3．肝発癌予防

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key words　hepatocellular carcinoma，hepatitis C virus，interferon，hepatitis B virus，retinoid

動　向

肝細胞癌（肝癌）が他の臓器の癌腫と大きく異なる点は，肝癌が慢性肝疾患を基礎疾患として発生することが多く，発生母地が明らかな点である．わが国では肝癌の80％以上がB型またはC型肝炎ウイルスに由来する肝硬変から発生している．このことは，ウイルス性慢性肝疾患や種々の原因による肝硬変が肝癌発生高危険群としての大規模なcohortを形成していることを意味し，肝癌早期発見を目指した腫瘍マーカー・画像診断による肝癌スクリーニングが効率的に施行されうることを意味している．癌発生のハイリスクグループが充分に設定でき，有効な肝癌拾い上げが可能なために，他臓器癌と比べて肝癌は小型の状態で腫瘤を発見できる有利な点がある一方，肝癌が再発しやすい（新規に発癌しやすい）という背景を有している特徴的な癌腫である．

このことは，大きなコホートに対して発癌予防治療を行う重要な根拠となり，すでに発生してしまった肝癌に対して行う肝切除・経皮的局所治療と並んで，発癌予防・再発予防はきわめて大切な肝癌診療の根幹となりつつある．これまでに肝細胞癌発癌予防として，インターフェロン，漢方薬（小柴胡湯），レチノイド，セレニウム，ビタミンD誘導体などが用いられており，ここでは最近1～2年間に出版された肝発癌予防の論文についてレビューする．

A．インターフェロンによる肝発癌予防

インターフェロンによる肝細胞癌発癌予防・再発予防に関する論文は，1990年代よりパイロット研究・レトロスペクティブ研究・非無作為化試験を中心にわが国から発信され始めた．

B型肝硬変に対する発癌予防としてのインターフェロン治療は，古くはOonら[1]，Ikedaら[2]のレトロスペクティブ研究，Linら[3]の無作為化比較試験により発癌抑制効果が示されているが，欧米の研究[4,5]では必ずしもインターフェロンによる発癌抑制効果は確認されていない．

Ikedaら[6]は，B型肝炎ウイルス量と肝発癌についてnested-case control studyの手法を用いて検討し，肝硬変であればHBV DNAが$10^{3.7}$コピー/ml未満の低濃度で持続している状態からの発癌はほとんどないことを示し，HBVウイルス量とB型発癌と強い関連を示した．このデータを受けてさらにIkedaら[7]は，長期にインターフェロン投与を行ったB型肝硬変からの発癌で，HBV DNAを良好に低下させることのできた症例で有効な発癌抑制が可能となることを示し，その早期

予測性についても述べている．一方Oonら[8]は，先に発表したB型肝癌切除後のインターフェロンの再発抑制効果を，14年にわたる長期経過観察で示している．肝硬変合併肝細胞癌の切除後化学療法を行った症例の検討で，天然型αインターフェロン1日300万単位10日間の投与を3カ月ごとに繰り返すという方法である．この治療方法で予定完遂した症例では再発例はなく，この間の化学療法単独群では全例再発した．治療期間中にインターフェロンを減量した3症例，中止した2症例，もしくは30MUを6カ月ごとに投与していた2症例では肝癌再発がみられたとしている．欧米系の研究は症例数が比較的少なく，統計的な有意差を証明するには至っていないが，アジア系の成績では，B型肝硬変に対するインターフェロンの発癌抑制効果を示す傾向がある．

C型慢性肝炎・肝硬変に対するインターフェロンの発癌抑制効果は，すでに1990年代から発表がなされている．慢性肝炎に対してインターフェロン治療を行った症例のうち，ウイルス排除に成功した例（ウイルス学的著効: sustained virological response[9,10]およびトランスアミナーゼの正常化が得られた例（生化学的著効: biochemical response)[10-12]で，有意に発癌率が低下することに関してはわが国からの報告がなされてきた．また，肝硬変に対する発癌抑制効果についても，Nishiguchiら[13]の無作為化比較試験によりインターフェロンが有意に発癌抑制的であることが示されている．

最近の論文では，Suzukiら[14]が，755例のインターフェロン施行C型慢性肝炎の治療例について発癌との関連を検討している．ウイルス学的著効，生化学的著効ともに肝細胞癌発癌率が低い結果は，これまでと同様であるが，生化学的著効達成のための唯一の独立因子はインターフェロン終了時のALT値であったとし，インターフェロン治療終了時のALTは発癌率予測因子になった．さらに生化学的著効になりやすい因子は，若年，肝炎活動性が低い，肝線維化が少ない，血小板数が多い，IFN投与期間が長いなどであるとしている．この論文では発癌抑制の観点から種々の要因を検討しているが，非治療群の対照をおいていないため，全体としてのインターフェロンのインパクトについては不明である．Testinoら[15]は，122例の代償期肝硬変を対象に，平均観察期間96カ月のレトロスペクティブな観察を行い，肝細胞癌発生に対するインターフェロンの効果をみている．このうち59例がインターフェロン施行（3MU週3回，12カ月），うち8例が副作用中止，71例はIFN施行しないというものであるが，インターフェロン投与は肝機能悪化・死亡・肝移植移行の観点からは有利だが，明らかな発癌抑制効果を示さなかった．本研究ではウイルス排除例からも11例中3例発癌しているなどがその理由としているが，肝硬変に進行していてすでに発癌過程が進んだ対象であることに加え，少数例の検討であることがそのような結論に至った原因と考えられる．

Nakajiら[16]は，αインターフェロンの肝癌発癌抑制メカニズムを検討した．Diethyl nitrosamine（DEN）でinitiationを起こし，2-acetylaminofluorene（2-AAF）と肝切除でpromotionを起こすもので，肝細胞癌は前癌病巣から炎症・線維化を伴わずに発癌する動物モデルである．化学発癌initiationの時点からインターフェロンを同時に開始し，腫瘍関連遺伝子，細胞周期関連遺伝子を免疫組織学的およびPCRで，preneoplastic fociについて検討したところ，αインターフェロン治療はpreneoplastic fociの数と平均の大きさ（volume）を小さくし，PCNAインデックス，G1 cylclinsの表出を低下させた．インターフェロン投与はpreneoplastic cellの細胞増殖を抑制することを介して40週での腫瘍進展を抑制し，長期のインターフェロン投与は腫瘍個

数・腫瘍volumeの両者を短期投与群よりも抑制する結果を得た．p21の表出はインターフェロン投与群で高かったが，p53表出は変わらなかったことなどより，そのメカニズムはp21誘導に一部依存しているが，p53とは独立していると推定している．インターフェロンの発癌抑制はこれまでは，抗ウイルス・抗炎症作用で説明されていたが，本研究のような抗癌作用のメカニズムについても今後の検討が待たれる．

B. ビタミンによる肝発癌予防

ビタミンによる肝癌発癌抑制・再発抑制についての論文は，これまで非環式レチノイドの論文を主として，基礎的・臨床的な成果が発表され，すでに無作為比較試験の長期成績も出されている．わが国でこの研究をリードしているOkunoら[17]，Kojimaら[18]は，ビタミンAの効果，基礎実験による作用メカニズムなどについて，最近のレビュー論文で詳細を記している．

同様な脂溶性ビタミンの中で，Meydaniら[19]は動物実験で認められる肝発癌抑制効果が抗酸化作用・免疫強化作用によると記載したが，Kakizakiら[20]はビタミンEが肝細胞癌発癌を抑制することを同様に動物実験で示している．Takagiら[21]は，83例のC型肝炎感染既往例・肝硬変例を2群に分け，44例はalpha-tocopherol群，39例は経過観察群として発癌率の比較を行った．5年間にわたり，alpha-tocopherol，アルブミン，ALT，コレステロール，血小板数を経時的に測定したところ，累積肝癌非出現率はalpha-tocopherol群で高い傾向であったが，統計学的有意差は得られなかった（$p = 0.07$）．インターフェロンや非環式レチノイドのデータ同様，肝硬変症例ではかなりの部分がすでに発癌過程の進行している例が含まれている可能性が考えられる．

C. その他の薬剤による肝発癌抑制

Cyclooxygenase-2（COX-2）inhibitorsは，悪性腫瘍ではアポトーシス的作用・抗血管新生作用があり，周囲組織に浸潤することを抑制する．COX-2はアラキドン酸代謝のキーエンザイムで，多くの悪性腫瘍で高発現しており，これら特性はCOX-依存性，COX-非依存性シグナル伝達を介している．ヒト肝細胞癌発癌におけるCOX-2の関与はまだ不詳であるが，種々の腫瘍悪性化作用を示す[22]．

肝炎ウイルスに対する持続的な免疫反応は肝細胞癌発癌のよく知られた危険因子である．しかし免疫応答の肝発癌促進については，その分子生物学的なメカニズムがわかっていない．Nakamotoら[23]は，慢性肝炎から肝発癌を起こす特徴的な動物モデルを用いて，抗FasLを用いて，Fasリガンド活性の中和を図ると肝細胞アポトーシス，増殖，肝の炎症，最終的には肝癌発癌過程までが抑制されることを見出した．この結果からは，Fasリガンドは直接の肝細胞傷害慢性肝炎に関係するのみならず，慢性肝炎における炎症と肝発癌過程にもかかわっていることが判明した．これにより慢性炎症をなんらかの治療で抑えると肝癌発癌を抑制することができることを示した．

Manigoldら[24]はこの論文に対する批評として，CTLによって誘導されたアポトーシスを抑制することによる発癌抑制について初めて論文化されたことを評価している．しかし，実際にこの実験モデルの内容を，慢性感染を起こしている患者についての肝癌抑制に当てはめるのは難しいと評している．具体的には，短期間の抗FasL治療がどの程度効くのかどうか疑問であること，しかし長期使用はFas/FasL相互作用が免疫監視に影響するであろうこと，活性化FasL表出リンパ球の超早期の細胞死をもたらすこと，ウイルス側のメカニズム（escape mutation，免疫システムの

積極的なdown regulationなど）が起きうること，HBxの意義が発癌には大きいことなどが問題であろう，と述べている．

Qianら[25]は，食事性・薬草性のGanfujian顆粒がdiethylnitrosamine（DEN)-誘発性のラット肝癌を抑制する効果があるかどうかをオスSDラットで検討したところ，Ganfujian顆粒は肝癌発癌率を低下させ生存率も高めた．Ganfujian顆粒は肝癌発癌の全過程におけるcyclin-dependent kinase（CDK4）の高発現を抑制し，16週でのcyclin D発現抑制，各発癌段階のPCNA陽性細胞数を抑制したことより，本剤が直接間接的に細胞周期に影響するとともに肝細胞の無制限増殖を抑制することによる肝癌抑制をきたすのではないかとしている．

これまでにもアフラトキシンによる肝発癌のリスクについては知られていたが，最近ではアフラトキシン曝露による生物的マーカーの確認，遺伝子学的な影響についていくつかの新知見が現れている．これらのbiomarkerについては，現在食餌内アフラトキシン曝露の毒性を薬理学的介入により変化させることができるかなどの作用について検索されている．Sudakinら[26]はアフラトキシンによる肝発癌性抑制に関するレビューを行い，Oltipraz によりアフラトキシンB1の遺伝子毒性を変化させることができるという臨床試験がpreliminaryにでていること，クロロフィリンによるさらに新しい臨床試験で食餌内のアフラトキシン曝露を防ぐなどの効果を紹介している．

D. 肝癌発癌抑制をレビューした論文

この2年間には，1990年代以後インターフェロンを主として発表された肝癌発癌抑制の論文について，多くのレビュー論文が出版された[27-41]．C型肝炎・肝硬変に対するインターフェロン治療に関するものをほとんどの論文は含んでいるが，切除後再発予防を含んでいるもの[28,36]，最近ではB型肝炎に対するラミブジンの効果について論じたもの[29]も発表されている．

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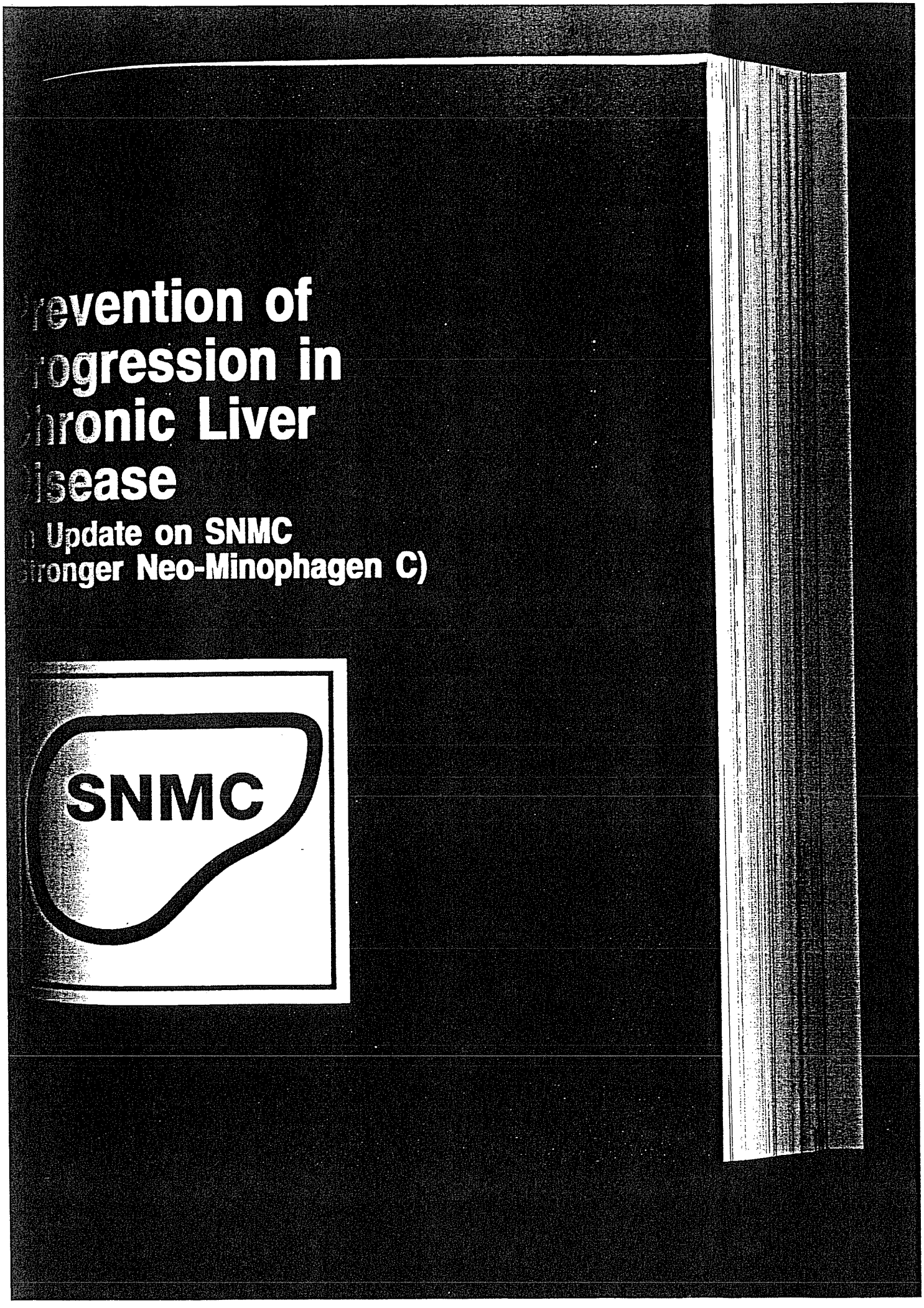
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9
SNMC in the prevention of cirrhosis and hepatocellular carcinoma – Japanese experience

K. IKEDA and H. KUMADA

INTRODUCTION

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

Interferon (IFN) is reported to be effective in reducing the rate of hepatocellular carcinogenesis through suppression of the necroinflammatory process of the disease and elevated serum alanine aminotransferase (ALT) values and/or through elimination of HCV in high-risk patients with chronic hepatitis C with or without cirrhosis. Although IFN has been proved to be valuable for reduction of the risk of carcinogenesis, it is not effective in every patient with HCV-related liver disease. Oka et al.[8] reported that in a randomized controlled trial a type of medicinal herb, *Sho-saiko-to*, significantly decreased the rate of hepatic carcinogenesis in patients with HBsAg-negative cirrhosis. Tarao et al.[9] showed that, in patients with HCV-related cirrhosis, HCC appearance rate was significantly higher in those with a high ALT value of 80 IU or more than in those with a lower ALT value of less than 80 IU, and suggested that treatment of cirrhosis and prevention of HCC should be directed to a suppression of the necroinflammation of HCV-related hepatitis.

In Japan a glycyrrhizin-containing preparation, Stronger Neo-Minophagen C™ (SNMC), is widely used for the treatment of chronic hepatitis. It is available in an injectable form for intravenous administration, containing 0.2% glycyrrhizin, 0.1% L-cysteine and 0.2% glycine in physiologic solution. It is made by dissolving glycyrrhizin (200 mg), L-cysteine (100 mg) and glycine (2 g) in 100 ml of physiologic saline. Glycyrrhizin is an aqueous extract of licorice root (*Glycyrrhizae radix*), which has anti-allergic, anti-inflammatory and

detoxicating effects. As has been reported, the anti-inflammatory action of SNMC is considered to be working to protect the liver cell membrane, which may explain its ability to lower serum aminotransferase levels in patients with chronic hepatitis. Since SNMC thus exerts a favourable effect on ALT and histology in patients with chronic viral hepatitis[10–16], we investigated how this product works on hepatocellular carcinogenesis in those patients.

STUDY I: A RETROSPECTIVE COHORT STUDY IN 1249 PATIENTS IN A SINGLE HOSPITAL

Background and purposes

In order to elucidate whether SNMC suppresses the carcinogenesis rate in patients with IFN-resistant chronic hepatitis C, we retrospectively assessed a cohort of 1249 patients without sustained virological response after IFN therapy.

Patients and methods

A total of 1249 consecutive Japanese patients with chronic hepatitis C with or without cirrhosis, in whom eradication of HCV-RNA was not attained under previous IFN therapy, were examined. The cohort consisted of 778 men and 471 women aged 18–81 years, with a median age of 53; diagnosis of cirrhosis was made by peritoneoscopy or liver biopsy or both between 1987 and 2002 at Toranomon Hospital, Tokyo, Japan.

Of the 1249 patients with IFN-resistant chronic liver disease, 453 patients underwent SNMC injection therapy and the remaining 796 patients did not receive SNMC therapy until the end of observation. The purpose of the introduction of SNMC therapy was to suppress high ALT values and to prevent disease progression in these high-risk patients. F1 stage hepatitis was significantly more often found in the untreated group than in the SNMC group ($p < 0.001$, chi-square test). The median AST and ALT values at the beginning of observation were significantly higher in the SNMC-treated group than in the untreated group ($p < 0.001$).

When SNMC was found effective in terms of suppression of aminotransferases, treatment was usually continued as long as possible. The 453 patients received a median daily dose of 100 ml of SNMC by intravenous injection three times a week during a median period of 4.3 years (range 0.1–14.5 years). Two (0.44%) of these 453 patients withdrew from therapy because of side-effects: one from hypertension and one from itching skin rash.

The number of cases which were excluded from the follow-up were 121 (9.7%): 28 patients (6.2%) in the SNMC group and 93 (11.7%) in the untreated group. Since the eventual outcome regarding appearance of HCC had not been identified in these patients, they were considered as constituting censored data in the following statistics. Death unrelated to HCC was also classified as a cause for withdrawal and the patient was 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.

HCC appearance rates were calculated from the period between the judgement of no response to IFN therapy and appearance of HCC, using Kaplan–Meier technique[17]. The differences in carcinogenesis curves were tested using the log-rank test.

Results

Crude hepatocellular carcinogenesis rates

During the observation period of 5.7 years, HCC appeared in 112 patients (9.0%): 70 (15.4%) in the SNMC treated group and 42 (5.3%) in the untreated group. Crude carcinogenesis rates were calculated from a period between the judgement of no response to IFN therapy and appearance of HCC. Hepatocellular carcinogenesis rates in the SNMC group and the untreated group were 5.1% and 3.0% at the end of the 3rd year, 11.6% and 5.0% at the end of the 5th year, 15.0% and 6.8% at the end of the 7th year, and 19.9% and 10.6% at the 10th year, respectively (Figure 1). The carcinogenesis rate in the SNMC-treated group was significantly higher than that in the untreated group (log-rank test, $p = 0.0001$).

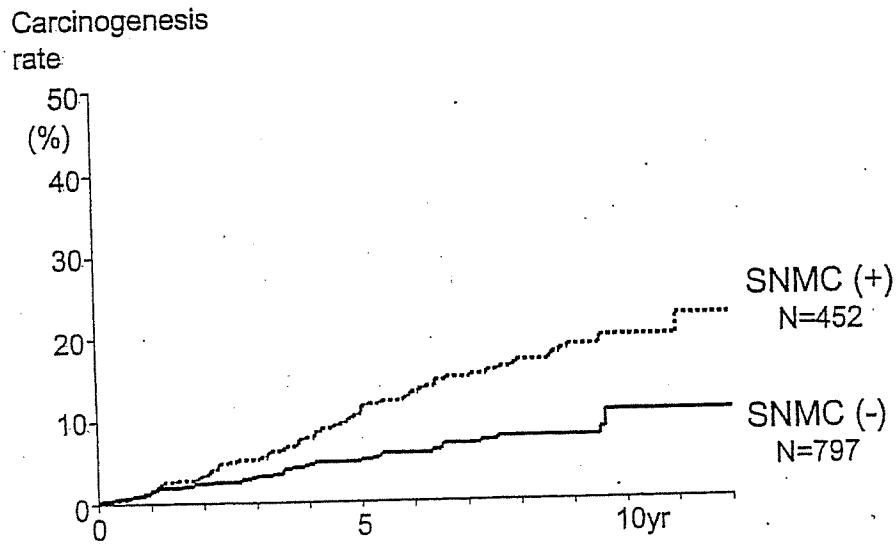

Figure 1 Study I: Crude hepatocellular carcinogenesis rates in patients with and without SNMC therapy. The carcinogenesis rate of the SNMC-treated group was significantly higher than that of the untreated group

Aminotransferase in patients with and without SNMC therapy

SNMC therapy was performed primarily in those patients with a high ALT value and high hepatitis activity. All patients with and without SNMC therapy were classified into six subgroups according to average ALT values during the

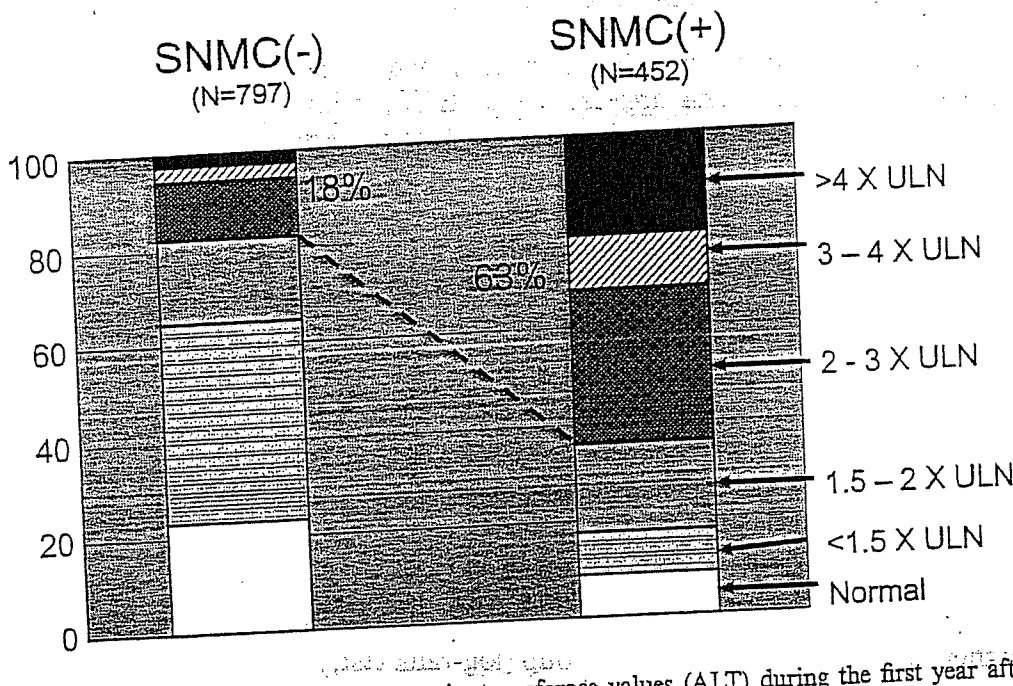

Figure 2 Study I: Average alanine aminotransferase values (ALT) during the first year after judgement of interferon ineffectiveness. The rate of a high ALT value of twice or more the upper limit of normal in the SNMC therapy group was significantly higher than that of the untreated group

first year following the respective IFN therapy: (1) with ALT value in normal range, (2) less than 1:5 times the upper limit of normal (ULN), (3) 1.5–2 times ULN, (4) 2–3 times ULN, (5) 3–4 times ULN and (6) more than 4 times ULN.

In this retrospective study the average ALT values were significantly different between the treated and untreated groups (Figure 2): (1) normal average ALT was found in 38 patients with SNMC therapy and in 188 patients without it, (2) an ALT value of less than 1.5 times ULN was found in 42 and 331, (3) 1.5–2 times ULN in 84 and 138, (4) 2–3 times ULN in 143 and 92, (5) 3–4 times in 53 and 29, and (6) more than 4 times ULN in 93 of the SNMC group and 18 of the untreated group, respectively. The rate of a high ALT value of twice ULN or more in the SNMC-treated group (64.2%, 289/453) was significantly higher than that in the untreated group (16.2%, 129/796).

Effect of SNMC therapy in patients with a high aminotransferase value

Among the 278 patients with SNMC therapy for a high average ALT value of twice or more ULN, 216 began SNMC injection therapy within 2 years after the judgement of no response to IFN therapy, and the remaining 62 began therapy after 2 years. Considering the median observation period of 5.7 years, the latter patients were not regarded as sufficiently treated during the complete observation period. Certain patients in the SNMC treatment group received the therapy for a short period of time within the observation period, following the judgement of no response to IFN therapy.

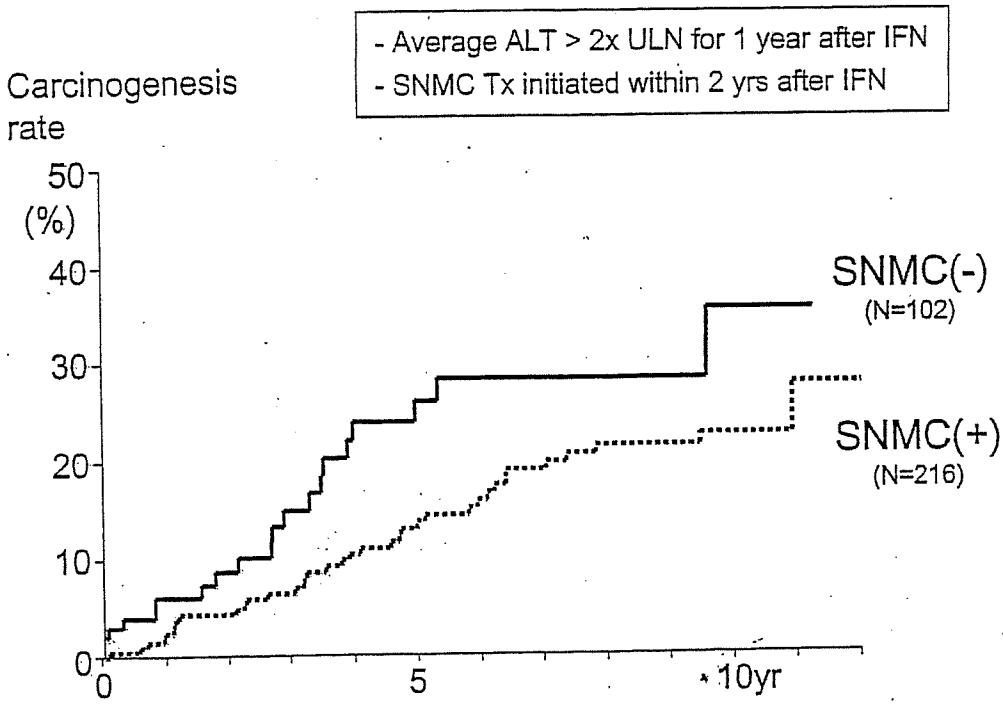

Figure 3 Study I: Carcinogenesis rates in patients with active chronic hepatitis showing a high average ALT value of twice or more the upper limit of normal. The carcinogenesis rate in patients with a sufficient period of SNMC treatment was significantly lower than that of untreated patients

Excluding those patients as having received 'insufficient treatment', we eventually compared the carcinogenesis rates between the untreated patients (n = 102) and the SNMC-treated patients who began SNMC therapy within 2 years after the judgement of no response to IFN therapy (n = 216). Cumulative carcinogenesis rates in the treated and untreated groups were 13.7% and 26.0% at the end of the 5th year, and 27.8% and 35.5% at the end of 10th year, respectively (Figure 3). The carcinogenesis rates were significantly lower in those patients with SNMC therapy (log-rank test, p = 0.038).

STUDY II: META-ANALYSIS OF INDIVIDUAL PATIENT DATA FROM 12 INSTITUTIONS

Background and purposes

In the patients with a history of ineffective interferon therapy for chronic hepatitis type C, an analysis was made on the effect of SNMC in prevention of carcinogenesis. Patient data were collected from 12 independent Japanese hospitals for the purpose of generalization of the results.

Patients and methods

This meta-analysis was initiated by Dr Suzuki H (Yamanashi Medical College, Japan) with a cooperation of Dutch and Japanese project teams: SW Schalm, B Hansen, BJ Veldt and E Verheij from Holland; and S Iino, H Kumada and K Ikeda from Japan. An advisory committee was also organized by experts from France (T Poynard), Holland (T Stijnen), and Japan (C Hirayama and N Hayashi). In order to gather reliable patient data, a total of 12 institutions were selected among large-scale, specialized hepatology centres throughout Japan: Sapporo Kousei Hospital (Sapporo), Hokkaido University (Sapporo), National Tokyo Hospital (Tokyo), Musashino Red Cross Hospital (Tokyo), Kiyokawa Hospital (Tokyo), East Hospital of Kitasato University (Kanagawa), Aikawa Hospital (Ibaraki), Yamanashi Prefecture Central Hospital (Yamanashi), Shinshu University (Nagano), Kawasaki Hospital of Kawasaki University(Okayama), Oono Gastroenterology Hospital (Ehime), and Kumamoto University (Kumamoto).

Patient data were collected from the 12 hospitals by eligibility criteria consisting of: chronic hepatitis or cirrhosis diagnosed by peritoneoscopy and/or liver biopsy, a history of interferon therapy from 1990 to 1995, non-sustained virological response under the therapy.

Individual patient data consisted of histological findings before IFN therapy, virological data, haematological and biochemical data before IFN therapy, details of IFN therapy, method of SNMC therapy, biochemical findings before and during SNMC therapy, development to cirrhosis, date of HCC development, and survival.

Data from 1093 patients were subjected to the same analysis as those of a retrospective cohort. A follow-up observation was made for a median period of 6.1 years with a range of 2.5–9.0 years.

Standard statistical methods, including chi-square test and Mann–Whitney *U*, were used for the analysis of differences of background factors between patients with and without SNMC treatment. A Kaplan–Meier method[17] was adopted to estimate cumulative carcinogenesis rate after cessation of interferon. Cox proportional hazards analysis[18] was performed to evaluate the independent predictors for future carcinogenesis rate in patients with chronic hepatitis.

Results

Background of patients

A total of 1093 patient data were collected from the 12 Japanese institutions. There were 634 men and 459 women, with a median age of 54 years, ranging from 17 to 81. Among the 1093 patients, 733 patients had HCV genotype 1, 210 had genotype 2, and 13 had genotype 3 or 4. At the beginning of the observation period, 451 patients had chronic hepatitis in a fibrosis stage 1 (F1) according to the classification of Desmet V, 372 in F2 stage, 202 in F3 stage, and the remaining 54 were in F4 stage or had cirrhosis.

Development to cirrhosis, carcinogenesis and death

During an observation period of 6.1 years, 138 (13.3%) of 1093 patients progressed to cirrhosis: 192 patients were finally diagnosed as having cirrhosis at the end of the observation period.

A total of 107 patients (9.8%) developed HCC and 33 patients (3.0%) died during the observation period. Of the 192 who eventually progressed to cirrhosis, 79 patients (41.1%) showed HCC by the end of the observation period, and 23 (12.0%) died with or without HCC. Cumulative hepatocellular carcinogenesis rates were 6% at the end of the 5th year, and 25% at the end of the 10th year.

The crude hepatocellular carcinogenesis rate of all 1093 patients was 6% at the end of the 5th year and 25% at the end of the 10th year (Figure 4). When carcinogenesis rates were calculated according to the fibrosis stages at the initiation of observation (Figure 5), HCC appeared in 10 (2.2%) of the 451 patients in F1 stage, 31 (8.3%) of the 372 patients in F2 stage, 42 (20.8%) of the 202 patients in F3 stage, and 20 (37.7%) of the 53 patients with cirrhosis, during a median observation period of 6.1 years. The more severe the hepatic fibrosis, the higher was the future risk of carcinogenesis.

Carcinogenesis curves were also generated according to initial ALT values. Initial ALT values were found to be clearly correlated with future carcinogenesis rates (Figure 6).

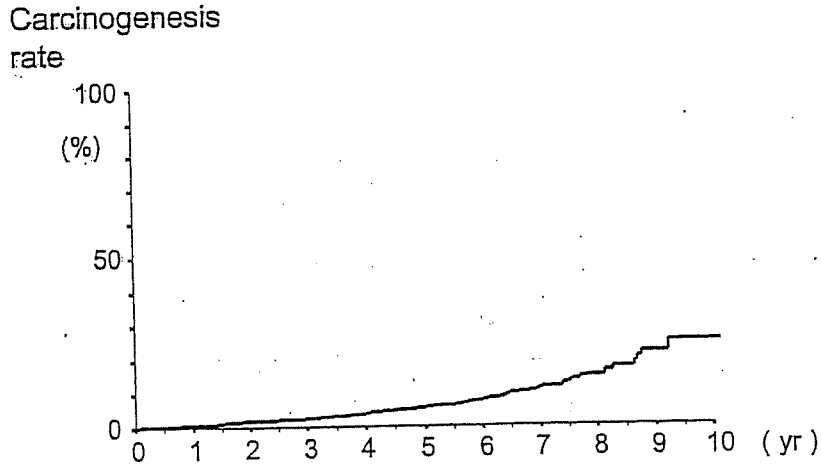

Figure 4 Study II: Carcinogenesis rate of entire patients with chronic liver disease caused by hepatitis C virus

SNMC therapy for patients with chronic hepatitis C

A total of 465 patients (42.5%) received SNMC therapy after ineffective IFN therapy: SNMC therapy was performed with or without other therapies (ursodeoxycholic acid and so on). A multivariate analysis revealed that SNMC therapy had been performed in older patients, in patients with advanced liver disease and in patients with high ALT values.

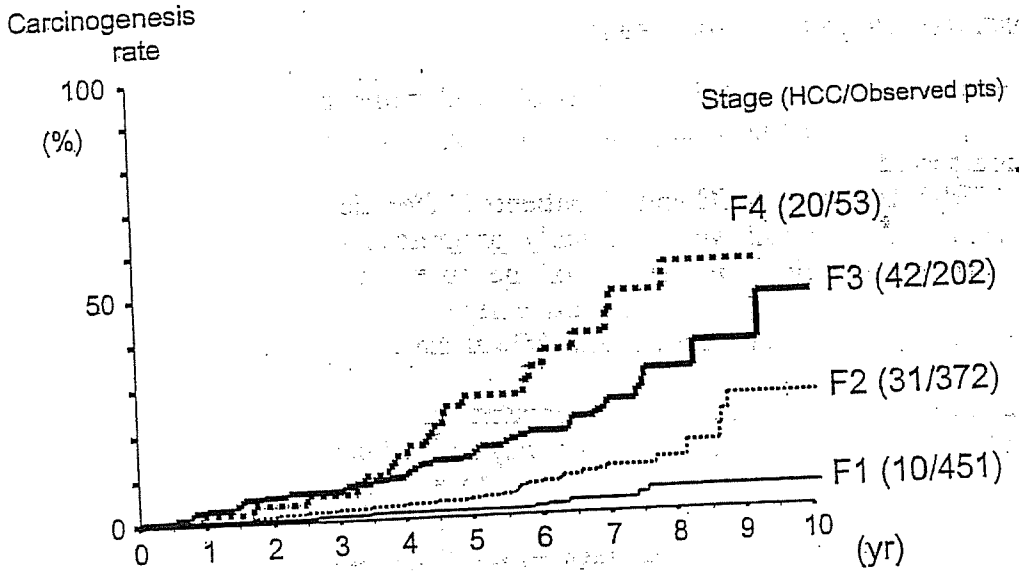

Figure 5 Study II: Carcinogenesis rates according to fibrotic stages at the initiation of observation

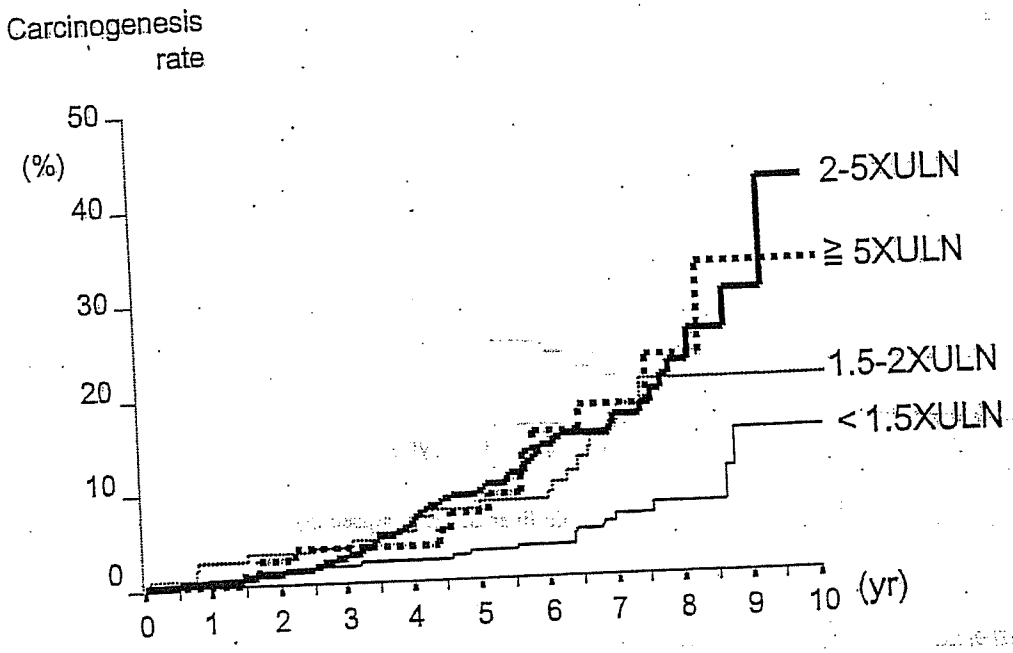

Figure 6 Study II: Carcinogenesis rates according to initial ALT value

Table 1 Study II: Independent predicting factors for future carcinogenesis rate in patients with F3 and F4 stage hepatitis (time-dependent Cox proportional hazards analysis)

Factor	*Category*	*Hazard ratio (95% confidence interval)*	*p-Value*
Age	+1 year	1.1 (1.0–1.1)	<0.001
Gender	Men	1	
	Women	0.3 (0.2–0.5)	<0.001
SNMC	No	1	
	Yes	0.4 (0.2–1.0)	0.04

The median dose of SNMC injection was 191 ml per week and the median duration of the therapy was 196 weeks.

Among the 395 patients who continued SNMC injection therapy for 16 weeks or longer, 169 (42.8%) showed a decrease of ALT values down to the level of less than 1.5 times ULN.

Influence of SNMC on carcinogenesis

A multivariate Cox proportional hazards analysis was performed only in patients in hepatitis stage F3 or F4, using the length of waiting time until the SNMC injection as a time-dependent variable. Older age ($p < 0.001$), female sex ($p < 0.001$) and use of SNMC ($p = 0.04$) were independently associated with carcinogenesis rate. In this analysis, after adjusting the background differences of the treated and untreated group with significant covariates (Table 1), SNMC was found to have significantly decreased the risk of cancer development.

DISCUSSION

Yamamoto et al.[10] first treated patients with chronic hepatitis with SNMC and found a remarkable improvement in their ALT levels. Suzuki et al.[12] confirmed the effect of SNMC to suppress serum aminotransferase in patients with chronic hepatitis in a randomized controlled trial. Hino et al.[13] and Yasuda et al.[14] also confirmed that SNMC was useful in the improvement of transaminase levels and liver histology. We previously reported that SNMC was beneficial for improvement of carcinogenesis rate in patients with chronic hepatitis C when it was administered for 10 years or longer[19]. In these retrospective studies, we assessed the role of SNMC therapy in the prevention of hepatocellular carcinogenesis in patients with chronic hepatitis C.

This retrospective study was undertaken to evaluate whether SNMC injection therapy could decrease the HCC rate in patients with HCV-related chronic hepatitis with or without cirrhosis not responding to IFN therapy. Since it requires at least 5 years to obtain a statistically valid finding in carcinogenesis

rate from hepatitis with or without cirrhosis between the glycyrrhizin-treated and the 'untreated' groups, a prospective randomized trial using untreated control patients is difficult from both ethical and medical viewpoints in Japan where SNMC injection therapy is covered by medical insurance, and it is already regarded as a usual choice of therapy as a salvaging procedure for IFN-ineffective patients. We therefore attempted to carry out a retrospective cohort study (study I) and meta-analysis of a large number of data from multiple hepatology centres (study II), with a statistical adjustment using possible covariates explored in multivariate analysis.

In both studies, when crude carcinogenesis rates were compared between the treated and untreated patient group, the hepatocellular carcinogenesis rate in the SNMC therapy group was found to be significantly higher than that in the untreated group. Since anti-inflammatory therapy using SNMC was usually performed for those patients with a high ALT value and high hepatitis activity, it seemed to be a plausible result that the carcinogenesis rate in the treated group was higher than that in the untreated group. Actually the treated group consisted of significantly more patients with a high ALT value, of twice ULN or more.

In study I, when carcinogenesis rates were assessed only in patients with a high ALT value of twice ULN or more, the rate in the SNMC-treated group came out slightly higher than that in the untreated group (data not shown). Some of the patients in the treated group received SNMC therapy several months or a few years after judgement of no response to IFN therapy. In order to elucidate the potential effect of SNMC to prevent carcinogenesis in patients with an active HCV-related liver disease, we further stratified the treated patients into two subgroups: early treatment subgroup of patients who received SNMC therapy within 2 years after judgement of no response to IFN therapy and late treatment subgroup of patients receiving treatment over 2 years after the event. Since the patients in the latter subgroup were observed without therapy for a considerable period, they were regarded as only partly and insufficiently treated with SNMC from the viewpoint of the entire observation period. We therefore compared carcinogenesis rates between treated and untreated patients, excluding those patients in the late treatment subgroup. The hepatocellular carcinogenesis rate of the patients who received SNMC therapy for a sufficient period of time was significantly lower than that in those who did not receive the therapy ($p = 0.038$). In the treated group the median ALT values decreased significantly after initiation of SNMC injection, suggesting that suppression of the necroinflammatory process of liver disease by SNMC therapy was working as a primary factor in reducing carcinogenesis in the high-risk patients. The current study dealing with a large cohort ($n = 1249$) showed that the carcinogenesis rate was reduced when SNMC therapy was started at an early time after judgement of no response to INF therapy.

In study II, background biases between the SNMC-treated group and the untreated group were adjusted by significant covariates using multivariate analysis. The final Cox proportional model showed that SNMC therapy reduced the hepatocellular carcinogenesis rate in F2–3 stage hepatitis patients, after statistical adjustment of differences of age and gender ratio. The reason why SNMC did not show an effect to reduce the carcinogenesis rate in F1 stage

hepatitis patients was presumed to be that the carcinogenesis rate in the untreated patients in F1 stage was too low to prove the effect of SNMC.

As carcinogenesis is not a single-step event, but a complex, multi-step process, the way in which SNMC works to suppress liver carcinogenesis still remains unclear. One of the principal roles played by SNMC in long-term administration seemed to be its anti-inflammatory actions, which would block the active carcinogenic process through a continuous hepatic necroinflammation and cell damage. SNMC may, however, only postpone the time of HCC appearance in the clinical course of cirrhosis. Since the entire process of hepatocellular carcinogenesis from the initial transformation of a hepatocyte to a detectable growth of cancer is considered to take several years, the influence of SNMC 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 underlying in liver disease, and the roles of long-term administration of SNMC in prevention of carcinogenesis in patients with cirrhosis caused by HCV.

In conclusion a long-term intermittent SNMC therapy over 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 SNMC therapy, is expected to confirm the effectiveness of this therapy in cancer prevention.

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