cellular and cholangiocarcinoma, in which well differentiated hepatocellular carcinoma contained cholangiocarcinoma in "nodules-in-nodules" fashion **(Figure 4A, 4B)**.

The Result of Immunohistochemical Staining of Ki-67

The average Ki-67 labeling index of the hepatocellular carcinoma component of combined hepatocellular and cholangiocarcinoma was 4.4±3.4% and the index of the cholangiocarcinoma component was 11.0±8.5%, significantly higher than that of the hepatocellular carcinoma component ($p<0.05$). As the control, we counted the Ki-67 labeling index of 11 cases of moderately differentiated hepatocellular carcinoma, and 8 cases of intrahepatic cholangiocarcinoma. The average Ki-67 labeling index of the ordinary hepatocellular carcinoma was 3.9±2.2%, and the index of the intrahepatic cholangiocarcinoma was 12.7±5.6%. In each case, the Ki-67 labeling index of the cholangiocarcinoma component was higher than the hepatocellular carcinoma component **(Figure 5)**.

p53 Immunohistochemical Staining

In the p53 immunohistochemistry, 5 of 18 cases (29.4%) were positive.

In one case, the cholangiocarcinoma component was positive for p53, but the hepatocellular carcinoma component was negative. In another case, both the hepatocellular carcinoma and cholangiocarcinoma component were positive. In the remaining 3 cases, the staining of the cholangiocarcinoma component was stronger than that of hepatocellular carcinoma.

DISCUSSION

In this study, of 1102 patients with primary liver cancers, 3 (0.3%) patients had both hepatocellular carcinoma and intrahepatic cholangiocarcinoma independently, and 18 (1.6%) patients had combined hepatocellular and cholangiocarcinoma. These were relatively lower than in the study of autopsy cases and other studies of surgical cases (4-9).

The mean age of patients in the present series was 56.7 years. Regarding the background of the liver, the positivity of the virus markers, hepatitis B virus surface antigen (HBsAg) and hepatitis C virus antigen, was 22.2% and 43.8% in combined hepatocellular and cholangiocarcinoma. The positivity of the HBsAg in the combined hepatocellular and cholangiocarcinoma was significantly higher than intrahepatic cholangiocarcinoma. However, the positivity of the HCVAb in the combined carcinoma was between ordinary hepatocellular carcinoma and intrahepatic cholangiocarcinoma.

Among 18 cases of combined hepatocellular and cholangiocarcinomas, in 5 cases (27.8%) there was no chronic liver disease in non-tumorous liver. In the report of the Liver Cancer Study Group of Japan, chronic liver disease was not identified in non-tumorous liver in 7.2% of hepatocellular carcinoma, and in 72.0% of intrahepatic cholangiocarcinoma. Then, the background of combined hepatocellular and cholangiocarcinoma was more similar to hepatocellular carcinoma than to cholangiocarcinoma

In our study, the 1- and 3-year survival rates after operation were 73.3% and 33.3%, respectively. The immunoreactivity of the p53 protein was 27.8% (5/18), and it was consistent with the previously reported values in Japanese poorly differentiated hepatocellular carcinoma and was lower than that of Japanese intrahepatic cholangiocarcinomas (10-15). Regarding the combined hepatocellular and cholangiocarcinoma, Maeda *et al.* (6) reported that only three of 29 cases were positive for p53 in combined hepatocellular and cholangiocarcinoma, and they were positive for the cholangiocarcinoma component.

In the previous reports about the Ki-67 labeling index of the Japanese primary liver cancer, the Ki-67 labeling index of hepatocellular carcinoma was 6-15.3%, and that of intrahepatic cholangiocarcinoma was 14-28.9% (13,15-18). In this study, the Ki-67 labeling indices of the intrahepatic cholangiocarcinomas and the cholangiocarcinoma part of the combined hepatocellular and cholangiocarcinomas were higher than those of the ordinary hepatocellular carcinomas and the hepatocellular carcinoma part of the combined hepatocellular and cholangiocarcinomas. Compared with the ordinary hepatocellular carcinoma and intrahepatic cholangiocarcinoma, the proliferative activity of the cholangiocarcinoma component in the combined hepatocellular and cholangiocarcinoma was similar to that of intrahepatic cholangiocarcinoma, and the activity of the hepatocellular carcinoma component was similar to that of ordinary hepatocellular carcinoma. This suggested that cholangiocarcinoma components might grow more rapidly than hepatocellular carcinoma parts, and this fact seemed to influence the survival rate of the combined hepatocellular and cholangiocarcinoma, which was similar to that of intrahepatic cholangiocarcinoma.

With regard to the pathogenesis of combined hepatocellular and cholangiocarcinoma, there are three

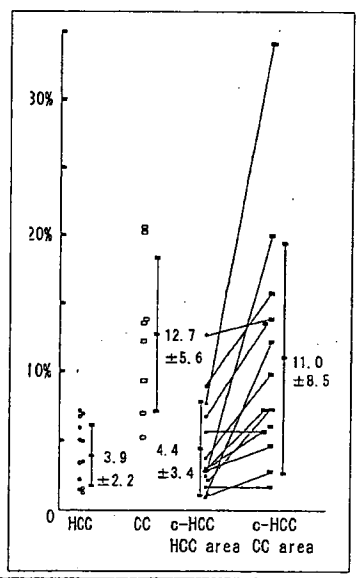

FIGURE 5
Comparison of the Ki-67 labeling indices of ordinary hepatocellular carcinomas, ordinary intrahepatic cholangiocarcinomas, hepatocellular carcinoma components, and cholangiocarcinoma components of combined hepatocellular and cholangiocarcinomas (mean±SD).
HCC: hepatocellular carcinoma;
CC: intrahepatic cholangiocarcinoma;
c-HCC: combined hepatocellular and cholangiocarcinoma.

hypotheses (19); 1) hepatocellular carcinoma and intrahepatic cholangiocarcinoma might have developed in the same liver coincidentally, 2) at first, either hepatocellular carcinoma or intrahepatic cholangiocarcinoma arises, and then is transformed to the other; 3) cancer arises from an intermediate cell between the hepatocytes and bile duct epithelium (stem cell), and then differentiates completely or incompletely into both components. From the standpoint of gene analyses, Imai *et al.* (7) showed the same mutational pattern in the hepatocellular carcinoma component and the cholangiocarcinoma component of the combined hepatocellular and cholangiocarcinoma, which was classified into Allen's type 2 and 3, and indicated the same origin of both components. Fujii *et al.* (20) reported that the majority of tumors classified into Allen's type 2 and 3 were derived from a single clone which shows bi-directional phenotypic diversity. This report supported the transformation hypothesis and the stem cell hypothesis. Yano *et al.* established a cell line designated as KYN-1, from AFP producing hepatocellular carcinoma cells, and reported that the cells, which were transplanted to nude mice, developed into adenocarcinoma within a few months (21).

In this study 6 out of 18 cases were microscopically classified type III, which exhibited lobular structures mimicking the hepatic lobular structure. Type III seems to be a feature of metaplasia or proliferation of bipotential progenitor cells. The background of the combined hepatocellular and cholangiocarcinoma is the middle of that of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. The cholangiocarcinoma component was more rapid in growth potential than the hepatocellular carcinoma component. In one case, we found that well-differentiated hepatocellular carcinoma contained cholangiocarcinoma in "nodules-in-nodules" fashion.

Therefore, it suggested that metaplasia of the hepatocellular carcinoma to the cholangiocarcinoma is one of the pathways in genesis of the combined hepatocellular and cholangiocarcinoma.

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FOOTNOTE

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Potential role of vitamin K_2 as a chemopreventive agent against hepatocellular carcinoma

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Vitamin K, a cofactor necessary for the production of several antihemorrhagic factors, can inhibit the growth of various types of cells derived from neoplasms. In hepatoma cells, vitamin K_2 causes cell-cycle arrest and apoptosis. Vitamin K_2 is widely used in Japan to treat osteoporosis. The safety, relatively low cost and ease of use of vitamin K_2 have led to good compliance with treatment. The result of preliminary clinical trials in patients with chronic liver diseases are intriguing and suggest that vitamin K_2 might reduce the risk of hepatocellular carcinoma (HCC) in patients with liver cirrhosis as well as prevent disease recurrence after curative therapy in patients with HCC. This article reviews the potential role of vitamin K_2 as a chemopreventive agent against HCC and discusses future directions for clinical trials.

Key words: hepatocellular carcinoma, vitamin K_2, viral hepatitis, liver cirrhosis, chemoprevention

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) arises almost exclusively in patients with chronic liver disease, especially hepatic cirrhosis. The annual incidence of HCC in patients with cirrhosis ranges 5–7%.[1–3] The rate of recurrence after curative treatment of primary HCC is high.[4–6] Epidemiological studies estimate that the number of deaths from HCC will increase by 2010–2015.[7] Decreased mortality from HCC requires preventive therapy. Prospective studies have been performed to evaluate the chemopreventive properties of interferon (IFN), "Sho-saiko-to", and an acyclic retinoid.[8–11]

The vitamin K family is known to inhibit the growth of human cancer cell lines. However, the mechanisms of this effect have yet to be fully explored. Recently, vitamin K_2 has attracted attention as a new chemopreventive agent against HCC.

BACKGROUND OF VITAMIN K

VITAMIN K IS a cofactor for the enzyme γ-glutamyl-carboxylase, which converts glutamate residues into γ-carboxy-glutamate. Vitamin K-dependent proteins include coagulation factors II (prothrombin), VII, IX, and X, protein C and S, osteocalcin, surfactant-associated proteins, and bone matrix protein. The vitamin K family of molecules comprises the natural forms vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinones) as well as the synthetic form vitamin K_3 (menadione). These naphthoquinone-containing molecules inhibit tumor cell growth in culture, with vitamin K_3 being more potent than either vitamin K_1 or K_2. Vitamin K_2 inhibits growth of human cancer cell lines and suppresses induction of differentiation in various human myeloid leukemia cell lines.[12,13] Clinically, myelodysplastic syndrome has been successfully treated with vitamin K_2.[14]

A number of findings indicate that vitamin K may have a role in controlling cell growth. Underlying mechanisms may involve redox cycling (as known for vitamin K_3), proteins with growth-inhibitory properties induced by vitamin K, such as prothrombin,[15] previously unidentified pathways involving arylation,[16] or growth arrest genes such as *gas 6*.[17] Geranylgeraniol (GGO), a side chain of vitamin K_2, strongly induces apoptosis of tumor cells, suggesting that GGO might inhibit cell growth.[18]

Recently, microarray analysis has shown that several genes are induced by treatment with vitamin K_2.[19] Protein kinase A (PKA) is a common activator of related

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Table 1 Baseline characteristics[21]

	Treatment ($n = 21$)	Control ($n = 19$)	*P*-value
Average age (years)	59.8 ± 8.7	61.4 ± 7.1	0.54
HBV/HCV	1/20	1/18	0.94
Albumin (g/dL)	3.9 ± 0.3	3.9 ± 0.3	0.87
Platelets (10^4/mm^3)	14.7 ± 5.4	12.1 ± 5.2	0.13
Total bilirubin (mg/dL)	0.8 ± 0.2	0.9 ± 0.4	0.47
ALT (IU/mL)	81.7 ± 42.7	70.4 ± 33.4	0.36
AFP (ng/mL)	13.4 ± 17.7	13.3 ± 8.7	0.99
IFN (+/−)	4/17	3/16	0.79

Mann–Whitney *U*-test for age, serum albumin, platelets, total bilirubin, alanine transferase (ALT) and α-fetoprotein (AF); χ^2 test for hepatitis B and C virus (HBV/HCV). IFN (+/−): Patents who received interferon(IFN) prior to enrollment; +, yes; −, no.

signaling pathways, identified by microarray analysis. Vitamin K_2 is thought to activate PKA, which inhibits RhoA activation. Alterations caused by high-dose treatment with vitamin K_2 result in cell-cycle arrest at the G1 and G2/M phases, accompanied by inhibition of tumor invasion. The effects of vitamin K_2 in doses used to treat osteoporosis are poorly understood, especially in the liver. However, the results of *in vitro* studies suggest that vitamin K_2 is one of the most promising agents for the chemoprevention of HCC.

PRIMARY CHEMOPREVENTION

WE PREVIOUSLY REPORTED a 2-year study showing that vitamin K_2 helps to prevent bone loss in women with viral cirrhosis of the liver.[20] Most of the subjects agreed to participate in an extended study designed to clarify the long-term effects of vitamin K_2 on bone loss associated with cirrhosis. The incidence of HCC was found to differ between women who received vitamin K_2 and those who did not.[21] In detail, the subjects of the initial 2-year study were 50 women with viral liver cirrhosis who were admitted to our department between 1996 and 1998. If the results of abdominal dynamic computed tomography and abdominal ultrasonography suggested the presence of HCC, abdominal angiography or needle biopsy was performed to confirm the diagnosis. Three patients in the treated group and four in the control group were confirmed to have HCC and were excluded from further study. The remaining 43 patients were randomly assigned by means of sealed envelopes to receive 45 mg/day of vitamin K_2 (Glakay; Eisai, Tokyo, Japan) p.o. (treated group) or no vitamin K_2 (control group). At the end of the first study (after 2 years of treatment), 21 patients in the treated group and 19 in the control group consented to participate in a longer trial. In a longer trial, all but one patient in each group had hepatitis C virus (HCV) infection; two other patients had hepatitis B infection. Seven patients, four in the control group and three in the treated group, had previously received IFN-α for their HCV infections, but HCV was not eradicated. No patient was given IFN therapy after study entry. Surveillance for HCC was done according to detailed guidelines for the follow up of patients with liver cirrhosis in Japan.[8] Compliance with vitamin K_2 in the treated group was good; no patient had adverse reactions or dropped out of the study. The two groups were similar with respect to age, virus type, platelets, alanine aminotransferase (ALT), α-fetoprotein (AFP) and other clinical findings (Table 1). After the first study commenced, HCC was detected in two of the 21 patients given vitamin K_2 and nine of the 19 controls; the cumulative proportion of patients with HCC was smaller in the treated group (log-rank test, $P = 0.024$; Fig. 1). On univariate analysis, the risk ratio for the development of HCC in the treated group versus the control group was 0.195 (0.042–0.913; $P = 0.038$). On multivariate analysis with adjustment for age, ALT activity, serum albumin, total bilirubin, platelet count, AFP, and history of treatment with IFN-α, the risk ratio for the development of HCC in patients given vitamin K_2 was 0.126 (0.016–0.992; $P = 0.049$) (Table 2).

The original goal of our trial was to assess the long-term effects of vitamin K_2 on bone loss in women with viral liver cirrhosis. Our trial thus had several important limitations when the data were used to assess the value of vitamin K_2 for the primary prevention of HCC in patients with liver cirrhosis. Factors limiting the value of our findings included the small study group, the inclusion of only women and the participation of only one

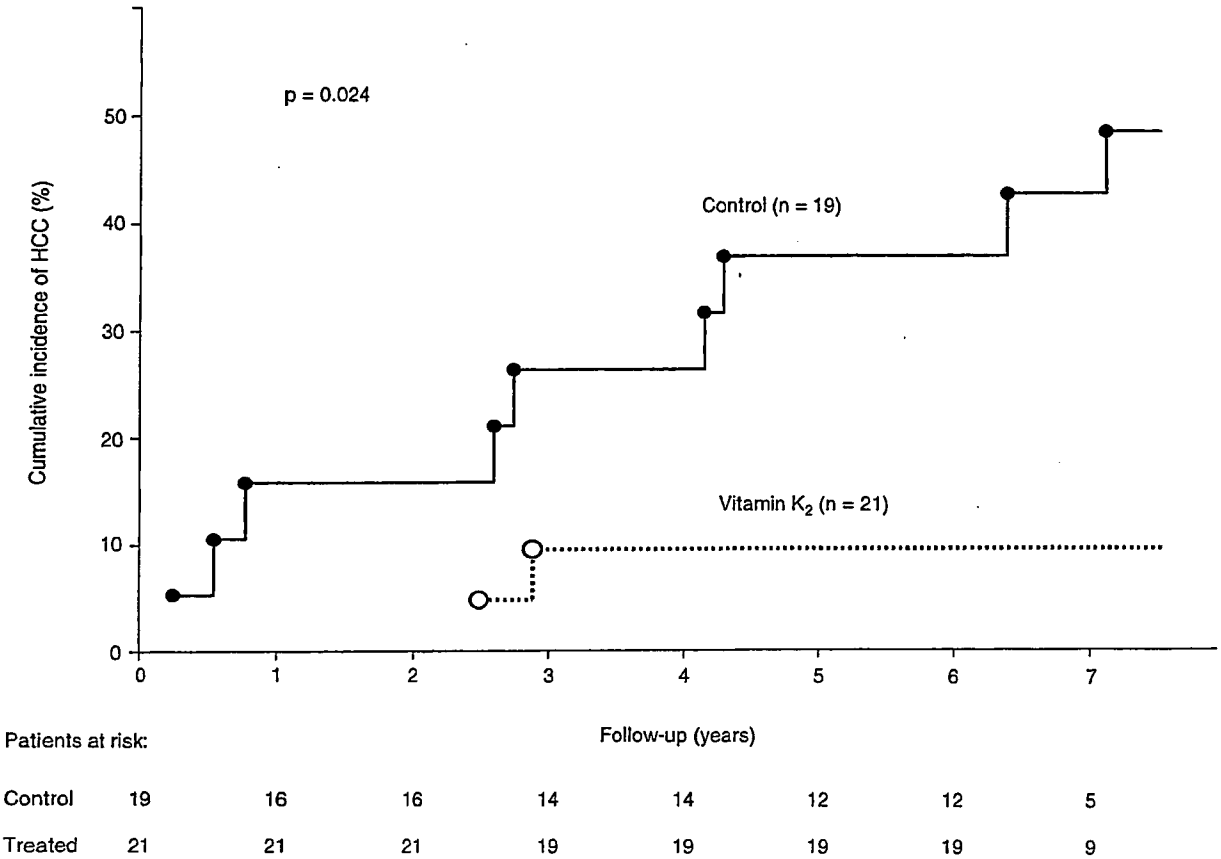

Figure 1 Cumulative incidence of hepatocellular carcinoma (HCC) diagnosed in patients treated with vitamin K_2 and in a control group.[21] All patients were followed up for at least 6 years. Vertical marks on curves show the latest follow-up to date for the 15 patients monitored for less than 7 years.

center. However, similar to previously reported randomized controlled studies of cirrhosis in which the primary end-point was the development of HCC, patients with evidence of HCC on highly sensitive imaging studies were excluded, and the two study groups were similar with respect to risk factors for HCC, including age, severity of cirrhosis, history of IFN therapy and type of hepatitis virus infection. Our results indicate that vitamin K_2

Table 2 Adjusted odds ratios for the development of hepatocellular carcinoma (HCC)[21]

	Odds ratio	95% CI	*P*-value
VK_2/control	0.126	0.016–0.992	0.0491
Total bilirubin (mg/dL) (1.0+/<1.0)	0.294	0.042–2.044	0.2161
Albumin (g/dL) (<3.5/3.5+)	33.434	2.362–473.352	0.0094
Platelets (10^4/mm^3) (<100/100+)	2.235	0.458–10.900	0.3200
ALT (IU/mL) (<80/80+)	0.393	0.071–2.164	0.2831
AFP (ng/mL) (20+/<20)	1.689	0.306–9.335	0.5477
IFN (+/–)	1.260	0.201–7.903	0.8053

Adjusted for age and all other variables in this table. IFN (+/–): Patents who received IFN prior to enrollment; +, yes; –, no. CI, confidence interval; VK_2, vitamin K_2.

decreases the risk of HCC to approximately 20% as compared with control, suggesting that vitamin K_2 may delay the onset of hepatocarcinogenesis.

SECONDARY CHEMOPREVENTION

THE RATE OF recurrence after curative therapy for HCC is high. Improved outcomes in HCC require inhibition of tumor recurrence. Before our study on primary chemoprevention, vitamin K_2 has been used to prevent the development of second primary malignancies after curative therapy for HCC. Koike *et al.* showed that the administration of vitamin K_2 to patients with HCC who have high levels of des-γ-carboxy prothrombin decreased the risk of portal vein invasion by tumor.[22] Preliminary results of a study being conducted by Mizuta *et al.* suggest that vitamin K_2 inhibits the recurrence of HCC, especially in patients with HCV (unpubl. results). However, this study is in progress; its results remain to be published.

COMBINATION THERAPY

PREVIOUS STUDIES HAVE evaluated the effectiveness of single agents for preventing HCC in patients with chronic liver diseases. To our knowledge, studies assessing the value of combination therapy for chemoprevention have not been reported. One of the reasons for the lack of studies evaluating combined treatment is concern about adverse effects associated with different agents. For example, adverse effects of IFN therapy include fever, leukopenia and thrombocytopenia. In contrast, vitamin K_2 has not been associated with serious side-effects in patients with osteoporosis. Vitamin K_2 may therefore be able to be used concomitantly with other chemopreventive agents, without increasing the risk of adverse reactions. Yoshiji *et al.* reported that a combination of vitamin K_2 and perindopril, an angiotensin-converting enzyme (ACE) inhibitor, was more effective for chemoprevention than either agent alone in a diethylnitrosamine-induced rat hepatocarcinogenesis model.[23] The number and size of enzyme-altered preneoplastic lesions were both significantly reduced, and the expression of CD31, a marker of neovascularization, was decreased in rats given combination treatment. Their findings suggested that a low dose of vitamin K_2 (1 μM) inhibits the proliferation of endothelial cells. Clinical trials examining whether vitamin K_2 plus an ACE inhibitor prevents HCC in patients with chronic liver diseases thus appear to be warranted.

CONCLUSION

AVAILABLE EVIDENCE SUGGESTS that vitamin K plays a role in controlling cell growth. The mechanisms responsible for the vitamin K_2-mediated inhibition of cell growth remain unexplained. Clinical studies have suggested that treatment vitamin K_2 reduces the incidence of HCC in patients with chronic liver diseases. Indeed, the annual incidence of HCC in control patients was 8.8%, similar to the incidence of HCC (7.9%; 32/107) in patients with liver cirrhosis in Japan,[3] as compared with only 1.6% in patients who received vitamin K_2 in our study. However, previous clinical studies of vitamin K_2 have focused on patients with specific characteristics or risk factors for HCC, including only women or patients with high levels of des-γ-carboxy prothrombin. Future investigations should attempt to define which patients would optimally benefit from chemopreventive therapy with vitamin K_2. The safety, relatively low cost and ease of use of vitamin K_2 have led to good compliance with treatment. These properties make vitamin K_2 a suitable candidate for clinical trials assessing the value of combination treatment for chemoprevention or chemotherapy in patients at risk for, or with a confirmed diagnosis of, HCC.

The results of preliminary trials are intriguing and suggest a potential role for vitamin K_2 in the prevention of primary and secondary hepatocarcinogenesis in patients with hepatic cirrhosis. However, currently available results must be verified by multicenter randomized controlled studies in which the primary end-point is the prevention of HCC by vitamin K_2.

CONFLICT OF INTEREST

NO CONFLICT OF interest has been received from the authors.

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症例報告

肝切除後に総肝動脈リンパ節転移を来した肝細胞癌の1例

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東野　正幸[2)]　久保　正二[1)]

肝癌切除後の孤立性リンパ節転移を摘除することで，術後2年6か月の現在，無再発生存中の症例を経験したので報告する．症例は58歳の男性で，C型慢性肝炎に伴う肝癌に対して肝切除術を2回施行されていた．経過観察中のCT像上，肝尾状葉に約4cm大の腫瘤性病変を認め，AFP，PIVKA-II値の著明な上昇がみられた．腹部血管造影像では腫瘤は中肝動脈および左胃動脈より栄養される腫瘍濃染像として描出され，肝癌の尾状葉再発と診断し開腹した．腫瘍は肝尾状葉に接するように総肝動脈の腹側に存在していたが，肝臓からは独立しており肝癌の総肝動脈幹リンパ節転移と考え摘除した．病理組織学的検査では中分化型肝癌のリンパ節転移と診断された．AFP，PIVKA-IIは術後2か月目に標準値範囲内へ低下し，以来，再発徴候を認めていない．原発巣がコントロールされた肝癌の孤立性リンパ節転移は摘除により良好な予後が得られる可能性が示唆された．

はじめに

肝細胞癌（以下，肝癌）のリンパ節転移は剖検例で約30％と比較的高率に認められるが[1)2)]，肝癌の臨床経過においてリンパ節転移が問題となることは比較的まれである．このため，肝癌リンパ節転移に対する治療選択および成績についての報告は少数である．今回，我々は肝癌切除後に総肝動脈幹リンパ節転移を孤立性に認め，摘除により長期間無再発生存しえた症例を経験したので報告する．

症　例

患者：58歳，男性

主訴：症状なし．

家族歴，既往歴：特記事項なし．

現病歴：十数年前よりC型肝炎のため近医にて経過観察されていた．2001年4月の超音波検査にて肝外側区域に約1cmおよび前区域に約2cmの肝腫瘤を認め，AFPは65.6ng/mlであった．腹部血管造影像上，同部位に腫瘍濃染像がみられたため，肝癌と診断し開腹した．術中超音波検査にて，前区域に新たに約1cm大の腫瘤を認めたため，それぞれに対して肝部分切除術を施行した．術後の病理組織学的検査において中分化型肝癌で，門脈腫瘍栓所見はなかった．非癌部肝組織は肝硬変像を呈していた．

術後リザーバー動注（5-FU 1,500mg＋CDDP5 mg＋leucovorin 12mg　計6回）を行っていたが，術後約1年目にAFPが71ng/mlと上昇し，CTにて肝内側区域に肝癌再発を認めたため，再度肝部分切除術を施行した．病理組織学的検査では広範な壊死を伴う低分化型肝癌と診断された．門脈腫瘍栓および肝内転移を認めなかった．再手術後2か月目にはAFPは正常範囲内となったが，再手術後1年目には，AFPは3,955ng/mlと再上昇したため，精査加療目的に入院となった．

入院時現症：身長161cm，体重61kg．皮膚，眼球結膜に黄染は認められず，腹部は平坦，軟で，肝脾とも触知しなかった．また，体表リンパ節は触知しなかった．

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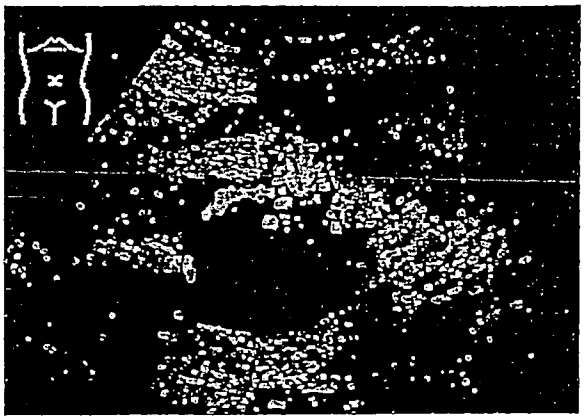
Table 1 Laboratory data on admission

WBC	3.93×10^3 /mm^3	BUN	11 mg/dl
Hb	13.6 g/dl	Creatinine	0.71 mg/dl
Ht	38.8 %		
Platelet	8.3×10^4 /mm^3	HBs Ag	(−)
TP	8.5 g/dl	HCV Ab	(−)
Albumin	4.3 g/dl		
T-Bil	0.9 mg/dl	AFP	3,955 ng/ml
AST	61 IU/l	AFP L3	6.4 %
ALT	55 IU/l	CEA	6.6 ng/ml
ALP	245 IU/l	CA19-9	21.8 U/ml
γ-GTP	71 IU/l	PIVKAII	5,780 AU/ml
ICGR15	20 %		
PT%	86.4 %		

AFP, α-fetoprotein ; CEA, carcinoembryonic antigen ; CA19-9, carbohydrate antigen19-9
PIVKAII, protein induced by vitamin K absence or antagonist II

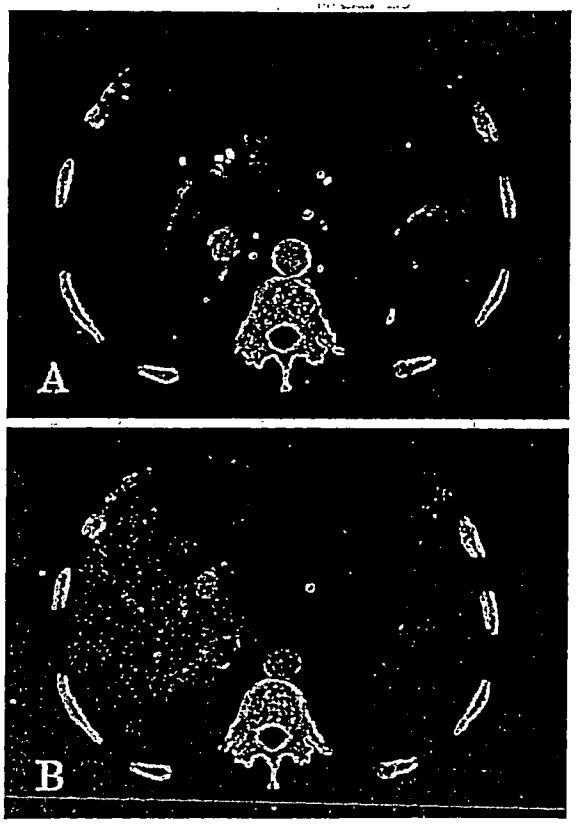
Fig. 1 Abdominal ultrasonography showed a hypoechoic lesion 4cm in a diameter fed by many arteries in the caudal side of caudate lobe.



入院時血液検査所見：血液生化学検査でAST, ALTが軽度上昇しており，アルブミン 4.4g/dl, 総ビリルビン 0.6g/dl, ICG15分値 20%，プロトロンビン活性 86.4% 以上と肝障害度はAであった．AFPは3,955ng/ml, PIVKA-IIは5,780AU/mlと著明に上昇し，CEAが軽度上昇していた (Table 1)．

腹部超音波検査：肝尾状葉より突出する径4cmの，辺縁整，内部均一な低エコーの腫瘤を認めた．また，栄養血管を多数認めた (Fig. 1)．

Fig. 2 Contrast-enhanced computed tomography showed the low density lesion about 4cm in a diameter that was enhanced at early phase (A), and that was washed out at late phase (B) in the caudal side of caudate lobe.



腹部ダイナミックCT所見：肝尾状葉より尾側に突出し，辺縁整，境界明瞭な約4cmの低吸収域を認めた．同病変は早期層にて造影効果をうけ (Fig. 2A)，後期層にて造影欠損像として認められた (Fig. 2B)．

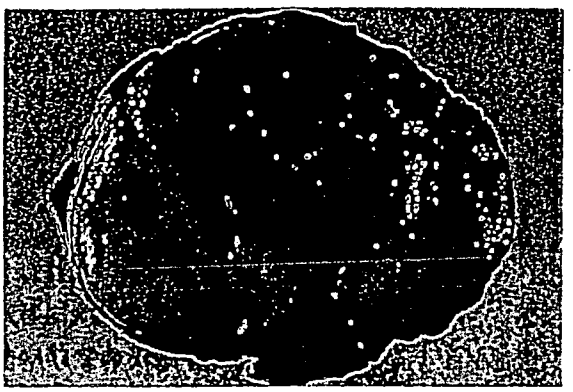
腹部血管造影検査所見：腫瘍は左胃動脈および中肝動脈より栄養血管を受ける腫瘍濃染像として描出された (Fig. 3)．

以上の所見より，肝癌の尾状葉再発と術前診断し開腹した．

手術所見：肝臓は表面凹凸不整，辺縁鈍，軽度弾性硬で中等度の線維化を認めた．腫瘍は総肝動脈の腹側に存在し，尾状葉に接していたが肝臓からは独立していた．総肝動脈幹リンパ節転移と診断し，リンパ節摘除術を施行した．なお，他のリ

Fig. 3　Selective angiography showed a tumor stain feeding from the middle hepatic artery (A) and the left gastric artery (B).

Fig. 4　Macroscopically, resected specimen showed the encapsulated white and soft tumor 6cm in a diameter.



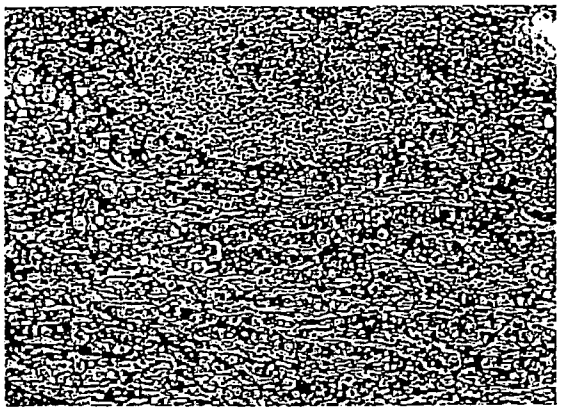
ンパ節に腫大は認められなかったため，リンパ節郭清は行わなかった．

切除標本肉眼検査所見：腫瘍は最大径 6cm，黄白色，膨隆性で線維性被膜を認めた（Fig. 4）．

病理組織学的検査所見：リンパ節内に太い索状構造を示す，核の大小不同を伴った中分化型肝細胞癌転移を結節状に認めた．腫瘍細胞はリンパ節髄内をびまん性に置換浸潤していたが，リンパ濾胞が腫瘍細胞間に残存していた（Fig. 5）．

術後経過：術後経過は良好で，術後 11 日目に退院した．術後 10 週目には AFP が 3.9ng/mg，

Fig. 5　Histopathological examination revealed moderately differentiated hepatocellular carcinoma in the lymph node (H.E.×200).



PIVKA-II が 17AU/ml と標準値範囲内まで低下した．術後 2 年 6 か月の現在，再発兆候なく生存中である．

考　察

肝癌の剖検例では，リンパ節転移は肺転移に次いで多く，20～30% に認められる[1)-4)]．また，Katyal ら[5)]は肝癌 403 例に CT を用いた肝外転移の検索を行い，148 例に肝外転移がみられ，そのうち 78 例（19.4%）にリンパ節転移を認めたとしており，その多くが T4 症例であったと報告している．一方，肝癌切除例でのリンパ節転移の頻度は諸家の報告では 0.7～4.9% と低率であるが[1)6)]，そのほとんどが門脈腫瘍栓および肝内多発病変を有する高度進行症例であるため，その予後は極めて不良である[7)8)]．我々も 504 例の肝切除症例のうち 6 例（1.2%）に同時性リンパ節転移を認め，肝切除時にリンパ節郭清を行ったが，全例が 2 年以内に死亡したことを報告している[9)]．また，過去の多変量解析を用いた研究でもリンパ節転移が肝癌症例の独立予後因子であったことから，一般的にリンパ節転移は肝癌の終末期像を反映していると考えられる[10)11)]．しかしながら，Une ら[6)]は原発巣切除後の孤立性リンパ節転移 2 例に対し外科切除を行い，4 年以上の予後を得ており，Ochiai ら[12)]も同様に孤立性リンパ節転移を切除後，7 年間無再発生存した症例を報告している．また，蒔田ら[13)]は 4

Table 2 Thirteen cases reported in Japan with lymphatic metastasis following surgery for hepatocellular carcinoma

Case No.	Referrence	(year)	Size (cm)	Primary tumor			Lymph node metastasis site	Treatment	Survival (mo)
				Histology	Liver parenchyma	* Interval (mo)			
1	Misawa [20]	1989	3.0	Ed II	Z1	28	#13	dissection	3 alive
2	Une [6]	1994	6.5	Ed III	Z1	12	#12p	dissection	46 died
3	Une [6]	1994	3.0	Ed II	Z1	10	#13	dissection	77 alive
4	Wakabayashi [10]	1997	5.0	poorly	Z1	14	#12a, b, c	none	27 alive
5	Fujimori [14]	1997	18	moderately	Z0	76	#12	radiation	15 alive
6	Saito [17]	1998	9.5	moderately	Z0	41	#5, 8a	dissection	?
7	Makita [13]	1999	5.0	poorly	?	6	#8, 12, 13a	dissection	24 died
8	Ochiai [12]	2000	2.9	Ed III	?	45	#12	dissection	39 alive
9	Hanawa [15]	2001	2.5	well ~ mod	Z1	20	#11p	dissection	30 alive
10	Koike [18]	2002	4.0	Ed I + II	?	108	#7	dissection	14 alive
11	Katagiri [16]	2003	3.0	moderately	Z1	6	#13	dissection	80 alive
12	Suzuki [19]	2003	2.5	Ed III	Z2	19	#16	dissection	13 alive
13	Y-E. Peng [21]	2005	3.7	poorly	Z1	21	#16a1 int	dissection	33 alive

* Interval is the period from the operation to recurrence.

例のリンパ節再発を切除することで比較的良好な成績を得たことから，転移巣が孤立性で肝内病変がコントロールされている症例では外科的摘除を含む集学的治療が有効であると報告している[14)~16)]．医学中央雑誌で「肝細胞癌」「孤立」「リンパ節転移」「肝切除術」をキーワードとして1983年から2005年までについて検討したところ，残肝再発のない肝切除後のリンパ節転移再発症例が13例報告されている（Table 2）[6)10)12)~21)]．今回，我々も肝癌切除後の孤立性リンパ節転移を切除することで良好な予後を得ており，肝癌のリンパ節転移は原発巣の進展とともに多発，系統的に出現した予後不良なリンパ節転移と，単発のまま増大し予後良好なリンパ節転移があるのではないかと考えている．肝臓のリンパ流には肝漿膜下リンパ管，肝小葉間結合織リンパ管および肝静脈系リンパ管が存在するが，肝硬変症例では線維化に伴い解剖学的系統的リンパ流とは異なる複雑な側副路が形成されると考えられている[22)23)]．このため，肝炎ウイルスによる慢性肝障害を発生母地する肝癌症例のリンパ節転移では，系統的なリンパ流に沿わないskip metastasisの報告が散見される[10)17)~19)]．本症例も総肝動脈リンパ節に孤立性リンパ節転移がみられたが，C型肝炎による肝硬変が存在していた．

肝癌切除例と剖検例のリンパ節転移頻度に大きな差がみられることより，リンパ節郭清を行わない肝切除例にリンパ節転移陽性例が潜んでいる可能性が示唆される．しかしながら，肝癌切除時の予防的リンパ節郭清はその意義が明らかでなく，肝癌の背景に慢性肝障害を有する症例が多く，安易なリンパ節郭清は難治性腹水などの危険を伴うため不必要と考えられる[9)]．一方，肝癌治療中にリンパ節転移がみられた場合，リンパ節転移が孤立性でその他の肝内肝外病変が十分にコントロールされていると，切除により良好な予後が得られるのではないかと推測できた．このため，手術適応の決定においてリンパ節転移個数の診断が重要と考えられる．近年，PET（positron emission tomography）は高分化型肝癌に対して検出率が低いとの報告も認められるが[24)]，リンパ節を含む遠隔転移を来すような分化度の低い肝癌であれば高率に検出しうるとの報告もあり[20)24)25)]，肝癌リンパ節転移の術前診断への応用が期待される．

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A Case of Solitary Lymph Node Metastasis of Hepatocellular Carcinoma after Hepatic Resection

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The presence of lymph node metastasis is rarely shown in surgical patients with hepatocellular carcinoma. A 58-year-old man who has undergone hepatic resection twice for hepatocellular carcinoma (HCC) and admitted to our hospital was found. During follow-up and abdominal computed tomography (CT) to have a low-density lesion 4cm in diameter in the caudate lobe. Angiography showed tumor staining fed by the left gastric artery and middle hepatic artery. The lesion was diagnosed during the surgery as lymphadenopathy in front of the common hepatic artery, we resected the lymph node. Histological examination showed lymph node metastasis of HCC. The patient remains alive without sign of tumor recurrence 30 months after surgery. Resection for lymph node metastasis from HCC is thus effective, when the metastatic lymph node is solitary and when the primary lesion is controlled.

Key words : solitary lymph node metastasis, hepatocellular carcinoma, hepatic resection

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症例報告

生体肝移植術後，タクロリムスおよびシクロスポリンにより脳症をきたした1例

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A Case of Encephalopathy caused by Tacrolimus and Cyclosporine after Living-Donor Liver Transplantation

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【Summary】

A 55-year-old man with non-compensatory cirrhosis secondary to chronic hepatitis C underwent living-donor liver transplantation. Immediately after transplantation, immunosuppressive therapy with tacrolimus (FK506) and steroid was started. On the 15th and 60th postoperative days, he experienced generalized seizures. At the time of his initial seizure, serum concentration of FK506 was within the therapeutic range. Computed tomography revealed no cerebral hemorrhage or infarction. An electroencephalogram revealed diffuse slow waves. Results of cerebrospinal fluid analysis were normal. FK506-induced encephalopathy was diagnosed and he recovered with withdrawal of FK506. After the second episode of encephalopathy, the immunosuppressive agent was changed from FK506 to oral cyclosporine (CsA). However, seizure occurred again 58 days after the start of CsA. The serum concentration of CsA was within the therapeutic range. Magnetic resonance imaging revealed high-intensity lesions in the white matter in the temporal and parietal lobes. He was diagnosed with CsA-induced encephalopathy and improved with withdrawal of CsA. Rejection can presently be controlled with a dosage of 0.8 mg/day of FK506, without any signs of encephalopathy. In summary, we experienced a rare case of encephalopathy caused by administration of both FK 506 and CsA after liver transplantation.

Keywords: encephalopathy, tacrolimus, cyclosporine, liver transplantation

I. はじめに

Cyclosporine (CsA) および tacrolimus (FK506) はともにカルシニューリン阻害剤であり，Tリンパ球の活性を阻害して強力な免疫抑制作用を発揮する。そのため，現在，臓器移植や自己免疫疾患治療に広く使用されており，これらカルシニューリン阻害剤の登場により臓器移植後の成績は著しく向上した。一方，カルシニューリン阻害剤による副作用のひとつに痙攣や意識障害等の神経学的徴候をきたす脳症が報告されており，注意が必要である。

今回，著者らは生体肝移植術後にFK506のみならずCsAによっても脳症を発症した症例を経験したので報告する。

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II. 症　例

症　例：55歳，男性。
家族歴：特記事項なし。
既往歴：6歳時，大腿骨骨髄炎に対して骨切り術が施行された。痙攣発作・意識消失発作の既往はない。
現病歴：45歳時にC型肝炎を指摘され，インターフェロン療法が施行されたが，効果なく経過していた。49歳時より食道静脈瘤に対する治療が繰り返されるようになり，1年前より難治性腹水が認められるようになった。そこで，非代償性肝硬変と診断され，生体肝移植目的で当科に紹介された。
臨床経過：入院時，黄疸および多量の腹水が認められ，特発性細菌性腹膜炎を併発していた。血液型はA型で，Child-Pugh分類C（12点），MELD scoreは17点であった。抗生剤投与による細菌性腹膜炎の改善を待ち，血液型O型の息子をドナーとし，中肝静脈付き右葉グラフトを用いた生体肝移植術を施行した（グラフト肝容積/レシピエント標準肝容積比＝53.8%，グラフト体重比1.1%）。

術翌日よりFK506およびステロイドホルモンによる免疫抑制療法を行った（図1）。ところが，術7日後に振戦が出現したため，FK506の副作用を疑い（トラフ値12.3 ng/ml），FK506の減量とともにmycophenolate mofetilを併用したところ軽快した。
第1回脳症発症：術15日後に突然，1〜2分間持続する全身強直性痙攣が出現した。非発作時の意識は保たれており，嘔吐や麻痺は認められなかった。その際，体温は37.3℃，最高血圧は160–180/mmHg（術後120–140/mmHgで推移）であった。血液検査では経時的に著明な変化は認められず，血糖値は287 mg/dl，血清コレステロール値は53 mg/dl，アンモニア値は32 μg/dl（表1），FK506トラフ値は6.7 ng/mlであった。脳

表1　血液検査成績

		脳症発症時		
	入院時	1回目	2回目	3回目
WBC（×10²/μl）	52	104	68	81
Hb（g/dl）	10.8	6.9	10	7.5
Ht（%）	30.4	19.8	27.6	20.1
Plt（×104/μl）	6.2	1.4	13.5	8.2
CRP（mg/dl）	5.9	3	1	5.8
T-Bil（mg/dl）	3.9	11.1	18.4	13.4
AST（IU/ℓ）	65	19	157	52
ALT（IU/ℓ）	47	26	285	50
ALP（IU/ℓ）	176	92	273	346
γ-GTP（IU/ℓ）	45	28	221	853
LDH（IU/ℓ）	265	331	682	780
ChE（IU/ℓ）	93	46	58	56
Cho（mg/dl）	117	53	136	275
Alb（g/dl）	3	4.1	3.1	2.8
PT-INR	1.42	1.55	2.74	1.29
BUN（mg/dl）	59	41	65	31
Cre（mg/dl）	1.2	0.74	1.09	1.10
NH3（μg/dl）	63	32	93	65
FBS（mg/dl）	156	287	251	129

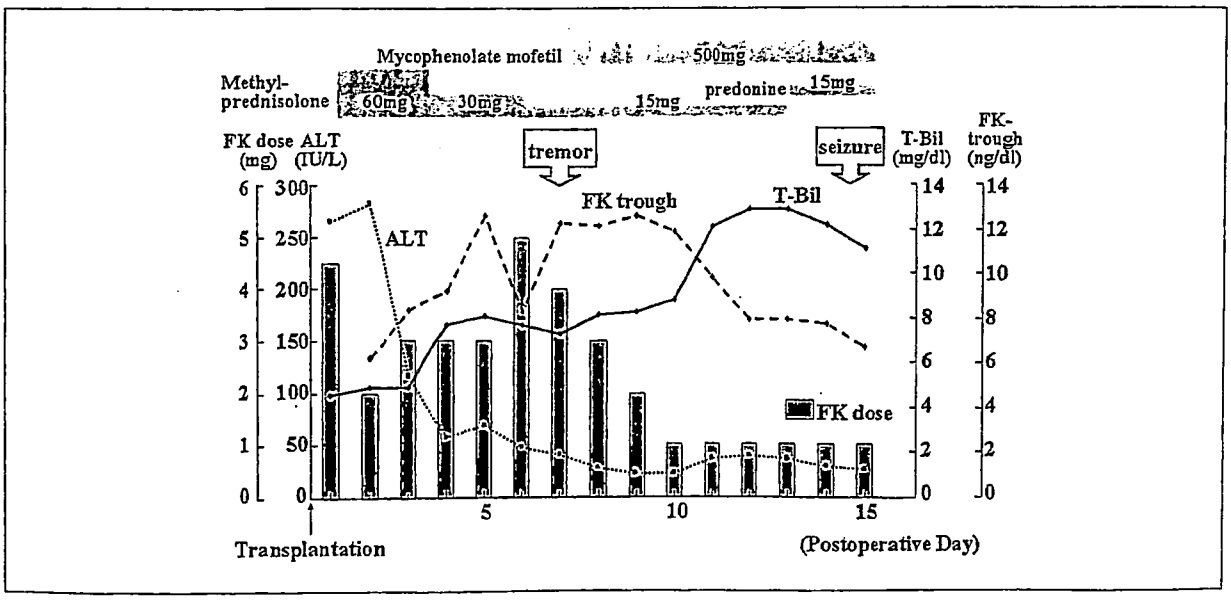

図1　肝移植術後から第1回目の脳症発症までの臨床経過

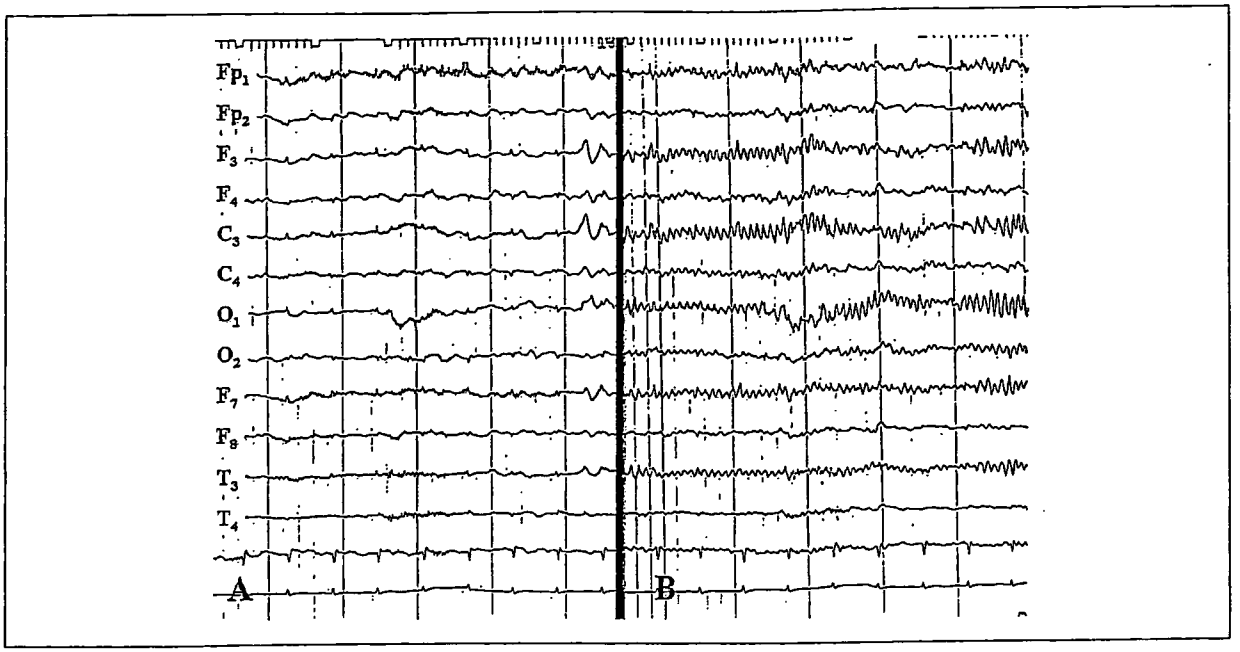

図2 第1回目脳症発症時の脳波所見
非痙攣発作時，C3dominant を中心に広汎性に徐波が認められ（A），
痙攣発作時には突発性律動波が左側中心に認められる（B）。

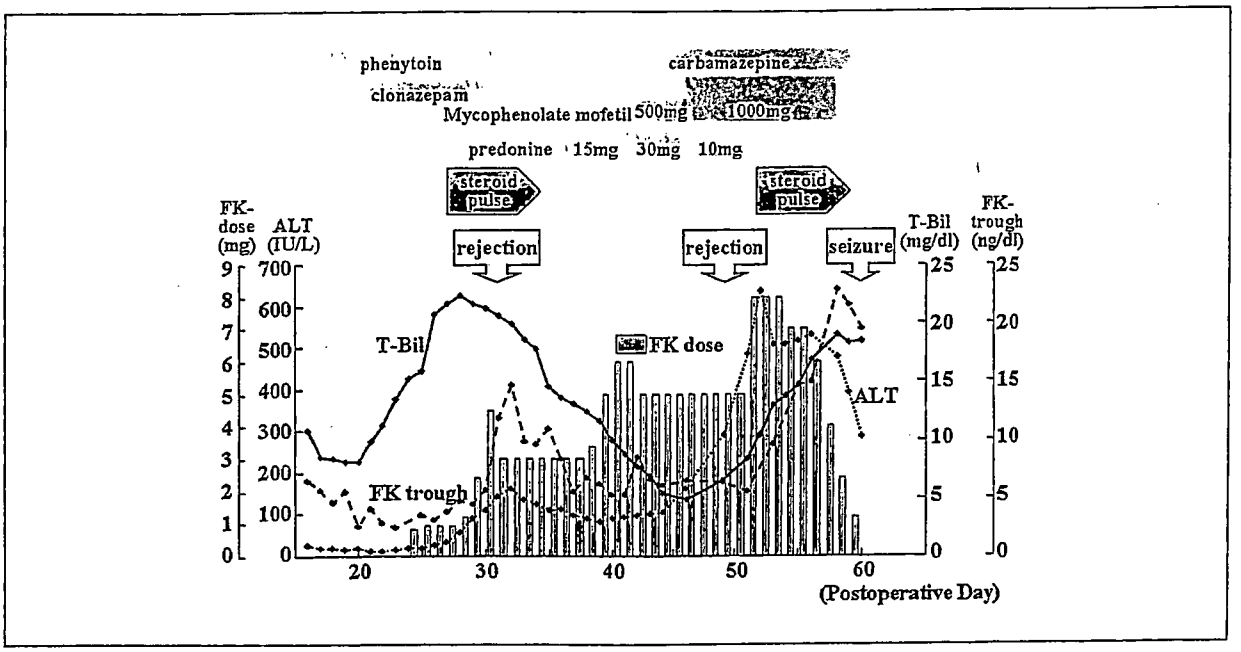

図3 第2回目の脳症発症までの臨床経過

CT 検査上，出血や梗塞などは認められず，髄液検査では白血球数は 2 個/μl であった。脳波検査では広汎性に徐波が認められた（図 2）。以上より，FK 脳症を疑い，FK506 の投与を中止したところ痙攣発作は消失し，発症 3 日後より次第に意識状態は改善した。拒絶所見がみられたため鎮痙剤（clonazepam 3 mg/日および phenytoin 250 mg/日）を予防的に使用しつつ，術 25 日後より FK506 を再開した。その後も拒絶に対してステロイドパルスの施行や各免疫抑制剤の投与量を適宜調節したが，拒絶が軽快しないため FK506 を増

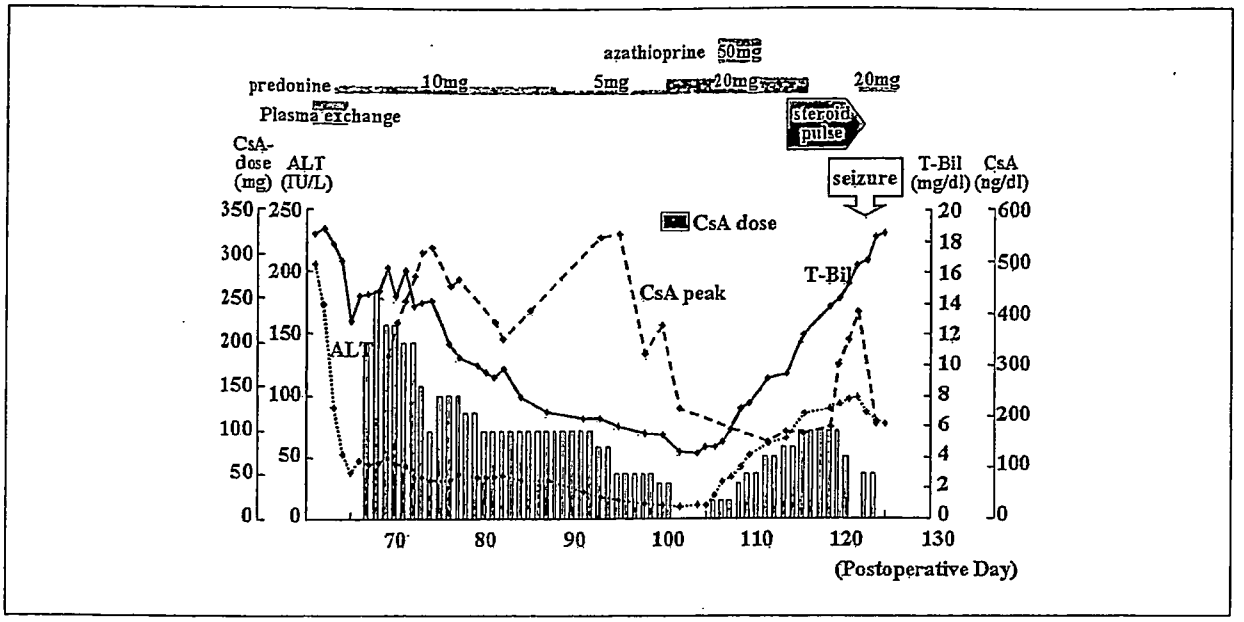

図4 第3回目の脳症発症までの臨床経過

量した（図3）。

第2回脳症発症：ステロイドパルスが終了した翌日の術60日後，突然，意識消失発作および約30秒間持続する全身硬直性痙攣が出現した。その際，体温は36.7℃，最高血圧は130-150/mmHgであった。血液検査上，血清アミラーゼ値の上昇（568 IU/ℓ）と肝機能障害が認められ，血糖値は251 mg/dl，アンモニア値は93 μg/dl（表1），FK506トラフ値は19.5 ng/mlであった。脳波検査では前回よりvoltageの低下があるものの同様の広汎性徐波が認められた。以上より，FK506による脳症および膵炎と診断し，FK506投与を中止したところ痙攣発作は消失し，発症2日後より意識状態は改善，膵炎も軽快した。そこでFK506の投与を断念し，術67日後よりCsAに変更した。投与量は1回100 mgの1日2回投与から開始し，ピーク値は297 ng/dlであった。腎機能障害の出現（最高：尿素窒素値49 mg/dl，血清クレアチニン値3.55 mg/dl）によりCsAの投与を一時中断したが，腎機能の回復を待ち，術106日後よりCsAを再開した。その後も拒絶に対してステロイドパルスの施行や各免疫抑制剤の投与量を適宜調節した（図4）。

第3回脳症発症：術125日後，全身状態は良好であったが，突然意識消失発作および約30秒間持続する全身強直性痙攣が出現した。その際，体温37.5℃，最高血圧160-180/mmHgであった。血液検査上，経時的な著明な変化は認められず，アンモニア値は65 μg/dl（表1），CsAトラフ値は101 ng/dl，CsAピーク（Cmax）値は183 ng/dlであった。脳波検査では左側中心に広汎性の徐波が認められ，脳MRI（FLAIR像）検査では左側頭葉内側と左頭頂葉に高信号域が認められた。出血や梗塞はみられなかった（図5）。以上より，CsA脳症と診断し，CsAの投与を中止したところ痙攣発作は消失し，発症2日後より意識状態は改善した。以後，CsAを再開したが，拒絶所見が改善しないため，再度FK506をトラフ値3.0-4.0 ng/mlを目標に維持しながら投与した。その結果，神経学的徴候をきたすことなく全身状態と拒絶反応が軽快したため，術307日後に退院した。

なお，術2年後の現在，FK506（0.8 mg/日）で拒絶反応は制御されているが，C型肝炎の再燃に対しインターフェロン療法を施行している。

III. 考 察

FK506およびCsAはともに優れた免疫抑制効果を有し，臓器移植後に汎用されているが，注意すべき副作用の1つとして脳症がある。脳症の臨床症状は頭痛，振戦，不眠などの軽度のものから，痙攣，言語障害，片麻痺などの局所症状を呈する重篤なものまである[1-3]。それら神経学的症状の発症頻度はFK506が5-31.1%，CsAが8-47%であり[1,3-5]，なかでも重篤な症

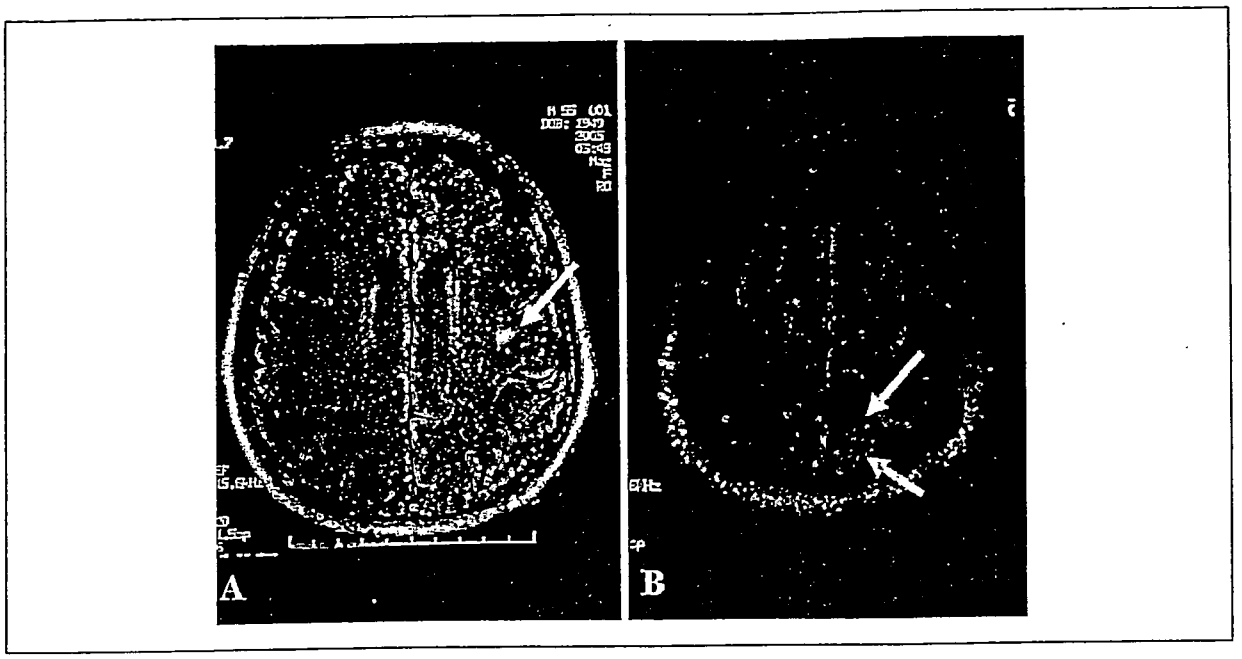

図5　第3回目脳症発症時のMRI-FLAIR像
左側頭葉内側（A：矢印）と左頭頂葉（B：矢印）に高信号域が認められる。

状を呈する頻度は5%程度と報告されている[1,6]。また，一般的にこの脳症は薬剤の減量および中止により後遺症なく軽快するとされている[6]。診断にはMRIが有用であり，T2強調像やFLAIR像で頭頂葉，後頭葉，後部側頭葉といった大脳半球の後部に高信号域を呈することが多いといわれており[1,7]，これら大脳の局所障害が起こると失認，記憶障害，精神運動発作などの巣症状をきたす。本症例でも側頭葉と頭頂葉に高信号域が認められ，意識消失発作や痙攣発作などの所見から薬剤による脳症と考えられた。また，FK506濃度が25 ng/ml以上になると重篤な中枢神経障害をきたしやすいとされているが，一般的には脳症発症と薬剤の血中濃度とは必ずしも関連がないと報告されている[5,8]。事実，本症例での脳症発症時のFK506やCsAの血中濃度は2回目でのみ高値であった。

一方，通常この脳症は投与開始後30日以内に発症することが多いと報告されており，肝移植術後数カ月経過した時点で発症することはまれである[9]。本症例では，第1回目の脳症は術15日後と早期に発症しているが，第2，3回目はそれぞれ術後約2あるいは4カ月経過してから発症していた。また，第1回目および2回目の脳症はFK506によって，第3回目の脳症はCsAによって引き起こされたと考えられたが，FK506あるいはCsA単独の免疫抑制剤による脳症の報告は散見されるものの，FK506およびCsA両者によって脳症を発症した症例はきわめてまれであると考えられた。

FK506やCsAによる脳症の発症機序の詳細は明確ではないが，①FK506やCsAは脂質親和性が高く脂質に富むミエリンと結合しやすいことから神経細胞に直接作用し軸索の腫大や浮腫をもたらす[8]，②細胞内に取り込まれたFK506やCsAは他の臓器と比べて神経系に高濃度で存在している[10]シクロフィリンやFK結合蛋白質などのイムノフィリンと選択的に高親和性結合し，複合体を形成する[11]，③血液脳関門を通過する機序は不明であるが，血管内皮障害により血管透過性が亢進し浮腫を引き起こし，エンドセリン-1の放出，そして脳虚血や白質浮腫を促進する[5,11]，などの機序が推測されている。この際，低コレステロール血症が存在すると，これら免疫抑制剤が脳のくも膜や星状細胞に結合し，傷害が助長されると想定されている[3]。また，脳症の危険因子として，薬剤自体の血中濃度上昇以外に，高血圧，低コレステロール血症，低マグネシウム血症，腎毒性およびステロイドパルス療法などが指摘されている[1,3]。本症例では，1回目の脳症発症時には血圧上昇と低コレステロール血症が，3回目の時は血圧上昇と腎機能障害がみられ，2回目の時はステロイドパルス直後であった。脳症は薬剤の血

中濃度上昇のみでも起こりうるが，正常治療域内であっても，これらの因子が複雑に絡みあい，発症すると考えられた。

カルシニューリン阻害剤による脳症発症の機序はいまだ不明な点が多く，今後，その機序の解明と予防策の開発が期待される。

IV. 結　語

今回，著者らはFK506およびCsAによって脳症を発症したまれな症例を経験した。脳症発症機序は不明な点が多く，その解明と対策樹立が期待される。

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主要治療⑥

肝動脈塞栓療法

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適応疾患

肝動脈塞栓療法（transcatheter arterial embolization；TAE）の適応疾患は，主として肝細胞がんです．肝細胞がんに対する治療には肝切除術，ラジオ波熱凝固療法やマイクロ波凝固療法などがあります．肝癌診療ガイドライン2005年度版によると，肝細胞がんに対する治療法は肝機能とがん進行度によって選択されますが，TAEは比較的肝機能が保たれており，多発肝細胞がんや切除適応とならない大型肝細胞がんが適応となります．また，肝切除や経皮的治療との組み合わせによる集学的治療の一つとして行われます．

肝細胞がんに対するTAEの効果は，正常肝と肝細胞がんにおける肝動脈および門脈血流の比率の差に基づいています．すなわち肝臓への流入血管は通常，肝動脈が20～30％，門脈が70～80％と門脈血流優位ですが，肝細胞がんでは肝動脈血流優位となります．そこでTAEにより正常肝に大きな影響を与えずに肝がん細胞を壊死させることができます．

合併症からみたTAEの適応は，原則として門脈血流の状態と肝機能面から決定されます．腫瘍栓により門脈本幹が閉塞しており，側副血行路がない場合は肝不全に陥る危険性があるため，TAEは禁忌です．血清総ビリルビン値が3.0mg/dL以上や難治性腹水例も禁忌です．一般に肝障害度CやChild-Pugh分類Cの肝硬変はTAEの適応から外れますが，近年，細径カテーテルの開発により，限局した肝動脈を塞栓することができるようになりTAEの適応範囲が広がっています．

その他の肝動脈血流に富む腫瘍に対してもTAEが試みられます．また，外傷や経皮経肝胆道ドレナージ（percutaneous transhepatic biliary drainage；PTBD）に伴う肝動脈損傷などに対しても，出血のコントロール目的にTAEが行われます．