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進行肝細胞癌の化学療法—Sorafenib placebo-control randomized study(SHARP trial)を中心に

古瀬純司

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話　題

進行肝細胞癌の化学療法—Sorafenib placebo-control randomized study (SHARP trial)を中心に*

古瀬純司**

Key Words : hepatocellular carcinoma, sorafenib, molecular-targeted therapy, systemic chemotherapy, placebo-control randomized study

はじめに

肝細胞癌は世界では5番目に多い癌であり，年間約626,000名の新規患者が診断されている[1]．地域別にみると，東アジア37,000名，日本40,000名，ヨーロッパ32,000名，米国19,000名の年間発症数が報告されている[2)~4)]．とくに米国，ヨーロッパではC型肝炎の増加に伴い，肝細胞癌の発症数が増加している．肝細胞癌の病因はB型，C型肝炎ウイルス感染，アルコール性肝硬変，アフラトキシン，非アルコール性脂肪性肝炎(non-alcoholic steatohepatitis：NASH)など多彩であり，東アジア諸国，アフリカ諸国ではB型肝炎，日本ではC型肝炎が主な病因であるなど地域による差が大きいのも特徴である[5)6)]．

肝細胞癌の治療は一般に癌進行度と肝障害度に応じて治療選択が行われ，肝切除などの局所療法や動脈塞栓療法から化学療法までその治療法は多岐にわたる．肝細胞癌に対する治療選択については日本では肝癌診療ガイドラインによる肝細胞癌治療アルゴリズムが公表されている[7)]．また，今回sorafenibによる大規模な第III相試験でも引用されたBarcelona groupによるBarcelona Clinic Liver Cancer (BCLC) staging classification[8)]がヨーロッパ中心に適応されている．これらの治療選択のガイドラインにおいて，肝切除やラジオ波(RFA)など局所壊死療法，肝移植，動脈塞栓化学療法(TACE)は適切な症例選択の下に標準治療として確立している．一方，化学療法はこれまで多くのレジメンが臨床試験として試みられてきたが，生存期間の改善が確認された標準治療もその位置づけも確立していない．

肝細胞癌に対する化学療法

肝細胞癌に対する化学療法は，肝動脈から注入する経動脈性化学療法(動注化学療法)と経静脈あるいは経口による全身化学療法に分けられる．肝細胞癌に対する化学療法は，肝切除，局所壊死療法，動脈塞栓(化学塞栓)療法の局所治療が無効あるいは適応困難な例(高度門脈腫瘍栓など)および遠隔転移例が適応となる．また肝細胞癌では肝硬変など慢性肝障害を背景にもつ例が多いことから，肝障害を助長するリスクも大きく，肝障害度C(Child-Pugh C)の肝機能不良例では化学療法は禁忌である．

わが国では肝動脈からの動注化学療法が盛んに行われている．動注化学療法剤としてepirubicin, mitomicin C, 5-FUが主に用いられてきたが，2004年7月，cisplatin(アイエーコール®)の保険適応が承認された．最近では5-FU＋cisplatinや5-FU＋interferon(IFN)で高い奏効率が報告されているが，いずれも前向きな臨床試験による検証は行われていない[9)10)]．

全身化学療法では，これまで肝細胞癌における無作為化比較試験としてdoxorubicin(DXR), tamoxifen, interferonなどいくつか行われてき

* Systemic chemotherapy of growth factor inhibitors for advanced hepatocellular carcinoma from the results of sorafenib placebo-control randomized study.

** Junji FURUSE, M.D.: 国立がんセンター東病院肝胆膵内科〔〒277-8577 柏市柏の葉6-5-1〕; Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Kashiwa 277-8577, JAPAN

表1　切除不能肝細胞癌におけるドキソルビシンと無治療との無作為化比較試験

	Doxorubicin	Best supportive care	
n	60	46	
Response	2 (3.3%)	−	
Median OS	10.6 weeks	7.5 weeks	P=0.036
Fetal complication	15 (25%)		
Cause of death			
Tumor progression with cachexia	60.0%	76.1%	
Sede effects of therapy	25.0%	0	
GI bleeding	6.6%	8.7%	
Ruputure of tumor	3.3%	6.5%	
Hypoglycemia	5.0%	4.3%	
Subarachnoid hemorrhage	0	2.3%	
Sicide	0	2.3%	

（文献[13]より引用）

表2　切除不能肝細胞癌におけるドキソルビシンとシスプラチン/インターフェロン/ドキソルビシン/フルオロウラシル併用療法(PIAF)との無作為化第III相試験

	Doxorubicin	PIAF	P-value
n	94	94	
Response	10.5%	20.9%	0.058
Median overall survival	6.83 months	8.67 months	0.83
Treatment-related mortality	3 %	9 %	0.194
Major toxicity*			
Neutropenia	63%	82%	0.003
Trhrombocytopenia	24%	57%	＜0.001
Vomiting	4 %	12%	0.058
Hypokalemia	0 %	7 %	0.007
Hyponatremia	1 %	6 %	0.054

* grade 3 or above

（文献[18]より引用）

た[11)~15)]．DXRでは無治療群に比べ有意に生存期間の延長が得られたが，25%の症例で致命的な合併症が認められている(表1)[11)]．TamoxifenやIFN-αでは生存期間の改善は認められておらず[12)~15)]，標準的治療法は確立していない．最近では多剤併用療法が試みられ，5-FU/mitoxantrone/cisplatin (FMP)，cisplatin/doxorubicin/5-FU/IFN-α (PIAF)などで25%を超える高い奏効率が報告されたが[16)17)]．しかし，DXRをcontrol armとしたPIAF regimenの第III相試験が行われたが，有意な生存期間の改善は示せず(表2)[18)]，既存の抗癌剤による化学療法は悲観的にとらえられている．

Sorafenibによる第III相試験 (SHARP trial)

SorafenibはRAFキナーゼ，VEGFR-1-3，PDGFR-βなどを標的とするマルチキナーゼ阻害薬である．肝細胞癌においてもRafキナーゼの高発現が認められ，RAF/MEK/ERKシグナル伝達経路が肝細胞癌発症に関与しているとの報告がある[19)]．またsorafenibの第I相試験では肝細胞癌例でpartial response (PR)が得られていた[20)]．以上の背景から，米国やヨーロッパなどでsorafenib 400mg, 1日2回経口投与量により進行肝細胞癌に対する有効性と安全性を確認する第II相試験が行われた[21)]．その結果，奏効率は2%と低率であったが，十分な忍容性が確認され，無増悪期間中央値(median TTP)4.2か月，生存期間中央値(median OS)9.2か月と有効性も期待される結果であった(表3)．わが国では日本人肝細胞癌患者での薬物動態，安全性，推奨用量などを明らかにする目的で第I相試験が行われた[22)]．

表3　肝細胞癌に対するSorafenibの臨床第I相，第II相試験

Study	Phase II study	Phase I study
n	137	25
Dose	400 mg bid	200, 400 mg bid
Response	2 %	4 %
Stable disease	39%	76%
Disease control rate	42%	80%
Median time-to progression	4.2 mo	4.9 mo
Median overall survival	9.2 mo	15.6 mo
Author	Abou-Alfa (JCO 2006)[21]	Furuse (EORTC 2006)[22]

表4　進行肝細胞癌患者におけるsorafenibとplaceboの無作為化第III相試験(SHARP Trial)：試験デザイン

主要評価項目	Overall survival Time to symptomatic progression
副次評価項目	Time to progression
デザイン	国際多施設共同 二重盲検化プラセボ対照ランダム化第III相試験(Sorafenib群 vs. プラセボ群)
割付因子	門脈腫瘍栓 and/or 肝外転移 ECOG PS 地域
仮　説	Median survival timeを7か月から9.7か月(40%)に改善 検出力90%，α=0.02(片側)，予定症例数560例，死亡数424例

その結果，他癌種，米国・ヨーロッパと同様の薬物動態および忍容性が確認され，推奨用量も400mg，1日2回と決定された(表3)．同試験では症例数は少ないものの，有効性も同等であった．

以上，sorafenibの肝細胞癌に対する前臨床データおよび第I，II相試験の結果をもとに今回のプラセボコントロールによる無作為化比較試験SHARP(Sorafenib HCC Assessment Randomized Protocol) trialが実施された[23]．本試験の試験デザインを表4にまとめた．主な患者選択基準は，組織学的な肝細胞癌の確認，進行肝細胞癌，ECOG PS 0-2, Child-Pugh A Class, 全身化学療法歴なし，などである．

2005年3月から2006年4月までにSorafenib群299例，プラセボ群303例が登録された．治療はsorafenib 400mg/回，1日2回内服，あるいはplacebo 1日2回内服に割り振られ，両群の患者背景に有意な差はみられなかった(表5)．

表5　進行肝細胞癌患者におけるsorafenibとplaceboの無作為化第III相試験(SHARP Trial)：患者背景

	Sorafenib	Placebo
n	299	303
Median age	67歳	68歳
Male	87%	87%
Region Europe	88%	87%
Etiology HCV/HBV /Alcohol/other	29/19 /26/26%	27/18 /26/29%
ECOG PS 0	54%	54%
Child-Pugh A	95%	98%
BCLC stage C	82%	83%

BCLC stage：Barcelona Clinic Liver Cancer staging classification

主要評価項目である全生存期間はsorafenib群10.7か月，placebo群7.9か月であり，ハザード比0.69(95%CI：0.55-0.87；P=0.0006)と両者間に明らかな統計学的有意差を認めた(表6)．もう一つの主要評価項目である症状増悪までの期間(time to symptomatic progression)では差は認められなかった．副次評価項目である無増悪期間(time to progression)はsorafenib群5.5か月，placebo群2.8か月であり，ハザード比0.0.58(95%CI：0.45-0.74；P=0.000007)と全生存期間と同様両者間に明らかな統計学的有意差を認めた．有害事象については両群に差はなく，主なGrade 3/4の有害事象は下痢(sorafenib vs. placebo：11% vs. 2 %)，手足皮膚反応(8 % vs. 1 %)，疲労感(10 % vs. 15%)，出血(6 % vs. 9 %)であった．Sorafenibは十分な忍容性があり，進行肝細胞癌患者の生存期間を延長した初めての全身治療である．臨床的に大きな意義のある結果であり，sorafenibはこれらの患者に対する第一選択の治療法として確立すると報告された．

解　説

肝細胞癌は比較的遠隔転移が少なく，肝機能低下による肝不全が主な死因となることが多いことから，肝内病変への局所治療が主な治療法として行われる．しかし，再発がきわめて多く，局所治療が抵抗性になった病態や肝外転移を有する場合，有効な全身治療がないのが現状であっ

表6　進行肝細胞癌患者におけるsorafenibとplaceboの無作為化第III相試験(SHARP Trial)：結果

	Sorafenib	Placebo	HR (sorafenib/placebo)	*P*-value
n	299	303		
Median overall survival	10.7 mo	7.9 mo	0.69	0.0006
Time to progression	5.5 mo	2.8 mo	0.58	0.000007
Overall response				
Partial response(PR)	2.3%	0.7%		
Stable disease(SD)	71%	67%		
Progressive disease(PD)	18%	24%		
Progression-free rate at 4 month	62%	42%		
Serious adverse event (SAE)	52%	54%		
Drug-related treatment emergent SAE	13%	9 %		

た．これまでいくつかの無治療と全身治療の無作為化比較試験が行われてきたが，確立した標準治療は認められていない．そのような状況で，sorafenibによるplacebo-control無作為化比較試験が行われた．本試験では，placebo群のmedian OSを7か月に設定し，40%の改善を見込むとの仮説が立てられたことは臨床的に妥当であり，本試験により仮説通りの結果が得られたことは肝細胞癌治療にとって画期的なことと考えられる．

本試験の解釈において，患者背景でヨーロッパからの登録が90%近くと偏っていること，対象をChild-Pugh Aのみに限ったことが問題点としてあげられた．わが国にこの結果をそのまま導入してよいかという点について考察が必要である．肝細胞癌の病因については，日本では70%程度でC型肝炎感染が関連しているが，B型肝炎は15%程度である[6]．今回の試験ではC型肝炎関連は30%弱であり，病因による治療効果や有害事象の差はないかどうか検証する必要がある．しかし，欧米ではC型肝炎が非常に増えており，日本の状況と類似してきていることも確かである．Child-Pugh Aのみで試験が実施されたことについては，国際試験であり，肝障害による影響を可能な限り除外し，sorafenibの効果をより確実に評価したいという考えがあったかと推測される．わが国で行われた肝細胞癌患者でのsorafenibの第I相試験では，同数のChild-Pugh AとBで治療を行ったが，両者で有効性や安全性に大きな差は認めなかった．実地診療ではChild-Pugh Bも十分忍容性があり，適応可能と考えられる．

肝細胞癌の全身化学療法は，一定の抗腫瘍効果が得られても肝障害や静脈瘤出血などの有害事象により生存期間の改善につながらないことが多くみられる．今回の試験では重篤な有害事象はsorafenib群54%，placebo群52%と高頻度に認められたが，治療関連と考えられる有害事象はそれぞれ13%と9%と低く，差も認められていない．肝細胞癌患者では全身化学療法実施時の有害事象は原病や肝硬変など背景障害肝から生じる合併症の関与がきわめて大きいものと考えられる．つまり，sorafenibは高い忍容性が確認されているが，肝細胞癌での適応においては，薬物有害反応以外のさまざまな症状・合併症に注意を払う必要がある．

まとめ

今後，欧米を初め多くの国々，地域でsorafenibの肝細胞癌に対する適応承認が認められることが予想される．わが国でも他に有効な全身治療薬がない状況であり，すみやかな実地医療での適応承認が期待される．さらにより有効な治療法の確立に向けて，sorafenibを参照治療とした比較試験やsorafenibへの上乗せ効果を期待する治療法の開発が考えられる．現在，日本では肝細胞癌の動脈塞栓化学療法後の無増悪期間の改善を目的とした補助療法としてのplacebo-control無作為化比較試験が行われている．今回のSHARP trialとはまったく異なった患者群とコンセプトであり，世界的にも大きく注目されていることから，早期に完遂されることが期待される．

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Randomized, double-blind, placebo-controlled trial of bovine lactoferrin in patients with chronic hepatitis C

Hideki Ueno,[1,16] Tosiya Sato,[2] Seiichiro Yamamoto,[3] Katsuaki Tanaka,[4] Shinichi Ohkawa,[5] Hitoshi Takagi,[6] Osamu Yokosuka,[7] Junji Furuse,[8] Hidetsugu Saito,[9] Akira Sawaki,[10] Hiroshi Kasugai,[11] Yukio Osaki,[12] Shigetoshi Fujiyama,[13] Keiko Sato,[14] Keiji Wakabayashi[15] and Takuji Okusaka[1]

[1]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo 104-0045; [2]Department of Biostatistics, Kyoto University School of Public Health, Kyoto 606-8501; [3]Biometric Research Section, Statistics and Cancer Control Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045; [4]Gastroenterological Center, Yokohama City University Medical Center, Kanagawa 232-0024; [5]Division of Hepatobiliary and Pancreatic Oncology, Kanagawa Cancer Center Hospital, Kanagawa 241-0815; [6]Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Gunma 371-8511; [7]Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, Chiba 260-0856; [8]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital East, Chiba 277-0882; [9]Department of Internal Medicine, Keio University Hospital, Tokyo 160-8582; [10]Department of Gastroenterology, Aichi Cancer Center Hospital, Aichi 464-8681; [11]Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka 537-8511; [12]Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Osaka 543-8555; [13]Department of Gastroenterology and Hepatology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-8556; [14]Genetic Counselling and Clinical Research Unit, Kyoto University School of Public Health, Kyoto 606-8501; [15]Cancer Prevention Basic Research Project, National Cancer Center Research Institute, Tokyo 104-0045, Japan

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Several studies have suggested that lactoferrin administration may decrease the serum level of hepatitis C virus (HCV) RNA in patients with chronic hepatitis C. The aim of the present study was to confirm the efficacy of orally administered bovine lactoferrin (bLF) in patients with chronic hepatitis C. The patients with chronic hepatitis C randomly received either oral bLF at a dose of 1.8 g daily for 12 weeks, or an oral placebo. The primary endpoint was the virologic response, defined as a 50% or greater decrease in serum HCV RNA level at 12 weeks compared with the baseline. The secondary endpoint was the biochemical response, which was defined as a 50% or greater decrease in the serum alanine aminotransferase (ALT) level at 12 weeks compared with the baseline. One hundred and ninety-eight of 199 patients were evaluable for efficacy and safety. bLF treatment was well tolerated and no serious toxicities were observed. A virologic response was achieved in 14 of 97 patients (14.4%) in the bLF group, and 19 of 101 (18.8%) in the placebo group. There was no significant difference in virologic response rates between the two groups (−4.4%, 95% confidence interval −14.8, 6.1). In addition, bLF intake did not have any favorable effect on the serum ALT level. The virologic responses were not different between two groups in any subgroup analysis. In conclusion, orally administered bLF does not demonstrate any significant efficacy in patients with chronic hepatitis C. (*Cancer Sci* 2006; 97: 1105–1110)

Hepatitis C virus is a leading cause of chronic liver disease in Japan, and nearly two million people are estimated to be infected.[1] It is well known that HCV infection frequently causes chronic hepatitis, and that chronic hepatitis eventually progresses to liver cirrhosis and HCC approximately 30 years after HCV infection.[2] In Japan, more than 30 000 people die of HCC annually, and approximately 80% of HCC patients are infected with HCV.[3] Therefore, effective anti-HCV therapy is necessary to reduce the number of patients suffering from cirrhosis or HCC. To date, interferon-based therapy is the only effective treatment used clinically for chronic hepatitis C. A sustained complete virologic response (loss of detectable serum HCV RNA) occurs in 15–20% of patients with chronic hepatitis C after interferon therapy.[4] Moreover, recent studies have demonstrated that interferon with ribavirin or peginterferon with ribavirin improves the sustained complete virologic response rate by up to 40–50%.[5,6] However, because more than half of patients do not respond to interferon therapy, and because interferon therapy sometimes induces strong adverse effects, further developments in the treatment of chronic hepatitis C are required.

Lactoferrin, a member of the transferrin family of iron-binding glycoproteins, is present mainly in breast milk and other exocrine secretions. Several biological activities of lactoferrin have been demonstrated, including regulation of iron absorption in the intestine and modulation of immunoreactions.[7] Lactoferrin also plays an important role in human innate defense mechanisms against bacteria, fungi and viruses.[8] *In vitro* studies to date have shown that lactoferrin has antiviral effects against human immunodeficiency virus-1 and human cytomegalovirus.[9] Recent experimental studies have suggested that lactoferrin has antiviral effect against HCV.[10–12] Yi *et al.* have reported that lactoferrin binds to HCV envelope proteins *in vitro*.[10] Ikeda *et al.* have reported that lactoferrin prevents HCV infection in cultured human hepatocytes, and suggested that the anti-HCV activity of lactoferrin might be related to its direct binding to viral surfaces.[11,12] In addition, recent clinical studies have demonstrated the potential efficacy of lactoferrin against chronic hepatitis C.[13,14] Tanaka *et al.* reported that 8-week oral administration of bLF at a dose of 1.8 or 3.6 g/day decreased the serum level of HCV RNA markedly in three of four patients with a low pre-treatment HCV RNA level (<100 Kcopy/mL).[13] Iwasa *et al.* administered bLF (3.6 g/day) orally to 15 patients with high viral loads (≥100 KIU/mL), and reported that the mean serum HCV RNA level decreased significantly from 1106 KIU/mL at entry to 612 KIU/mL after 6 months of treatment ($P < 0.01$).[14] Based on these promising findings, we planned to investigate the efficacy of orally administered bLF in patients with chronic hepatitis C. First, we conducted a dose-finding study in 45 patients with chronic hepatitis C.[15] In that study, three dose levels of bLF (1.8, 3.6 and 7.2 g/day) were scheduled, and 15 patients at each dose level received the determined dose of bLF for 8 weeks. bLF treatment was well tolerated up to 7.2 g/day, and no serious adverse events were observed. Although no relationship between bLF dose and efficacy was recognized, a 50% or greater decrease in the serum HCV RNA level was seen in four of 45 patients (8.9%). Furthermore, the HCV RNA level was decreased by 50% or more in eight patients (17.8%) at week 8 after the end of treatment. These results encouraged us to conduct further investigations, and the present randomized

[16]To whom correspondence should be addressed. E-mail: hiueno@ncc.go.jp
Abbreviations: ALT, alanine aminotransferase; bLF, bovine lactoferrin; CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IL, interleukin; NK, natural killer.

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trial was designed to clarify the anti-HCV activity of bLF in patients with chronic hepatitis C.

Patients and Methods

Patients. Each patient was required to meet the following eligibility criteria: 20–74 years of age; positivity for anti-HCV antibody; an HCV RNA level of 0.5–850 KIU/mL evaluated within 1 month before entry; a sustained elevation of serum ALT level for at least 6 months; a serum ALT level of at least twice the upper normal limit evaluated within 1 month before entry; no evidence of HCC on the basis of ultrasonography or computed tomography carried out within 3 months before entry; and adequate bone marrow function (white blood cell count $\geq 4000/mm^3$, platelet count $\geq 100\,000/mm^3$, and hemoglobin level ≥ 11 g/dL), liver function (total bilirubin level ≥ 2.0 mg/dL, serum albumin level ≥ 3.5 g/dL, and serum aspartate aminotransferase and ALT level ≥ 200 IU/L) and renal function (normal serum creatinine and blood urea nitrogen levels).

The exclusion criteria were: positivity for hepatitis B surface antigen; interferon therapy within 6 months before entry; immunomodulatory or corticosteroid therapy within 3 months before entry; intravenous glycyrrhizin therapy within 1 month before entry; past or present history of bLF tablet intake; pregnant or lactating females; severe hepatic disease (e.g. autoimmune hepatitis and primary biliary cirrhosis); other serious medical conditions (e.g. gastrointestinal bleeding, active infection, severe pulmonary disease and psychiatric disorders).

Methods. This double-blind, placebo-controlled phase III trial was conducted at 11 centers in Japan. The study was approved by the institutional review board at each center, and all the participants provided written informed consent. Eligible participants were assigned randomly to one of two treatment groups in equal proportions using permutation blocks stratified by centers. A randomization list was drawn up using the SAS random number generator at the data center (Quintiles Transnational Japan K. K. Tokyo, Japan). The treatments consisted of bLF at a dose of 1.8 g/day or a placebo, administered orally twice daily for 12 weeks. In the current study, bLF at 1.8 g/day was selected on the basis of the previous dose-finding study, which indicated that there was no significant relationship between bLF dose (range, 1.8–7.2 g/day) and anti-HCV activity.[15] After the treatment allocation, the data center sent a numbered container of bLF or placebo tablets to a participant. During treatment, combined use of interferon, immunomodulatory therapy, corticosteroid and intravenous glycyrrhizin was prohibited. bLF (450 mg/tablet) and placebo tablets were provided by Morinaga Milk Industries (Tokyo, Japan).

In the current study, we tested the hypothesis that oral administration of bLF would: (1) reduce the serum HCV RNA level; and (2) reduce the serum ALT level in patients with chronic hepatitis C. In addition, we investigated the influence of orally administered bLF on systemic immune response in a small group of participants. The participants were evaluated every 4 weeks as outpatients until 4 weeks after completion of treatment. Serum HCV RNA level and serum ALT level were measured before treatment, during treatment at weeks 4, 8 and 12, and at 4 weeks after treatment. Serum HCV RNA level was determined by reverse transcription–polymerase chain reaction using the Amplicor-HCV monitor V 2.0 kit with a sensitivity of 0.5 KIU/mL (Roche Diagnostics, Tokyo, Japan). Anti-HCV antibody was determined by chemiluminescent enzyme immunoassay (Ortho-Clinical Diagnostics, Tokyo, Japan). HCV serotyping was carried out as described previously.[16] HCV serotype 1 corresponds to genotypes 1a and 1b of the Simmonds classification, and HCV serotype 2 corresponds to genotypes 2a and 2b.[17] Serum concentration of IL-18 was measured in participants at two institutions (National Cancer Center Hospital and Osaka Red Cross Hospital), and the percentage of $CD4^+$, $CD8^+$, $CD16^+$ and $CD56^+$ peripheral blood lymphocytes was measured in participants at the National Cancer Center Hospital. IL-18 and all lymphocytes were measured before treatment, during treatment at weeks 4, 8 and 12, and at 4 weeks after completion of treatment. Serum concentration of IL-18 was assayed with a human IL-18 enzyme-linked immunosorbent assay kit (Medical and Biological Laboratories, Nagoya, Japan). Lymphocyte surface phenotypes of CD4, CD8, CD16 and CD56 were determined by flow cytometry.

Adverse events were graded for severity according to the Japan Society for Cancer Therapy criteria,[18] which are similar to the National Cancer Institute Common Toxicity criteria. During treatment, participants were asked to record in a daily journal both compliance and any adverse events they experienced.

Assessment of efficacy and statistical analysis. Analyses were carried out on an intention to treat basis. The primary endpoint was a virologic response. In the current study, we defined a virologic response as a 50% or greater decrease in the serum HCV RNA level at 12 weeks compared with the baseline. Secondary endpoints were a biochemical response, as were changes in serum HCV RNA level and serum ALT level. If the serum ALT level at 12 weeks showed both a ≥50% decrease compared with the baseline and was ≤ twice the upper normal limit, we considered it a biochemical response. Response rate was calculated as the number of responders divided by the total number in each group. Participants whose HCV RNA (or ALT) data at 12 weeks were missing were included only in the denominator. Change in HCV RNA level (or ALT level) was calculated as the logarithm of the HCV RNA level (or ALT level) at 12 weeks minus the logarithm of these at the baseline. Differences in the virologic or biochemical response rates between two groups were analyzed using a test for the difference between two proportions. Differences in the change in HCV RNA level or ALT level between two groups were analyzed using a test for the difference between two means. In addition to the above planned analyses, subgroup analyses for virologic response were carried out based on pretreatment variables including age, serum HCV RNA level and HCV serotype. In a small group of participants, change in the serum concentration of IL-18 and changes in the percentage of $CD4^+$, $CD8^+$, $CD16^+$ and $CD56^+$ peripheral blood lymphocytes during the study period were investigated. Analyses were carried out using JMP4.0 and PC SAS Release v.8.02 (SAS Institute Japan Ltd, Tokyo, Japan). All P-values are two-tailed, and differences at $P < 0.05$ were regarded as statistically significant.

We estimated that a total of 250 participants would be the maximum to enroll for a 2-year enrollment period. Subsequent power analysis revealed that 125 participants per group would have 75% power to detect a 10% difference in the virologic response rate (15 vs 5%) at the 5% level of significance. An interim analysis by the independent data monitoring committee was planned after the first 125 participants had been enrolled. All trial personnel and participants were blinded to treatment assignment for the duration of the trial. Only the trial statistician and the independent data monitoring committee saw unblinded data. In the interim analysis of the primary endpoint, the O'Brien–Fleming method was used.[19]

Results

Patients. Enrollment began at seven institutions in April 2001. Because 250 participants were not enrolled for the 2 years planned originally, we extended the registration period for one more year and increased the number of participating institutions from seven to 11. An interim analysis was carried out in March 2004 with the data from the first 125 participants. Because the results of the interim analysis indicated that it was highly unlikely that a significant difference in treatment efficacy between

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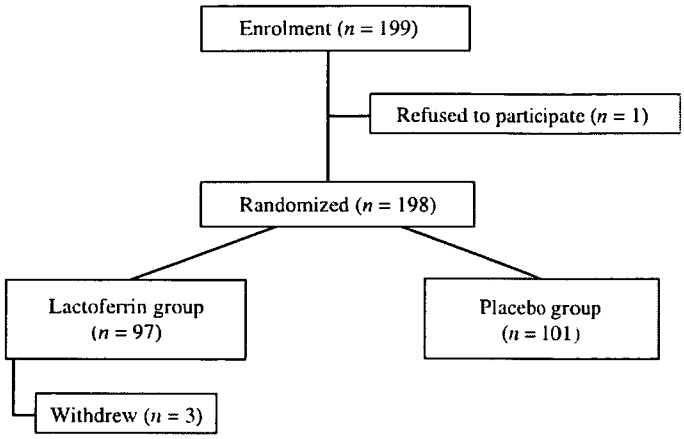

Fig. 1. Flow diagram of participant enrolment.

Table 1. Baseline characteristics of the patients

Characteristic	Bovine lactoferrin	Placebo
No. patients	97	101
Age (years)†	61 (29–74)	58 (31–74)
Sex (male/female)	53/44	55/46
History of interferon therapy	25	29
ALT level (IU/L)†	91 (41–340)	98 (27–250)
HCV RNA level (KIU/mL)†	378 (8.8–960)	452 (8.0–1560)
HCV serotype (1/2/ND)	78/17/1	76/22/3

†Median (range). ALT, alanine aminotransferase; HCV, hepatitis C virus; ND, not determined.

the two groups would be observed with the planned full enrollment of 250 participants, the data monitoring committee recommended discontinuation of further enrollment. Therefore, enrollment was stopped on 31 March 2004, at which point 199 participants had been enrolled. Because one patient refused to participate in the study before randomization, efficacy and safety were analyzed in the remaining 198 participants (97 bLF and 101 placebo) (Fig. 1). Although three participants in the bLF group discontinued treatment for reasons other than an adverse event, the remaining 195 participants completed the scheduled 12 weeks of treatment. The baseline characteristics of the 198 participants are shown in Table 1. There was no significant difference between the bLF and placebo groups regarding the pretreatment characteristics including age, sex, serum ALT level and serum HCV RNA level.

Virologic efficacy. Virologic response, the primary endpoint, was assessed in all 198 participants who received at least one dose of treatment. Virologic response was observed in 14 of 97 participants (14.4%) in the bLF group, and in 19 of 101 (18.8%) in the placebo group (Table 2). No complete virologic response (loss of detectable serum HCV RNA) was seen in either of the groups. There was no significant difference in the virologic response rate with bLF treatment in comparison with the placebo (−4.4%, 95% CI −14.8, 6.1). Change in the HCV RNA level at 12 weeks compared with the baseline was assessed in 190 participants (93 bLF group, 97 placebo group), excluding eight participants for whom HCV RNA data at 12 weeks were lacking. The change in the mean logarithm of the HCV RNA level was −0.09 in the bLF group and −0.09 in the placebo group, indicating no significant difference between the groups ($P = 1.00$).

Biochemical efficacy. Biochemical response was assessed in 198 participants. Biochemical response was seen in six of 97 participants (6.2%) in the bLF group, and in four of 101 participants (4.0%) in the placebo group (Table 2). No significant difference in the biochemical response rate was seen between the groups (2.2%, 95% CI −3.9, 8.3). Change in the serum AST level was assessed in 192 participants (93 bLF group, 99 placebo group), excluding six participants for whom ALT data at 12 weeks were lacking. The change in the mean logarithm of the ALT level was −0.085 in the bLF group and −0.080 in the placebo group, indicating no significant difference ($P = 0.93$).

Subgroup analysis. The rates of virologic response with respect to pretreatment variables are presented in Table 3. Among participants with a low HCV RNA level (<100 KIU/mL), the virologic response rate was 29.4% in the bLF group and 15.4% in the placebo group, indicating no significant difference between the groups (14.0%, 95% CI −15.2, 43.2). The virologic responses were also not different between two groups in other subgroup analyses such as age, sex and HCV serotype.

Analysis of IL-18 and lymphocytes. The serum concentration of IL-18 was measured in 73 participants enrolled at the National Cancer Center Hospital and Osaka Red Cross Hospital (36 bLF, 37 placebo). Figure 2 shows the changes in the mean IL-18 levels in the bLF group and placebo group. The mean IL-18 levels in the bLF and placebo groups were 293.9 pg/mL and 309.9 pg/dL at the baseline and 280.7 pg/mL and 291.5 pg/mL at 12 weeks, respectively. The corresponding changes in the mean IL-18 level at 12 weeks were −14.5 pg/mL and −15.9 pg/mL, respectively, indicating no significant difference between the groups ($P = 0.91$). Similarly, there were no significant differences between the groups at any other points during the study period. The percentage of lymphocyte was measured in 46 participants at the National Cancer Center Hospital (bLF 23, placebo 23), and the results are shown in Fig. 3. The percentage of $CD4^+$, $CD8^+$, $CD16^+$ and $CD56^+$ peripheral blood lymphocytes remained almost unchanged throughout the study in both groups, and the differences between them were not significant.

Safety. Safety was assessed in 198 participants who received at least one dose of bLF or placebo during the study. The bLF treatment was well tolerated, and no serious complications occurred during the treatment. Although minor adverse events including neutropenia, γ-GTP elevation and hyperglycemia were observed in participants treated with bLF, their frequency and intensity did not differ from those in the placebo group. HCC was detected in one participant in the bLF group and in one participant in the placebo group during the study period.

Discussion

The present study was carried out to confirm the anti-HCV activity of orally administered bLF in patients with chronic hepatitis C. A virologic response (a 50% or greater decrease in the serum level of HCV RNA at 12 weeks compared with the baseline) was observed in 14 of 97 participants (14.4%) in the bLF group, and 19 of 101 (18.8%) in the placebo group, the difference between the groups being non-significant. The virologic responses were not different between two groups in any subgroup analysis. Furthermore, bLF intake did not have any favorable effect on the serum ALT level. On the basis of these results, we concluded that orally administered bLF did not have any efficacy, including anti-HCV activity, in patients with chronic hepatitis C.

The virologic response rate of 14.4% observed in the bLF group was somewhat higher than that reported in the previous dose-finding study,[15] in which four of 45 patients (8.9%) showed a virologic response at the end of bLF treatment. Nevertheless, the current study failed to demonstrate any anti-HCV activity of bLF, because a similar virologic response rate to that in the bLF group was seen in the placebo group. Having designed this randomized study, we assumed that a virologic

Table 2. Virologic and biochemical efficacy

Characteristic	Bovine Lactoferrin	Placebo	Difference (95% CI)	*P*-value
Virologic efficacy				
Response rate (%)	14.4	18.8	−4.4 (−14.8, 6.1)	
Change in HCV RNA level†	−0.09	−0.09		1.00
Biochemical efficacy				
Response rate (%)	6.2	4.0	2.2 (−3.9, 8.3)	
Change in ALT level†	−0.085	−0.080		0.93

†Mean logarithm. ALT, alanine aminotransferase; CI, confidence interval; HCV, hepatitis C virus.

Table 3. Virologic response rate as a function of baseline variables

Variable	Bovine lactoferrin (*n* = 97)		Placebo (*n* = 101)		Difference	
	Response/total	%	Response/total	%	%	95% CI
Age						
<65 years	12/62	19.4	14/77	18.2	1.2	−11.9, 14.2
≥65 years	2/35	5.7	5/24	20.8	−15.1	−33.1, 2.9
Sex						
Male	10/53	18.9	10/55	18.2	0.7	−14.0, 15.3
Female	4/44	9.1	9/46	19.6	−10.5	−24.7, 3.8
ALT level						
<100 IU/L	6/57	10.5	7/51	13.7	−3.2	−15.6, 9.2
≥100 IU/L	8/40	20.0	12/50	24.0	−4.0	−21.1, 13.1
HCV RNA level						
<100 KIU/mL	5/17	29.4	2/13	15.4	14.0	−15.2, 43.2
≥100 KIU/mL	9/80	11.3	17/88	19.3	−8.0	−18.8, 2.7
HCV serotype†						
1	11/78	14.1	16/76	21.1	−7.0	−18.9, 5.0
2	3/18	16.7	2/22	9.1	7.6	−31.4, 28.6

†Hepatitis C virus serotype was not measured in four patients. ALT, alanine aminotransferase; CI, confidence interval; HCV, hepatitis C virus.

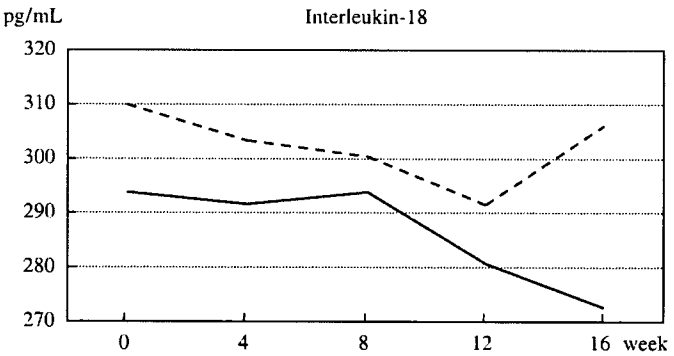

Fig. 2. Changes in the mean serum concentration of interleukin-18 in the bovine lactoferrin group (straight line, *n* = 36) and the placebo group (dotted line, *n* = 37).

response rate of around 5% would be seen in the placebo group due to spontaneous remission of viral activity. However, contrary to our expectation, 19 of 101 participants (18.8%) in the placebo group showed a ≥50% decrease in the HCV RNA level at 12 weeks, indicating that our assumption was inappropriate. Our results suggested that in order to assess the reduction of the HCV RNA level, periodic evaluation would be necessary to exclude the influence of spontaneous fluctuation of HCV RNA.

Several experimental studies have suggested that lactoferrin has some activity against HCV. Yi *et al.*[10] reported that lactoferrin binds to the HCV E1 and E2 envelope proteins *in vitro*, and Ikeda *et al.*[11,12] reported that lactoferrin prevents HCV infection in cultured human hepatocytes. They suggested that the anti-HCV activity of lactoferrin might be due to a neutralizing efficacy, in which the administered lactoferrin became bound directly to the HCV virion, thus inhibiting adsorption of the HCV-lactoferrin complex into human hepatocytes. Therefore, intravenous administration of lactoferrin might improve the viremic state in patients with chronic hepatitis C. However, for practical application, administration of lactoferrin directly into blood does not seem to be a suitable approach because lactoferrin is a large glycoprotein molecule (80 kDa) that may cause allergic reactions. Therefore, oral administration of bLF was selected for the present study, even though the metabolism and mechanism of ingested lactoferrin are yet to be clarified. As to absorption, it has been reported that intact lactoferrin and its fragments are present in the urine of human milk-fed preterm infants.[20] However, in adult rats, lactoferrin and its fragments are not detectable in portal blood after bLF ingestion,[21] and in adult humans, the serum lactoferrin level does not increase after oral administration of recombinant human lactoferrin.[22] However, several studies have suggested that orally administered lactoferrin might enhance immune responses via cytokine production.[23,24] It has been reported that oral administration of bLF to mice enhances the production of IL-18 and interferon-γ in the mucosa of the small intestine, and increases the number of $CD4^+$, $CD8^+$ and NK cells in the small-intestinal epithelium.[25,26] Varadhachary *et al.* reported that oral administration of recombinant human lactoferrin to mice stimulates IL-18 production from gut enterocytes, and augments the NK activity of spleen cells and production of blood $CD8^+$ cells.[27] Furthermore, a recent clinical study has demonstrated that oral administration of bLF (0.6 g/day) for 3 months in 36 patients with chronic hepatitis C increased the serum IL-18 level significantly compared with the

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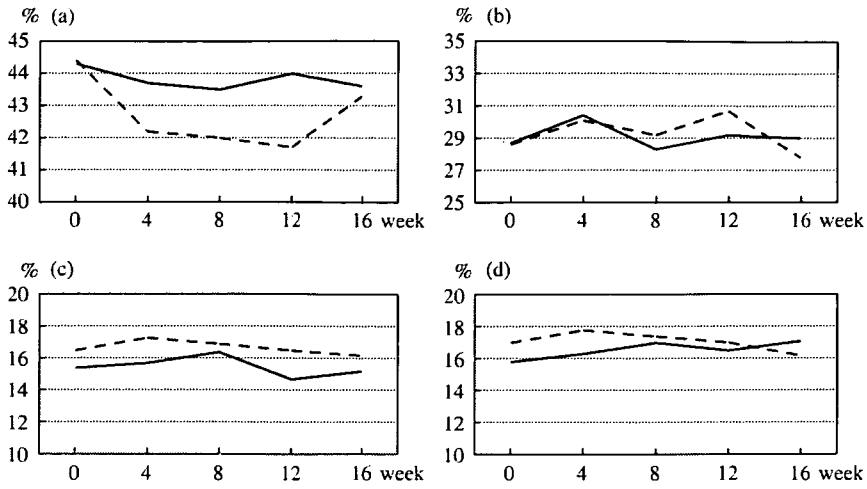

Fig. 3. Changes in the mean percentages of (a) $CD4^+$, (b) $CD8^+$, (c) $CD16^+$ and (d) $CD56^+$ peripheral blood lymphocytes in the bovine lactoferrin group (straight line, $n = 23$) and the placebo group (dotted line, $n = 23$).

baseline.[28] However, our study found no evidence that oral administration of bLF influences the serum concentration of IL-18 or the percentage of $CD4^+$, $CD8^+$, $CD16^+$ and $CD56^+$ lymphocytes. Further investigations are required to clarify the peripheral and systemic effects of orally administered lactoferrin. In addition, as many *in vitro* studies have suggested that lactoferrin has direct binding neutralizing efficacy against HCV,[29–31] further investigations are needed to devise a means of delivering lactoferrin or its fragment into the bloodstream safely and effectively.

Recently, several studies have investigated the value of adding lactoferrin to interferon therapy for chronic hepatitis C. Hirashima *et al.* randomly assigned 21 patients with chronic hepatitis C to either a consensus interferon plus oral lactoferrin (3.0 g/day) group or a consensus interferon monotherapy group.[32] Three of 10 patients in the consensus interferon plus lactoferrin group showed a sustained complete virologic response, as did four of 11 patients in the consensus interferon group, indicating no statistically significant difference between the groups. Ishibashi *et al.* conducted a randomized controlled trial to investigate the efficacy of interferon α-2b and ribavirin plus oral lactoferrin (0.6 g/day) compared with interferon α-2b and ribavirin plus placebo in 36 patients with chronic hepatitis C.[33] A sustained complete virologic response was seen in six of 18 patients in the lactoferrin group and in five of 18 patients in the placebo group, there being no statistically significant difference between the groups ($P = 0.7$). Although the numbers of patients recruited in the two randomized trials were small, these results suggested that the additional value of oral lactoferrin combined with interferon therapy would be negative for the treatment of chronic hepatitis C.

In summary, oral administration of bLF at a dose of 1.8 g/day for 12 weeks showed an acceptable safety profile in patients with chronic hepatitis C. However, there was no significant difference in the virologic responses between patients who received oral bLF and those receiving placebo. In addition, bLF intake did not have any favorable effect on the serum ALT level. These findings do not support the practical use of oral bLF in patients with chronic hepatitis C.

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肝内胆管癌の化学療法（動注を含む）

古瀬純司* 鈴木英一郎* 仲地耕平*
清水怜* 石井浩* 吉野正曠*

索引用語：肝内胆管癌，全身化学療法，肝動注化学療法，臨床試験

1 はじめに

肝内胆管癌に対する治療は，切除が唯一の根治治療であり，化学療法は一般に切除不能進行例や術後再発例に行われる．これまで肝内胆管癌のみを対象とした化学療法の臨床試験はなく，標準的治療法は全く確立していないのが現状である．

肝内胆管癌は取扱い規約上原発性肝癌として扱われているが，その大部分を占める肝細胞癌とは治療戦略も化学療法の感受性も異なる．胆管癌の多くは腺癌であり，化学療法は胆道癌の一部として行われることが多い．一方，肝外胆管癌と異なり，肝内胆管癌は肝内に腫瘤を形成し，切除不能例では，肝内病変の進行による肝不全が予後決定因子となる場合も少なくない．したがって，肝内病変のコントロールが予後改善に寄与する場合もあることから，肝細胞癌で広く行われているような肝動脈注入化学療法も治療戦略のひとつとして考えられる．

本稿では肝内胆管癌の化学療法として胆道癌における臨床試験や実際の治療成績について概説し，肝動注化学療法についてもその可能性について述べる．

2 胆道癌に対する全身化学療法の臨床試験

2000年以降に報告された主な化学療法の治療成績を示す（表1，表2）．フッ化ピリミジン系やプラチナ系薬剤が多く用いられ，最近ではゲムシタビンを中心とした化学療法が多く行われている[1~38]．一般に単剤では治療効果に限界がみられるため，多くの多剤併用治療が試みられてきた．単剤に比べ多剤併用では一般に奏効率は高く，生存期間も長い傾向がみられる．しかし，これらはほとんど第Ⅱ相試験であり，背景因子の違いが治療成績に大きく影響する．ゲムシタビン＋カペシタビンによる第Ⅱ相試験では，胆嚢癌患者の生存期間中央値（MST）6.6カ月に対し，胆管癌では19カ月と有意差を認め，生物学的違

Jyunji FURUSE et al: Chemotherapy for intrahepatic cholangiocarcinoma
*国立がんセンター東病院肝胆膵内科［〒277-8577 柏市柏の葉6-5-1］

表1　胆道癌に対する全身化学療法の治療成績(単剤)

Regimen	n	Response	MST (mo)	Author	year	
5FU/LV	28	32%	6.0	Choi	2000	[1]
5FU/FA	30	7%	14.8	Malik	2003	[2]
5-FU	29		5	Ducreux	2005	[3]
UFT/LV	16	0%	4.5	Chen	2003	[4]
UFT	19	5%	8.8	Ikeda	2005	[5]
S-1	19	21%	8.3	Ueno	2004	[6]
Capecitabine	26	19%	9.0	Patt	2004	[7]
Gemcitabine (1,000 mg/m^2)	25	36%	7.0	Gallardo	2001	[8]
Gemcitabine (2,200 mg/m^2)	32	22%	11.5	Penz	2001	[9]
Gemcitabine (1,000 mg/m^2)	24	13%	7.2	Lin	2003	[10]
Gemcitabine (800 mg/m^2)	30	30%	14.0	Tsavaris	2004	[11]
Gemcitabine (FDR 1,500 mg/m^2)	15	0%	4.6	Eng	2004	[12]
Gemcitabine (1,000 mg/m^2)	40	18%	7.6	Okusaka	2006	[13]
Docetaxel	24	20%	8.0	Papakostas	2001	[14]
CPT-11	36	8%	6.1	Alberts	2002	[15]
Erlotinib	42	8%	7.5	Philip	2006	[16]

いがあると推測されている[32]．わが国で行われたUFT＋ドキソルビシンの第Ⅱ相試験でも胆嚢癌のMST 5.0カ月に対し，肝内胆管癌・肝外胆管癌・乳頭部癌では11.0カ月と有意差を認めた[20]．すなわち，同じ化学療法が適応される胆道癌でも，胆嚢癌では予後が特に不良であり，胆嚢癌，胆管癌，乳頭部癌の含まれる比率によりその臨床試験のMSTはかなり差が出ると考えられる．胆道癌における治療成績の適切な評価には無作為化比較試験が必要である．

表3に胆道癌に用いられる主な抗癌剤を示す．これらの内，わが国で胆道癌に保険適応が承認されている薬剤は，UFT，ドキソルビシン，キロサイド(他の抗腫瘍剤と併用)に限られていた．しかしこれらの薬剤で十分な有効性は認めておらず，胆道癌に適応できる有効な薬剤の開発が急務であった．2002年以降，わが国でゲムシタビンとS-1の治験が行われ[6,13]．2006年6月，ゲムシタビンが胆道癌に保険適応の承認を得た．なんと23年ぶりの胆道癌に対する新しい抗癌剤であった．しかし，ゲムシタビンの治験では胆道癌を対象に行われたため，肝内胆管癌は含まれていない[13]．肝内胆管癌での治療成績を明らかにしていくことが今後の課題である．

3 肝内胆管癌における治療成績

当院では切除不能肝内胆管癌に対する化学療法は，進行胆道癌と同様のレジメンを用いてきた．1992年から2006年6月まで，シスプラチン＋エピルビシン＋5-FU (CEF)療法，5-FU＋ドキソルビシン＋マイトマイシンC (FAM)療法，UFT単独およびUFT＋ドキソルビシン(UFD)療法を40例に施行した．奏効例はFAM療法とUFD療法により3例に認められ(図1)，全体の奏効率は8%であった(表4)．全40例の生存期間中央値は4.7カ月，6カ月生存率37.6%，1年生存率10.7%と極めて予後不良である．今後，ゲムシタビンやS-1など新しい抗癌剤による治療成績の改善が期待される．

表2　胆道癌に対する全身化学療法の治療成績(多剤併用)

Regimen	n	Response	MST (mo)	Author	year	
Fluoropyrimidine-based						
MMC/5FU/LV	19	26%	6.0	Chen	2001	[17]
Capecitabine/MMC	26	31%	9.3	Kornek	2004	[18]
5FU/etop/LV	27	—	12.0	Rao	2005	[19]
UFT/DXR	24	13%	7.6	Furuse	2006	[20]
Platina-based						
DXR/CDDP/5FU/IFN	38	21%	14.0	Patt	2001	[21]
CDDP/5FU/LV	29	34%	9.5	Taieb	2002	[22]
CDDP/5FU/FA	29	—	8.0	Ducreux	2005	[3]
CDDP/capecitabine	42	21%	9.1	Kim TW	2003	[23]
Oxaliplatin/5FU/LV	16	19%	9.5	Nehls	2002	[24]
EPI/CDDP/5FU	37	19%	5.9	Morizane	2003	[25]
EPI/CDDP/5FU	27	—	9.0	Rao	2005	[19]
EPI/CDDP/UFT/LV	40	23%	7.9	Park KH	2005	[26]
EPI/CDDP/capecitabine	43	40%	8.0	Park SH	2006	[27]
Gemcitabine-based						
Gemcitabine/docetaxel	43	9%	11.0	Kuhn	2002	[28]
Gemcitabine/MMC	25	20%	6.7	Kornek	2004	[18]
Gemcitabine/5FU	27	33%	5.3	Knox	2004	[29]
Gemcitabine/5FU/LV	42	12%	4.7	Hsu	2004	[30]
Gemcitabine/5FU/LV	42	12%	9.7	Alberts	2005	[31]
Gemcitabine/capecitabine	45	31%	14.0	Knox	2005	[32]
Gemcitabine/capecitabine	45	32%	14.0	Cho	2005	[33]
Gemcitabine/oxaliplatin	33	33%	15.4	Andre	2004	[34]
Gemcitabine/CDDP	30	38%	4.6	Doval	2004	[35]
Gemcitabine/CDDP	40	28%	8.4	Thongprasert	2005	[36]
Gemcitabine/CDDP	29	35%	11.0	Kim ST	2006	[37]
Gemcitabine/CDDP	27	33%	10.0	Park BK	2006	[38]

表3　胆道癌に対して用いられる主な抗癌剤

・代謝拮抗剤	UFT, gemcitabine cytarabine	5-FU*, (2006/6-) (他剤との併用)	S-1*,	capecitabine*,
・抗癌性抗生物質	doxorubicin,	epirubicin*	mitomycin C*	
・その他	cisplatin*,	oxaliplatin*,		

*保険適応なし

4　肝内胆管癌に対する肝動注化学療法

遠隔転移がない，あるいは肝内病変が広範囲に進行した例では肝内病変をコントロールすることにより，予後の改善が得られる可能性がある．これが肝動注化学療法もひとつの選択肢として挙げられる理由である．これまで少数例での検討であるが，5-FU単独あるいは5-FUにシスプラチン，ドキソルビシン，

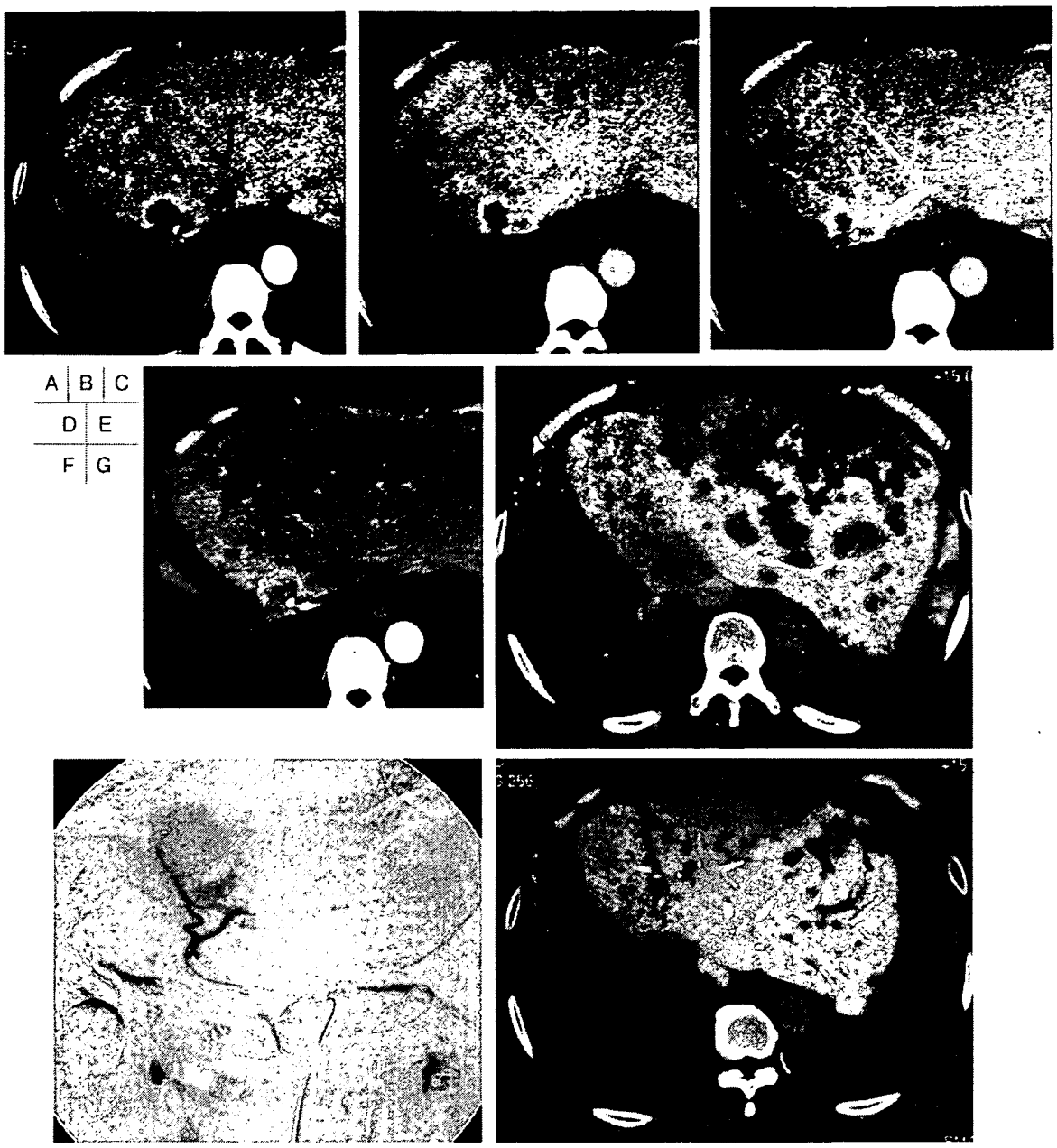

図1　64歳，男性．肝内胆管癌の診断にて肝右葉切除術を施行．1年4カ月後，CTにて下大静脈浸潤を伴う再発と診断された．

A：再発時のダイナミックCT早期相．

B：切除不能と診断され，全身化学療法(UFT + DXR)を施行．2カ月後，54%の縮小を認めた．

C：全身化学療法(UFT + DXR)を継続．4カ月後も68%縮小が持続し，PRと判定．

D：全身化学療法(UFT + DXR) 6カ月(10コース後)のCTにて病巣の増大を認め，PDと判定し，治療中止．その後，増大した病変に放射線療法を行うも，3カ月後肝内多発病変による病状増悪を認め，CDDP動注化学療法を施行した．

E：動注前の上腸間膜動脈造影CTであり，肝全体に多発する病変を認める．

F：固有肝動脈造影．同部位からCDDP 100mgを動注した．

G：4回目動注前の上腸間膜動脈造影CT．明らかな病変の縮小を認める．

表4　肝内胆管癌に対する化学療法の治療成績

	n	PR	SD	PD	NE
CEF	2	0	1	1	0
FAM	12	1	4	6	1
UFT	3	0	0	3	0
UFD	23	2	15	6	0
Total	40	3（8%）	20（50%）	16（40%）	1（3%）

CEF: CDDP + epirubicin + 5-FU
FAM: 5-FU + doxorubicin + mytomicin C
UFD: UFT + doxorubicin

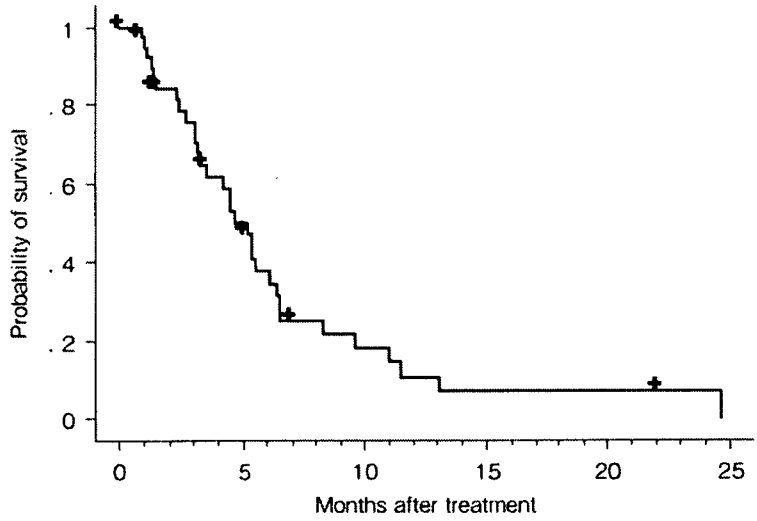

図2　化学療法により治療された肝内胆管癌患者の生存曲線
生存期間中央値　4.7カ月，6カ月生存率37.6%，1年生存率10.7%

エピルビシンなどを組み合わせたレジメンにより効果がみられた症例が報告されている[39~42]．しかし，多数例による臨床試験など信頼性の高い成績は報告されておらず，十分なエビデンスはないのが現状である．

当院では肝内病変の高度進行例や全身化学療法不応例に5-FUあるいはシスプラチンの肝動注化学療法を施行した．図1のように動注化学療法により著明な縮小が得られた症例もあり，今後動注化学療法の前向きな臨床試験を行う意義があると考えられる．

5 最後に

第16回全国原発性肝癌追跡調査報告では，肝内胆管癌の全生存率は1年48.8%，5年20.3%，非切除例では1年30.9%，5年7.4%と極めて不良である[43]．切除不能例や切除後再発例に対する治療戦略を考える上で，化学療法は重要な役割を果たしている．多くの臨床試験の結果，胆道癌に有効性が期待できる治療法も報告されつつあり，最近では分子標的薬による治療も試みられている．肝内胆管

癌を含めた胆道癌おいても質の高い臨床試験を積極的に実施し，有効な治療法の開発と標準的治療法の確立を急ぐ必要がある．

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