noninvasive tests were compared as described by DeLong et al. [21]. Values of $P < 0.05$ were considered to indicate statistical significance.

Results

Patient characteristics

The clinical characteristics of the patients are summarized in Table 1. In the NASH group, body mass index, total cholesterol, triglycerides, fasting plasma glucose, and platelet count were significantly higher than those in the HCV group. The proportion of patients with a clinical diagnosis of diabetes or hyperlipidemia was also significantly higher in the NASH group.

Table 1 Patient characteristics

	NASH ($n = 50$)	HCV ($n = 100$)	
Age (years)[a]	55.8 ± 15.2	56.7 ± 13.6	Matched
Female[b]	37 (74%)	74 (74%)	Matched
Body mass index (kg/m^2)[a]	27.1 ± 3.8	22.9 ± 3.7	<0.0001
Diabetes[b]	22 (44%)	8 (8%)	<0.0001
Hypertension[b]	23 (46%)	31 (31%)	0.0721
Hyperlipidemia[b]	31 (62%)	3 (3%)	<0.0001
AST (IU/l)[c]	72 (33–332)	64 (26–340)	0.5946
ALT (IU/l)[c]	106 (24–368)	79 (20–314)	0.0858
Alkaline phosphatase (IU/l)[a]	213.6 ± 64.8	210.3 ± 85.7	0.8044
Total bililubin (mg/dl)[a]	0.94 ± 0.35	0.87 ± 0.33	0.2325
Total cholesterol (mg/dl)[a]	209 ± 42.2	172 ± 28.3	<0.0001
Triglyceride (mg/dl)[c]	123 (49–536)	92 (34–436)	0.0005
Fasting plasma glucose (mg/dl)[a]	124.2 ± 45.7	102.3 ± 25.5	0.0002
INR[a]	1.06 ± 0.17	1.07 ± 0.13	0.4301
Platelet count ($\times 10^9$/l)[a]	192 ± 85	151 ± 59	0.0008

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *INR* international normalized ratio

[a] Mean ± SD

[b] Number (%)

[c] Median (range)

Stages of fibrosis

Among the 50 patients with NASH, the stage of fibrosis according to the classification of Brunt et al. [19] was stage 1, 14 (28%); stage 2, 14 (28%); stage 3, 13 (26%); and stage 4, 9 (18%). Of the 100 patients with HCV, fibrosis was stage 1, 45 (45%); stage 2, 20 (20%); stage 3, 18 (18%); and stage 4, 17 (17%) according to the classification of Desmet et al. [20].

ROC curves

The ROC curves of the AAR, AP index, APRI, CDS, and HALT-C model for the prediction of cirrhosis in NASH and HCV are shown in Fig. 1, and the AUROC values of these noninvasive predictive tests are shown in Table 2. In both NASH and HCV, 4-variable analyses, such as the CDS and HALT-C model, had higher AUROC values for the prediction of cirrhosis than did 2-variable analyses, such as the AAR, AP index, and APRI. For all tests, the AUROC value was higher in patients with NASH than in age- and sex-matched patients with HCV.

Cutoffs

The sensitivity, specificity, PPV, and NPV of CDS and HALT-C model for predicting cirrhosis in NASH with use of various cutoff values are shown in Table 3. For CDS, a cutoff predicted value of less than 5 misclassified none of the 9 patients with cirrhosis, while a cutoff predicted value of more than 8 misclassified none of the 41 patients without cirrhosis. When these cutoff values were used, cirrhosis could be excluded in 27 patients without cirrhosis and diagnosed in 3 patients with cirrhosis, meaning that liver biopsy for predicting the risk of cirrhosis could be avoided in 30 of the 50 (60%) patients with NASH. With the HALT-C model, when a cutoff value of less than 0.6 was used to exclude cirrhosis, none of the nine patients with

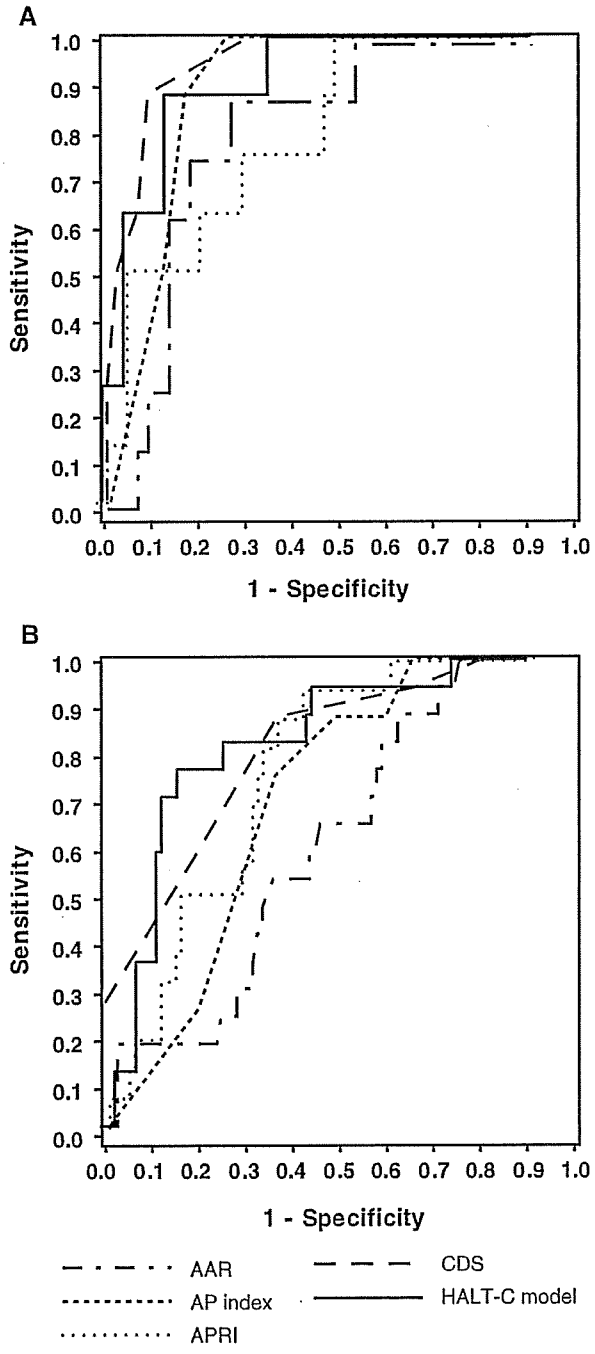

Fig. 1 ROC curves of five simple noninvasive tests for the prediction of cirrhosis constructed **a** for 50 patients with NASH and **b** for 100 age- and sex-matched patients with HCV. *AAR* AST/ALT ratio, *AP-index* age-platelet index, *APRI* AST-to-platelet ratio index, *CDS* cirrhosis discriminant score, *HALT-C model* the Hepatitis C Antiviral Long-term Treatment against Cirrhosis model

cirrhosis would have been misclassified. When a cutoff value of 0.97 or higher was used to confirm cirrhosis, none of the 41 patients without cirrhosis were mistakenly predicted to have cirrhosis. Our results indicate that use of this model might have obviated the need for a liver biopsy aimed to predict the risk of cirrhosis in 24 of the 50 (48%) patients with NASH (22 without cirrhosis and 2 with cirrhosis) (Table 4).

Discussion

Several noninvasive indices, scores, and models have been proposed for the prediction of hepatic fibrosis in patients with chronic hepatitis C [12–17]. However, most tests were based on data from white or African-American patients with HCV and remain to be validated in other ethnic groups. In our cohort of Japanese patients with HCV, the AUROC value of CDS or HALT-C model for predicting cirrhosis was 0.782, suggesting that these indices are also useful for predicting the risk of cirrhosis in Japanese patients with HCV. More importantly, the AUROC value was higher in patients with NASH than in age- and sex-matched patients with HCV. This finding suggests that the diagnostic accuracies of these noninvasive tests, originally designed for patients with HCV, were at least comparable when used in patients with NASH.

The AUROC values of the AAR, AP index, APRI, CDS, and HALT-C model for predicting advanced fibrosis (stage 3–4) were also high (0.787, 0.795, 0.838, 0.811, and 0.808; data not shown) in patients with NASH. However, we cannot directly compare the accuracies for predicting advanced fibrosis between patients with NASH and those with HCV, because the histological definition of stage 3 differs between the classification of Brunt for NASH and that of Desmet for HCV.

Negative correlations between platelet count and the degree of hepatic fibrosis have been noted previously in patients with chronic hepatitis C [22, 23]. The decreased platelet count is associated with portal hypertension, resulting in increased pooling of platelets in the spleen [24] and, to a lesser extent, with reduced production of thrombopoietin in hepatocytes [25]. Shimada et al. [26] reported that a low platelet count was a significant predictor of severe hepatic fibrosis in patients with NASH. In our study, the AUROC value of the platelet count was 0.893 in patients with NASH (data not shown). In contrast, the AUROC value of 2-variable analyses that included platelet count (such as the AP index and APRI) was smaller than that of the platelet count per se, and the addition of age or AST did not increase the predictive value.

In 1988, Williams et al. [12] reported that an AAR of 1 or more strongly suggests the presence of cirrhosis in patients with various forms of chronic hepatitis. Progression of hepatic fibrosis may reduce AST clearance, leading to increased AST levels in serum [27]. In addition,

Table 2 AUROC of noninvasive tests for the prediction of cirrhosis in patients with NASH and in patients with HCV

	NASH (n = 50)		HCV (n = 100)	
	AUROC	Confidence intervals	AUROC	Confidence intervals
AAR	0.813	(0.674–0.952)	0.555	(0.416–0.694)
AP index	0.877	(0.785–0.968)	0.652	(0.538–0.767)
APRI	0.786	(0.625–0.947)	0.761*	(0.654–0.868)
CDS	0.949*†	(0.889–1.008)	0.782**‡	(0.665–0.898)
HALT-C score	0.908	(0.811–1.004)	0.782**‡	(0.667–0.898)

AUROC the area under the receiver-operating characteristic curve

* <0.05, **<0.01 versus AAR

†<0.05 versus APRI

‡<0.05 versus AP index

Table 3 Sensitivity, specificity, PPV, and NPV of CDS for predicting cirrhosis in patients with NASH

Predicted values	No. of patients	No. (%) with cirrhosis	No. (%) without cirrhosis	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
0	2	0 (0%)	2 (100%)	100	5	19	100
1	1	0 (0%)	1 (100%)	100	7	19	100
2	7	0 (0%)	7 (100%)	100	24	23	100
3	7	0 (0%)	7 (100%)	100	41	27	100
4	10	0 (0%)	10 (100%)	100	66	39	100
5	11	1 (9%)	10 (81%)	89	90	67	97
6	3	2 (67%)	1 (33%)	67	93	67	93
7	3	1 (33%)	2 (67%)	56	98	83	91
8	3	2 (67%)	1 (33%)	33	100	100	84
9	2	2 (100%)	0 (0%)	11	100	100	84
10	1	1 (100%)	0 (0%)				
Total	50	9 (18%)	41 (82%)				

PPV positive predictive value, *NPV* negative predictive value

Table 4 Sensitivity, specificity, PPV, and NPV of HALT-C model for predicting cirrhosis in patients with NASH

Predicted values	No. of patients	No. (%) with cirrhosis	No. (%) without cirrhosis	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<0.3	1	0 (0%)	1 (100%)	100	2	18	100
0.3–0.4	3	0 (0%)	3 (100%)	100	10	20	100
0.4–0.5	8	0 (0%)	8 (100%)	100	29	24	100
0.5–0.6	10	0 (0%)	10 (100%)	100	54	32	100
0.6–0.7	7	1 (14%)	6 (86%)	89	68	38	97
0.7–0.8	6	0 (0%)	6 (100%)	89	83	53	97
0.8–0.9	10	5 (50%)	5 (50%)	33	95	60	87
0.9–0.97	3	1 (66%)	2 (34%)	22	100	100	85
0.97≦	2	2 (100%)	0 (0%)				
Total	50	9 (18%)	41 (82%)				

advanced liver disease may be accompanied by mitochondrial injury, resulting in more marked release of AST, which is present in mitochondria and cytoplasm, than of ALT [28]. Although many researchers have examined the usefulness and diagnostic ability of AAR in patients with HCV, results have been conflicting [13, 29–32]. Some

studies have demonstrated that AAR is useful for predicting cirrhosis in NASH [26, 33, 34]. In our study, AAR was a better predictor of cirrhosis in patients with NASH than in patients with HCV.

In 1995, Teran et al. [35] originally described a CDS based on two clinical variables: vascular spiders and ascites. In 1997, Bonacini et al. [26] modified this score and included only objective laboratory variables, such as platelet count, AAR, and INR. As mentioned above, platelet count and AAR can be useful for predicting cirrhosis in patients with NASH. INR is directly related to the synthetic function of the liver, and worsens with progression of fibrosis and loss of hepatocyte mass. In our study, the AUROC value of CDS for predicting cirrhosis was the highest among the five noninvasive tests evaluated; with use of this score, liver biopsy for predicting the risk of cirrhosis can be avoided in 60% of patients with NASH.

The HALT-C model is also derived from platelet count, AAR, and INR [17]. It was based on data collected prospectively from more than 1,100 patients of various ethnic backgrounds. However, unlike CDS, the HALT-C Trial was not designed specially to identify predictors of cirrhosis, and the study group consisted of patients with advanced fibrosis. The HALT-C model had the second highest AUROC value for predicting cirrhosis; use of this model might obviate the need for liver biopsy for predicting the risk of cirrhosis in 48% of patients with NASH.

Fibrotest is an algorithm based on five biochemical markers: bilirubin, gamma-glutamyl transpeptidase, gammaglobulin, haptoglobin, and alpha2-macroglobulin [36]. It has been validated in patients with chronic hepatitis B, hepatitis C, and NAFLD/NASH [36–38]. Fibrotest was found to have an AUROC value of 0.80–0.85 and could prevent the need for liver biopsy in about 40% of patients. However, the algorithm includes the biomarkers that are costly to evaluate and not measured routinely.

Transient elastography is an emerging technology that is more sensitive than currently available radiologic techniques for staging hepatic fibrosis [39]. In patients with HCV, cirrhosis was differentiated from milder stages of fibrosis with an AUROC value of 0.97 [40]. Similar results were obtained from patients with primary biliary cirrhosis [41] and NASH [42]. However, elastography is not suitable for use in patients with morbid obesity, which often accompanies NASH. Moreover, the equipment is very expensive and is not available at most hospitals.

In conclusion, we demonstrated that noninvasive tests proposed for the prediction of cirrhosis in patients with HCV can also be used with a high degree of accuracy in patients with NASH. In particular, CDS and HALT-C model, which utilize four routine laboratory variables, such as platelet count, AST, ALT, and INR, can distinguish between the presence or absence of cirrhosis with sufficient reliability to be used to predict the risk of cirrhosis in patients with NASH.

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Industrial Info.

Ⅰ型インターフェロンの肝線維化改善メカニズム

The mechanism of action of type I interferon to improve liver fibrosis

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Key words
interferon, liver fibrosis
Hepatic Stellate Cell

要　約

C型慢性肝炎やC型代償性肝硬変に対するインターフェロン（IFN）治療において，ウイルス駆除に至らなくとも，肝線維化の進展や肝癌の発症が抑えられることが示されている。しかし，その詳細な分子機構は不明である。我々は，肝線維化に重要な役割を果たしている肝星細胞（HSC）に対するIFNの直接的作用を明らかにすることを目的として検討を行った。

その結果，Ⅰ型IFNはヒトHSCに対し，①サイクリン依存性キナーゼ阻害因子であるp21の誘導を介して細胞周期のG_1期からS期への移行を抑制し，細胞増殖抑制作用を示すこと，②マトリックスメタロプロテアーゼ（MMP）-1の産生亢進作用およびTGF-βのシグナル抑制作用を示すことを明らかにした。これらのことから，Ⅰ型IFNはHSCに対して直接的に多様な作用を引き起こし，抗線維化作用を示すことが明らかとなった。

はじめに

Ⅰ型インターフェロン（interferon, 以下IFN）は，慢性C型肝炎の治療方法としてウイルス駆除を行う目的で広く用いられている。慢性C型肝炎の治療においては，ウイルス駆除とともに，肝線維化→肝硬変→肝癌への進行をくい止めることが重要である。IFN治療により慢性C型肝炎患者において肝線維化が改善されることは，多数の報告により示されている[1]。しかしながら，IFNによる肝線維化改善作用のメカニズムは明らかにされておらず，一般的にはウイルス排除より肝炎が沈静化した結果による二次的なものであると考えられている。しかし，IFN治療によりC型肝炎ウイルス排除に至らない患者についても，肝線維化の進行が遅延する例があるとの報告[2]があり，IFNによる肝線維化の改善は，ウイルス排除による二次的な機構以外にも，抗線維化メカニズムが存在することが示唆されている。その一候補として，*in vitro*の系において，IFNが細胞外マトリックス産生・分解の中心的な役割を担っている肝星細胞（Hepatic Stellate Cell, 以下HSC）に対して，その増殖や細胞外マトリックスの主要構成成分であるコラーゲンの産生を抑制するとの報告[3]があり，IFNが直接的にHSCに作用して肝線維化を改善する可能性が考えられる。しかし，その詳細なメカニズムは，明らかになっていない。そこで，我々は，ヒトHSC株を用い，Ⅰ型IFNの抗線維化メカニズムを解析した。

1．肝線維化の分子生物学的機構とIFNの作用点（図1）

正常なHSCは，類洞の内皮細胞と肝細胞の間のDisse腔に存在し，pericyteとして類洞の血流の調節を行っていると考えられている。C型肝炎ウイルスなどによる慢性的な肝障害により，肝細胞の一部が，変

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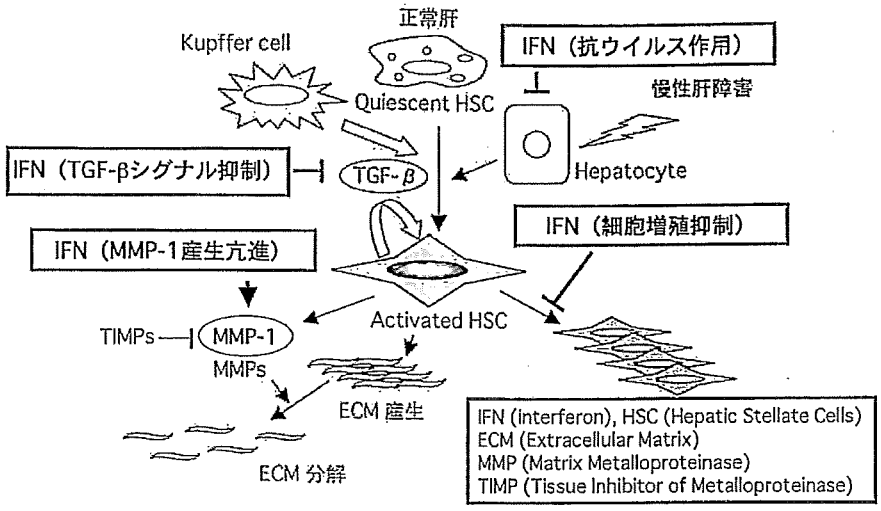

図1　肝線維化の分子生物学的機構とIFNの作用点（文献[4]より引用改変）

性，壊死，アポトーシスに陥ると，マクロファージ系のクッパー細胞が活性化され，TGF-β，PDGFおよびTNF-αなどのサイトカインを放出するようになる。これらのサイトカインによりHSCは活性化され，筋線維芽細胞（myofibroblast）様に形質転換する。活性化されたHSCは自ら増殖し，細胞外マトリックスを産生し，TGF-β，PDGFなどのサイトカインをautocrineで産生し，肝の線維化が進行していく。したがって，HSCの活性を抑制し，細胞外マトリックスの産生を抑えることが肝線維化の治療に有効であると考えられている。HSCの制御をターゲットとした肝線維化阻害剤の研究の例としては，gliotoxin[5]などの細胞毒によるHSCのアポトーシス誘導，コラーゲン分子のシャペロンであるHSP47 siRNAのHSC特異的な導入[6]などの報告があり，動物レベルで有効性が示されている。

IFNの肝線維化抑制における作用点としては，前述のようにC型肝炎ウイルスが感染した肝細胞（hepatocyte）とHSCが考えられる（図1）。IFNは，hepatocyteに作用してウイルスを排除することにより，二次的に肝線維化を改善する。また我々の今回の研究から，IFNはHSCに直接作用し，①細胞増殖を抑制して肝線維化の進行を抑制，②マトリックスメタロプロテアーゼ-Matrix Metalloproteinase-1，以下MMP-1）の産生を促して細胞外マトリックスの分解を促進，③線維化促進因子であるTGF-β シグナルを抑制，することが明らかとなった。

2．HSCに対するⅠ型IFNの細胞増殖および細胞周期への作用

我々が解析したIFNの抗肝線維化メカニズムのひとつとして，まずHSCの増殖抑制作用について詳しく述べる。培養可能なヒトHSC株をヒトⅠ型IFNであるIFN-αあるいはIFN-βで処理し，細胞増殖に対する作用をMTS法にて検討した。また常法により細胞周期に対する作用を解析した。次いで細胞周期に関連するサイクリン依存性キナーゼ（Cyclin Dependent Kinase，以下CDK）阻害因子*p21*遺伝子およびp21蛋白の発現への影響をそれぞれ定量PCR法およびウェスタンブロット法により検討した。

IFN-α またはIFN-β は，HSCに対し細胞増殖抑制作用を示し，同じ生物活性濃度で処置した場合，IFN-β はIFN-α よりも作用が強力であることが明らかとなった（図2a）。IFN-β のHSCの細胞周期に対する作用を解析した結果，IFN-β は，細胞周期のG_0/G_1期からS期への移行を遅らせることで，細胞分裂を抑制することが明らかとなった（図2b）。細胞周期が，G_0/G_1期で停まっていることからCDK阻害因子，特に*p21*遺伝子に着目して定量PCRを行ったところ，IFN-β は*p21*遺伝子の発現を2倍上昇させ(図は示していない)，更に蛋白レベルでもp21の発現を増加させることが確認された（図2c）。一方で他のCDK阻害因子であるp15には影響しないことが示された。

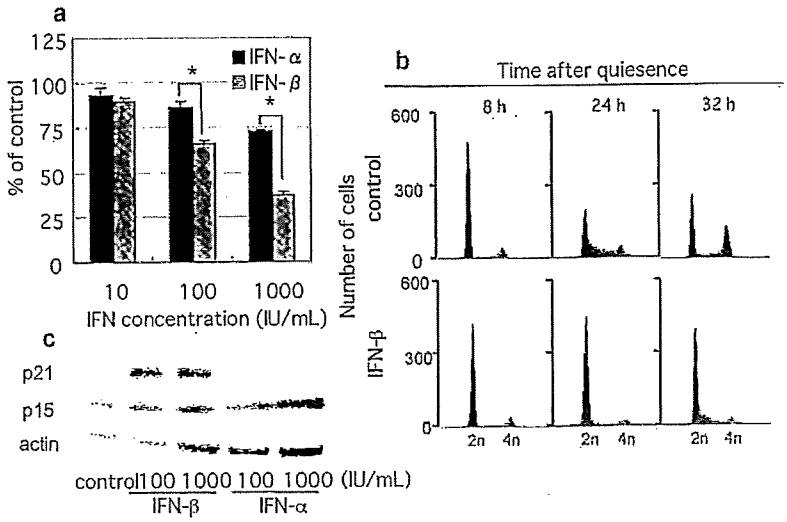

図2　HSCに対するI型IFNの細胞増殖または細胞周期への作用

a：ヒトHSCであるTWNT-4細胞[7]をIFN-βまたはIFN-α 10-1000 IU/mLで処置して5日間培養後，細胞増殖をMTS cell proliferation assayにより測定した。平均値±標準偏差 (n=5)　*p <0.05 (t-test)

b：細胞周期解析は，TWNT-4細胞を無血清培地で培養することによりG_0/G_1期に同調した後，IFN-β 100 IU/mLで処置し，一定時間後にフローサイトメトリーにより細胞内のDNA量を測定した。

c：TWNT-4細胞をIFN-βまたはIFN-α 100，1000 IU/mLで処置して16時間培養後，p21およびp15蛋白の発現をウエスタンブロッティング法により解析した。

3. IFN-βの細胞外マトリックス分解酵素（MMP）に対する作用

HSCは，活性化に伴い細胞外基質を分解するMMPやそれらの阻害因子であるTissue Inhibitor of Metalloproteinase（TIMP）などを産生分泌するようになる。そこで，上述した細胞増殖抑制作用の検討でより強力な作用を示したIFN-βについて，ヒトHSC用いて各種*MMP*遺伝子および*TIMP*遺伝子の発現への影響を定量PCR法にて検討した。その結果，IFN-β は*MMP-1*遺伝子の発現を有意に増加させることが明らかとなった（図3a）。有意な変動が認められたMMP-1に注目し，線維化促進因子としての機能が知られているTGF-β 共存下におけるIFN-β の作用を続

a

Gene Name	Time		
	8h	24h	72 h
MMP-1	→	↗1.3folds	↑2 folds
MMP-2	→	→	→
MMP-3	→	→	→
MMP-9	→	→	→
MMP-14	→	→	→
TIMP-1	↗1.2 folds	↗1.2 folds	↗1.3 folds
TIMP-2	→	→	→

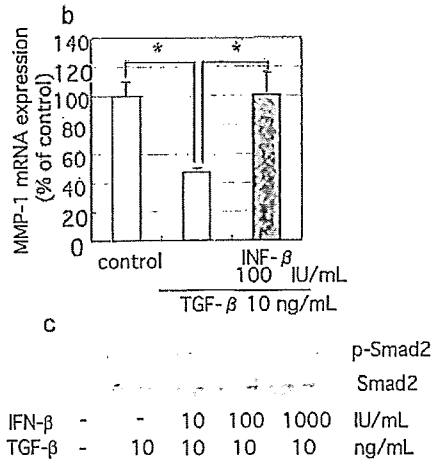

図3　IFN-βの細胞外マトリックス分解酵素（MMP）およびTGF-βシグナルに対する作用

a：ヒトHSCであるLI90細胞[8]をIFN-β 100 IU/mLで処置して一定時間培養後，total RNAを抽出し，MMP-1, -2, -3, -9, -14およびTIMP-1, -2 mRNAの発現レベルをリアルタイムPCR法により定量した。

b：LI90細胞をTGF-β 10 ng/mLとIFN-β 100 IU/mLで同時に処置し，72時間後にMMP-1 mRNAの発現レベルを定量した。平均値±標準偏差 (n=3) *p <0.05 (t-test)

c：LI90細胞をTGF-β 10 ng/mLとIFN-β 10-1000 IU/mLで同時に処置し，24時間後のリン酸化Smad2（p-Smad2）とSmad2蛋白の発現を，ウェスタンブロッティング法により解析した。

いて解析した。TGF-β 処置により *MMP-1* 遺伝子の発現量は低下するが，IFN-β はその発現量を正常レベルにまで回復させることが明らかとなった（図3b）。これら遺伝子レベルでの変動は蛋白レベルでも同様の変化が確認された（図は示していない）。

MMPは細胞外基質の構築や分解に深く関与することが知られており，コラゲナーゼ群（MMP-1, -8, -13），ゼラチナーゼ/Ⅳ型コラゲナーゼ群（MMP-2, -9），ストロメライシン群（MMP-3, -10），細胞膜貫通型MMP群（MMP-14,-15,-16）に分類される。肝線維症/肝硬変で顕著に増加するのはⅠ型コラーゲンであるので，肝線維化改善には，Ⅰ型コラーゲンを特異的に分解するコラゲナーゼであるMMP-1の発現および活性の調節が重要とされる。このことは，MMP-1を発現するアデノウイルスベクターを肝硬変ラットに導入することで線維化が抑制されるという報告[9]からも支持され，IFN-β によるMMP-1発現の促進は，その抗線維化メカニズムのひとつとして非常に重要であると考えられる。

MMP-1に関する上述の検討から，IFN-β が，TGF-β 刺激時のヒトHSCに対して抑制的に働くことが示唆されたため，TGF-β シグナル伝達分子について更に解析を進めた。その結果，HSCにおいてIFN-β 処置によりSmad2のリン酸化が濃度依存的に抑制されることが示された。（図3c）。よってIFN-β によるMMP-1発現の亢進はHSCからautocrineに産生されているTGF-β のシグナルをブロックすることによる作用である可能性が考えられた。

おわりに

Ⅰ型IFNが動物レベルおよび臨床においても肝線維化を抑制することについては，多くの知見が得られている。また，これまで一方向性と考えられてきた線維化の進行がリバースする可能性があり，Ⅰ型IFNにその作用を有する可能性が示唆されている[1]。

今回，我々の検討により，Ⅰ型IFNが，肝線維化において重要な役割を果たしているHSCに対して直接的に，細胞増殖抑制作用，細胞周期抑制作用，MMP-1産生亢進作用，TGF-β 抑制作用などの多彩な作用を引き起こすことが明らかとなった。現在，我々はⅠ型IFNを処置したHSCにおける，高感度DNAチップ（*3D-Gene*：東レ）を用いた網羅的miRNAおよびmRNAの発現解析を実施しており，今後，両結果を関連づけながら詳細な解析を進め，得られた情報からIFNの線維化抑制機構を分子レベルで解明していく予定である。

肝線維化に対するⅠ型IFNの作用分子機構を詳細に再検討し，明確にすることは，肝線維化を制御する薬物の創薬研究の基盤となり，類似の作用を有する低分子化合物の探索方針を新たに提示する可能性を秘めている。IFN-β や肝線維化に関しては，まだまだ不明な点が多く，新しい手法を用いた細胞分子生物学的なアプローチによって，これらの分子機構を改めて研究しなおすことは非常に意義あることと思われた。

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