●看護に活かすQOLの視点

疾患別QOL向上に向けた実践

肝臓病とQOL

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要旨　慢性肝炎・肝硬変・肺がんは言うに及ばず，肝炎ウイルス感染状態のみでもQOLの低下が起こりうることを理解し，そのうえで，インターフェロン・核酸アナログ製剤の特性を理解した慢性肝炎治療，内科的・外科的特徴を把握した肝がん治療を考慮する必要がある。

Key Words　肝炎ウイルス，ペグインターフェロン，自己注射，ラジオ波凝固療法，QOL，慢性肝炎

はじめに

QOL (quality of life)の概念は，1948年にKarnofskyが患者の日常動作を定量化し，「performance scale」として報告したことに端を発している。欧米では1976年にCampbellがQOLを「個人のすべての経験からもたらされる健康状態に関する主観」と定義し，わが国でも医療の有用性を評価する指標の一つとして重要視されるに至っている。この「個人の主観」であるQOLを評価するためにさまざまの質問票が，包括的あるいは疾患特異的尺度として欧米を中心に開発されてきた。

1984年にはSchipperらがQOL評価のための質問票であるFunctional Living Index-Cancer (FLIC)を発表した。一方，Torranceらが1970年代より提唱したutility theory (効用値理論)のQOL評価への応用は，1980年代にQuality-Adjusted Life Years (QALYs)の確立へと発展した。1980年代後半に開発されたMedical Outcomes Study 36-Item Short-Form Health Survey (SF-36)は，各国語訳が完成して国際的に汎用されている健康関連QOL尺度である。がん治療におけるQOLの評価法としては，1993年にEuropean Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core Module (EORTC QLQ-C30)やFunctional Assessment of Cancer Therapy(FACT)が発表されており，肝がん患者の質問票としても使用されている。

肝疾患におけるQOL評価は，これら質問票による主観的な尺度をもとにした研究のほか，実際の臨床で行われている通院診療・受ける診療手技・治療内容・診療に要する時間などを分析した「客観的」指標の分析からも行われている。

慢性肝炎・肝硬変・肝がん患者のQOL

慢性肝疾患患者を対象としたQOLの調査では，質問票としてSF-36を用いたprospectiveな研究が多数報告されている。

三輪ら[1]はQOLの調査で慢性肝炎においてQOLが低下していることを報告している。福原ら[2]は，C型慢性肝疾患480例を対象にSF-36を用いてQOLを調査し，Child Bの肝硬変例では慢性肝炎やChild Aの肝硬変症例に比較して，6項目でのスコアが有意に低下していることを明らかにした。C型慢性肝炎患者は健常人と比較し

て，SF-36の８項目でスコアが低下していることも報告されている[3)-5)]。Younossi ら[6)]は，慢性肝疾患症例について，疾患特異的なアンケートを作成し，health-related QOL の低下について報告している。

肝移植を施行した原発性胆汁性肝硬変および原発性硬化性胆管炎患者157例の調査は，肝移植後に QOL が改善することが明らかになっている[7)]。

厚生労働省研究班(班長：藤原研司)による肝がん患者の QOL 研究(平成14～16年度)では，治療前後および治療の内容により QOL の経過観察が行われた。全国10施設178例に行われた Prospective 研究で経時的な SF-36および追加的アンケートが行われた。肝がん治療後３カ月で，手術(肝切除)群の RP(日常役割機能)スコアは低下し，ラジオ波凝固療法(RFA)群との間に有意差がみられた。PF (身体機能)・BP (体の痛み)スコアは各治療法で低下し，GH (全体的健康感)は改善する傾向を示した。手術群では治療前に比して治療後の BP (体の痛み)が有意に低値であった。治療後の皮膚症状のスコアは，手術群に比べ RFA 群では有意に高値であった。肝がん治療後，TAE 群の「経済的負担感」スコアは低下し，手術群との間に有意差を認めた。治療後３カ月までの QOL に関しては，治療時の痛みをコントロールできれば，RFA 治療後の QOL は他の治療法に比べて良好である可能性が示された[8)]。

C型肝炎とうつ病との関連

C型肝炎ウイルス感染(慢性C型肝炎)があって，これに罹患していることを患者に知らしめると，患者の「疲労感」や「うつ状態」が発生することが知られている[9)]。

初回献血者のなかで HCV 感染を知っていた人と知らなかった人の間には「疲労感」「うつ状態」の頻度に差がないことが知られており，HCV に感染しているから慢性的な疲労感を起こすわけではない[10)]。また，原因としてウイルス感染が疑われる慢性疲労症候群患者の HCV 感染頻度は，正常人より高くないことも知られている[11)]。C型肝炎患者に「うつ状態」の患者はしばしばみられる。この原因として，病原体を体内に保有していているという自覚，感染からくる社会での疎外感や差別，感染離脱が容易ではなく肝硬変・肝細胞がんに進行するという知識など多くの因子が関連している。

C型肝炎に対するインターフェロン治療の際の副作用としての「うつ病」は極めて重大な問題であり，これまでこの発症頻度や危険因子などを報告した論文は多い[12)]。これまでに報告されているうつ病の頻度は，３～45%と幅があり，その診断基準の相違なども少なくないので評価は難しい。

わが国でのインターフェロン治療に伴ううつ病の頻度は欧米に比べてやや低いと推定されている。ベータ型インターフェロンによる治療はアルファ型インターフェロンによる治療よりも，うつ病発生率が著しく低いため，うつ状態やその既往のある患者にインターフェロン治療を行う場合には，α型は避けるべきである。

慢性肝炎に対するインターフェロン治療と QOL

1. インターフェロンの種類による副作用の相違

わが国では，B型・C型慢性肝炎に対して使用できるインターフェロンとして，大きく分けるとα(アルファ)型とβ(ベータ)型の２種類が使用されている。ペグインターフェロンはα型に属する。インターフェロン治療を行う際に，治療初期にみられるインフルエンザ様症状にα・β両者の間に大差はなく，QOL の点で比較すべき副作用はない。しかし，βインターフェロンは，副作用としての精神症状・うつ病の発生，および治療数カ月後に目立つ脱毛の発生頻度が明らかに少ない。うつ状態やその既往のある患者にインターフェロン治療を行う場合には，α型は避けるべきである。QOL を損なわない観点から，勤務内容や生活習慣上，精神的な問題が起こることが想定される場合には，β型を積極的に採用する必要がある。一方，脱毛も女性患者を中心に非常に苦痛な症状となることが多く，QOL に直接影響する重大副作用であることも考慮すべきである。

2. ペグインターフェロンによる治療

ペグインターフェロンは，従来のアルファ型インターフェロンにポリエチレングリコール(PolyEthyleneGlycol)を結合させた，「高分子型」のインターフェロンであり，その頭文字を

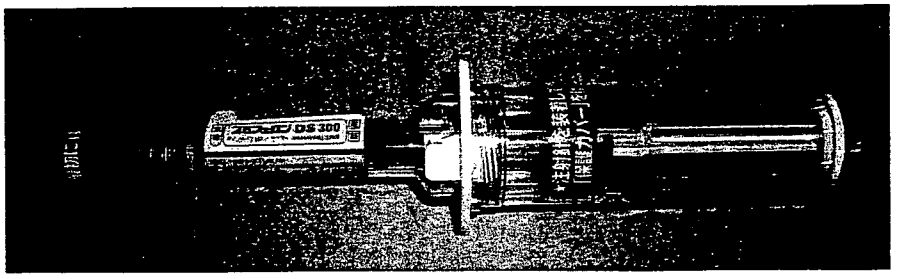


図1●自己注射用のインターフェロン製剤

図2●インターフェロン注射器の廃棄ボックス

とってペグ(Peg)インターフェロンといわれる。わが国では，ペグの分子量12kDaのインターフェロン(商品名：ペグイントロン)と40kDaのペグインターフェロン(商品名：ペガシス)が使用可能である。いずれもリバビリン併用治療として使用されることが多く，ウイルス排除をめざす「根治目的」で使用される場合がほとんどである。わが国で行われた治験の成績では，ウイルス排除効果は後者がやや優れているが，副作用(間質性肺炎・血小板減少)もやや強いため頻回の通院が義務づけられているため，個々の患者について，いずれのタイプのインターフェロンを使用するかの適応が決められることが多い。

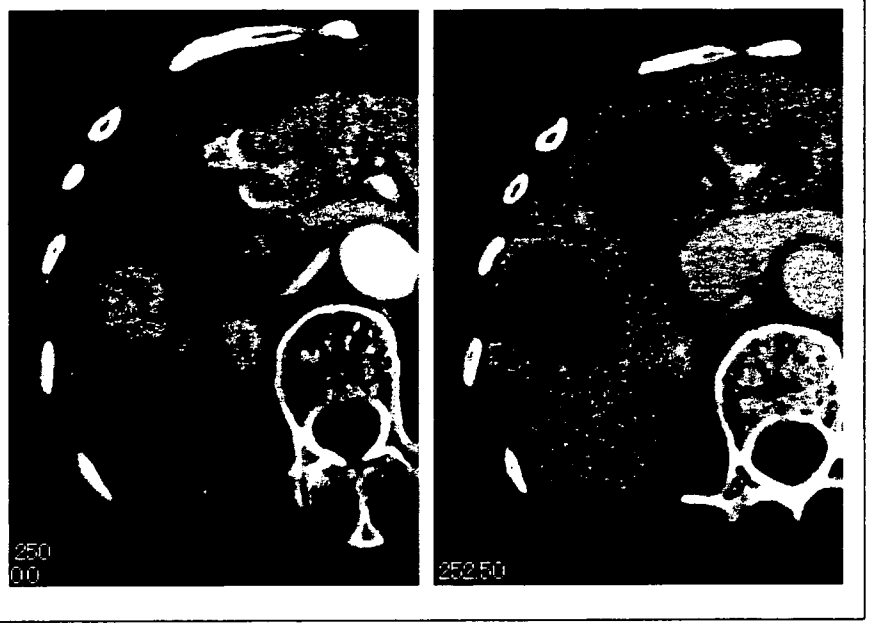
3．インターフェロン自己注射

C型慢性肝炎では，インターフェロン自己注射が保険診療可能で，広く行われるに至っている。自己注射は，最低限の注射手技が行える患者であれば，素人にも行えるように注射器に工夫がなされている(**図1**)。現在行えるインターフェロン自己注射は，インターフェロン単独(リバビリン併用ができない)のみであり，1b型高ウイルス量の難治性肝炎に対するウイルス排除効果は不良である。インターフェロン自己注射そのものは，多発性骨髄腫・腎がんその他の疾患で1990年より認可されている治療法であり，C型慢性肝炎に対する認可は2005年と15年遅れた保険承認となっている。C型慢性肝炎に対するインターフェロン自己注射の保険承認が遅れた理由の一つとして，感染性医療廃棄物処理の問題があげられる。

QOLの視点からみたインターフェロン自己注射のメリットは大きく2つある。1つは言うまでもなく，病院に通院しなくてもインターフェロンを注射できるという「時間的」「経済的」な面であり，職場を離れなくても治療継続できるという勤労者にとっての利便性は大きい。

もう1つのメリットは「医学的」理由で，夜間・就寝前にインターフェロンを注射することによる副作用の軽減である。就寝前にインターフェロン注射を行うと，発熱・インフルエンザ様症状・精神的影響その他の副作用が著しく少なくなることが明らかとなっている。コーチゾルの日内変動・脳内アミン・メラトニンなどの観点から種々の研究がなされているが，その詳細な理由はまだ明らかではない。インターフェロンの自己注射は，夜間・就寝前に射ってこそ，QOLからの観点で価値があることを，医師・看護師は患者に十分知らせる必要がある。

自己注射によって損なわれるQOLは，「看護師・医師によらない注射なので，どこか心配」という漠然とした不安があるが，約1カ月の注射続行でほぼ解決できる。また，やや大型の注射器の廃棄ボックス(**図2**)を月2回の



a) スクリーニングで発見された小型肝癌(ダイナミックCT像)。

b) ラジオ波凝固療法で良好な壊死に陥った小型肝癌(ダイナミックCT像)。

図3●ラジオ波凝固療法

病院通院時に運ばなければならない「面倒くささ」を患者が訴える場合もあるが,「社会的」「医学的」メリットの大きさを考えると問題にならない。

2007年7月現在,インターフェロン自己注射は,B型肝炎患者には認可されていない。

B型肝炎に対するインターフェロンと核酸アナログ製剤治療

B型慢性肝炎に対する原因療法としての抗ウイルス治療には,インターフェロン注射と核酸アナログ製剤(エンテカビル,ラミブジン,アデホビル)内服の,2つの方法がある。厚生労働省の示しているB型慢性肝炎治療ガイドラインでは,35歳以上の患者では核酸アナログ製剤長期内服,35歳未満ではインターフェロン治療と核酸アナログ製剤の治療のいずれかを選択することが指針となっている。

年齢によってインターフェロン治療を推奨しない理由にはいくつかある。インターフェロンによるe抗原陰性化・HBVDNAの安定低値化率は若年で有意に高率で,治癒確率が高い。また,核酸アナログ製剤は数年以上の長期内服をせねばならないのに比し,余命の長い若年者では,6カ月～1年の比較的短期で治療が完結するインターフェロン治療のほうが適している場合もある。

一方,DNA陰性化率・ALT正常化率では,一般に核酸アナログ製剤のほうが高率であり,また,インフルエンザ様症状やうつ病などの副作用もないため,これ以上の年齢では,インターフェロンより核酸アナログ製剤を第一に選択することが推奨される。

肝がん診療をめぐるQOLの問題

1. 肝がんスクリーニングのための定期検診

C型慢性肝炎からは年率1～2%,C型肝硬変からは年率5～8%の肝がん発がんが起こる。小型肝がんであれば,侵襲の少ない経皮的局所治療で十分な治療が行えるため,肝がんが発生しやすい高危険群を設定し,これらの患者に超音波検査を主とする画像診断と腫瘍マーカーを定期的に測定することによる「肝がんスクリーニング」が行われる。このスクリーニングが励行されて肝がんが発見された場合には,肝切除・経皮的局所治療などの「根治的治療」の行える確率が増す[13]。発見された肝がんが3cm・3個以内程度の比較的「早期」であった場合には,侵襲の低い経皮的局所治療が行える機会が増す。

定期的なスクリーニング検査自身は,通院・医療費出費などの負担を患者にもたらすが,肝疾患の直接の生命予後に最も関連の強い肝細胞がん発見のためであることを十分に患者に理解してもらう必要がある。肝がん早期発見の臨床は,もともと患者QOLの立場からは心地よいものではないことを十分に周知すべきである。

2. 肝がん診断・告知に伴うQOLの問題

定期的画像スクリーニングで肝がんの早期発見をめざしていた患者では,肝がん出現に対する心の準備状態がで

きていることもあるが，肝がん発見に伴う心の痛手はやはり少なくない。無症状で検診を受けていなかった患者や，若年者，肝疾患に対する予備知識のない患者などでは，一時的な抑うつ状態になることもありうる。一般的に難治性疾患・悪性腫瘍に罹患したことを告知するのと同様の精神的ダメージになりうることを考慮する。さらに，慢性肝疾患を基礎に発がんした場合には，その後も再発を繰り返し予後が必ずしも良くないという知識を有している患者もいるため，時間をかけた対処が必要になることもある。

肝がん治療法別にみたQOLの相違

肝がんが3cm以下の小型で発見された場合には，根治的治療法として，外科的切除術もしくはラジオ波凝固療法が選択される場合が多い。経皮的ラジオ波凝固療法では，局所麻酔下に経皮的に施行可能であるため広く全国で行われているが，壊死を免れた残存肝がんの状態とならないため，多くの施設では腫瘍周囲5mm以上の安全域を確保して焼灼・凝固することをめざして行われている(**図3a，b**)。

根治性・再発率の点では，2cm以下の肝がん治療では，外科切除・ラジオ波凝固療法の両者は互角であるが，2cmを超える腫瘍では，ラジオ波凝固療法での局所再発率がわずかに高いのではないかと考えられている。QOLはともかく，「腫瘍の完全除去」を追求するのであれば外科切除のほうが「医学的な根治性」はやや優位である。実際には，3cm以内の肝がんにラジオ波凝固療法を行ったあと，局所的に腫瘍が残存しただけでは，追加治療により容易に対処可能であり，生命予後に影響することはまれである。医療経済的な側面も患者のQOLにかかわってくるが，小型肝がんの治療であれば治療効果を勘案しても，RFAが十分に安価で行えることがわかっている[14]。

今後は，慢性肝疾患からの発がん過程，小型肝がんの時期の根治療法，再発してからの繰り返し治療など，長い臨床経過のなかでの肝がん治療のQOLが総合的に評価される必要がある。

●文　献●

1) 三輪佳行，森脇久隆：慢性肝疾患におけるQOLの評価．臨床成人病，31：78-82，2001.
2) 福原俊一，日野邦彦，加藤孝治，他：C型肝炎ウイルスによる慢性肝疾患のHealth Related QOLの測定．肝臓，38：587-595，1997.
3) Foster, G. R., Goldin, R. D., Thomas, H. C.：Chronic hepatitis C virus infection causes a significant reduction in quality of live in the absence of cirrhosis. Hepatology, 27：209-12. 1998.
4) Bonkovsky, H. L., Wooley, J. M.：Reduction of health-related quality of life in chronic hepatitis C and improvement with interferon therapy. The Consensus Interferon Study Group, Hepatology, 29：264-270, 1999.
5) Hussain, K. B., Fontana, R. J., Moyer, C. A., et al.：Comorbid illness is an important determinant of health-related quality of life in patients with chronic hepatitis C. Am. J. Gastroenterol., 96：2737-2744, 2001.
6) Younossi, Z. M., Guyatt, G., Kiwi, M., et al.：Development of a disease specific questionnaire to measure health related quality of life in patients with chronic liver disease. Gut., 45：295-300, 1999.
7) Gross, C. R., Malinchoc, M., Kim, W. R., et al.：Quality of life before and after liver transplantation for chronic cholestatic liver disease. Hepatology, 29：356-364, 1999.
8) 厚生労働科学研究費補助金・肝炎等克服緊急対策研究事業(主任研究者，藤原研司)：「肝がん患者のQOL向上に関する研究」平成14～16年度総合研究報告書．2005年4月．p.19.
9) Wessely, S., Pariante, C.：Fatigue, depression and chronic hepatitis C infection. Psychol. Med., 32：1-10, 2002.
10) Hoofnagle, J. H.：Hepatitis C：The clinical spectrum of disease. Hepatology, 26：15-20, 1997.
11) Dale, J. K., Di Bisceglie, A. M., Hoofnagle, J. H.：Chronic fatigue syndrome：Lack of association with hepatitis C virus infection. J. Med. Virol., 34：119-121, 1991.
12) Asnis, G. M., DeLa Garza, R.：Interferon-induced depression in chronic hepatitis C：a review of its prevalence, risk factors, biology, and treatment approaches. J. Clin. Gastroenterol., 40：322-335, 2006.
13) 科学的根拠に基づく肝癌診療ガイドライン作成に関する研究班：肝癌診療ガイドライン2005年版．金原出版，東京，2005，p.168.
14) Ikeda, K., Kobayashi, M., Hosaka, T., et al.：Cost-effectiveness of radiofrequency ablation and surgical therapy for small hepatocellular carcinoma of 3cm or less in diameter. Hepatol. Res., 33：241-249, 2005.

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Glycyrrhizin injection therapy prevents hepatocellular carcinogenesis in patients with interferon-resistant active chronic hepatitis C

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Aim: There is no useful and effective treatment for patients with non-sustained response to interferon, from the viewpoint of cancer prevention. Our aim was to elucidate the influence of a glycyrrhizin therapy on hepatocarcinogenesis rate in interferon-resistant hepatitis C

Methods: We retrospectively analyzed 1249 patients with chronic hepatitis with or without cirrhosis. Among 346 patients with high alanine transaminase values of twice or more of the upper limit of normal, 244 patients received i.v. glycyrrhizin injection and 102 patients did not, after judgment of interferon resistance.

Results: Crude carcinogenesis rates in the treated and untreated group were 13.3%, 26.0% at the fifth year, and 21.5% and 35.5% at the 10th year, respectively ($P = 0.021$). Proportional hazard analysis using time-dependent covariates disclosed that fibrotic stage, gender and glycyrrhizin treatment were significantly associated with future carcinogenesis. A long-term glycyrrhizin injection therapy decreased the hepatocarcinogenesis rate (hazard ratio, 0.49; 95% confidence interval, 0.27–0.86, $P = 0.014$) after adjusting the background features with significant covariates. Cancer preventive activity was also found in a subgroup of older patients of 60 years or more.

Conclusions: Glycyrrhizin injection therapy significantly decreased the incidence of hepatocellular carcinoma in patients with interferon-resistant active chronic hepatitis C, whose average aminotransferase value was twice or more of the upper limit of normal after interferon.

Key words: cancer prevention, chronic hepatitis, glycyrrhizin, hepatitis C virus, hepatocellular carcinogenesis

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers in the world. Until recently, hepatitis C virus (HCV) has been reported to be a causative agent of HCC aside from hepatitis B virus (HBV).[1–5] The annual incidence of HCC in patients with HCV RNA-positive cirrhosis ranges 5–7%.[5–7] The carcinogenesis rate was higher in those patients with cirrhosis caused by HCV than in those with HBV-related cirrhosis.[5]

Interferon (IFN) is effective in reducing HCC rate through suppression of necroinflammatory process serum alanine aminotransferase (ALT) and in eliminating HCV in some patients with chronic HCV and cirrhosis. Although IFN proves to be valuable in suppression of the risk of carcinogenesis, it is not effective in every patient with HCV-related disease. Oka *et al.*[8] reported in a randomized controlled trial that a kind of medicinal herb, "Sho-saiko-to", could significantly decrease hepatic carcinogenesis rate in patients without hepatitis B surface antigen (HBsAg)-negative cirrhosis. Tarao *et al.*[9] showed that the HCC appearance rate was significantly higher in HCV-related cirrhotics with a high ALT value of 80 IU or more than that of those with lower ALT value, and also suggested that treatment of cirrhosis and prevention of HCC should be directed to suppress the necroinflammation of HCV-related hepatitis.

In Japan, a glycyrrhizin-containing herbal medicine, Stronger Neo-Minophagen C (SNMC), is widely used in Japan for the treatment of chronic hepatitis. It is used in the form of an i.v. solution, comprised of 0.2% glycyrrhizin, 0.1% cysteine and 0.2% glycine in physiological solution. It is made by dissolving glycyrrhizin (200 mg),

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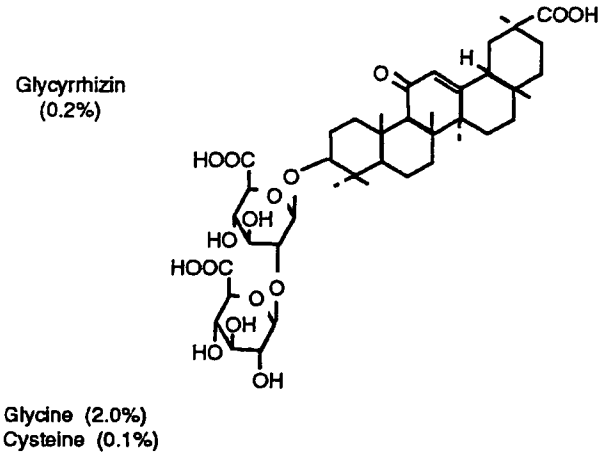

Figure 1 Chemical structure of glycyrrhizin and Stronger Neo-Minophagen C in physiological saline solution.

cysteine (100 mg), glycine (2 g) in 100 mL of physiological saline (Fig. 1). Glycyrrhizin is an aqueous extract of licorice root (*Glycyrrhizae radix*), which is anti-allergic and has detoxicating effects. As has been reported, the anti-inflammatory mechanism of glycyrrhizin is believed to be due to its protective effect on the hepatic cellular membrane, which may explain its ability to lower the serum transaminase level in patients with chronic hepatitis. Because glycyrrhizin has an anti-inflammatory action and favorable effect on ALT and histology in patients with chronic viral hepatitis,[10–15] we analyzed its effect on HCC in those patients with chronic HCV.[16]

In order to elucidate whether glycyrrhizin suppress the carcinogenesis rate in patients with IFN-resistant chronic hepatitis C, we retrospectively assessed a cohort of 1249 patients without sustained virological response.[17]

METHODS

Patients

A TOTAL OF 1249 consecutive Japanese patients with chronic hepatitis or cirrhosis type C were examined, who could not eradicate HCV RNA with previous IFN therapy. There were 778 men and 471 women aged 18–81 (median age, 53 years) in the study. They were diagnosed as having liver cirrhosis by peritoneoscopy, liver biopsy or both between 1987 and 2002 at Toranomon Hospital, Tokyo, Japan. All the patients had a history of receiving IFN therapy once or more. A total of 347 patients showed a normal ALT for at least 6 months after cessation of IFN (biochemical responders), and the other 902 patients showed abnormal ALT at 6 months after the end of IFN therapy.

Glycyrrhizin therapy

Of 1249 patients with IFN-resistant chronic liver disease, 453 patients underwent glycyrrhizin injection therapy (SNMC) and the remaining 796 patients did not receive glycyrrhizin therapy until the end of observation. The purpose for the introduction of glycyrrhizin therapy was to suppress high ALT levels and to prevent disease progression in all the patients. F1 stage hepatitis was significantly more often found in the untreated group than in the glycyrrhizin group ($P < 0.001$, χ^2 test). Both AST and ALT medians were significantly higher in the glycyrrhizin group than in the untreated group ($P < 0.001$).

When glycyrrhizin was regarded as effective from an aminotransferase viewpoint, treatment was usually continued for as long a period as possible. As a result, a median daily dose of 100 mL of glycyrrhizin was administered thrice weekly during a median period of 4.3 years (range, 0.1–14.5 years) in the treated group. Two (0.44%) of the 453 treated patients withdrew from glycyrrhizin injection therapy because of side-effects: one from hypertension and one from skin rash without itching.

Background and laboratory data of patients with and without therapy

Table 1 summarizes the profiles and data of the patients at the time of diagnosis of chronic hepatitis, with or without cirrhosis.

The male : female ratio was not different between the two groups. Median age was older by 2 years in the treated group than in the untreated group ($P < 0.001$). F1-stage hepatitis was found significantly more often in the untreated group than in the glycyrrhizin group ($P < 0.001$, χ^2 test). Median levels of both AST and ALT were significantly higher in the treated group than in the untreated group ($P < 0.001$). The rate of HCV serological group 1 was significantly higher in the glycyrrhizin group than in the untreated group ($P = 0.032$).

Follow up of the patients

Follow up of the patients was made on a monthly basis after the judgment of IFN resistance by monitoring hematological, biochemical, and virological data.

Table 1 Patients profiles and laboratory data at the time of judgment of interferon-resistance

	Glycyrrhizin group ($n = 453$)	Untreated group ($n = 796$)	*P*-value
Demography			
Sex (M/F)	283/170	495/301	0.92‡
Age (year)†	54 (25–81)	52 (18–77)	<0.001
Liver histology			
F1/F2/F3/F4	146/193/38/69	502/192/52/38	<0.001‡
Laboratory data†			
Aspartic transaminase (IU/L)†	81 (19–446)	54 (11–355)	<0.001
Alanine transaminase (IU/L)†	122 (12–630)	83 (10–822)	<0.001
HCV serological group 1/2	360/73	582/165	0.032‡

†Expressed by median (range). ‡χ^2 test or Mann–Whitney *U*-test.

Imaging diagnosis with ultrasonography (US) and/or computerized tomography (CT) was made three or more times per year in a majority of patients with cirrhosis, and once a year in patients without cirrhosis.

The numbers of cases lost to follow up were 121 (9.7%): 28 patients (6.2%) in the glycyrrhizin group and 93 (11.7%) in the untreated group. Because the eventual outcomes regarding appearance of HCC were not identified in these patients, they were dealt as censored data in the following statistics. Death unrelated to HCC was also classified as withdrawal and regarded as a censored case. The median observation period of the total number of patients was 5.7 years with a range of 0.1–16.1 years.

Statistical analysis

Non-parametric procedures were employed for the analysis of background characteristics of the patients, including Mann–Whitney *U*-test and χ^2 method. HCC appearance rates were calculated from a period between the judgment of IFN ineffectiveness and the appearance of HCC in each group, using the Kaplan–Meier technique.[17] The differences in carcinogenesis curves were tested using the log-rank test. Independent factors associated with the appearance rate of HCC were studied using time-dependent Cox regression analysis.[18] An interaction term of IFN treatment and "waiting time" to the therapy was introduced in the analysis as a time-dependent covariate. The independence of treatment factor from "waiting time" was also confirmed by a log-minus-log plot of a proportional hazard model.

All data analysis was performed using the computer program SPSS version 11 (SPSS, Chicago, IL, USA).

RESULTS

Initial aminotransferase and carcinogenesis rates

BECAUSE AMINOTRANSFERASE LEVEL is likely to affect future disease progression, entire patients of the cohort were classified into six categories according to average ALT value during the first year after cessation of IFN therapy: (i) normal ALT; (ii) less than 1.5 times of upper limit of normal (ULN); (ii) 1.5–2 times of ULN; (iv) 2–3 times of ULN; (v) 3–4 times of ULN; and (vi) more than 4 times of ULN. Hepatocellular carcinogenesis rates were 2.5%, 5.0%, 8.1%, 11.8%, 12.0% and 12.7% at the end of the fifth year, and 6.6%, 7.2%, 19.6%, 15.1%, 21.0% and 39.3% at the 10th year, respectively. There was a significant statistical difference among the six subgroups (log-rank test, $P < 0.0001$). The higher the average ALT, the higher the carcinogenesis rate was.

Glycyrrhizin therapy was usually performed in patients with a high ALT value and high hepatitis activity. In this retrospective study, average ALT values were significantly different between the treated and the untreated groups: (i) normal average ALT was found in 38 among patients with glycyrrhizin therapy and in 188 among patients without therapy; (ii) ALT of less than 1.5 times of ULN was found 42 and 331; (iii) 1.5 times to 2 times of ULN 84 and 138; (iv) 2–3 times of ULN in 143 and 92; (v) 3–4 times in 53 and 29; and (vi) ALT of more than 4 times of ULN in 93 of the glycyrrhizin group and 18 of the untreated group, respectively. The rate of a high ALT value of twice or more of ULN in the glycyrrhizin treated group (64.2%, 289/453) was significantly higher than that of the untreated group (16.2%, 129/796).

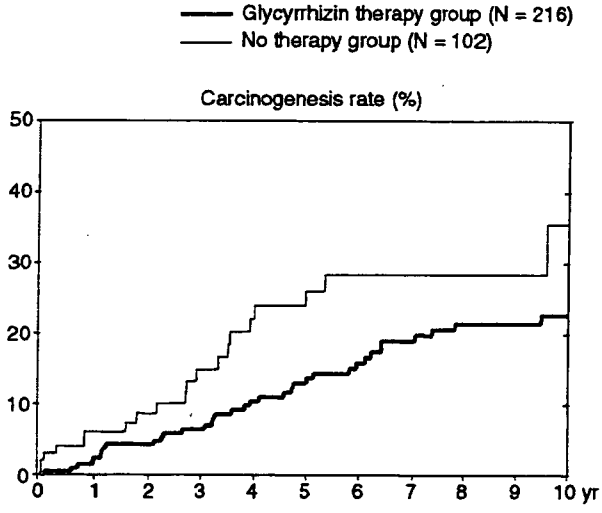

Figure 2 Carcinogenesis rates in patients with active chronic hepatitis showing high average alanine transferase (ALT) values of twice or more of upper limit of normal. Carcinogenesis rate in patients with a sufficient period of glycyrrhizin treatment was significantly lower than that of the untreated patients (log-rank test, $P = 0.021$).

Carcinogenesis in patients with high aminotransferase

Of the 418 patients with a high average ALT in both groups, 68 patients showed a normal ALT value for at least 6 months just after IFN therapy (biochemical response). because biochemical response with normal ALT for a certain period after IFN was likely to affect carcinogenesis rates in those patients, biochemical responders were excluded in the following analyses on the influence of glycyrrhizin on carcinogenesis: after all, 244 patients with glycyrrhizin therapy and the 102 patients without therapy were assessed.

Cumulative hepatocellular carcinogenesis rates were calculated in these 346 patients with high average ALT values, excluding biochemical responders from both groups. Carcinogenesis rates in the glycyrrhizin group and the untreated group were 6.5% and 13.3% at the end of the third year, 13.3% and 26.0% at the end of the fifth year, 17.7% and 28.3% at the end of the seventh year, and 21.5% and 35.5% at the 10th year, respectively (Fig. 2). In the stratified and selected patient group, the carcinogenesis rate of the glycyrrhizin-treated group was significantly lower than that of the untreated group (log-rank test, $P = 0.021$).

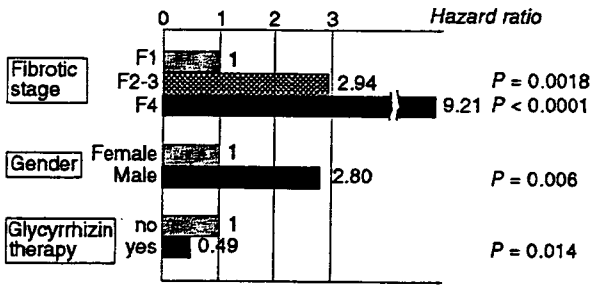

Figure 3 Independent risk factors affecting hepatocellular carcinogenesis (time-dependent Cox proportional hazard analysis).

Impact of glycyrrhizin therapy on carcinogenesis

In the selected patients with active hepatitis with an average ALT value of twice ULN or higher, multivariate analysis was performed to explore associating factors with carcinogenesis, using a time-dependent Cox proportional hazard model. Time between the judgment of IFN ineffectiveness and initiation of glycyrrhizin therapy was set as a time-dependent variable, in order to clarify the significance of glycyrrhizin therapy in the clinical course of HCV-related chronic liver diseases. Patients with biochemical response with a normal ALT value sustained for at least 6 months after IFN therapy were also excluded in the analysis.

In multivariate analysis, the following three factors influenced the carcinogenesis: (i) fibrotic staging; (ii) sex ($P = 0.006$); and (iii) glycyrrhizin therapy ($P = 0.014$) (Fig. 3). When a hazard of F1-stage fibrosis for carcinogenesis was set as 1 in the model, the hazard ratio of F2 to F3 stage fibrosis was calculated as 2.94 ($P = 0.018$), and that of F4 (cirrhosis) was estimated as 9.21 ($P < 0.001$). Similarly, the hazard ratio for carcinogenesis of male gender was 2.80, compared to female. Use of glycyrrhizin independently decreased the carcinogenesis rate with a hazard ratio of 0.49, in patients with active chronic hepatitis after IFN therapy. The following factors did not affect the HCC appearance rate significantly: age, association of diabetes mellitus, serological grouping of HCV, HCV RNA concentration, AST, ALT at the time before IFN therapy, and bilirubin.

Carcinogenesis in elderly patients

Cumulative carcinogenesis rates were compared between patients with and without glycyrrhizin therapy, in a subgroup of older patients of 60 years old or more. Carcinogenesis rates in the treated ($n = 58$) and

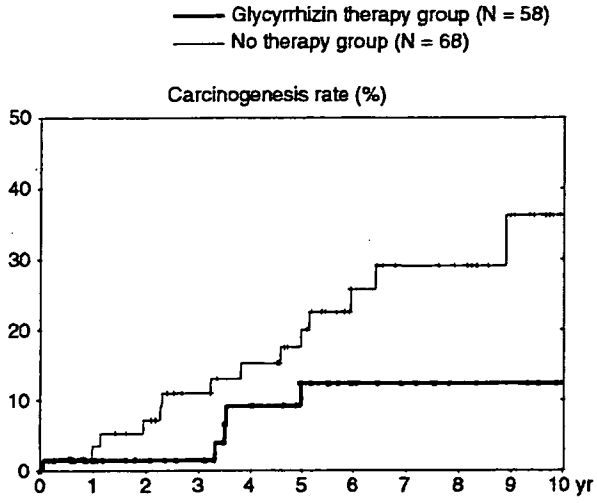

Figure 4 Carcinogenesis rates in elderly patients of 60 years or more.

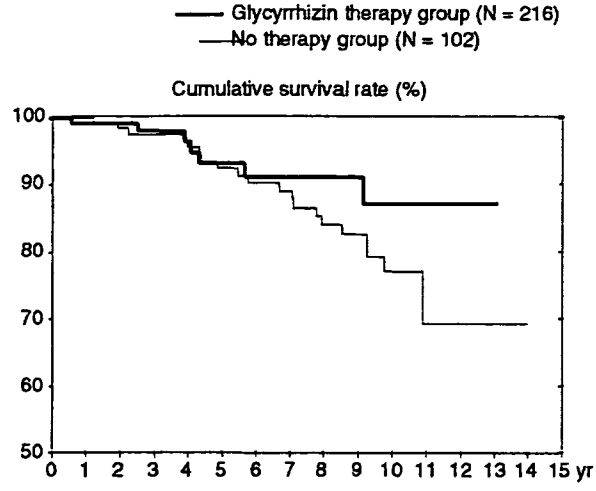

Figure 5 Crude survival rates in patients with and without glycyrrhizin treatment.

untreated groups ($n = 68$) were 12.4% and 20.0% at the end of the fifth year, and 12.4% and 36.2% at the 10th year, respectively (Fig. 4). The carcinogenesis rate in the glycyrrhizin injection group apparently decreased, but marginal statistical difference was observed (log-rank test, $P = 0.052$).

Survival rate

Cumulative survival rates after cessation of IFN therapy were calculated in the treated and untreated groups. Five-year survival rates in patients with and without glycyrrhizin injection therapy were 93.3% and 92.5%, and 10-year rates were 87.2% and 77.1%, respectively (Fig. 5). Although statistical significance was not obtained in the survival rates between the two groups, it showed higher rates in the treated group than in the untreated group.

DISCUSSION

YAMAMOTO *ET AL.*[10] first treated patients with chronic hepatitis with glycyrrhizin (SNMC) and found a distinct improvement in their ALT levels. Suzuki *et al.*[12] confirmed its ability to suppress serum aminotransferase in patients with chronic hepatitis in a randomized controlled trial. Hino *et al.*[13] and Yasuda *et al.*[14] also proved glycyrrhizin to be useful in the improvement of transaminase and liver histology. We once reported that glycyrrhizin was beneficial in carcinogenesis rate in patients with chronic hepatitis type C when it was administered for 10 years or longer.[19] In this study,[20] we assessed the role of glycyrrhizin in the prevention of hepatocellular carcinogenesis in patients with INF-resistant chronic hepatitis C.

Because it requires at least 5 years to show a statistical difference in carcinogenesis rate from hepatitis or cirrhosis between glycyrrhizin-treated and "untreated" groups, a prospective randomized trial using untreated control patients is actually difficult from both ethical and medical viewpoints in Japan, where glycyrrhizin injection therapy is covered by standard medical insurance and it is already regarded as a usual salvaging procedure for IFN-ineffective patients. Therefore, we attempted to carry out a retrospective cohort study, with a statistical adjustment using possible covariates explored in multivariate analysis.

When crude carcinogenesis rates were compared between the treated and untreated patient group, the hepatocellular carcinogenesis rate in the glycyrrhizin therapy group was higher than that of the untreated group (data not shown). Because anti-inflammatory therapy using glycyrrhizin was usually performed for those patients with high ALT values and more active hepatitis, it seemed a quite convincing result that the carcinogenesis rate of the treated group was higher than that of the untreated group. Actually, the treated group consisted of significantly more numbers of patients with high ALT values of twice or more of ULN. When carcinogenesis rates were assessed only in patients with high ALT values of twice or more ULN, the rate of the treated group became slightly higher than that of the

untreated group. Of patients in the treated group, some of them received glycyrrhizin injection therapy several months or a few years after judgment of IFN ineffectiveness. In order to elucidate the cancer preventive activity of glycyrrhizin in active HCV-related liver disease, we further stratified the treated patients into two groups: (i) early treatment group of glycyrrhizin within 2 years after judgment of IFN ineffectiveness; and (ii) late treatment group after 2 years. Because the latter patients were observed without therapy for a considerable period inspite of the "treated group", they were regarded as partly and insufficiently treated with glycyrrhizin from a viewpoint of the entire observation period. We therefore compared the carcinogenesis rates between the treated and untreated patients, excluding those patients of a late treatment group.

The hepatocellular carcinogenesis rate of the patients with a sufficient period of glycyrrhizin injection was significantly lower than that of those without therapy ($P = 0.038$). In the treated group, median ALT values significantly decreased after initiation of the glycyrrhizin injection, suggesting that suppression of the necroinflammatory process was the principal mechanism of the anti-carcinogenic activity of the medicine. The current study dealing with a large cohort ($n = 1249$), showed that the carcinogenesis rate reduces when glycyrrhizin therapy is started at an early time after judgment of IFN ineffectiveness. Cancer preventive activity of glycyrrhizin was also found in a subgroup of elderly patients 60 years or older. Because glycyrrhizin therapy has few side-effects, it should be taken into account for the treatment of aged patients with chronic hepatitis C, from the viewpoint of cancer prevention. Survival rate is likely to increase in those patients undergoing long-term glycyrrhizin injection therapy through suppression of aggressive necroinflammatory process and suppression of liver-related morbidity and mortality.

CONCLUSIONS

AS CARCINOGENESIS IS not a single-step event, but a complex, multistep process, the exact mechanism of the glycyrrhizin activity in suppression of liver carcinogenesis still remains unknown. One of the principal roles of long-term administration of glycyrrhizin in decreasing the carcinogenesis rate seemed to be anti-inflammatory ones, which would retrieve an active carcinogenic process with ALT elevation and continuous hepatic necroinflammation. Glycyrrhizin may only postpone the time of HCC appearance in the clinical course of cirrhosis. Because the entire process of hepatocellular carcinogenesis from initial transformation of a hepatocyte to detectable growth is considered to take at least a few years, the influence of glycyrrhizin on the carcinogenesis rate will not be evaluated in a short period of a few years. Future studies should therefore be aimed at defining the basic oncogenic mechanisms and roles of long-term administration of glycyrrhizin in carcinogenesis in patients with cirrhosis caused by HCV.

In conclusion, a long-term intermittent glycyrrhizin therapy for a few years or more successfully reduced hepatocellular carcinogenesis in patients with HCV-related chronic liver disease. A randomized control study with a larger number of cases, with or without glycyrrhizin therapy, is expected to confirm the effectiveness of this therapy.

CONFLICT OF INTEREST

NO CONFLICT OF interest statement has been received from the author.

REFERENCES

1 Bruix J, Calvet X, Costa J *et al.* Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989; 2: 1004–6.
2 Colombo M, Kuo G, Choo QL *et al.* Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989; 2: 1006–8.
3 Hasan F, Jeffers LJ, Medina MD *et al.* Hepatitis C-associated hepatocellular carcinoma. *Hepatology* 1990; 12: 589–91.
4 Kew MC, Houghton M, Choo QL, Kuo G. Hepatitis C virus antibodies in southern African blacks with hepatocellular carcinoma. *Lancet* 1990; 335: 873–4.
5 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis – A prospective observation of 795 cases with viral and alcoholic cirrhosis. *Hepatology* 1993; 18: 47–53.
6 Oka H, Kurioka N, Kim K *et al.* Prospective study of early detection of hepatocellular carcinoma with cirrhosis. *Hepatology* 1990; 12: 680–7.
7 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; 328: 1797–801.
8 Oka H, Yamamoto S, Kuroki T *et al.* Prospective study of chemoprevention of hepatocellular carcinoma with Sho-saiko-to (TJ-9). *Cancer* 1995; 76: 743–9.
9 Tarao K, Rino Y, Ohkawa S *et al.* Association between high serum alanine aminotransferase levels and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. *Cancer* 1999; 86: 589–95.

10 Yamamoto S, Maekawa Y, Imamura M, Hisajima T. Treatment of hepatitis with the antiallergic drug, Stronger Neo-Minophagen C [in Japanese]. *Clin Med Pediatr* 1958; 13: 73.
11 Fujisawa K, Watanabe Y, Kimura K. Therapeutic approach to chronic active hepatitis with glycyrrhizin. *Asian Med J* 1980; 23: 745–56.
12 Suzuki H, Ohta Y, Takino T, Fujisawa K, Hirayama C. Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis. Double blind trial. *Asian Med J* 1983; 26: 423–38.
13 Hino K, Miyakawa H, Takahashi J *et al.* Effect of large dose of SNMC on the liver histology of chronic active hepatitis [in Japanese]. *Kan-Tan-Sui* 1986; 13: 797.
14 Yasuda K, Hino K, Fujioka S *et al.* Effects of high dose therapy with Stronger Neo-Minophagen C (SNMC) on hepatic histography in non-B chronic active hepatitis. In: *Proceedings of International Symposium on Viral Hepatitis and Liver Disease*. ed. Nishioka K *et al.* Excerpta Medica (Amsterdam) 1991; 205–9.
15 Wildhirt E. Experience in Germany with glycyrrhizinic acid for the treatment of chronic viral hepatitis. In: *Viral Hepatitis and Liver Disease*. ed. Nishioka K *et al.* Springer, New York 1994; 658–61.
16 Rossum TGJ, Vulto AG, Hop WCJ, Brouwer JT, Niesters HGM, Schalm SW. Intravenous glycyrrhizin for the treatment of chronic hepatitis C: a double-blind, randomized, placebo-controlled phase I/II trial. *J Gastroenterol Hepatol* 1999; 14: 1093–9.
17 Ikeda K, Arase Y, Kobayashi M *et al.* A long-term glycyrrhizin injection therapy reduces hepatocellular carcinogenesis rate in patients with interferon-resistant active chronic hepatitis C. *Dig Dis Sci* 2006; 51 (3): 603–9.
18 Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. *J Am Stat Assoc* 1958; 53: 457–81.
19 Cox DR. Regression models and life tables. *J R Stat Soc* 1972; 34: 248–75.
20 Arase Y, Ikeda K, Murashima N *et al.* The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; 79: 1494–500.

Significance of glucose intolerance and SHIP2 expression in hepatocellular carcinoma patients with HCV infection

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Abstract. Glucose intolerance frequently is found in hepatocellular carcinoma (HCC) patients with hepatitis C virus (HCV) infection; however, the significance of glucose intolerance remains unclear. In addition, SH2 domain-containing inositol phosphatase (SHIP) 2 is a negative regulator of intracellular insulin signaling; however, changes in SHIP2 expression have not been investigated in HCC. To assess the significance of glucose intolerance, we analyzed 118 HCC patients with HCV infection. Twenty HCC specimens were used for immunoblotting and immunostaining for SHIP2. Patients were classified into two groups: a glucose intolerance group (n=39) and a normal glucose tolerance group (n=79). There was no significant difference in the disease-free survival (P=0.838) or long-term survival (P=0.091) between the groups. However, for males, the cumulative survival rate was significantly lower in the glucose intolerance group (n=22) than that in the normal glucose tolerance group (n=52) (P=0.036). In multivariate analysis, Child-Pugh class (P=0.0003) and glucose intolerance (P=0.036) were identified as statistically significant and independent prognostic factors in males. SHIP2 expression level decreased in HCC compared to that in nontumor tissues. In conclusion, this study is the first to demonstrate the significance of glucose intolerance in prognosis of male HCC patients and down-regulation of SHIP2 expression in HCC.

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Key words: hepatocellular carcinoma, hepatitis C virus, SH2 domain-containing inositol phosphatase 2, insulin resistance, prognosis

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. The incidence of HCC has increased in Eastern Asia and Africa during the past several decades and has also increased in the US (1). This trend has been attributed to hepatitis C virus (HCV) infection. In many areas of the world, HCV infection accounts for more than half of the cases of HCC, and in Japan, ~75% of all HCC are associated with chronic liver disease and HCV infection (2). In order to prevent and treat this malignancy, it is important to understand the pathogenesis of HCC in patients with HCV infection.

Liver is one of the major organs regulating glucose metabolism and patients with chronic liver diseases frequently show glucose intolerance which is called hepatogenous diabetes (3). Although its pathogenesis involves various factors, insulin resistance and hyperinsulinemia are thought to play major roles (4,5). In chronic liver disease associated with HCV infection, the prevalence of glucose intolerance is higher than in other chronic liver diseases, including hepatitis B infection (6). We previously reported that down-regulation of insulin receptor substrate 1/2, central molecules for intracellular insulin signaling, was seen in livers from HCV core-transgenic mice as well as patients with HCV infection (7). In HCV hyperendemic areas, anti-HCV-positive subjects were nearly three-fold as likely as anti-HCV-negative subjects to develop diabetes mellitus with insulin resistance (8). Collectively, these findings suggest that HCV directly causes hepatic insulin resistance and subsequent hyperinsulinemia (9).

Glucose intolerance is frequently observed in HCC patients with chronic liver disease. In addition, glucose intolerance has been suggested as a potential risk factor for the incidence of HCC. Several large population-based cohort studies showed that the incidence of HCC was increased 2-4-fold in patients with diabetes mellitus (10,11). However, it is unclear how glucose intolerance is linked to the incidence of HCC in patients. Moreover, it is unclear whether the presence of glucose intolerance has an impact on the prognosis in HCC patients with HCV infection.

Insulin is known as one of the most important factors not only for a variety of metabolic pathways, but also for cell growth (12). Insulin stimulates activation of the tyrosine kinase activity of the insulin receptor and subsequently causes the phosphorylation of insulin receptor substrate families. Upon tyrosine phosphorylation, these proteins interact with signaling molecules through their Src homology 2 (SH2) domains (13), resulting in a diverse series of signaling pathways, including activation of phosphatidylinositol 3-kinase (PI3K) and Akt cascade (14), and Ras and mitogen-activated protein (MAP) kinase cascade (15). These cascades regulate cell proliferation, differentiation, and apoptosis. Changes in these insulin signaling cascades are involved in cell growth.

SH2-containing inositol phosphatase (SHIP)-2 plays an important role in the negative regulation of insulin sensitivity (16,17). In insulin signaling, PI3K produces phosphatidylinositol 3,4,5-trisphosphate (PIP3) from phosphatidylinositol 3,4-bisphosphate (PIP2) (14). PIP3 mediates insulin signals to downstream molecules including Akt (18). SHIP2 hydrolyses the PI3K product PIP3 to PIP2, leading to decreased level of this phospholipid and, simultaneously, reduced activation of PI3K and Akt signaling cascade (19). In addition, SHIP2 causes down-regulation of Ras and MAPK signaling cascade (20). Thus, SHIP2 suppresses cell growth through regulating intracellular insulin sensitivity. However, changes in expression of SHIP2 in patients with HCC have not been investigated.

The aim of this study was to evaluate the long-term impact of glucose intolerance and to examine the expression of SHIP2 in HCC patients with HCV infection.

Materials and methods

Materials. All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise indicated.

Patients. Between January 1994 and December 2000, 330 Japanese patients with HCV infection at the Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine were diagnosed with HCC. These patients had a single tumor ≤5 cm, and three or fewer tumors each ≤3 cm. HCC with HCV infection was defined as HCC with positive hepatitis C virus antibody and negative hepatitis B virus surface antigen. Among these patients, 120 patients were randomly selected and their blood samples analyzed. Blood samples were obtained from each patient on admission and stored at -20°C for later analysis. Plasma glucose levels were measured by a glucose oxidase method. Serum insulin levels were measured using a sandwich enzyme immunoassay kit (Eiken Chemical, Tokyo, Japan).

Insulin resistance was calculated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method. The formulas for the HOMA model are: Insulin resistance (HOMA-IR) = fasting glucose (mg/dl) x fasting insulin (μU/ml)/405. All patients were classified either into the glucose intolerance group or the normal glucose tolerance group based on the presence of hyperinsulinemia and/or insulin resistance. The glucose intolerance group was defined by fasting insulin level ≥15 μU/ml and/or HOMA-IR value ≥3, and the normal glucose tolerance group was defined by fasting insulin level <15 μU/ml and/or HOMA-IR value <3 according to previous studies (21,22). We enrolled 120 patients in this study, however, 2 patients were excluded because of type 1 diabetes mellitus. The remaining 118 patients were retrospectively examined.

The diagnosis of HCC was histologically confirmed by needle biopsy under ultrasonographic guidance in 78 of 118 patients. In the remaining 40 patients, the diagnosis of HCC was made based on the findings of typical radiological features on ultrasonography, contrast enhanced dynamic computed tomography, magnetic resonance imaging, and hepatic angiography along with elevated alpha-fetoprotein levels. The pretreatment hepatic functional reserve was determined using the Child-Pugh scoring system (23). Tumor staging of HCC was determined using the tumor node metastasis (TNM) classification (24). The measurement of tumor size was based on the largest dimension observed on ultrasonography and computed tomography. Alcohol drinking was defined as an average daily consumption of an amount exceeding 60 g per day of pure ethanol over a period of >5 years based on the report of Donato *et al* (25). Body mass index (BMI) was calculated as body weight in kg divided by the square of height in meters (kg/m^2). The study protocol was approved by the institutional review board, and informed consent for participation in the study was obtained from each subject and conformed to the guidelines of 1995 Declaration of Helsinki.

Treatment and follow-up. As treatment for HCC, 22 patients underwent hepatic resection, 80 patients received percutaneous ethanol injection therapy, microwave coagulation therapy or percutaneous radiofrequency ablation therapy, and 16 received transcatheter arterial chemoembolization. No patient underwent liver transplantation.

After initial treatment, the condition of each patient was followed carefully. Serum biochemistries, alpha-fetoprotein levels, and Child-Pugh score were measured and ultrasonography was performed monthly. Contrast enhanced dynamic computed tomography was performed every 3 months until 6 months post-treatment and every 6 months beyond 6 months post-treatment. Magnetic resonance imaging was performed as a supplemental examination. The closing date of this study was December 2004 or the time of the patient's death. Follow-up ranged from 12 to 128 months (median, 57 months). During follow-up, 74 patients died of HCC. No patient died of complications of cirrhosis or diabetes.

Measurement of HCV core. Serum HCV core levels were evaluated using a newly developed HCV core antigen enzyme linked immunosorbent assay test system (Ortho-clinical Diagnostics K.K., Tokyo, Japan) as previously described (26). This assay has high stability and reproducibility under all conditions and the detection limit is 50 fmol/l.

Human HCC tissues. Tumor and nontumor tissues were obtained from 20 patients with HCC who underwent partial hepatectomy [15 men and 5 women, mean age 67.7±6.7 years (range, 53-76 years)]. Tissue sections were stained with hematoxylin-eosin, and each HCC was histologically graded into one of three categories: well-, moderately- or

poorly-differentiated, according to criteria proposed by the Liver Study Group of Japan (27). The tumors included one well-differentiated, 18 moderately differentiated and one poorly differentiated HCCs. All sections were examined by immunostaining and four tissues from men were used for immunoblotting.

Immunoblotting. Tumor and nontumor tissues were obtained from patients with HCC who underwent partial hepatectomy, and were homogenized on ice in RIPA buffer (150 mmol/l NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mmol/l/Tris-HCl, pH 7.5) containing 100 ng/phenylmethylsulfonyl fluoride, 4 μg/ml aprotinin, 2 μg/ml leupeptin, 1 μg/ml pepstatin, 10 μg/ml antipain, 10 μg/ml soybean trypsin inhibitor, and 2 mmol/l ethylenediaminetetraacetic acid. Then, sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer was added and immediately boiled for 5 min. An equal amount of protein (50 μg) of tumor or nontumor liver homogenates was applied to each lane and was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 7.5% acryl amide gels. The resolved proteins were transferred to polyvinylidene difluoride membranes (Amersham International, Buckinghamshire, UK). The membranes were incubated with an anti-human SHIP2 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:100 (vol/vol) with PBS at room temperature for 1 h, and then incubated with a horseradish peroxidase-conjugated goat anti-mouse IgG (Nichirei, Tokyo, Japan) diluted 1:1000 at room temperature for 1 h. After several washes, the membranes were incubated with chemiluminescence reagents (ECL-kit; Amersham International) for 1 min and immediately exposed on radiograph film.

Immunohistochemistry. Tumor and nontumor tissues were obtained from patients with HCC who underwent partial hepatectomy. Paraffin-embedded sections (3-μm) were deparaffinized and incubated in 0.2% (vol/vol) hydrogen peroxide in methanol for 15 min to inhibit endogenous peroxidase activity. To prevent nonspecific binding, sections were incubated with protein block serum-free (Dako, Kyoto, Japan) at room temperature for 30 min. Sections were then incubated with an anti-human SHIP2 polyclonal antibody (Santa Cruz Biotechnology) diluted 1:50 (vol/vol) with phosphate-buffered saline (pH 7.4, 130 mmol/l NaCl, 2 mmol/l NaH_2PO_4, and 7 mmol/l Na_2HPO_4, PBS) overnight at 4°C. Sections were washed 3 times for 5 min in PBS and incubated with a horseradish peroxidase-conjugated anti-goat antibody (Nichirei) diluted 1:50 (vol/vol) with PBS at RT for 1 h. After several washes with PBS, peroxidase activity was visualized using 3,3'-diaminobenzidine-tetrahydrochloride. Finally, sections were counterstained with hematoxylin.

Quantitation of SHIP2 expression. Immunoblotting and immunostaining for SHIP2 were quantified as previously described (28). Briefly, immunoblotting intensity and immunostaining intensity were quantified by measuring pixel intensities by an investigator without any information regarding the group assignment of the patients. This analysis was carried out on a Macintosh computer (PowerBook G4; Apple Computer, Cupertino, CA) using the public domain NIH Image-J program for Mac OS X (developed at the National Institutes of Health and available by anonymous FTP from http://rsb.info.nih.gov/ij/download.html).

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 the Chi-square test or the Fisher exact test for discrete variables. Cumulative overall survival and disease-free survival were estimated by the Kaplan-Meier method. Any significant differences in cumulative overall survival or disease-free survival rates were determined using the log-rank test. A Cox proportional hazard model was used for univariate and multivariate analysis to identify any independent variables that are related to cumulative overall survival. The variables analyzed were age at HCC diagnosis, gender, body mass index, alcohol drinking, Child-Pugh class, glucose intolerance, alpha-fetoprotein, tumor stage, and maximal tumor size. All P-values were two-tailed, and a level of <0.05 was considered to be statistically significant. Statistical analysis was performed using Stat View software (version 5.0: SAS Institute Inc., Cary, NC).

Results

Patient characteristics. A total of 118 patients were enrolled. The mean age was 66.9±6.8 years (range, 50-87 years). The mean BMI value was 22.6±2.6 (range, 14.7-32.3). Twenty-seven patients (23%), were considered alcohol drinkers. Among the 118, 79 patients (67%) were classified as the normal glucose tolerance group while 39 patients (33%) were classified as the glucose intolerance group. This classification was based on analysis of blood samples obtained at initial admission. Of the 39, 14 (36%) had already been diagnosed as having diabetes mellitus. The 14 had been treated as follows: 2 with insulin, 8 with oral hypoglycemics, and the rest were managed using diet only. The baseline clinical characteristics of both the glucose intolerance and normal glucose tolerance groups are summarized in Table I. BMI values (P=0.0008), bilirubin levels (P=0.048) and HCV core levels (P=0.049) in the glucose intolerance group were significantly higher compared to those of the normal glucose tolerance group. Albumin levels in the glucose intolerance group were significantly lower than that of the normal glucose tolerance group (P=0.007).

Disease-free survival rate and survival rate based on glucose intolerance. The comparison of the disease-free survival rate based on glucose intolerance was not significantly different between the groups (Fig. 1A) (P=0.838) nor was cumulative survival rate (Fig. 1B) (P=0.091). The 1-, 3-, and 5-year cumulative survival rates were 100, 64.1, and 42%, respectively, in the glucose intolerance group, and 97.4, 78.2, and 57.2%, respectively, in the normal glucose tolerance group.

Univariate and multivariate analysis in all patients. Cox proportional hazard regression analysis was performed to determine which of the 9 variables were independently associated with cumulative overall survival. The results of

univariate analysis are shown in Table II. Child-Pugh class [hazard ratio (HR): 2.81, 95% confidence interval (CI): 1.74-4.54, P<0.0001], and tumor stage (HR: 1.75, 95% CI: 1.09-2.82, P=0.021) were found to be significant factors affecting survival. In multivariate analysis, Child-Pugh class (HR: 3.37, 95% CI: 1.95-5.82, P<0.0001) was identified as an independent prognostic factor (Table II).

The difference in glucose intolerance between male and female. This study included 74 males and 44 females. Among patients who were glucose intolerant, there was no significant difference in the number of males and females [22 males (29.7%) vs. 17 females (38.6%); P=0.482]. There was also no significant difference in plasma glucose levels between males and females (87.6±29.8 vs. 92.8±39.6; P=0.264). However, the mean fasting insulin level in males (15.2±23.63 μU/ml) was significantly higher than in females (15.1±13.8 μU/ml) (P=0.045). Thus, glucose intolerance in males was more severe than that in females. We evaluated further the ability of glucose intolerance to influence long-term outcomes in male patients with HCC.

Male patient characteristics. Baseline clinical characteristics of male patients are summarized in Table III. Although BMI value in the glucose intolerance group was significantly higher compared to those in the normal glucose tolerance group (P=0.009), the mean value of BMI was within normal range. There were no significant differences in other clinical characteristics.

Table II. Univariate and multivariate analyses of survival for hepatocellular carcinoma by Cox proportional hazard model.

Variable	HR	95% CI	P-value
Univariate analysis			
Gender	1.12	0.70-1.80	0.637
Age	1.26	0.78-2.02	0.341
BMI	1.16	0.66-2.05	0.609
Alcohol drinking	1.32	0.77-2.27	0.319
Child-Pugh class[a]	2.81	1.74-4.54	<0.0001
Glucose intolerance	1.53	0.93-2.50	0.093
AFP	0.92	0.58-1.46	0.725
Tumor stage[b]	1.75	1.09-2.82	0.021
Maximal tumor size	0.97	0.48-1.95	0.932
Multivariate analysis			
Child-Pugh class[a]	3.37	1.95-5.82	<0.0001

BMI, body mass index; AFP, alpha-fetoprotein. [a]Child-Pugh scoring system (23). [b]TNM classification (24).

Disease-free survival rate and survival rate based on glucose intolerance in male patients. Disease-free survival rates were not significantly different between the two groups (Fig. 2A) (P=0.378). The 1-, 2-, and 3-year disease-free survival rates were 45.5, 27.3, and 18.2%, respectively, in the glucose intolerance group and 61.5, 32.7, and 26.9%, respectively, in

Table I. Comparison of patient characteristics based on glucose intolerance.

	Glucose intolerance group (n=39)	Normal glucose tolerance group (n=79)	P-value
Gender (male/female)	22/17	52/27	0.320
Age (yrs.)	67.7±7.0	66.5±6.8	0.339
BMI	23.8±2.6	22.1±2.4	0.0008
Alcohol drinking (+/-)	9/30	18/61	0.972
Child-Pugh class (A/B/C)[a]	25/13/1	60/18/1	0.392
AST (IU/l)	79.4±31.9	76.0±35.6	0.355
Bilirubin (mg/dl)	1.2±0.4	1.0±0.4	0.048
Albumin (g/dl)	3.4±0.4	3.6±0.4	0.007
AFP (ng/ml)	224.4±992.0	414.8±2363.2	0.567
HCV core	5031.3±5335.1	3235.1±3798.7	0.049
Tumor stage (I/II)[b]	23/16	54/25	0.423
Maximal tumor size (mm)	20.0±8.1	21.0±10.3	0.777
(≤30 / >30 to ≤50)	36/3	66/13	0.258

Data are expressed by mean ± SD. BMI, body mass index; AST, glutamine oxaloacetic transaminase; AFP, alpha-fetoprotein; HCV, hepatitis C virus. [a]Child-Pugh scoring system (21). [b]TNM classification (22).

Table III. Comparison of the characteristics of male patients based on glucose intolerance.

	Glucose intolerance group (n=22)	Normal glucose tolerance group (n=52)	P-value
Age (yrs.)	65.6±6.9	66.7±6.8	0.586
BMI	23.4±2.2	21.7±2.4	0.009
Alcohol drinking (+/-)	9/13	18/34	0.609
Child-Pugh class (A/B/C)[a]	15/6/1	44/8/0	0.133
AST (IU/l)	82.0±32.5	77.0±36.8	0.303
Bilirubin (mg/dl)	1.1±0.4	1.0±0.3	0.052
Albumin (g/dl)	3.5±0.4	3.7±0.4	0.079
AFP (ng/ml)	337.4±1321.0	183.9±554.2	0.404
Tumor stage (I/II)[b]	12/10	33/19	0.647
Maximal tumor size (mm)	21.1±8.6	21.0±10.3	0.776
(≤30 / >30 to ≤50)	20/2	44/8	0.725

Data are expressed by mean ± SD. BMI, body mass index; AST, glutamine oxaloacetic transaminase; AFP, alpha-fetoprotein. [a]Child-Pugh scoring system (23). [b]TNM classification (24).

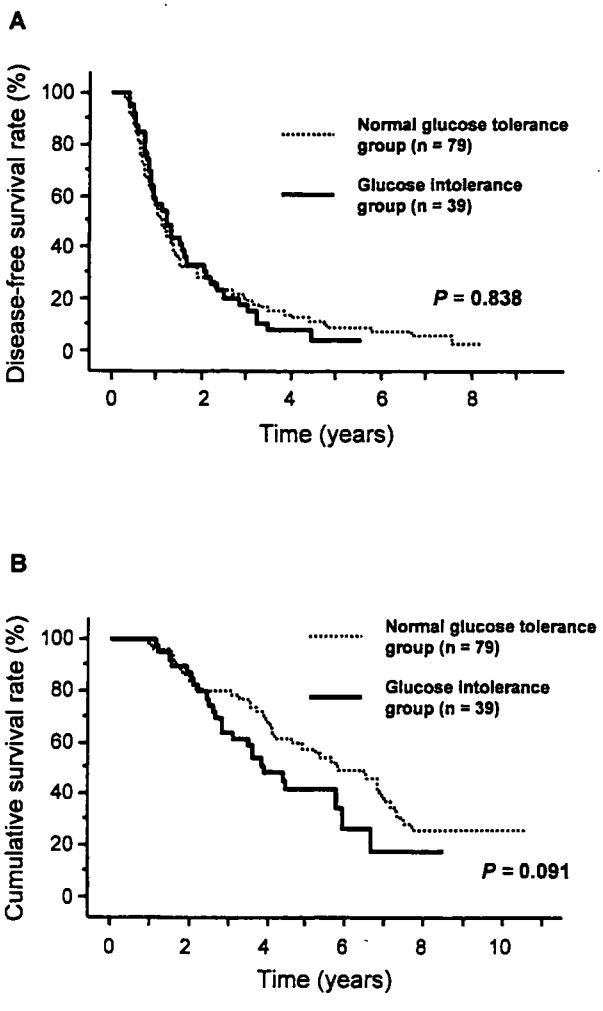

Figure 1. Comparison of disease-free survival rate (A) and cumulative survival rate (B) for all HCC patients between the normal glucose tolerance and the glucose intolerance groups.

Table IV. Univariate and multivariate analyses of survival for hepatocellular carcinoma in males by Cox proportional hazard model.

Variable	HR	95% CI	P-value
Univariate analysis			
Age	1.14	0.64-2.04	0.649
BMI	0.96	0.43-2.15	0.920
Alcohol drinking	1.27	0.70-2.31	0.428
Child-Pugh class[a]	3.79	1.96-7.14	<0.0001
Glucose intolerance	1.95	1.03-3.62	0.039
AFP	1.15	0.65-2.05	0.628
Tumor stage[b]	2.05	1.11-3.80	0.023
Maximal tumor size	0.94	0.40-2.21	0.880
Multivariate analysis			
Child-Pugh class[a]	4.26	1.95-9.34	0.0003
Glucose intolerance	2.30	1.05-5.03	0.036

BMI, body mass index; AFP, alpha-fetoprotein. [a]Child-Pugh scoring system (23). [b]TNM classification (24).

the normal glucose tolerance group. On the other hand, the cumulative survival rate in the glucose intolerance group was significantly poorer than that in the normal glucose tolerance groups (Fig. 2B) (P=0.036). The 1-, 3-, and 5-year cumulative survival rates were 100, 50, and 36.4%, respectively, in the glucose intolerance group and 98, 84.3, and 56.5%, respectively, in the normal glucose tolerance group.

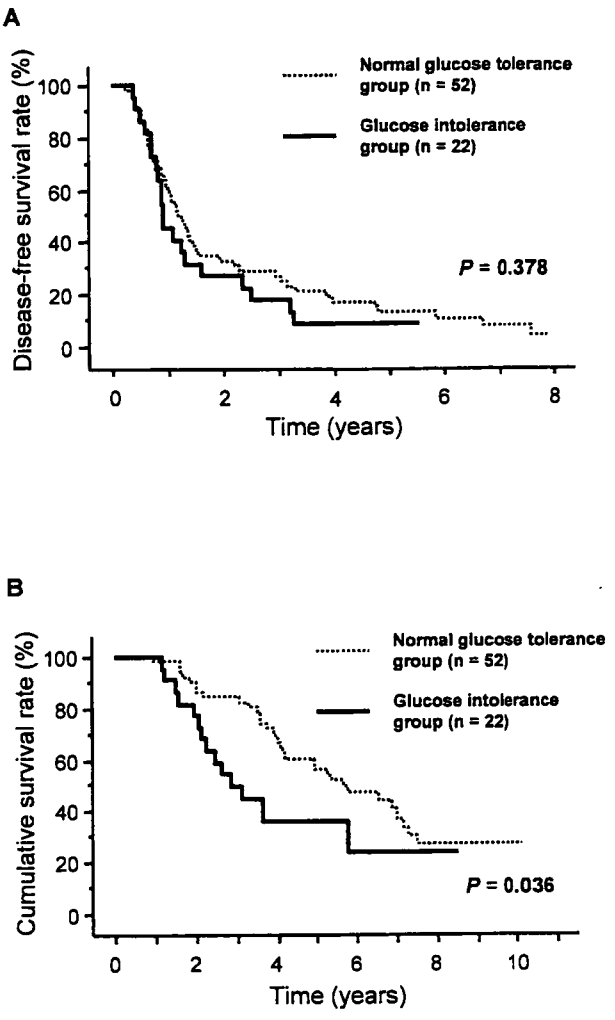

Figure 2. Comparison of disease-free survival rate (A) and cumulative survival rate (B) for male HCC patients between the normal glucose tolerance and the glucose intolerance groups.

Univariate and multivariate analysis in male patients. The results of univariate analysis of male patients are shown in Table IV. Child-Pugh class (HR: 3.79, 95% CI: 1.96-7.14, P<0.0001), glucose intolerance (HR: 1.95, 95% CI: 1.03-3.62, P=0.039) and tumor stage (HR: 2.05, 95% CI: 1.11-3.80, P=0.023) were identified as being statistically independent prognostic factors. In the multivariate analysis, Child-Pugh class (HR: 4.26, 95% CI: 1.95-9.34, P=0.0003) and glucose intolerance (HR: 2.30, 95% CI: 1.05-5.03, P=0.036) were identified as independent prognostic factors.

Protein expression levels of SHIP2 in the tumor and nontumor livers from HCC patients. Protein expression levels of SHIP2 in tumor and nontumor livers from patients with HCC were examined by immunoblotting and immunostaining. Immunoblotting revealed that SHIP2 expression was decreased in HCC tissues compared to that in nontumor tissues (Fig. 3A). Quantitation of immunoblotting intensity confirmed that SHIP2 expression was significantly down-regulated in HCC tissues compared to that in nontumor tissues (48.5±17.2 vs. 151.2±35.3 arbitrary units, P<0.05) (Fig. 3B). Immunostaining demonstrated cytoplasmic SHIP2 expression in nontumor hepatocytes, but not in peripherally located well-differentiated

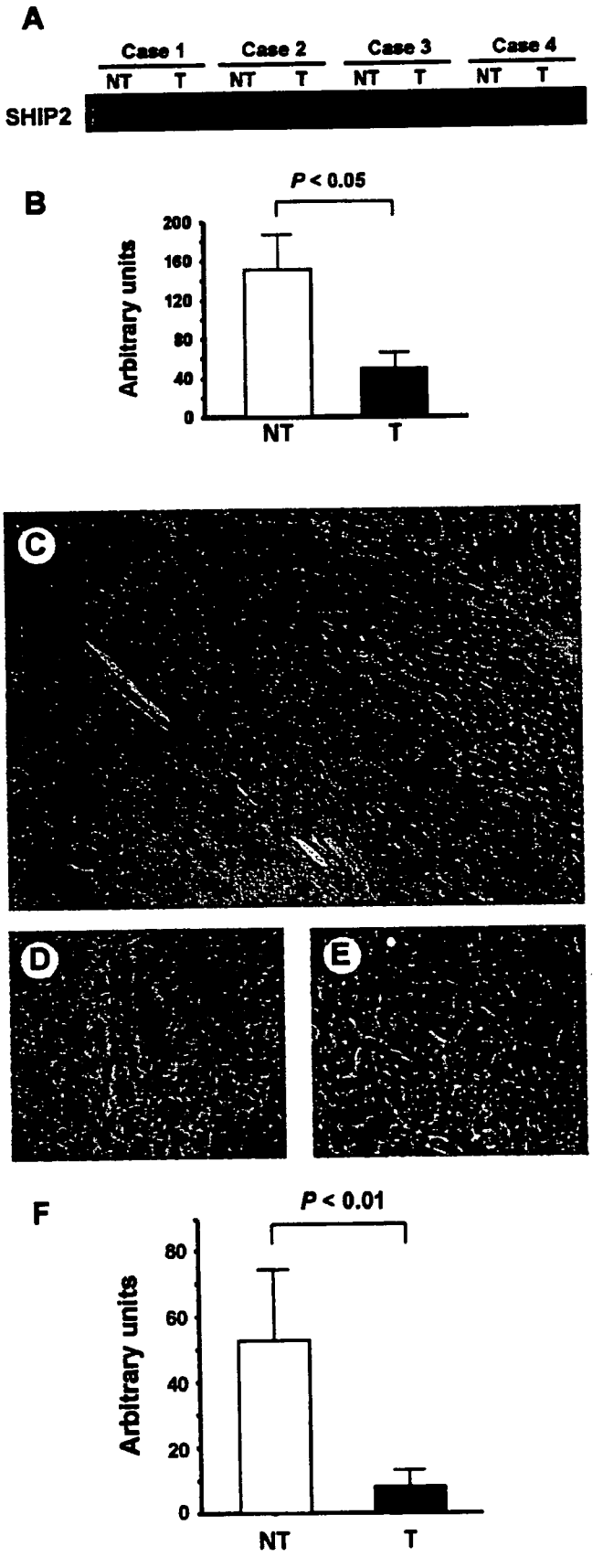

Figure 3. Expression levels of SHIP2 in tumor and nontumor livers from patients with HCC. (A) Immunoblotting for SHIP2. Proteins from tumor and nontumor extracts were immunoblotted with anti-SHIP2 antibodies. (B) Quantitation of immunoblotting intensity for SHIP2 in tumor and nontumor tissues. (C) Immunostaining for SHIP2. SHIP2 staining of liver sections from border areas between HCC tissue (T) and nontumor tissue (NT), (D) tumor tissue, and (E) nontumor tissue. (F) Quantitation of immunostaining intensity for SHIP2 in tumor and nontumor sections.

HCC cells (Fig. 3C-E). Quantitation of immunostaining intensity confirmed that SHIP2 expression was significantly decreased in HCC cells compared to that of nontumor hepatocytes (13.5±3.1 vs. 192.2±15.9 arbitrary units, $P<0.01$) (Fig. 3F).

Discussion

In Japanese HCC patients, survival is poorer in patients with diabetes mellitus compared to those without diabetes mellitus (29). On the other hand, two other cohort studies had reported that diabetes mellitus does not have a significant influence on the perioperative outcome or prognosis after hepatic resection for HCC (30,31). Thus, the impact of glucose intolerance for HCC patients remains controversial. A major contrast between their studies is that the majority of Japanese patients had HCV infection, whereas the patients of other studies had predominantly HBV infection. In a large population based cohort study, the relative risk for the development of HCC among those with HBV infection was 9.6, and the relative risk among those with HCV infection was 2.7. This result suggests that HBV itself, compared to HCV, may have greater potential for being carcinogenic (32). Furthermore, we previously reported that more severe insulin resistance was present in patients with HCV infection than in patients with HBV infection. HCV core protein has been reported as a responsible factor to the development of glucose intolerance (9). Similarly, HCV core protein level was associated with glucose intolerance. Therefore, glucose intolerance may have a significant influence on survival of HCC patients with HCV infection.

In this study, we investigated the impact of glucose intolerance in HCC patients with HCV infection. We observed no change in either disease-free survival or long-term survival. Barbara *et al* reported that Child-Pugh class was independently correlated with survival in the natural history of small untreated HCC among patients with cirrhosis (33). In agreement with the previous study, Child-Pugh class was an independent prognostic factor in the multivariate analysis of this study. Thus, our data imply that the ability to change the clinical course of the disease is strongly influenced by liver function.

Although the effect of glucose intolerance as a survival predictor was not significant, the significance of glucose intolerance in HCC patients was disclosed by stratification of gender. In male HCC patients, glucose intolerance was associated with significant decrease in long-term survival rate, although no difference was observed in disease-free survival between patients with and without glucose intolerance. It is presently uncertain why a gender difference was observed in the negative relationship between glucose intolerance and long-term survival of HCC patients with HCV. However, we showed that fasting insulin level was significantly higher in males than in females. It is possible that increased insulin resistance in male patients is associated with long-term survival of HCC patients.

In order to examine risk factors for long-term survival of male HCC patients, we performed univariate and multivariate analyses. In both analyses, the Child-Pugh class and glucose intolerance was identified as statistically independent prognostic factors. It remains unclear how glucose intolerance is involved in survival. It could be explained as insulin resistance contributing to fibrotic progression in chronic hepatitis with HCV infection (34). Furthermore, diabetes mellitus is an independent prognostic factor associated with the occurrence of hepatic decompensation in HCC patients (35). Thus, our findings suggest that the presence of glucose intolerance in HCC patients causes a more severely impaired liver function, which results in poor long-term survival. An alternative mechanism is that glucose intolerance could be a significant factor contributing to the growth rate of HCC. Saito *et al* reported the effect of hyperinsulinaemia on growth of human HCC (36). In addition, clinical studies have reported that high cell proliferation activity is an important survival predictor for HCC patients (37). Collectively, increased insulin levels may stimulate the growth of HCC, and survival may be reduced in male HCC patients.

Suppression of insulin signaling is also related to growth of HCC. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a suppressor of insulin signaling and it is frequently mutated or deleted in a variety of human cancers (38). Decreased PTEN expression levels are involved in the pathogenesis of HCC, and this decrease was correlated with tumor progression and poorer prognosis (39). SHIP2 is also known to be a negative regulator of insulin signaling (16,17). In chronic myeloid leukemia cells, overexpression of SHIP2 results in decreased insulin sensitivity, which strongly reduces cell proliferation (40). Thus, although the important role of SHIP2 as a tumor suppressor has been clarified in insulin signaling, the changes in SHIP2 on HCC has not been investigated. In this study, immunoblotting and immunostaining showed significant decreases in SHIP2 expression level in HCC tissues compared to nontumor tissue. These results suggest that down-regulated SHIP2 expression leads to increased sensitivity to insulin, which is linked to cell proliferation in HCC.

In conclusion, in this study we showed that glucose intolerance is an independent factor of poor prognosis in male HCC patients with HCV infection and expression of SHIP2 is significantly down-regulated in human HCC.

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References

1. Bosch FX, Ribes J, Diaz M and Cleries R: Primary liver cancer: worldwide incidence and trends. Gastroenterology 127: S5-S16, 2004.
2. Kiyosawa K, Umemura T, Ichijo T, *et al*: Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 127: S17-S26, 2004.
3. Megyesi C, Samols E and Marks V: Glucose tolerance and diabetes in chronic liver disease. Lancet 2: 1051-1056, 1967.
4. Marchesini G, Pacini G, Bianchi G, Patrono D and Cobelli C: Glucose disposal, beta-cell secretion, and hepatic insulin extraction in cirrhosis: a minimal model assessment. Gastroenterology 99: 1715-1722, 1990.

5. Petrides AS, Vogt C, Schulze-Berge D, Matthews D and Strohmeyer G: Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. Hepatology 19: 616-627, 1994.
6. Mason AL, Lau JY, Hoang N, *et al*: Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology 29: 328-333, 1999.
7. Kawaguchi T, Yoshida T, Harada M, *et al*: Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 165: 1499-1508, 2004.
8. Kawaguchi T, Nagao Y, Tanaka K, *et al*: Causal relationship between hepatitis C virus core and the development of type 2 diabetes mellitus in a hepatitis C virus hyperendemic area: A pilot study. Int J Mol Med 16: 109-114, 2005.
9. Shintani Y, Fujie H, Miyoshi H, *et al*: Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 126: 840-848, 2004.
10. Adami HO, Chow WH, Nyren O, *et al*: Excess risk of primary liver cancer in patients with diabetes mellitus. J Natl Cancer Inst 88: 1472-1477, 1996.
11. El-Serag HB, Tran T and Everhart JE: Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 126: 460-468, 2004.
12. Gruppuso PA, Boylan JM, Bienieki TC and Curran TR Jr: Evidence for a direct hepatotrophic role for insulin in the fetal rat: implications for the impaired hepatic growth seen in fetal growth retardation. Endocrinology 134: 769-775, 1994.
13. Virkamaki A, Ueki K and Kahn CR: Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Invest 103: 931-943, 1999.
14. Cantley LC: The phosphoinositide 3-kinase pathway. Science 296: 1655-1657, 2002.
15. Boulton TG, Nye SH, Robbins DJ, *et al*: ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 65: 663-675, 1991.
16. Pesesse X, Deleu S, De Smedt F, Drayer L and Erneux C: Identification of a second SH2-domain-containing protein closely related to the phosphatidylinositol polyphosphate 5-phosphatase SHIP. Biochem Biophys Res Commun 239: 697-700, 1997.
17. Clement S, Krause U, Desmedt F, *et al*: The lipid phosphatase SHIP2 controls insulin sensitivity. Nature 409: 92-97, 2001.
18. Milburn CC, Deak M, Kelly SM, Price NC, Alessi DR and Van Aalten DM: Binding of phosphatidylinositol 3,4,5-trisphosphate to the pleckstrin homology domain of protein kinase B induces a conformational change. Biochem J 375: 531-538, 2003.
19. Taylor V, Wong M, Brandts C, *et al*: 5' phospholipid phosphatase SHIP-2 causes protein kinase B inactivation and cell cycle arrest in glioblastoma cells. Mol Cell Biol 20: 6860-6871, 2000.
20. Ishihara H, Sasaoka T, Hori H, *et al*: Molecular cloning of rat SH2-containing inositol phosphatase 2 (SHIP2) and its role in the regulation of insulin signaling. Biochem Biophys Res Commun 260: 265-272, 1999.
21. Monzillo LU and Hamdy O: Evaluation of insulin sensitivity in clinical practice and in research settings. Nutr Rev 61: 397-412, 2003.
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419, 1985.
23. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC and Williams R: Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60: 646-649, 1973.
24. Sobin LH and Witteking CH: TNM Classification of malignant tumors. 5th edition, John Wiley & Sons, Inc., New York, 1997.
25. Donato F, Tagger A, Gelatti U, *et al*: Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 155: 323-331, 2002.
26. Aoyagi K, Ohue C, Iida K, *et al*: Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. J Clin Microbiol 37: 1802-1808, 1999.
27. Liver Cancer Study Group of Japan: Primary liver cancer in Japan. Clinicopathologic features and results of surgical treatment. Ann Surg 211: 277-287, 1990.
28. Kawaguchi T, Sakisaka S, Sata M, Mori M and Tanikawa K: Different lobular distributions of altered hepatocyte tight junctions in rat models of intrahepatic and extrahepatic cholestasis. Hepatology 29: 205-216, 1999.
29. Ikeda Y, Shimada M, Hasegawa H, *et al*: Prognosis of hepatocellular carcinoma with diabetes mellitus after hepatic resection. Hepatology 27: 1567-1571, 1998.
30. Poon RT, Fan ST and Wong J: Does diabetes mellitus influence the perioperative outcome or long term prognosis after resection of hepatocellular carcinoma? Am J Gastroenterol 97: 1480-1488, 2002.
31. Huo TI, Wu JC, Lui WY, *et al*: Differential mechanism and prognostic impact of diabetes mellitus on patients with hepatocellular carcinoma undergoing surgical and nonsurgical treatment. Am J Gastroenterol 99: 1479-1487, 2004.
32. Yang HI, Lu SN, Liaw YF, *et al*: Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 347: 168-174, 2002.
33. Barbara L, Benzi G, Gaiani S, *et al*: Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. Hepatology 16: 132-137, 1992.
34. Hui JM, Sud A, Farrell GC, *et al*: Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. Gastroenterology 125: 1695-1704, 2003.
35. Huo TI, Lui WY, Huang YH, *et al*: Diabetes mellitus is a risk factor for hepatic decompensation in patients with hepatocellular carcinoma undergoing resection: a longitudinal study. Am J Gastroenterol 98: 2293-2298, 2003.
36. Saito K, Inoue S, Saito T, *et al*: Augmentation effect of postprandial hyperinsulinaemia on growth of human hepatocellular carcinoma. Gut 51: 100-104, 2002.
37. Donato MF, Arosio E, Del Ninno E, *et al*: High rates of hepatocellular carcinoma in cirrhotic patients with high liver cell proliferative activity. Hepatology 34: 523-528, 2001.
38. Li J, Yen C, Liaw D, *et al*: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275: 1943-1947, 1997.
39. Hu TH, Huang CC, Lin PR, *et al*: Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. Cancer 97: 1929-1940, 2003.
40. Giuriato S, Blero D, Robaye B, Bruyns C, Payrastre B and Erneux C: SHIP2 overexpression strongly reduces the proliferation rate of K562 erythroleukemia cell line. Biochem Biophys Res Commun 296: 106-110, 2002.