図6　定期的に開催されている消化器病教室の勉強会

改善するための情報公開や相談窓口の設置なども必要であろう。

更に、医療従事者が患者の説明に使う医療用語が、患者の理解と判断の障害にならないように、分かりやすく話すことも非常に重要である。独立行政法人国立国語研究所の「病院の言葉」委員会は、2009年3月に患者にとって難しい医療用語を分かりやすく説明するための最終報告を発表した。書籍としても発刊された（『病院の言葉を分かりやすく―工夫の提案―（勁草書房）』）。患者中心の医療の実現には、医療従事者が分かりやすく説明するという基本的な姿勢を忘れてはならない。

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医療従事者の皆様へ

ご存じですか？
ビブリオ・バルニフィカス感染症

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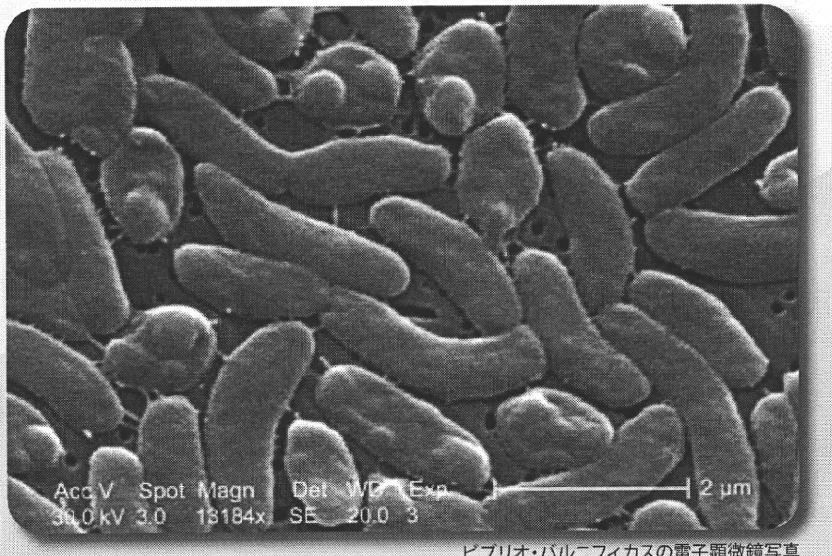


ビブリオ・バルニフィカスの電子顕微鏡写真

ビブリオ・バルニフィカスとは？

ビブリオ・バルニフィカスは、コレラ菌や腸炎ビブリオ菌などと同じビブリオ科に属するグラム陰性桿菌です。ただし、他のビブリオ属菌が主に消化管感染症の原因になるのに対し、ビブリオ・バルニフィカスは肝疾患などの慢性疾患を有する患者に、原発性敗血症や創傷部感染を引き起こすのが特徴です。ビブリオ・バルニフィカス感染症は、いったん発症すると急速に悪化し、きわめて死亡率が高いため、注意を要する細菌感染症です。

ビブリオ・バルニフィカスは一端に鞭毛を持ち、活発な運動性を有しています。「ビブリオ（*Vibrio*）」とは「振動する」という意味で、「バルニフィカス（*vulnificus*）」とは「創傷に関係ある」という意味を持ちます。

主に暖かい海水や海泥の魚介類や甲殻類などに付着しつつ増殖し、周囲の海水中に広く分布しています。ビブリオ・バルニフィカスは、低い塩分濃度を好んで増殖しますので、とくに河口に近い海水と真水が交わる汽水域に存在します。また、海水温度が20℃を超す夏から秋に活発に増殖するため、発症も7月から9月に集中しています。

肝臓病の方は、夏場の海産魚介類の生食には注意が必要です。

発生件数は？

日本のビブリオ・バルニフィカス感染症は、1978年に長崎県で報告されて以来、約200例の誌上報告があります。しかし本感染症は、届け出義務のある感染症に指定されていないため、未報告の国内発生事例は多いと考えられています。ビブリオ・バルニフィカス感染症のサーベイランス調査結果では、年間推定発生患者は425例とされています。

感染経路は？

感染経路は、ビブリオ・バルニフィカスに汚染した生の魚介類を食べることで感染する経口感染型（原発性敗血症型）と、創傷部位へ海水中の菌が侵入することで感染する経皮感染型の2種類があります。日本では、欧米と異なり前者の経口感染型が圧倒的に多いのが特徴です。これは、わが国では刺身や寿司などを好んで食べるという食習慣の違いが原因として考えられています。元来、欧米人は魚を生で食べる習慣を持っていませんが、生ガキを食べますので、欧米における経口感染の大部分は生ガキが原因です。ただわが国では、カキを食べるシーズンは海水温が低い冬場が多いため、カキが原因となる事例は少ないようです。

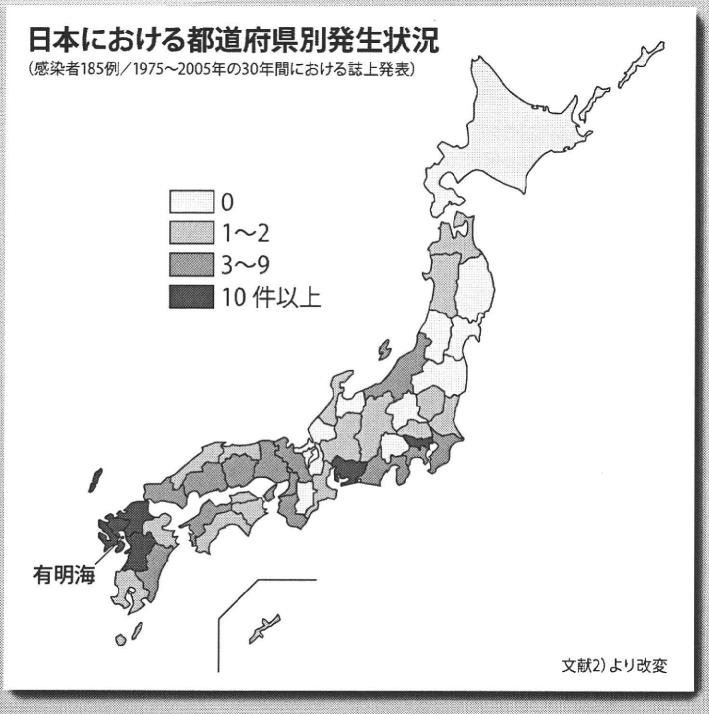
なお、まな板や包丁などの調理器具を介して感染することもありますので注意が必要です。

どんな人が感染しやすいのですか？

ビブリオ・バルニフィカスに汚染された食品を食べたとしても、健康な人であれば下痢や腹痛がみられる程度です。肝臓病の方（とくに肝硬変やアルコール性肝臓病）、糖尿病の方、免疫機能が低下している方、ステロイド剤を飲んでいる方、貧血などで鉄剤を飲んでいる方は重症化しやすく、感染した場合には、死亡することもあります。致死率は、60～80%です。

日本における都道府県別発生状況

ビブリオ・バルニフィカス感染症の発生地域は、熊本県がもっとも多く、福岡県、佐賀県など九州北部での発生が過半数を占めています。九州に感染者が多い原因として、海水温が高いこと（ビブリオ・バルニフィカスは海水温が20℃以上で活発に増殖する）や肝疾患患者が多いこと（佐賀県は肝臓がん死亡者が日本で一番多い）、大きな河川が湾に流れ込み汽水域が広いことなどが影響しています。



感染したときにみられる症状は？

この感染症の本態は、経口感染から敗血症を生じ、その後に菌が下肢の軟部組織に着床し、壊死性筋膜炎を生じるものです。生の魚介類を食べて数時間から1日の間に、突然高熱や悪寒が発現します。まれに腹痛や吐き気、下痢がみられることもあります。血圧が下がり、全身の臓器機能が低下し致命的になります。四肢（とくに下肢）の激痛に加え、皮膚の腫脹、水疱形成、紫斑を伴う紅斑、血疱などの皮膚症状が出現し、皮膚や筋肉が壊死に陥るのが特徴です。入院後平均約2日で死亡するというきわめて急激な経過をたどります。急激に進む壊死を形容して、本菌を「人食い菌」「人食いバクテリア」と呼ぶこともあります。

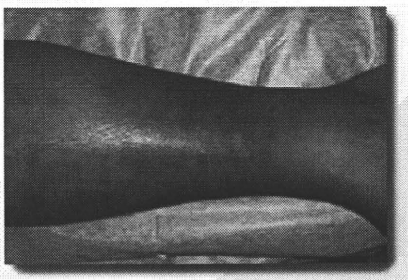


写真1 下腿の著しい腫脹と暗紫色斑

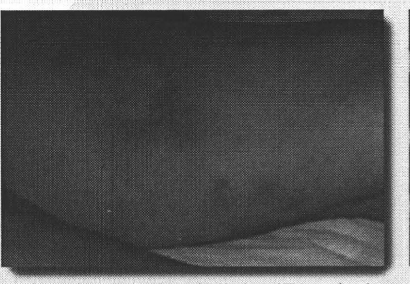


写真2 大腿にみられた紫斑を混じる紅斑

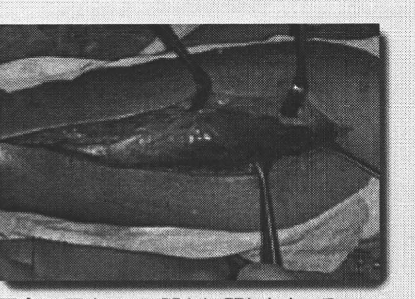
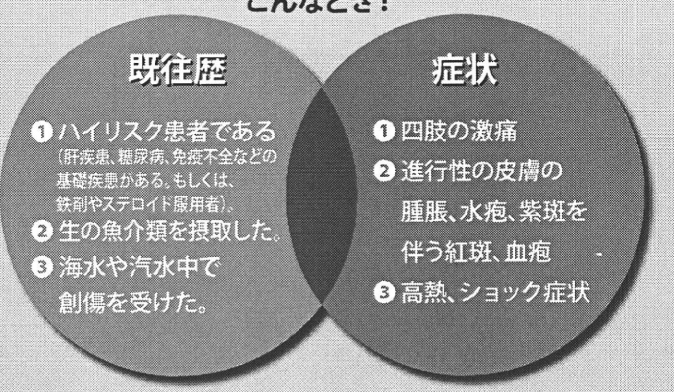


写真3 写真1の下腿を切開したところ
脂肪組織が壊死している

治療は？

治療には、第3世代セフェム系やテトラサイクリン系の抗生剤の投与と的確な対症療法が不可欠です。また、下肢に痛みや紅斑が生じている場合はすでに壊死性筋膜炎を発症しており、これに対して早急で徹底したデブリードマン（壊死組織切除術）が必要です。ショックやDICを合併し多臓器不全となる場合が多く、厳重な全身管理が必要になります。治療開始が遅れれば遅れるほど死亡率は高くなり、発症から2〜3日で死亡することもあるため、初期治療が非常に重要です。ビブリオ・バルニフィカス感染を疑った場合は、急いで救急医療機関に紹介してください。

ビブリオ・バルニフィカス感染症を疑うのは、こんなとき！



日頃の患者さんへの指導は？

ハイリスク患者とくに肝臓病や糖尿病などの患者には、ビブリオ・バルニフィカス感染症について正しい知識と予防法を説明することが大事です。夏場の生の魚介類を控えるか、十分に加熱したものを食べるように指導をお願いします。手足にキズがあるときは、海水浴を避けるといった助言も大事です。

また、万一感染してしまった可能性があるときは、急いで救急医療機関を受診するよう指導してください。地域の医療連携病院や肝疾患診療連携拠点病院などがあれば、日頃から患者さん自身に緊急連絡先（夜間や休日時の体制）を控えさせておくことも大切です。

正しい知識と予防法についての理解を広めましょう！

予防法は？

ハイリスク患者には、下記のような注意点を指導してください。

① 海水温度が20℃を超えると、細菌が増殖します。
夏場に、生や加熱不足の魚介類を食べないようにしましょう。

② ビブリオ・バルニフィカスという細菌は、加熱することで死滅します。
魚介類は十分に加熱して食べましょう。中心温度が70℃・1分間（100℃であれば数秒間）で死滅します。

③ 貝を煮る場合は、貝が開いてからも5分間、蒸す場合は9分以上の調理が必要です。
開かない貝は食べてはいけません。

④ カラを取ったカキ（むき身）の場合には、少なくとも3分間煮ることが必要です。
フライにする場合は、油の中で191℃以上・10分間加熱しましょう。

⑤ 貝を調理するときは、手にケガをしないように、丈夫な防護手袋をしましょう。
生ものを触ったら、よく手を洗いましょう。

⑥ 生ものを調理するときは、流水で魚の表面や調理器具を十分に洗浄することも大切です。

⑦ 手足にキズのある場合は、夏場に海水に入らないことも予防になります。

⑧ 素足で海岸や岩場を歩くと、ケガをしやすく、傷口から感染することがありますので、
海辺を素足で歩くことは避けましょう。

肝疾患患者へのビブリオ・バルニフィカス感染症に関する啓発は？

厚生労働省や農林水産省、また国立感染症研究所は、ビブリオ・バルニフィカス感染に関する正しい知識と予防策等について理解を深めるために、Q＆Aを作成しています。しかし残念ながら、日本では肝疾患患者におけるビブリオ・バルニフィカス感染の認知度は低いのが現状です。2008年に、肝臓専門医が常勤している全国14施設で、肝臓病の方を対象に調査が行われました。1,336名の患者においてビブリオ・バルニフィカス感染を認知している患者の割合は14.5%、肝硬変患者304名では17.4%の認知率でした。肝疾患患者への正しい知識の普及には、かかりつけ医と肝臓専門医を中心とした患者教育が必要です。

健康な方は過敏になる必要はありません。海産魚介類は一般の方の健康に有益ですので、摂食の減少につながらないよう、正確なご指導をお願いします。

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A Randomized Study of Extended Treatment With Peginterferon α-2b Plus Ribavirin Based on Time to HCV RNA Negative–Status in Patients With Genotype 1b Chronic Hepatitis C

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OBJECTIVES: **The treatment of patients with hepatitis C virus (HCV) genotype 1 with peginterferon plus ribavirin treatment for more than 48 weeks demonstrated high sustained virological response (SVR) rates. Although many studies extended the duration of therapy from 48 weeks to 72 weeks, the optimal duration has not yet been determined.**

METHODS: **A total of 113 genotype 1b patients with high viral load were randomized at baseline to the standard (n=56) or extended (n=57) treatment group. The standard group patients received 48 weeks of peginterferon plus ribavirin treatment. In the extended group, the treatment was performed for 44 weeks after patients became negative for HCV RNA (total duration 48–68 weeks).**

RESULTS: **The SVR rate of the standard and extended group was 36% (20 of 56) and 53% (30 of 57; P=0.07). However, the extended group patients who became negative for HCV RNA between weeks 16 and 24 had a significantly higher SVR rate (78%; 7 of 9) than that of standard group (9%, 1 of 11; P=0.005). The predictive factors for the SVR were the treatment regimen (the standard vs. extended treatment) and the time to HCV RNA negative–status.**

CONCLUSIONS: **The extended treatment significantly increased the SVR rate in patients who were HCV RNA negative at 16–24 weeks.**

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INTRODUCTION

The combination of peginterferon plus ribavirin is recognized as a standard treatment for patients with chronic hepatitis C. Specifically, a 48-week regimen of treatment is currently the recommended therapy for hepatitis C virus (HCV) genotype 1-infected patients (1,2). However, there is only a 40–50% likelihood of achieving a sustained virological response (SVR) (3–6). Therefore, to increase the rate of SVR, trials extending the duration of treatment have thus been conducted (7–12). These studies indicated that the extension of treatment significantly increased the rate of SVR in patients with slow virological response. However, as these studies extended the treatment uniformly, it is therefore desirable to tailor the treatment regimen to achieve the SVR more efficiently.

HCV genotype 1-infected patients who become HCV RNA negative after 4 weeks of treatment have been reported to achieve an excellent SVR rate of almost 90% (3,13,14). This means that these patients had 44 weeks of treatment with a disappearance of HCV RNA. In addition, a recent analysis based on a mathematic model suggested that the rate of SVR in patients infected with HCV genotype 1 directly correlates with the duration of treatment once HCV RNA has been cleared from serum (15).

Following this concept, an extended therapy regimen was therefore designed to ensure the patients become HCV RNA negative for 44 weeks. HCV genotype 1b patients with high viral loads were randomized into two groups receiving the treatment for 48 weeks (standard group) or 48–68 weeks (extended group) and then the outcomes were evaluated.

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METHODS

Patients

Male and female patients aged 20–75 years with a compensated chronic HCV genotype 1b infection were eligible for enrollment. The eligible patients tested positive for HCV RNA by a quantitative reverse-transcription polymerase chain reaction (PCR; Amplicor Monitor HCV version 2.0 using the 10-fold dilution method; Roche Diagnostics, Tokyo, Japan; lower limit of detection 5KIU/ml) with a concentration >100KIU/ml, and had at least one elevated serum alanine aminotransferase level at the time of screening or entry into the trial. Patients with an HCV genotype other than 1b infection, hepatitis B surface antigen, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, decompensated cirrhosis (Child–Pugh class B or C), or with evidence of hepatocellular carcinoma were excluded. The patients with platelet counts of 8×10^4 per mm^3 or less, leukocyte counts of 2,500 per ml or less, or hemoglobin levels of 12 g/dl or less were also excluded from the study. HCV genotyping was performed according to Okamoto's method (16), and the genotypes were classified according to Simmonds' classification system (17). Chronic hepatitis was diagnosed based on the histological scoring system of Desmet *et al.* (18).

Study design

This randomized controlled trial was carried out from January 2005 to November 2006. All patients provided their written informed consent. The study was in accordance with the principles of the 1975 Declaration of Helsinki.

A total of 113 protocol-eligible patients were assigned randomly using sealed envelopes with a 1:1 randomization ratio. All patients received both 1.5 μg/kg per week s.c. peginterferon α-2b (Pegintron; Schering-Plough, Kenilworth, NJ) and oral ribavirin (Rebetol; Schering-Plough) at 600 mg per day (body weight <60 kg), 800 mg per day (body weight between 60 and 80 kg), or 1000 mg per day (body weight >80 kg) according to the manufacturer's drug information for ribavirin. All patients were monitored every 4 weeks. A total of 56 patients (standard group) received a 48-week course of peginterferon plus ribavirin combination therapy. The other 57 patients (extended group) received a 48–68 weeks of the combination therapy. The extended treatment was performed for 44 weeks after HCV RNA first became negative (e.g., if HCV RNA became negative at week 16, total treatment duration was 60 weeks; **Figure 1**). If the patient was HCV RNA positive at week 24, the patient discontinued this trial design treatment. If a patient wished to continue the treatment, the treatment could be given for 48 weeks. Serum HCV RNA was determined using the standardized automated qualitative PCR (Cobas Amplicor Hepatitis C Virus Test, version 2.0; Roche Diagnostics; detection limit: 50 IU/ml). Dose modification followed standard procedures in principle. Therefore, according to the intensity of the adverse event or when laboratory results showed hemoglobin <10 g/dl in subjects with no cardiac disease, the dose of ribavirin was thus decreased by 200 mg per day. When the neutrophil count was <750 per mm^3 or the platelet count was <80,000 per mm^3, the dose of peginterferon was decreased by 50%. When the hemoglobin was <8.5 g/dl, the neutrophil count was <500 per mm^3, or the platelet count was <50,000 per mm^3, then both drugs were discontinued.

After the end of the treatment protocol, patients from both groups were followed up for a further 24 weeks. The primary aim of this study was to assess the SVR, which was defined as undetectable serum HCV RNA levels by qualitative PCR at the end of the 24-week post-treatment follow-up period. The others were classified as non-SVR.

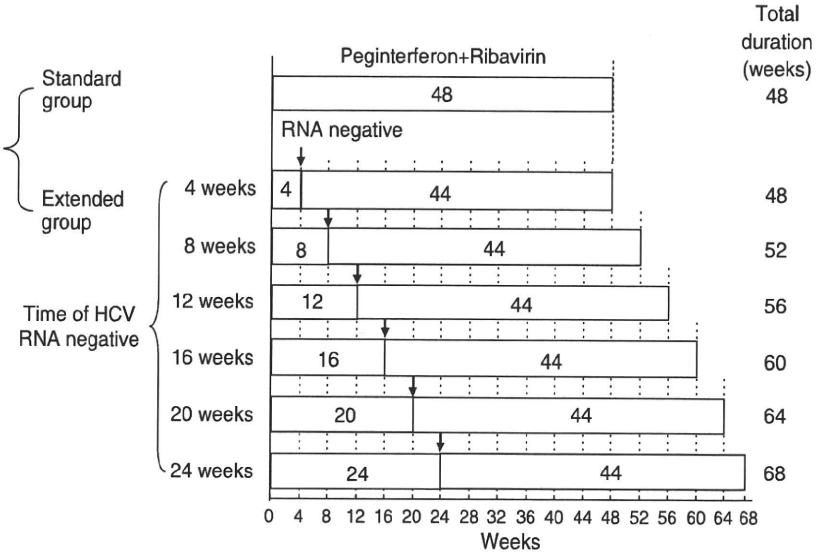

Figure 1. Trial design. The standard group received 44 weeks of treatment. The duration of the extended treatment was determined based on the time to hepatitis C virus (HCV) RNA negative–status.

Statistical analysis

At the time of conceiving this study plan no published reports were available for efficacy of extended treatment for patients with genotype 1. The sample size calculation was based on the assumption that SVR rate of late viral responder (HCV RNA of the patients become negative between weeks 12 and 24) was 30% in the standard group and 60% in the extended group. On the basis of two-sided tests with a significance level of 0.05 and a statistical power of 80%, recruiting of 90 patients in the two arms was planned. To compensate for dropped out patients, we planned to enroll 110 patients.

Frequency was compared between the groups using the χ^2-test with Yates' correction, or Fisher's exact test. The group means, presented as the mean values±the standard deviations, were compared using the Mann–Whitney *U*-test. All reported *P* values were two sided, and *P* values of <0.05 were considered to be significant. A multivariate analysis was carried out using a multiple logistic regression analysis. Statistical analyses were performed using the JMP software (SAS, Cary, NC).

RESULTS

Patient profiles

Patients were enrolled between January 2005 and June 2006. A total of 113 patients (mean age: 54.9±10.2-year old, men/women: 56/57) were included in this study. The patients in the two treatment groups were well matched for the baseline characteristics (**Table 1**). The trial profile is shown in **Figure 2**. Seven patients in the standard group and six in the extended group discontinued treatment because of adverse events. Thirteen patients in the standard group and twelve in the extended group discontinued this trial design treatment due to positive HCV RNA at week 24. There were two patients (one in the standard and the other in the extended group) who became HCV RNA negative after 25 weeks and completed the 48-week treatment. However, the HCV RNA of these patients was relapsed after the treatment. In total, 32 patients in the standard group and 36 patients in the extended group became HCV RNA negative within 24 weeks and thus completed the treatment. The mean duration of the total treatment in the extended group was 56.1±5.0 weeks.

The on-treatment virological response rates (intention-to-treat analysis) between the standard and the extended group were not significantly different at week 12 (26 of 56 (46%) patients vs. 29 of 57 (51%) patients, respectively) or at the end of treatment (39 of 56 (70%) patients vs. 40 of 57 (70%) patients, respectively).

SVR rates

On the basis of an intention-to-treat analysis, the SVR rate of standard group and extended group were 36% (20 of 56) and 53% (30 of 57; $P=0.07$). **Figure 3** shows the SVR rates in patients in the standard and extended groups whose HCV RNA became negative within 24 weeks and the planned therapy was completed. In the patients who became HCV RNA negative within week 8, more than 90% of the patients achieved an SVR in both groups. In the standard group, as the time of HCV RNA negative was later, the SVR rate was lower. However, in the extended group, the patients who became HCV RNA negative after 16 also had high SVR rates. The SVR rates of the patients who became HCV RNA negative at 16, 20, and 24 weeks were 33% (1 of 3), 0% (0 of 0), 0% (0 of 2) in the standard group and 100% (5 of 5), 0% (0 of 1), and 67% (2 of 3) in the extended group, respectively. Therefore, the extended group patients who became HCV RNA negative from week 16 to 24 demonstrated a significantly higher SVR rate (78%, 7 of 9) than those of the standard group (9%, 1 of 11; $P=0.005$, 95% CI: 0.002–0.38) (**Figure 3**).

As the number of patients who became HCV RNA negative from week 16 to 24 was small, we performed a multiple regression analysis of the patients who became HCV RNA negative from week 12 to 24. The independent predictive value of the treatment regimen (standard vs. extended group), age, sex, body mass index, pretreatment levels of alanine aminotransferase, platelet, HCV RNA, grade of activity, fibrosis stage, received daily doses of ribavirin and weekly dose of peginterferon, the time of first HCV RNA negative, and duration of the treatment was determined using a multiple logistical regression analysis. The independent predictors of SVR were the treatment

Table 1. Characteristics of patients at baseline

	Standard group	Extended group	*P* value
Age (years)	55.3±11.6	54.6±8.7	0.69
Sex (M:F)	26:30	30:27	0.51
Body mass index (kg/m^2)	23.1±3.1	23.4±3.4	0.72
ALT (IU/l)	69.8±45.3	71.1±34.6	0.37
Platelet count (×10^4/mm^3)	16.1±4.4	15.8±4.7	0.53
Grade of activity[a]			
1	17	13	
2–3	32	28	0.76
Fibrosis stage[a]			
1–2	38	30	
3–4	11	11	0.63
HCV RNA level (K IU/ml)	2056±1482	2211±1461	0.51
Initial received dose of peginterferon (μg/kg/week)	1.46±0.17	1.47±0.11	0.81
Initial received dose of ribavirin (mg/kg/day)	11.5±1.1	11.1±1.9	0.31

M, male; F, female; ALT, alanine aminotransferase; HCV, hepatitis C virus.
Note: Continuous variables are presented as the mean±s.d. [a]Data were unavailable from 23 patients, including 7 and 16 from the standard and extended groups, respectively.

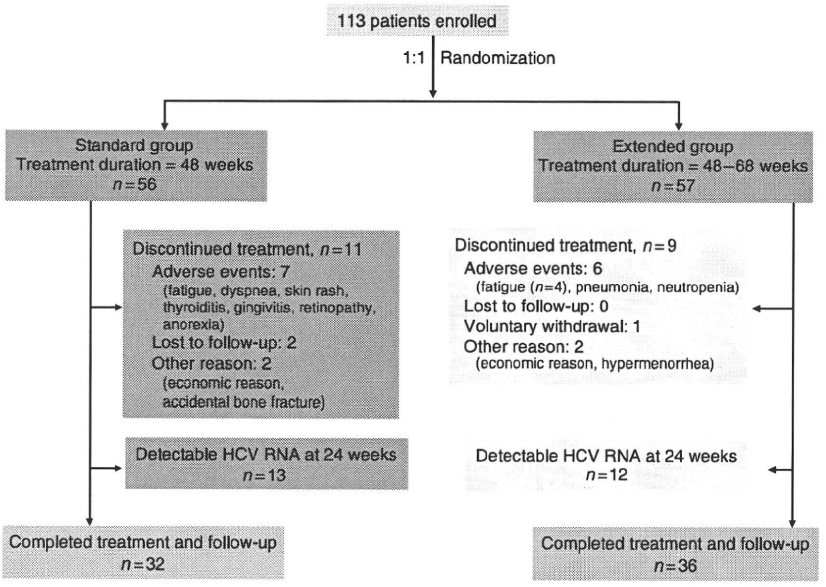

Figure 2. Study participant flow.

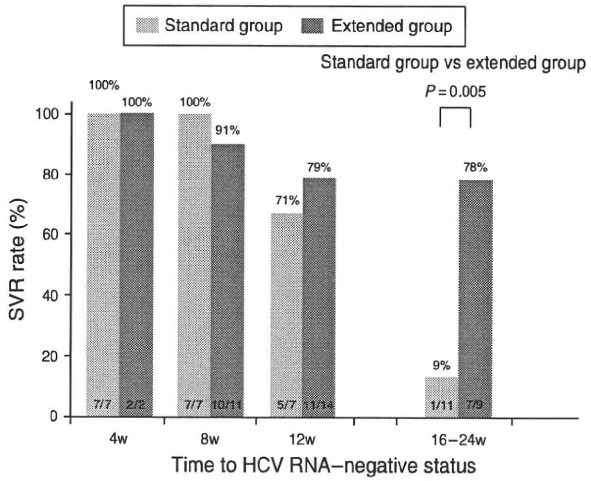

Figure 3. The SVR rate in patients who became hepatitis C virus (HCV) RNA negative within 24 weeks and the planned therapy was completed.

regimen (odds ratio, 4.8; 95% CI, 0.4–3.4, $P=0.031$) and the time of HCV RNA negative (odds ratio, 1.4; 95% CI, 0.04–0.7, $P=0.0495$).

Adherence

Peginterferon plus ribavirin combination therapy was discontinued in 7 of 56 (12.5%) patients of the standard group and 6 of 57 (10.5%) patients of the extended group because of side effects (**Figure 2**). The growth factors were not used in any patients. There were no differences in the discontinuation rate between the standard group and the extended group ($P=0.78$).

Table 2. Outcome of treatment

	Standard group	Extended group	*P* value
Duration of therapy (week)[a]	48	56.1±5.0	<0.0001
Treatment completion, *n* (%)	32 (57)	36 (63)	0.56
On full-dose therapy	18 (38)	21 (37)	0.69
With any dose reduction[b]	14 (29)	15 (26)	1.00

[a]Patients who completed the planned therapy. [b]Patients who had dose reduction of peginterferon, ribavirin, or both are included.

All therapy discontinuation occurred within 48 weeks; none of the therapy discontinuation in the extended group occurred between 48 and 68 weeks.

The dose of peginterferon and/or ribavirin was reduced in 14 of 56 (29%) patients of the standard group and 15 of 57 (26%) patients of the extended group (**Table 2**).

DISCUSSION

A 48-week regimen of peginterferon ribavirin combination therapy is the main therapeutic regimen for chronic hepatitis C patients with genotype 1 with a high viral load. In 48-week regimen of this therapy, the efficacy of treatment varies according to the time when HCV RNA first became negative. Specifically, in clinical trials that have been conducted in Japan, patients who became HCV RNA negative within 4 weeks

exhibited SVR rates of almost 100%, patients who became negative within 5–12 weeks exhibited SVR rates of approximately 70%, and patients who became negative within 13–24 weeks exhibited SVR rates of approximately 30%. Therefore, there have been attempts to increase the efficacy of treatment by extending the duration of treatment. Previous reports have shown that the efficacy of treatment can be increased by extending administration to 72 weeks for late viral responder that become RNA negative between weeks 12 and 24 (7–10). From these reports, it is believed that extended administration to late viral responder can assure increases in treatment efficacy. However, considering the physical and financial burdens to the patients, we believe that it would be important to establish the shortest possible treatment duration that exerts maximum efficacy (19). We thus believe that it is necessary to establish more detailed individualized treatment duration rather than extending treatment from 48 to 72 weeks. On the other hand, Drusano *et al.* (15) have reported that in previously untreated genotype 1 patients at least 32–36 weeks of undetectable HCV RNA by quantitative PCR is needed to attain the sustained clearance of HCV. We established the duration of our treatment so that the administration would be performed for 44 weeks after HCV RNA became negative, because the SVR rate of patients who became HCV RNA negative at week 4 was almost 100%. HCV RNA was only measured at weeks 4, 12, and 24 in the previous reports, but in Japan, HCV RNA is often measured every 4 weeks, and treatment is administered while checking the treatment efficacy, so we believe that it is possible to establish shorter periods of durations.

Among the patients in the present study, almost all patients in the standard group that became HCV RNA negative at weeks 4 and 8 exhibited SVR, so we therefore considered 48 weeks of administration to be sufficient. It is reported that high SVR rates have been obtained even with 24 weeks of administration in patients who became RNA negative at week 4 (20,21), 48 weeks may be too long. In the extended group, the patients who became HCV RNA negative at weeks 12 showed response rates of 79%, which were almost the same as those in the standard group (71%). As a comparatively sufficiently high response rate has been obtained in the standard group, our extended therapy may not benefit these patients.

On the other hand, the SVR rate of patients who became negative from weeks 16 to 24 was 9% in the standard group and 78% in the extended group.

Our extended therapy had maximal benefit in these patients. However, as the SVR rate of patients who became HCV RNA negative from weeks 20 and 24 was low (50%), a slightly higher response rate may be obtained through an extended administration of over 68 weeks in such patients, and this will be the subject of future study.

In terms of factors that contribute to SVR, the duration of administration and the time of HCV RNA negative were extracted as the most important factors according to the results of multivariable analysis. As there were no differences in the amount of ribavirin that was administered per day or the amount of peginterferon that was administered per week between the SVR and non-SVR groups, we believe that the duration of administration is important.

We believe that, in the future, it will be important to analyze patients who do not exhibit SVR despite extended administration. In the present study, one of the patients who became HCV RNA negative at week 12 was administered very small dosages because of the occurrence of side effects, but many patients were given full doses of both drugs, and the number of non-SVR patients was small, so the characteristics of the non-SVR patients could not be elucidated (data not shown).

The side effects were the same as those reported in previous reports. Moreover, regarding the time of discontinuation due to side effects, in all patients discontinuation occurred within 48 weeks from the start of administration, and no patients were discontinued during the extended period after 48 weeks. Therefore, we believe that extending administration to 48 weeks or longer would not cause any significant problems in terms of safety.

In conclusion, we believe that our extended administration method is a unique and economical treatment method that is able to achieve a high treatment efficacy.

CONFLICT OF INTEREST

Guarantor of the article: Tatsuya Ide, MD.

Specific author contributions: conception: Tatsuya Ide; study design: Tatsuya Ide, Teruko Arinaga, Michio Sata; participation in patient management and data collection: all authors; contribution to the data acquisition, responsibility for writing the paper, and statistical analysis: Tatsuya Ide. All authors reviewed the paper and approved the final version.

Financial support: None.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Forty-eight week regimen of peginterferon/ribavirin treatment is currently recommended therapy for HCV genotype 1-infected patients. However, there is only a 40–50% likelihood of achieving a sustained virological response (SVR).
- ✓ The efficacy of treatment can be increased by extending administration to 72 weeks for late viral responder that become RNA negative between weeks 13 and 24.
- ✓ It is desirable to tailor the treatment regimen to achieve the SVR more efficiently.

WHAT IS NEW HERE

- ✓ The extended therapy regimen was designed to ensure the patients become HCV RNA negative for 44 weeks.
- ✓ This treatment significantly increased the SVR rate in patients who were HCV RNA negative at 16–24 weeks.
- ✓ This treatment is a unique and economical treatment method.

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CLINICAL STUDIES

Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection

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Keywords
hepatitis C virus – hepatocellular carcinoma – diabetes mellitus – insulin – sulphonylurea

Abbreviations
ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HbA1c, haemoglobin A1c; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment of insulin resistance; IGF, insulin-like growth factor; LDH, lactate dehydrogenase; OR, odds ratio.

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Abstract

Background: Diabetes mellitus is frequently seen in hepatitis C patients and is often treated with antidiabetic agents that increase serum insulin levels. Because insulin is a growth-promoting hormone, antidiabetic agents could pose a risk for hepatocellular carcinoma (HCC). *Aim:* The aim of this study was to investigate an association between antidiabetic therapies and the incidence of HCC in hepatitis C patients with diabetes mellitus. *Methods:* A nested case–control study was conducted. Participants were recruited from a cohort study, in which patients with hepatitis C were consecutively registered. Participants were assigned to an HCC group ($n = 138$) or a non-HCC group ($n = 103$). To identify independent factors, variables including use of antidiabetic agents were analysed by logistic regression analysis. *Results:* Besides ageing, being male, cirrhosis and hypoalbuminaemia, use of exogenous insulin and a second-generation sulphonylurea were significant independent factors associated with an incidence of HCC [odds ratio (OR) 2.969, 95% confidence interval (CI) 1.293–6.819, $P < 0.0103$ and OR 6.831, 95% CI 1.954–23.881, $P < 0.0026$ respectively). In stratified analyses, the impact of these antidiabetic agents was more evident in patients who were non-cirrhotic than in those who were cirrhotic. *Conclusions:* Exogenous insulin and a second-generation sulphonylurea were independent variables associated with an incidence of HCC in hepatitis C patients with diabetes mellitus. This association was evident in patients who were non-cirrhotic. To verify a causal relationship between these antidiabetic agents and the development of HCC, a prospective cohort study is required.

Type 2 diabetes mellitus is a common metabolic abnormality worldwide and is associated with various complications. For example, cardio-vascular complications are well known for diabetes mellitus (1), while recent epidemiological studies have shown that patients with type 2 diabetes mellitus are highly predisposed to cancer (2). Type 2 diabetes mellitus has been implicated in the development of cancer of the pharynx, oesophagus, colorectum, pancreas, cervix uteri, breast and prostate (2–5). In addition, type 2 diabetes mellitus is also known to be associated with the development of hepatocellular carcinoma (HCC) (6–8).

Type 2 diabetes mellitus is also frequently seen in patients with chronic hepatitis C virus (HCV) infection (9–11). Factors that are involved in the development of type 2 diabetes mellitus include not only life-style choices such as diet and exercise but also hepatic inflammation, fibrosis, steatosis, iron deposition and HCV core protein (10, 12–15). A high prevalence of HCC is seen in patients with HCV infection compared with patients with other

chronic liver diseases. Although the mechanism for HCV-related hepatocarcinogenesis is unclear, an association between type 2 diabetes mellitus and HCV infection could be responsible for the high prevalence of HCC in patients with HCV infection.

Hyperglycaemia is a common feature of type 2 diabetes mellitus. Hyperglycaemia increases oxidative stress, which causes oxidative DNA damage, an initial step in carcinogenesis (16). Moreover, hyperglycaemia is a factor leading to carcinogenesis because of immune suppression through the regulation of T-cell function (17). Thus, hyperglycaemia itself might stimulate hepatocarcinogenesis.

Hyperinsulinaemia combined with insulin resistance is another common feature of type 2 diabetes mellitus. Insulin is a growth-promoting hormone with mitogenic effects (18), and therefore could stimulate hepatocarcinogenesis. An increase in circulating insulin levels is also seen in diabetic patients treated with sulphonylureas or exogenous insulin. Indeed, exogenous insulin injection promotes colonic carcinogenesis in rats (19) and use of exogenous insulin significantly increases the risk of colorectal cancer among diabetic patients (20). Similarly, patients with type 2 diabetes exposed to sulphonylureas and exogenous insulin have a significantly increased risk of cancer-related mortality compared with patients exposed to metformin, a insulin sensitizer (21). Moreover, metformin reduces the risk of cancer in patients with type 2 diabetes (22). These findings suggest that pharmacologic effects of antidiabetic agents on circulating insulin levels play an important role in carcinogenesis. Despite the recognition of this potential link between type 2 diabetes mellitus and hepatocellular carcinoma, a role for antidiabetic agents in hepatocarcinogenesis has not been established.

Accordingly, in this study, we examined a possible association between antidiabetic therapies and an incidence of HCC in patients with HCV infection.

Methods

Study design and participants

A nested case–control study was conducted. Hepatitis C patients with diabetes mellitus were culled from the HCV-related diabetes mellitus study (HDMS), a hospital-based, prospective, multi-centre cohort. Patients with hepatitis C were consecutively recruited from three Japanese hospitals specialized for liver diseases (Kurume University Hospital, Nagata Hospital, and Chikugo City Hospital) from January 2004 to December 2008. Eligible participants were identified from all patients who were aged $\geq$40 years old, and had both a positive result for anti-HCV antibodies and a diagnosis of type 2 diabetes mellitus. The diagnosis of type 2 diabetes mellitus was based on the 2004 American Diabetes Association criteria (23) or use of any anti-diabetic agent. Participants in whom diabetes was diagnosed before age 30 with a positive result for pancreatic beta-cell autoantibodies (antiglutamic acid decarboxylase, anti-insulinoma-associated protein-2 or anti-islet-cell antibodies) were categorized as having type 1 diabetes mellitus and were excluded.

A total of 265 participants provided baseline data. The analysis was performed on the data of 241 participants, after excluding 24 participants because of unavailability of data on glucose metabolism, coincidence with other causes of liver disease such as chronic hepatitis B, autoimmune hepatitis, a metastatic liver tumour, or cholangiocellular carcinoma, taking corticosteroids or a history of pancreatitis or a pancreatic tumour. HCC was diagnosed by ultrasonic-guided biopsy, the non-invasive European Society of Study of the Liver criteria for the diagnosis of HCC or superparamagnetic iron oxide-enhanced magnetic resonance imaging (24, 25). All patients were classified into an HCC or a non-HCC group according to the incidence of HCC.

Informed consent for participation in this study was obtained from each participant. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in prior approval by the Ethics Committee of the Kurume University School of Medicine. None of the participants was institutionalized.

Measurements

Clinical data including age, sex and alcohol intake were collected at the first medical examination. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in metres (kg/m^2).

The diagnosis of cirrhosis was based on a liver biopsy or the aspartate aminotransferase (AST) to platelet ratio index (APRI); serum AST level (U/L)/upper limit of normal AST (33 U/L) $\times$ 100/platelet count ($\times$ 10^4/ml) (26). APRI is one of the models for predicting the stage of liver fibrosis. Patients with APRI values higher than 2.0 were diagnosed as having cirrhosis (26). In stratification analysis, the severity of liver disease was classified into non-cirrhosis or cirrhosis.

Venous blood samples were taken in the morning after a 12-h overnight fast. The following were measured using standard clinical methods: platelet count, plasma glucose, haemoglobin A1c (HbA1c), serum AST, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), albumin and total bilirubin levels (Department of Clinical Laboratory, Kurume University Hospital, Nagata Hospital and Chikugo City Hospital).

Classification of antidiabetic agents

Antidiabetic agents were classified into exogenous insulin (any type of insulin preparation), second-generation sulphonylurea (gliclazide or glybenclamide), third-generation sulphonylurea (glimepiride), α-glucosidase inhibitor (acarbose, voglibose or miglitol), glinide (nateglinide or mitiglinide), biguanide (metformin) or

thiazolidine (pioglitazone) according to each characteristic of these agents.

Statistical analysis

All data are expressed as mean ± standard deviation. Comparisons between the two groups were performed using the Mann–Whitney *U*-test for continuous variables and univariate analysis for discrete variables. In order to evaluate the possible importance of variables, a univariate analysis was performed initially. The relevant variables with univariate *P* values < 0.1 were selected for inclusion in the initial step of logistic analysis. Then, logistic regression analysis was used to identify any independent variable that was related to the incidence of HCC. The variables analysed were age (≥60 years old), sex, BMI (≥25 kg/m^2), alcohol intake (≥50 g/day), the incidence of cirrhosis, use of exogenous insulin, use of a second-generation sulphonylurea, use of biguanide, total bilirubin (≥2 mg/dl), albumin (< 3.5 g/dl), AST (≥40 U/L), ALT (≥40 U/L), LDH (≥230 U/L), ALP (≥360 U/L), γ-GTP (≥50 U/L), platelet count (< 10 × 10^4/μl), fasting plasma glucose (≥126 mg/dl) and HbA1c (≥5.9%). Stratification analysis was conducted to examine the impact of use of exogenous insulin and a second-generation sulphonylurea on the incidence of HCC. Stratification factors were severity of liver disease (non-cirrhotic patients, cirrhotic patients), hypoalbuminaemia (≥3.5 g/dl or < 3.5 g/dl) and sex (male or female). All the statistical tests were two-sided, and a *P* value of < 0.05 was considered to be statistically significant.

Results

Comparison of the characteristics and use of antidiabetic agents between hepatocellular carcinoma and non-hepatocellular carcinoma groups in hepatitis C patients with diabetes mellitus

Age, prevalence of males, the incidence of cirrhosis and fasting plasma glucose were significantly higher in the HCC group compared with those in the non-HCC group (Table 1).

There were no significant differences in the HbA1c levels between two groups. However, use of anti-diabetic agents was more frequent in the HCC group than that in the non-HCC group ($P = 0.0030$; Table 1). In order to investigate an association between HCC and the pharmacologic effects of anti-diabetic agents, anti-diabetic agents were classified into seven subgroups according to the characteristics of each agent: exogenous insulin, second-generation sulphonylurea, third-generation sulphonylurea, α-glucosidase inhibitor, glinide, biguanide or thiazolidine. There were no significant differences in the use of a third-generation sulphonylurea, α-glucosidase inhibitor, glinide, biguanide or thiazolidine between HCC and non-HCC groups. However, use of exogenous insulin and a second-generation sulphonylurea were significantly more frequent in the HCC group than those in the non-HCC group ($P = 0.0020$ and $P = 0.0003$, respectively; Table 1).

Table 1. Comparison of characteristics and use of anti-diabetic agents between hepatocellular carcinoma and non-hepatocellular carcinoma groups in hepatitis C patients with diabetes mellitus

	HCC ($n = 138$)	Non-HCC ($n = 103$)	*P* value
Age (year)	68.8 ± 8.0	64.7 ± 10.3	0.0032
Male	103	60	0.0083
BMI (kg/m^2)	22.9 ± 3.2	22.8 ± 3.5	0.4595
Excess alcohol intake (≥50 g/day)	29	12	0.0590
Cirrhosis	101	34	< 0.0001
Total bilirubin (mg/dl)	1.37 ± 1.96	1.09 ± 1.46	0.1171
Albumin (g/dl)	3.39 ± 0.53	3.82 ± 0.56	< 0.0001
AST (U/L)	65.4 ± 33.0	55.4 ± 32.8	0.0027
ALT (U/L)	56.9 ± 32.6	56.4 ± 40.5	0.3534
LDH (U/L)	229.6 ± 70.1	214.7 ± 65.9	0.0286
ALP (U/L)	392.0 ± 213.3	343.0 ± 161.3	0.0547
γ-GTP (U/L)	130.6 ± 239.5	74.8 ± 80.4	0.0046
Platelet count (× 10^4/μl)	10.5 ± 5.2	12.9 ± 5.2	0.0002
Fasting plasma glucose (mg/dl)	163.4 ± 70.1	146.1 ± 60.5	0.0230
HbA1c (%)	6.6 ± 1.1	7.1 ± 1.7	0.0905
Use of antidiabetic agents	98	53	0.0030
Exogenous insulin	43	14	0.0020
Second-generation sulphonylurea	26	4	0.0003
Third-generation sulphonylurea	22	20	0.4969
α-glucosidase inhibitor	25	15	0.4898
Glinide	8	11	0.2266
Biguanide	4	5	0.5024
Thiazolidine	1	4	0.1670

All data are expressed as mean ± standard deviation.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GTP, glutamyl transpeptidase; HCC, hepatocellular carcinoma; LDH, lactate dehydrogenase.

Variables associated with the incidence of hepatocellular carcinoma in hepatitis C patients with diabetes mellitus

In the univariate analysis, age (≥60 years old), being male, cirrhosis, albumin (< 3.5 g/dl), AST (≥40 U/L), LDH (≥230 U/L), ALP (≥360 U/L), platelet count (< 10 × 10^4/μl) and HbA1c (≥5.9%) were significant variables associated with the incidence of HCC (Table 2). In addition, use of exogenous insulin and a second-generation sulphonylurea were significant variables associated with the incidence of HCC [odds ratio (OR) 2.877, 95% confidence interval (CI) 1.474–5.617, $P < 0.0020$ and OR 5.746, 95% CI 1.938–17.038 respectively: Table 2).

In the logistic regression analysis, age (≥60 years old), being male, cirrhosis and albumin (< 3.5 g/dl) were independent factors associated with a greater incidence of HCC (Table 2). Moreover, use of exogenous insulin

Table 2. Univariate and logistic regression analyses for the incidence of hepatocellular carcinoma in all patients ($n = 241$)

	Univariate analysis			Logistic regression analysis		
Variable	HR	95% CI	*P* value	HR	95% CI	*P* value
Age (≥60 years)	2.270	1.221–4.222	0.0096	2.781	1.231–6.283	0.0139
Male	2.109	1.219–3.649	0.0076	3.075	1.511–6.258	0.0019
BMI (≥25 kg/m^2)	1.085	0.559–2.105	0.8092			
Excess alcohol intake (≥50 g/day)	2.018	0.974–4.179	0.0588			
Cirrhosis	5.540	3.173–9.672	< 0.0001	3.366	1.465–7.731	0.0042
Total bilirubin (≥2 mg/dl)	2.537	0.801–8.028	0.1133			
Albumin (< 3.5 g/dl)	5.078	2.843–9.070	< 0.0001	3.008	1.373–6.591	0.0059
AST (≥40 U/L)	2.447	1.403–4.267	0.0016	1.690	0.797–3.582	0.1709
ALT (≥40 U/L)	1.441	0.856–2.427	0.1694			
LDH (≥230 U/L)	1.919	1.099–3.350	0.0220	0.936	0.436–2.008	0.8650
ALP (≥360 U/L)	1.912	1.100–3.325	0.0216	1.044	0.499–2.187	0.9084
γ-GTP (≥50 U/L)	1.565	0.929–2.637	0.0926			
Platelet count (< 10×10^4/μl)	2.000	1.177–3.398	0.0104	0.913	0.401–2.079	0.8285
Fasting plasma glucose (≥126 mg/dl)	1.559	0.9296–2.624	0.0945			
HbA1c (≥5.9%)	0.451	0.219–0.929	0.0308	0.654	0.268–1.596	0.3511
Use of exogenous insulin	2.877	1.474–5.617	0.0020	2.969	1.293–6.819	0.0103
Use of second-generation sulphonylurea	5.746	1.938–17.037	0.0016	6.831	1.954–23.881	0.0026
Use of third-generation sulphonylurea	0.787	0.404–1.535	0.4823			
Use of α-glucosidase inhibitor	1.298	0.646–2.609	0.4641			
Use of glinide	0.515	0.199–1.330	0.1702			
Use of biguanide	0.585	0.153–2.235	0.4332			
Use of thiazolidine	0.181	0.020–1.641	0.1285			

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GTP, glutamyl transpeptidase; HR, hazard ratio; LDH, lactate dehydrogenase.

and a second-generation sulphonylurea were also identified as independent variables associated with an incidence of HCC (OR 2.969, 95% CI 1.293–6.819, $P = 0.0103$, OR 6.831, 95% CI 1.954–23.881, $P = 0.0026$ respectively; Table 2). Use of a second-generation sulphonylurea showed the highest OR among all the variables (Table 2).

Variables associated with the incidence of hepatocellular carcinoma in stratification analysis by severity of liver disease

All patients were stratified into two subgroups: a non-cirrhosis or a cirrhosis group. In patients with cirrhosis, age (≥60 years old) and albumin (< 3.5 g/dl) were identified as independent factors associated with the incidence of HCC in the logistic regression analysis (Table 3). However, use of any antidiabetic agent was not an independent factor associated with the incidence of HCC in patients with cirrhosis (Table 3).

In non-cirrhotic patients, not only being male but also use of exogenous insulin and a second-generation sulphonylurea were determined to be independent variables associated with a greater incidence of HCC (Table 3).

Table 3. Logistic regression analysis for the incidence of hepatocellular carcinoma by stratification according to severity of liver disease

	Logistic regression analysis		
Variables	OR	95% CI	*P* value
Chronic hepatitis ($n = 107$)			
Male	6.150	1.705–22.185	0.0055
Use of exogenous insulin	4.142	1.072–16.007	0.0393
Use of second-generation sulphonylurea	4.822	0.963–24.144	0.0556
AST (≥40 U/L)	1.506	0.530–4.285	0.4423
γ-GTP (≥50 U/L)	2.234	0.759–6.578	0.1444
Albumin (< 3.5 g/dl)	3.632	0.940–14.031	0.0614
Cirrhosis ($n = 134$)			
Age (≥60 years)	3.357	1.335–8.440	0.0100
Albumin (< 3.5 g/dl)	2.402	1.061–5.436	0.0355

AST, aspartate aminotransferase; CI, confidence interval; GTP, glutamyl transpeptidase, OR, odds ratio.

Variables associated with the incidence of hepatocellular carcinoma in stratification analysis by hypoalbuminaemia

All patients were stratified into two subgroups: ≥3.5 g/dl of albumin or < 3.5 g/dl of albumin group. In patients with < 3.5 g/dl of the albumin, being male and age (≥60 years old) were associated with the incidence of HCC in the logistic regression analysis (Table 4). Use of exogenous insulin or a second-generation sulphonylurea was not determined to be an independent variable, while use of biguanide was negatively associated with the incidence of HCC (Table 4).

In patients with ≥3.5 g/dl of albumin, not only being male and cirrhosis but also use of a second-generation sulphonylurea were determined to be independent variables associated with a greater incidence of HCC (Table 4). Use of a second-generation sulphonylurea showed the

Table 4. Logistic regression analysis for the incidence of hepatocellular carcinoma by stratification according to hypoalbuminaemia

	Logistic regression analysis		
Variable	OR	95% CI	*P* value
≥3.5 g/dl of albumin (*n* = 139)			
Male	2.536	1.066–6.034	0.0353
Cirrhosis	2.830	1.096–7.308	0.0317
Use of exogenous insulin	2.557	0.973–6.718	0.0567
Use of second-generation sulphonylurea	5.195	1.338–20.171	0.0173
AST (≥40 U/L)	1.602	0.696–3.691	0.2682
LDH (≥230 U/L)	1.777	0.696–4.535	0.2289
Platelet count (< 10×10^4/μl)	1.200	0.445–3.237	0.7183
< 3.5 g/dl of albumin (*n* = 102)			
Male	4.922	1.562–15.502	0.0065
Age (≥60 years)	3.357	2.178–28.454	0.0016
Use of biguanide	0.060	0.004–0.846	0.0371

AST, aspartate aminotransferase; CI, confidence interval; LDH, lactate dehydrogenase; OR, odds ratio.

highest OR in patients with ≥3.5 g/dl of albumin (OR 5.195, 95% CI 1.338–20.171, $P = 0.0173$; Table 4).

Variables associated with the incidence of hepatocellular carcinoma in stratification analysis by sex

All patients were stratified into two subgroups: a male or a female group. In male patients, age (≥60 years old) and albumin (< 3.5 g/dl) were identified as independent factors associated with the incidence of HCC in the logistic regression analysis (Table 5). Moreover, use of a second-generation sulphonylurea was also identified as an independent variable associated with a greater incidence of HCC (OR 4.267, 95% CI 1.046–17.412, $P = 0.0431$; Table 5).

In female patients, cirrhosis was identified as an independent factor associated with an incidence of HCC in the logistic regression analysis (Table 5). Use of a second-generation sulphonylurea was also identified as an independent variable associated with a higher incidence of HCC in female patients and its OR was higher than that in male patients (Table 5).

Discussion

We conducted a hospital-based nested case–control analysis in order to identify variables associated with an increasing incidence of HCC. Besides known variables such as ageing, being male, cirrhosis, and hypoalbuminaemia, we found that use of exogenous insulin and a second-generation sulphonylurea that increase circulating insulin levels were independent variables associated with a greater incidence of HCC. In addition, the impact of the use of exogenous insulin and a second-generation sulphonylurea was more evident in patients who did not have cirrhosis or showed ≥3.5 g/dl of albumin.

Table 5. Logistic regression analysis for the incidence of hepatocellular carcinoma by stratification according to sex

	Logistic regression analysis		
Variable	OR	95% CI	*P* value
Male (*n* = 163)			
Age (≥60 years)	2.807	1.114–7.073	0.0286
Cirrhosis	2.298	0.805–6.564	0.1201
Use of exogenous insulin	2.195	0.827–5.825	0.1143
Use of second-generation sulphonylurea	4.267	1.046–17.412	0.0431
AST (≥40 U/L)	1.665	0.678–4.087	0.2656
LDH (≥230 U/L)	1.094	0.434–2.756	0.8486
ALP (≥360 U/L)	1.482	0.605–3.630	0.3890
Platelet count (< 10×10^4/μl)	0.717	0.249–2.067	0.5383
Albumin (< 3.5 g/dl)	3.516	1.299–9.518	0.0133
Total bilirubin (≥2 mg/dl)	0.986	0.095–10.199	0.9905
Female (*n* = 78)			
Cirrhosis	16.710	2.743–101.785	0.0023
Use of exogenous insulin	4.985	0.868–28.618	0.0716
Use of second-generation sulphonylurea	50.993	3.011–863.633	0.0065
HbA1c (≥5.9%)	0.472	0.083–2.682	0.3968
LDH (≥230 U/L)	0.753	0.169–3.366	0.7107
Platelet count (< 10×10^4/μl)	1.800	0.393–8.247	0.4491
Albumin (< 3.5 g/dl)	1.740	0.348–8.700	0.4998

ALP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval; GTP, glutamyl transpeptidase; LDH, lactate dehydrogenase; OR, odds ratio.

Use of antidiabetic agents was a variable associated with a greater incidence of HCC in this study. Thus, we found a possible association between antidiabetic agents and the risk of HCC. Among antidiabetic agents, use of exogenous insulin and a second-generation sulphonylurea were significant variables associated with the incidence of HCC. Hyperinsulinaemia combined with insulin resistance is considered as a promoter for hepatocarcinogenesis and tumour growth (27, 28). Because exogenous insulin and a second-generation sulphonylurea increase circulating insulin levels, we hypothesize that the use of these antidiabetic agents accelerates the development of HCC in patients with HCV infection. Use of a third-generation sulphonylurea or an α-glucosidase inhibitor was not a variable associated with the incidence of HCC in this study. A third-generation sulphonylurea is categorized as a sulphonylurea because it contains a sulphonylurea structure. However, a third-generation sulphonylurea is known to improve hyperinsulinaemia through extra-pancreatic effects (29). In fact, a preliminary study in our HDMS cohort showed that treatment of the third generation sulphonylurea causes a reduction of serum insulin levels in hepatitis C patients with diabetes mellitus (data not shown). α-glucosidase inhibitors reduce post-prandial glucose elevation by delaying the release of glucose from disaccharides and complexes of carbohydrates and do not promote insulin secretion

(30). In addition, use of biganide, an insulin sensitizer, was negatively associated with the incidence of HCC in hepatitis C patients with < 3.5 g/dl of albumin. Furthermore, subanalysis of our database showed that serum fasting insulin levels and the homeostasis model assessment of insulin resistance (HOMA-IR) value, an index for insulin resistance, were significantly higher in the HCC group compared with that in the non-HCC group [fasting insulin levels; HCC group 16.1 ± 16.8 μIU/ml ($n = 90$); non-HCC group 12.3 ± 11.9 μIU/ml ($n = 88$); $P = 0.0036$, HOMA-IR value; HCC group 4.72 ± 4.95; non-HCC group 3.94 ± 4.35; $P = 0.0404$; data not shown], although serum insulin levels were not routinely measured in all patients. In good agreement with our findings, the significance of hyperinsulinaemia or the use of exogenous insulin has been associated with the development of colon cancer (20, 31). Thus, the characteristics of antidiabetic agents, subanalysis for serum fasting insulin levels and previously reported facts support our hypothesis.

Recently, in patients with type 2 diabetes mellitus, a direct association of HCC with insulin and sulphonylurea treatment has been reported by Donadon *et al.* (32, 33). Our results were in good agreement with the previous report and further provided new information for the impact of the use of exogenous insulin and a second-generation sulphonylurea. By focusing on hepatitis C patients with diabetes mellitus, a more homogenous group, we found that the use of exogenous insulin and a second-generation sulphonylurea were more strongly associated with an incidence of HCC in patients who were not cirrhotic or showed ≥ 3.5 g/dl of albumin than in patients who were cirrhotic or showed < 3.5 g/dl of albumin. Severity of liver disease is also one of the strongest factors of hepatocarcinogenesis. Therefore, in cirrhotic patients, carcinogenic activity of exogenous insulin and a second-generation sulphonylurea may be drowned out by the carcinogenic activities of cirrhosis. Similar findings were obtained in patients with hypoalbuminaemia (< 3.5 g/dl of albumin), a parameter for severity of liver disease. On the other hand, risk for developing HCC is decreased in patients with non-cirrhotic liver disease (34, 35) and ≥ 3.5 g/dl of albumin (36). Thus, we assume that hepatocarcinogenic activities of exogenous insulin and a second-generation sulphonylurea stand out in patients who have such negative factors for the development of HCC. Although sex affects the development of HCC and females are less prone to HCC than males (34, 37), the numbers of the female subset were small and the confidence intervals were large in this study. Therefore, we have to be cautious when interpreting the data and further elucidation is required for sex differences in an association between antidiabetic agents and an increased incidence of HCC.

Kath *et al.* (38) examined an association between the incidence of malignancy and the use of exogenous insulin and reported that insulin treatment is not a risk factor for developing malignancy. Although the reason for this discrepancy is unclear, it might be explained by the fact that only 28 patients developed cancers from 2720 patients in their study. A relatively small number of patients with cancers is one possible reason. More recently, Colhoun and the Scottish Diabetes Research Network (SDRN) Epidemiology Group reported that exogenous insulin use is not associated with an increased risk of site-specific cancers (39). The reason for this discrepancy is also unclear. However, a possible explanation is that the surveyed cancers are different from HCC. In their study, breast, prostate, colorectal, lung and pancreatic cancers were examined. Accordingly, use of exogenous insulin and a second-generation sulphonylurea may only have a significant role in the development of cancer when patients have carcinogenic factors such as an HCV infection. In support of this hypothesis, Donadon *et al.* (32, 33) recently reported a direct association of HCC with insulin and sulfonylurea treatment.

Mechanisms underlying the association of use of exogenous insulin and a second-generation sulphonylurea with HCC risk are uncertain. However, several possibilities exist. Firstly, insulin is an important mitogen and stimulates cell proliferation (18). Insulin directly upregulates intracellular molecules involved in cell proliferation such as mitogen-activated protein kinase by binding to insulin receptors (40). In addition, suppressors of intracellular insulin signalling such as tensin homology deleted on chromosome 10 (41) and SH2 domain-containing inositol phosphatase-2 (7) are downregulated in HCC and therefore insulin effects are considered to be potentiated in HCC. Secondly, insulin also binds insulin-like growth factor (IGF)-1 receptor (42), resulting in the activation of tyrosine kinase and a cascade of intracellular responses. Moreover, insulin inhibits the binding of IGF-1 to IGF-binding proteins and the subsequent increase in IGF-1 levels. The IGF system is a potent growth regulator closely associated with carcinogenesis (43).

Use of a second-generation sulphonylurea was more associated with the incidence of HCC than use of exogenous insulin in all analyses of this study. The reason for the difference in the incidence of HCC between these antidiabetic agents is uncertain. However, one would think that the route of insulin delivery (portal vein or subcutaneous tissue) is a possible reason, because insulin actions in the liver depend on the insulin concentration in the portal vein rather than that in the peripheral vein. Alternatively, sulphonylurea increases not only endogenous insulin secretion but also its precursors which might have mitogenic effects by themselves (44). Similarly, Donadon *et al.* (33) reported that the prevalence of sulphonylurea treatment is higher than that of insulin treatment in patients with HCC.

The main limitation of this study is the study design. A nested case–control analysis is not ideally suited to examine the causal relationship between anti-diabetic agents and the development of HCC. However, the incidence of HCC has rapidly increased over the past 20 years, making HCC one of the fastest-growing causes of

cancer-related death (34). Thus, possible factors associated with the incidence of HCC should be urgently determined. A nested case–control analysis has the advantages of prompt elucidation for association between antidiabetic agents and the development of HCC, and we used a nested case–control analysis in this study. However, some confounding factors including a selection bias may exist in this study and prospective long-term cohort studies are needed. Another limitation of this study is that we did not clarify the types of exogenous insulin. The mitogenic potency of insulin glargine (21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin) is eight-fold higher than that of human insulin (45). Insulin glargine has recently been reported to increase the incidence rate of cancer (39, 46, 47). However, the carcinogenic activity of glargine is still controversial (48–50) and further study should be focused on an association between types of insulin analogues and the incidence of HCC.

In conclusion, we found that the use of exogenous insulin and a second-generation sulphonylurea was an independent variable associated with an incidence of HCC using a hospital-based nested case–control analysis. In addition, an association between the use of these antidiabetic agents and the incidence of HCC was more evident in patients who were non-cirrhotic or showed ≥ 3.5 g/dl of albumin.

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Branched-Chain Amino Acids and Pigment Epithelium-Derived Factor: Novel Therapeutic Agents for Hepatitis C Virus-Associated Insulin Resistance

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Abstract: Recent clinical studies have shown that patients with chronic liver disease are insulin resistant. Of all etiologies of chronic liver disease including non-alcoholic fatty liver disease, the one that causes the most sever insulin resistance is hepatitis C virus (HCV) infection. Since insulin resistance promotes inflammatory and fibrogenic reactions in the liver, thus leading to the development of liver cirrhosis and hepatocellular carcinoma (HCC) in patients with HCV infection, amelioration of insulin sensitivity may inhibit the progression of HCV-associated liver disease, and could improve the survival of these patients. HCV directly causes insulin resistance through HCV core protein-elicited proteasomal degradation of insulin receptor substrates and subsequent inactivation of intracellular insulin signaling molecules such as Akt. Furthermore, tumor necrosis factor-alpha (TNF-α) and/or triglyceride accumulation-induced nuclear factor-κB (NF-κB) activation in the liver is shown to play a role in insulin resistance in patients with HCV-related chronic liver disease as well. We, along with others, have recently found that branched-chain amino acids (BCAAs) and pigment epithelium-derived factor (PEDF) could improve the HCV-associated insulin resistance *via* suppression of NF-κB and preservation of insulin signaling pathway. In this review, we discuss the mechanisms for the actions of BCAAs and PEDF, and their clinical implications in insulin resistance of chronic liver disease in patients with HCV infection. We also discuss here which chemical structures could contribute to insulin-sensitization in patients with HCV infection.

Keywords: Hepatitis C virus, insulin resistance, branched-chain amino acids, pigment epithelium-derived factor, insulin receptor substrate, suppressor of cytokine signaling, nuclear factor-kappaB, peroxisome proliferator-activated receptor.

INTRODUCTION

Worldwide, more than 170 million people are infected with hepatitis C virus (HCV) [1-3]. HCV infection causes chronic liver diseases such as liver cirrhosis and hepatocellular carcinoma (HCC), and HCV-associated liver disease is currently one of the most common reasons for receiving liver transplantation [4]. Therapeutic options, including a combination therapy with pegylated interferon and ribavirin, are far from satisfactory. They lead to a successful outcome in only about 50% of patients with chronic HCV infection because of severe adverse effects [5, 6]. In addition, patients with HCV infection are not always candidates for interferon-based therapies [7]. Thus, HCV is still a main cause of death in chronic liver disease patients [8, 9] and, therefore, the development of new therapeutic approaches is urgently desired.

Recent clinical studies have shown that patients with chronic liver disease are insulin resistant. Of all etiologies of chronic liver disease including non-alcoholic fatty liver disease, the one that causes the most sever insulin resistance is HCV infection [10-17]. Insulin resistance decreases response to antiviral treatment [18-21], promotes progression of hepatic fibrosis [22-24] and esophageal varices [25], increases the risk for the development of HCC [26-29], and is also a sign for poor prognosis in patients with HCV infection [30]. Furthermore, insulin resistance is associated with extrahepatic manifestations of HCV infection such as lichen planus, abnormal thyroid function, and rheumatoid arthritis as well [31, 32]. These observations suggest that insulin resistance plays a pathological role in various intrahepatic and extrahepatic derangements in patients with HCV infection and is a novel therapeutic target for improving the survival and quality of life in these patients.

Although awareness of the health risk of insulin resistance and the development of diabetes has increased, no common pharmaceutical agents are yet available for treating HCV-associated insulin resistance. Exogenous insulin injection and oral hypoglycemic agents such as sulfonylureas are not suitable remedies for the treatment of HCV-associated insulin resistance because (1) most of the patients with HCV infection are hyperinsulinemic, rather than hypoinsulinemic [10, 15-21, 24, 25, 31-37], and (2) insulin is a growth–promoting hormone [38, 39] and use of exogenous insulin or sulfonylureas may be associated with an increased incidence of HCC [35, 40-43]. Although biguanides and thiazolidinediones could improve insulin resistance, and a recent clinical trial shows that metformin improves rapid viral response rate of patients with HCV infection treated by combination therapy with peginterferon and ribavirin [44], they are not always recommended for patients with HCV infection. Indeed, biguanides could predispose to lactic acidosis in patients with severe liver dysfunction [45]. Thiazolidinedione may cause overproduction of hydrogen peroxide and subsequent severe hepatotoxicity in some patients [46]. Lactic acidosis and severe hepatotoxicity are rare adverse effects, however, they sometimes become life-threatening complications in patients with chronic liver diseases.

HCV directly causes insulin resistance through HCV core protein-elicited proteasomal degradation of insulin receptor substrates and subsequent inactivation of intracellular insulin

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