表5　NVR に寄与する因子（多変量解析）

Factor	Odds ratio (95%C.I)	P
HCV Core 70番目 アミノ酸置換あり	6.6 (2.006-21.719)	0.002

NVR ; non－virological response

で，IFN 単独療法から IFN＋リバビリン併用療法，PEG－IFN 単独療法，PEG－IFN＋リバビリン併用療法へと大きく変貌した．2004年に PEG－IFN＋リバビリン併用療法が保険認可され，serotype 1 型かつ高ウイルス量の IFN 難治例に対しても，約40～50％程度に HCV 排除が得られるようになった[5)-8)]．当科においても，保険認可と共にC型肝炎治療法が変遷していった．特に，PEG－IFN＋リバビリン併用療法が保険認可された2005年以降は，本治療法が大半をしめていた．IFN 単独治療から PEG－IFN＋リバビリン併用療法へと治療法が進歩した結果，著効率も2.4倍へと飛躍的な向上を成し遂げていた．

IFN 治療効果を規定する因子については，これまでにいくつかの因子が報告されている．PEG－IFN＋リバビリン併用療法における治療効果を規定する因子として，①宿主因子（年齢，人種，体重，インスリン抵抗性，脂肪肝，肝硬変，IL-28B 領域の遺伝子型），②治療因子（薬剤アドヒアランス，治療期間，副作用），③ウイルス側因子（HCV 遺伝子型，ウイルス量）などが報告されている[9)-11)]．この10年間における当科の331例において，C型肝炎治療において SVR に寄与する因子を多変量解析にて検討した結果，①宿主側因子として“年齢”，②治療側因子として“PEG－IFN＋リバビリン併用療法”，③ウイルス側の因子として“HCV 遺伝子型”と“ウイルス量”，が独立因子として抽出された．年齢と IFN 治療効果に関しては，57歳未満では著効率55.1％に対して，57歳以上では44.9％と有意に低下していた（P＝0.016）．高齢者への IFN 投与に関する問題点は，(1)肝線維化進行例が多い，(2)副作用による薬剤減量や治療脱落例が多い，(3)他疾患の合併症保有率が高い，(4)IFN 療法に対して著効となりにくい，といった問題点がある．実際，近年の傾向として，当科のC型肝炎患者年齢の高齢化が確実に進行していた．高齢C型慢性肝炎症例に対しては，肝硬変，肝癌を発症する前に，高齢者への IFN 投与に関する問題点を解消するために，抗ウイルス療法をどう行うべきかを検討していかなければならない．

現在の標準治療である PEG－IFN＋リバビリン併用療法の著効率は，欧米における大規模臨床試験において，genotype 1 型症例（48週投与）では42～52％[5)6)8)]，genotype 2 / 3 型（24週投与）では81～84％[6)7)] と報告されている．当科における PEG－IFN＋リバビリン併用療法の治療成績も，これらの報告とほぼ同等であった．しかしながら，現在の標準治療である PEG－IFN＋リバビリン併用療法を行ったとしても，serotype 1 型高ウイルス量症例では約半数の症例において治療が奏功しない症例が存在するのも事実であった．今後は，治療が奏功しなかった症例に対して，肝硬変，肝癌へ進展させないために，どのような治療およびマネージメントを行っていくかが課題である．

近年では，IFN 治療開始前にウイルス側および宿主側の遺伝子解析を行うことで，治療効果を予測できることが報告されている[3)11)]．当科で PEG－IFN＋リバビリン併用療法を行った症例に対して，HCV Core aa70 置換の有無を検討した結果，IFN 治療経過中，一度も HCV RNA が陰性化しない超難治例が，HCV Core aa70 置換を有する症例で有意に多く存在していた．多変量解析にても，HCV Core aa70 置換が存在すると，NVR となる独立因子として抽出された．一方，この HCV Core aa70 置換があると，治療抵抗性になる機序は未だ不明である．可能性として，HCV Core 蛋白の置換が，PEG－IFN＋リバビリン併用療法の治療抵抗性となる宿主要因（肥満，インスリン抵抗性，肝細胞の脂肪化）に影響を与えている可能性が示唆されている．HCV Core 蛋白は，脂肪酸β酸化に関わる PPARα発現を抑制し[12)]，増加する中性脂肪の細胞外へのくみ出しを VLDL の分泌低下とともに抑制して肝細胞の脂肪化に深く関与したり[13)]，細胞内のインスリン受容体蛋白である insulin receptor substrate－1（IRS－1）や IRS－2 の発現を低下させインスリン抵抗性を惹起する[14)] ことが報告されている．その他に，Jak－STAT シグナル伝達を阻害することで IFN シグナル伝達経路を阻害する[15)] ことも知られている．また，HCV Core 領域に細胞障害性T細胞（CTL）エピトープが存在していることも知られている[16)]．従って，HCV Core 蛋白置換があると，細胞内における脂質代謝やインスリン抵抗性の発現，あるいは IFN 伝達系や細胞性免疫に影響を与える可能性が考えられており治療抵抗性になることが推測されている．

この10年間における IFN 治療法の変遷とともに，治療効果は飛躍的に向上したが，serotype 1 型高ウイルス量症例では PEG－IFN＋リバビリン併用療法を行ったとしても約半数の症例において治療に奏功しない症例が存在する．このような難治例および近年の患者高齢化に対して，HCV 遺伝子型，ウイルス量，年齢，治療法，

ウイルス側の遺伝子多型を検討することにより，治療によるリスクを回避，根治の見込めない患者を選別，無用な苦痛や経済的負担から免れることができる，といったテーラーメード医療の可能性が考えられ，個々の症例に最適な治療ができると思われる．実際，当科では，高齢かつIFN超難治（HCV serotype 1型，高ウイルス量，HCV Core aa70置換あり）が予測される場合，PEG－IFN＋リバビリン併用療法を行うのではなく，IFN単独少量長期療法や肝庇護療法を行って肝線維化進行，発癌を抑制すること目指すように提案している．最後に，近年，宿主側の遺伝子多型（IL-28B）の解析[11]などを組み合わせることによって，より正確なIFN治療効果予測が可能になることも報告されており，これらの情報をもとに可能な限りウイルス学的治癒に導いていく必要があると思われる．

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CASE REPORT

A case of hepatitis C-associated osteosclerosis that was improved with the combination therapy of peginterferon alfa-2b and ribavirin

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Abstract Hepatitis C-associated osteosclerosis (HCAO) is a rare disorder characterized by a marked increase in skeletal mass in patients who are infected with hepatitis C virus (HCV). The clinical presentation is an acquired deep bone pain with increased serum alkaline phosphatase (ALP) activity. We present a case of a patient with HCAO who was treated with antiviral therapy. A 42-year-old Japanese man presented with severe, stabbing pain in his lower limbs. He was diagnosed with hepatitis C secondary to intravenous drug use 20 years earlier. Serum biochemical studies revealed markedly elevated ALP activity and osteocalcin levels. Skeletal radiographs showed diffuse bony sclerosis with marked cortical thickening in the long bones. The bony findings and clinical symptoms were attributed to HCAO. The HCV RNA viral load was high and the genotype was 2a. The patient was treated with peginterferon alfa-2b and ribavirin for 24 weeks. After 24 weeks of the combination therapy, the patient had a sustained virological response and clinical remission of bone pain and a decrease in the level of serum ALP. In conclusion, HCAO was improved by the combination therapy of peginterferon alfa-2b and ribavirin when the patient achieved sustained virological response. It was confirmed that HCAO was one of the extrahepatic manifestations of HCV.

Keywords Peginterferon alfa-2b · Ribavirin · Hepatitis C-associated osteosclerosis

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Introduction

Hepatitis C virus (HCV) infection leads not only to liver disease, but also to extrahepatic manifestations [1, 2]. Hepatitis C-associated osteosclerosis (HCAO) is a rare disorder characterized by a marked increase in skeletal mass in patients who are infected with HCV [3–15]. The clinical presentation of such patients is an acquired deep bone pain with increased serum alkaline phosphatase (ALP) activity. However, it is uncertain whether HCV is the causative agent of this skeletal disease. We present a new case of a patient with HCAO who was treated with antiviral therapy, and consider HCAO as an extrahepatic manifestation of HCV.

Case report

A 42-year-old Japanese man was referred to us at Fukuoka University Hospital for severe, stabbing pain in his lower limbs. The pain had begun bilaterally in his knees 6 months previously and had progressed to involve the pre-tibial regions and thighs. There was no history of fracture or skeletal deformity. Serum biochemical studies revealed

markedly elevated bone specific ALP activity and osteocalcin levels, as well as a mild to moderately increased alanine aminotransferase (ALT) level (Table 1). Serum calcium levels were reduced. In urine, deoxypyridinolin (DPD) excretion and crosslinked *N*-telopeptide of type I collagen (NTX) were markedly elevated. Hematology, renal function, serum protein, electrophoresis, and screening for fluorosis as well as heavy metal poisoning were all normal. Family histories were negative. The patient was seropositive for antibody against HCV. The HCV RNA viral load was high and the genotype was 2a. There was a history of intravenous drug abuse 20 years previously. Liver biopsy revealed the histological finding of F2/A2 according to the New Inuyama classification [16]. Skeletal radiographs showed diffuse bony sclerosis with marked cortical thickening in the long bones and with cancellous osteosclerosis in the spine and pelvis (Fig. 1). Bone mineral density of lumbar vertebrae (L2, 3, 4) was 1.991 g/cm^2, approximately twice the mean value for controls. Helical computed tomography imaging showed osteosclerosis with marked cortical thickening along the diaphyses of the bilateral shank (Fig. 2). A 99mTc-methylene diphosphonate bone scan demonstrated diffusely increased radionuclide activity, a finding consistent with markedly increased bone turnover (Fig. 3).

The diagnostic criteria for HCAO have not yet been established. This patient had chronic hepatitis C and osteosclerosis with severe bone pain. Although markers of bone formation such as serum ALP activity and osteocalcine were invariably high, markers of bone resorption such as urinary DPD and NTX were also increased. The radiographic finding indicated osteosclerosis as a result of bone formation and bone resorption. Furthermore, there were no diseases causative of osteosclerosis such as fractures, skeletal deformity or bone tumor. The patient was diagnosed with hepatitis C secondary to intravenous drug use, and the bony findings and clinical symptoms were attributed to HCAO.

Although the patient received non-steroidal anti-inflammatory drugs (NSAIDs) for the alleviation of bone pain, his bone pain was not improved. The patient was treated with peginterferon alfa-2b (PegIntron, Schering-Plough) subcutaneously once weekly at a dose of 100 μg (1.5 μg/kg) and ribavirin (Rebetol, Schering-Plough) was administered orally at a dose of 800 mg/day. This combination therapy was continued for 24 weeks. Clinical biochemical laboratory values and serum HCV RNA were assessed before therapy, at 4-week intervals throughout the 24-week treatment period and during a post-treatment follow-up period. After the 4 weeks of the combination therapy, ALT was normalized and serum HCV RNA was negative. A serum bone specific ALP level was markedly decreased. Because the level of serum calcium in the patient was decreased after the combination therapy, calcium at a dose of 3–6 g/day was administrated orally to the patient (Fig. 4). The combination therapy of peginterferon alfa-2b and ribavirin for 24 weeks obtained sustained virological response in the patient. After 24 weeks of the combination therapy, the patient had a clinical remission of bone pain and a decrease in the level of serum bone specific ALP. Because the patient had no bone pain after the combination therapy, the use of NSAIDs was not necessary. Table 2 shows the change in laboratory data. After the HCV clearance, bone mineral metabolisms were improved.

We recommended a computed tomography or bone scintigram to the patient for determining whether bone hypertrophy was improved. However, the patient has not accepted more radiological examination. Because we were not able to get the informed consent of our patient, we could not present the radiological change after the combination therapy of peginterferon alfa-2b and ribavirin.

Discussion

The bone pain of the patient with HCAO improved when the patient attained a sustained virological response with the combination therapy of peginterferon and ribavirin. This is the first report to prove that HCAO is an extrahepatic manifestation of HCV.

In 1990, Beyer et al. [3] reported a case of idiopathic acquired diffuse osteosclerosis. Thereafter, several cases of painful diffuse osteosclerosis after drug abuse were reported [4, 5, 7]. Patients were found to be seropositive for HCV. In 1997, Whyte and Reasner [8] described this new skeletal disorder as HCAO. HCAO is characterized by a generalized increase in bone mass in adults who are infected with HCV. These patients have a deep, mild or severe pain and tenderness of the limbs, with no record of previous fractures. Radiographs show diffuse bone sclerosis with marked cortical thickening. Bone scintigraphy shows a diffuse increased uptake of radionuclide; however bone biopsy shows normal lamellar bone with accelerated rates of skeletal formation [9, 14]. Although markers of bone formation such as serum ALP activity and osteocalcine are invariably high, markers of bone resorption such as urinary DPD and NTX are also increased. Furthermore, areal bone mineral density is abnormally high. However, HCAO is thought to be rare among HCV seropositive patients, because a study looking at the skeletal radiographs of 107 randomly selected HCV patients failed to demonstrate dense bones in these patients [17].

This patient had the clinical hallmarks of HCAO. However, hypocalcemia was found and serum PTH level along with 1,25-hydroxyvitamin D level were increased. We

Table 1 Laboratory data on admission

Hematology	
WBC	5300/μl
RBC	477 × 10^4/μl
Hb	14.7 g/dl
Ht	44.4%
PLT	24.6 × 10^4/μl
Biochemistry	
T-Bil	0.7 mg/dl
AST	84 IU/l
ALT	123 IU/l
Alb	3.4 g/dl
LDH	225 IU/l
GGT	27 IU/l
ALP	2910 IU/l
ALP isozyme	
1	0
2	16%
3	84%
4	0
5	0
BUN	15 mg/dl
Cr	0.6 mg/dl
Na	139 mEq/l
K	4.0 mEq/l
Cl	103 mEq/1
CRP	0.2 mg/dl
Coagulation	
PT	100%
Virus marker	
HBs-Ag	(−)
HCV-Ab	(+)
HCV genotype	2a
Quantitative HCV RNA	2900 KlU/ml

		Normal range
Bone and mineral metabolism		
Ca	78.8 mg/dl	8.7–10.3
IP	4.1 mg/dl	2.5–4.5
Bone–specific ALP	653 U/l	13.0–33.9
Osteocalcin	90 ng/ml	2.5–13.0
Vitamin D3	113 klU	20.0–60.0
TSH	1.052 μl U/ml	0.350–4.94
F-T4	1.08 ng/dl	0.70–1.48
Intact PTH	224 pg/ml	10–65
Urine		
DPD	582.0 nmol/nmol Cr	2.8–7.6
NTX	7540 nmolBCE/l	9.3–54.3

PTH parathyoid hormone, *DPD* deoxypridinolin, *NTX* crosslinked N-telopeptide of type I collagen

considered that these findings pointed to secondary hyperparathyroidism apparently resulting from skeletal hyperaccretion of calcium.

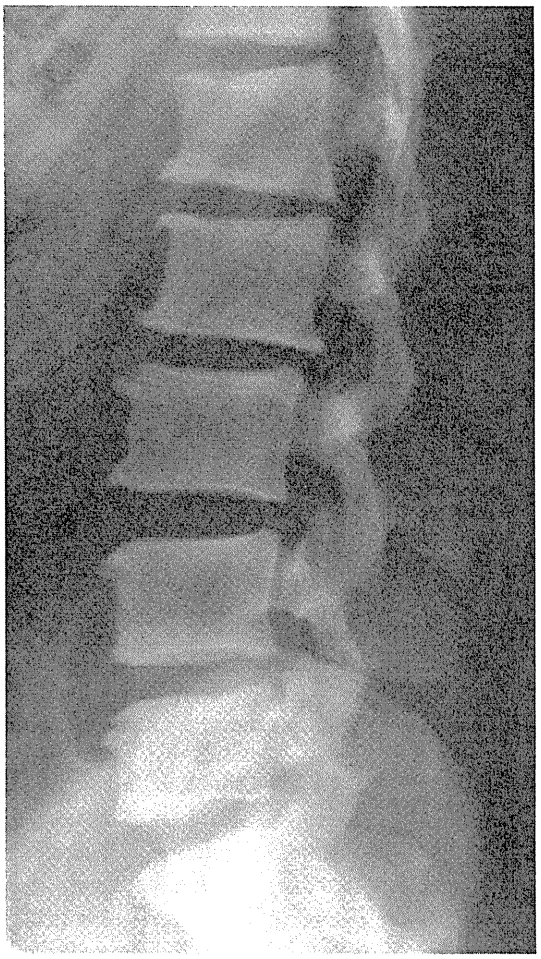

Fig. 1 Lateral view of lumbar spine shows generalized osteosclerosis of cancellous bone

Treatment of HCAO with vitamin D, calcitonin, pamidronate and etidronate that inhibits osteoclast function may decrease serum ALP activity (Table 3). However, subjective response to pharmacological treatment appears to vary. In our case, the administration of peginterferon alfa-2b and ribavirin obtained a sustained virological response and the severe bone pain improved. Approximately 55% of patients with chronic hepatitis C obtain a sustained virological response after treatment with peginterferon alfa and ribavirin [18, 19]. However, those with genotype 1 infection have a sustained virological response rate of 40–55 versus 70–90% among those with genotype 2 or 3 infection. Because our patient was infected with HCV genotype 2a, 24-week treatment of peginterferon alfa-2b and ribavirin induced a sustained viral clearance in our patient. Both interferon alfa and beta suppress bone resorption in osteoclasts and increase bone mineral density [20, 21]. Patients with HCAO who are treated with interferon experience greater bone formation. In our patient, it was thought that the function of osteoclasts was suppressed by the interferon treatment, resulting in increased bone

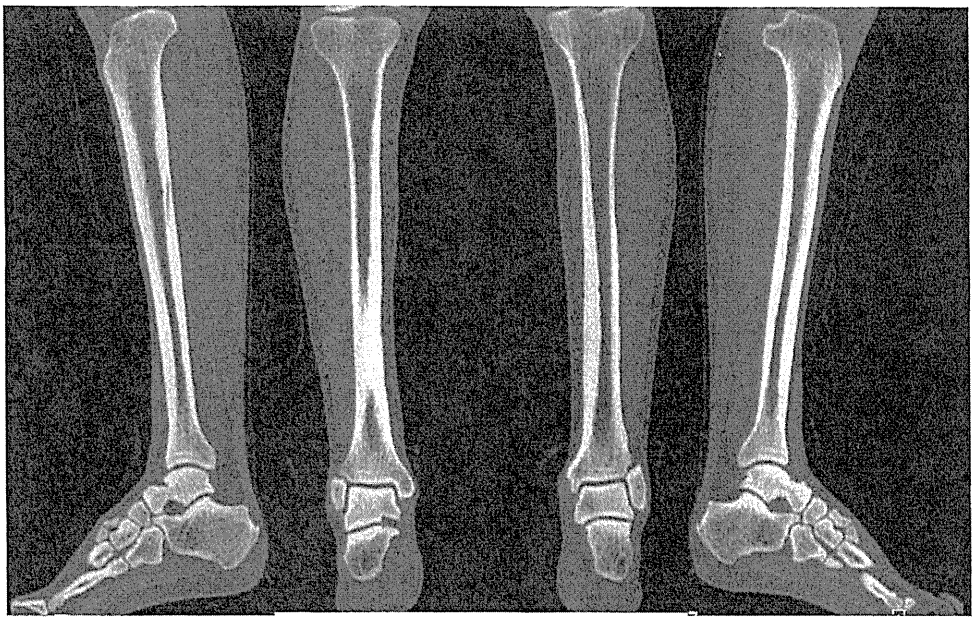

Fig. 2 Helical CT imaging shows osteosclerosis with marked cortical thickening along the diaphyses of the bilateral shank

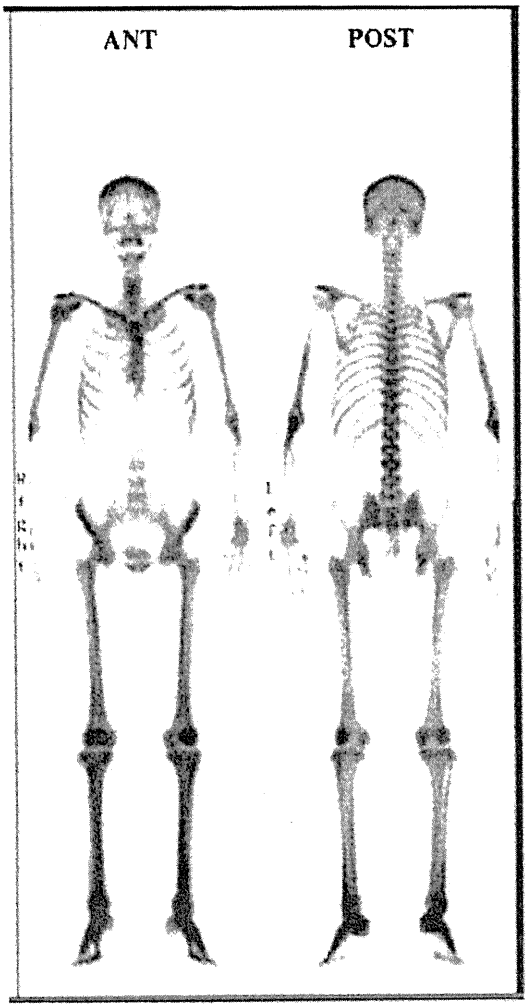

Fig. 3 A 99mTc-methylene diphosphonate bone scan demonstrates diffusely increased radionuclide activity in the spine and long trunk bones. This is consistent with markedly increased bone turnover

formation and worsening hypocalcemia. The patient was given calcium orally for hypocalcemia. However, the severity of the bone pain was non-progressive during interferon treatment. After the end of treatment with peginterferon alfa-2b and ribavirin, hypocalcemia was improved and bone pain was further improved. Therefore, when physicians treat HCAO patient with interferon, they must monitor the level of serum calcium and administer the calcium to correct hypocalcemia. However, this case report may suggest that interferon treatment is safe for the patient with HCAO because there was no osteocopic exacerbation.

The disorder is almost certainly related to HCV; however, the pathogenesis remains incompletely understood. Fiore et al. [13] documented an increase of circulating osteoprotegerin (OPG) in a patient with HCAO, and a concentration of circulating receptor activator for nuclear factor-kappa-B ligand (RANKL) below the lower limit of the reference range. OPG is an inhibitor of osteoclast differentiation and RANKL is an osteoclast differentiation factor [22, 23]. OPG is produced mostly by osteoblasts, so if there is an increased osteoblast function in HCAO, one would expect higher OPG levels in HCAO than in healthy individuals. OPG acts as a decoy receptor for RANKL and prevents its interaction with receptor activator for nuclear factor-kappa-B (RANK), a cell surface receptor on pre-osteoclasts and osteoclasts [24, 25]. Fiore et al. suggested that the abnormalities of the OPG/RANKL system might contribute to the maintenance of the positive balance of bone remodeling that characterizes patients with HCAO. Tanaka et al. [14] hypothesized three explanations for the pathogenesis of HCAO: HCV directly infects bone cells, another unknown-infective agent, and cytokines or growth factors that hepatocytes or other tissues may produce. Kaji

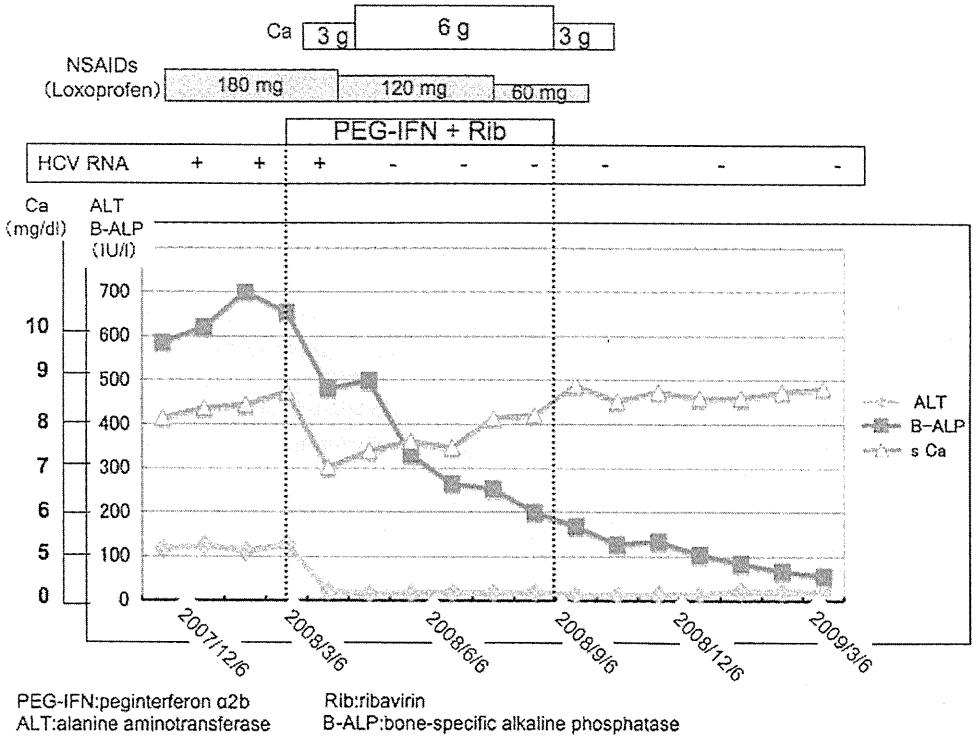

Fig. 4 Clinical course of the patient

Table 2 Change in Laboratory data

	Before	After	Normal range
Biochemistry			
AST (IU/l)	84	34	6–3
ALT (IU/l)	123	23	6–30
ALP (IU/l)	2,910	454	115–359
Ca (mg/dl)	7.8	9.1	8.7–10.3
IP (mg/dl)	4.1	4.5	2.5–4.5
Virus marker			
HCV RNA (KIU/ml)	2,900	Negative	
Bone and mineral metabolism			
Bone-specific ALP (U/l)	653	55.3	13.0–33.9
Osteocalcin (ng/ml)	90	20	2.5–13.0
Vitamin D3 (KIU)	113	62.4	20.0–60.0
Intact PTH (pg/ml)	224	119	10–65
Urine DPD (nmol/nmol Cr)	582.0	197.8	2.8–7.6

ALP alkaline phosphatase, *PTH* parathyroid hormone, *DPD* deoxypyridinolin

et al. [26] reported that serum soluble factors in patients with HCAO induce cell proliferation, ALP activity and transforming growth factor-beta signal in mouse osteoblastic cells. Khosla et al. [27] have also previously found that the insulin-like growth factor (IGF)-II E in HCAO patients circulates bound to insulin-like growth factor binding protein (IGFBP)-2. Moreover, they indicated that upon binding IGF- II, IGFBP-2 has greatly enhanced avidity for the osteoblast extracellular matrix. Thus, they have postulated that the IGF-II E/IGFBP-2 complex accumulates in bone in HCAO patients, and subsequently results in the stimulation of bone formation and osteosclerosis observed in these patients. Conover et al. [28] reported that in a rat model of osteoporosis, subcutaneous administration of IGF-II/IGFBP-2 complex stimulates bone formation and prevents loss of bone mineral density.

Because we did not investigate IGF-II, IGFBP-2, OPG and RANKL in our patient, we could not ascertain whether the levels of cytokines and growth factors were altered by the combination therapy with peginterferon alfa-2b and ribavirin. Previous reports have identified that these agents may play a role in the pathogenesis of HCAO. Also it was not clarified whether peginterferon alfa-2b and ribavirin affect cytokines and growth factors. However, we considered that cytokines and/or growth factors produced by hepatocytes or other tissues induced HCAO, and that in the HCV clearance state induced by peginterferon alfa-2b and ribavirin, these agents improved to normal levels. Therapy with drugs that inhibit osteoclast function may decrease serum ALP activity, but subjective response varies. There have been reports of patients benefiting from calcitonin injections or pamidronate infusions, whereas other patients have not improved (Table 3). We considered that HCV clearance by treatment with peginterferon alfa-2b and ribavirin was the best therapy for patients with HCAO. Because the HCV genotype in our patient was 2a, we treated our patient with peginterferon alfa-2b and ribavirin. HCV genotype has been shown to be the best parameter on which to base the individualization of therapy. In patients

Table 3 Review of previous case reports

Case	Age	Gender	ALP	OC	s-Ca	IPTH	Vit.D	HCAO therapy	Efficacy	HCV genotype	HCV therapy	References
1	28	F	↑			↑	↑	Non therapy	Improve			[3]
2	27	F	↑	↑	↓	→	↑	Calcitonin	Improve			[4]
3	38	M	↑		↓	→	↑	Calcitonin	Improve			[4]
4	38	M										[5]
5	52	M										[6]
6	37	M	↑	↑	↓	↑	↑	Pamidronate	Improve			[7]
7	37	M	↑	↑	→	→	→	Vit. D, pamidronatee, calcitonin	Progress			[8]
8	73	M	↑	↑	→	↑	→	Pamidronate, etidronate	Improve			[9]
9	38	M	↑	↑	→	→	→	Pamidronate	Improve			[9]
10	69	F	↑	↑	→	↑	↑					[10]
11	45	F	↑	→	→	→				2a		[11]
12	74	F										[12]
13	65	F	↑	↑	→	↑				1b		[13]
14	72	F	↑	↑	→	↑	↑	Vit. D, Ca	Improve	2		[14]
15	28	M	↑		→	→		Pamidronate	Progress			[15]
16	42	M	↑	↑	↓	↑	↑	PEG-IFN + RBV	Improve	2a	PEG-IFN + RBV	※

Ca calcium, *OC* osteocalcine, *IPTH* intact PTH, *Vit.D* 1,25(OH)2Vit.D3, *PEG-IFN* peginterferon alfa 2b, *RBV* ribavirin, ※ present case

with genotype 2, sustained virological response rates of 70–90% were obtained with 24 weeks of treatment [18, 19]. Unfortunately, interferon treatment is often associated with adverse effects such as depression and hematological abnormalities. Recent reports indicate one host-related factor could be genetic variations near the IL28B gene (rs8099917, rs12979860) on chromosome 19, which encodes interferon-λ-3; these variations are pretreatment predictors of virological response to therapy with peginterferon and ribavirin [29–31]. Physicians should consider HCV-genotype, viral load, age, complications and IL28B SNPs before starting treatment, and identify the patients with HCAO who are unlikely to benefit from this treatment.

It is possible that the actual prevalence of HCAO among HCV patients may be underestimated. We propose that physicians caring for patients with HCV who have invariably high levels of ALP activity should check the bone mineral density of their patients.

In conclusion, HCAO is improved by the combination therapy of peginterferon alfa-2b and ribavirin when the patients achieved sustained virological response. It was confirmed that HCAO was one of the extrahepatic manifestations of HCV.

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福岡大学消化器内科における10年間の急性肝障害患者の臨床的検討（2000-2009）

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A Clinical Study of Acute Liver Injury Over the Last Decade (2000-2009) in the Department of Gastroenterology and Medicine, Fukuoka University School of Medicine

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Abstract：A total of 112 patients with acute liver injury in the Department of Gastroenterology and Medicine, Fukuoka University School of Medicine, during a 10-year period (from 2000 to 2009), were analyzed to identify any association risk factors. Acute hepatitis B was the most frequent cause, being involved in 36 cases (32.1%). Unidentified liver damage was document in 32 (28.6%) patients, and drug-induced hepatitis was observed in 18 (16.1%) patients. Acute hepatitis C was only found in four cases during this period (3.6%). In the last five years, the number of patients with acute hepatitis B and drug-induced hepatitis increased. Among the 13 cases of severe hepatitis, ten cases (76.9%) were caused by hepatitis B virus, thus strongly suggesting that the establishment of a universal vaccination against the hepatitis B virus is urgently required in Japan. In addition, physicians and patients should be aware of the risk of acute liver injury due to both pharmaceutical agents and health foods.

Key words：Acute liver injury, Acute hepatitis B, Drug induced hepatitis, Clinical study, Fukuoka University

福岡大学消化器内科における10年間の急性肝障害患者の臨床的検討（2000-2009）

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要旨：この10年間に，急性肝障害の診断で，福岡大学消化器内科で入院加療を受けた患者の臨床的検討を行った．症例は112例であった．B型急性肝炎が36例（32.1%）と最も多く，原因不明肝障害が32例（28.6%），薬剤性肝障害が18例（16.1%）であった．C型急性肝炎はこの10年でわずか4例（3.6%）であった．2000年～2004年の前期5年間と2005年～2009年の後期5年間の比較検討では，B型肝炎ウイルスと薬

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剤による肝障害が増加していた．また，重症肝炎・劇症肝炎13例中，B型肝炎ウイルスが原因であるものが10例（76.9%）と最も多かった．B型肝炎による重症肝炎例では，劇症肝炎発症率が40%であった．また，B型肝炎ウイルスによる劇症肝炎では死亡率は50%と高率であった．国全体でのB型肝炎に対するユニバーサルワクチン開始を考慮する必要性が考えられた．また，近年増加している薬剤性肝障害には，健康食品による薬剤性肝障害も散見されるため注意が必要である．

索引用語：急性肝障害，急性B型肝炎，薬剤性肝障害，臨床的検討，福岡大学

はじめに

平成12年4月，福岡大学医学部に新規講座として第3内科（現消化器内科）が誕生した．第3内科は消化管疾患と肝胆膵疾患を専門とする消化器内科学講座である．開講10周年にあたり，この10年間で当科に入院した急性肝障害患者の臨床統計を行い，急性肝障害患者における治療の現状と問題点を臨床的に検討した．

対象と方法

平成12年（2000年）から平成21年（2009年）までの10年間に，当科へ入院となった急性肝障害患者を対象とした．対象症例の病歴，生活歴，生化学検査所見より，急性肝障害の原因を明らかにした．急性肝障害の診断は，血液生化学検査による肝炎ウイルスマーカー測定と，問診によって得られた飲酒歴，薬剤服用歴によって診断した．いずれにも該当しない症例は，原因不明の急性肝障害として集計した．当科の診断基準を表1に示す．各年度ごとの原因別急性肝障害の発症数を明らかにするとともに，前期5年間と後期5年間の2群に分けて，原因別急性肝障害の推移を検討した．なお，重症肝炎は，経過中にプロトロンビン活性40%未満に至った症例とし，劇症肝炎はプロトロンビン活性40%未満かつ肝性昏睡Ⅱ度以上の出現が確認された症例とした．

結果

表2に，この10年間の急性肝障害による入院患者の一覧を示す．総数は112例であった．2002年の1年間は，急性肝障害で入院する症例数が3例と少なかったが，平均して年10例ほどが，急性肝障害の診断で当科へ入院し加療を受けていた．最も頻度の高い疾患は，B型急性肝炎の36例（32.1%）であった．次いで，原因不明急性肝障害の32例（28.6%），薬剤性肝障害が18例（16.1%）であった．B型急性肝炎は，各年度において，高頻度に発生しており（平均3.6症例/年），薬剤性肝障害（平均1.8症例/年）の2倍の発生率であった．

ウイルス性肝炎の年度別の患者数を図1に示す．ほとんどの年度において，B型肝炎の症例が最多であった．C型急性肝炎はこの10年間でわずか4例（3.6%）であった．A型急性肝炎は，最近の3年間では，当科に入院する症例は無かった．E型急性肝炎は，2004年の1例だけであった．2000年～2004年の前期と2005年～2009年の後期の2群に分けて，各疾患別の入院患者数を比較すると，B型肝炎と薬剤性肝障害が後期5年間で増加していた（図2）．薬剤性肝障害と診断された症例においては，多剤併用症例や，健康食品・サプリメント併用症例が多く，原因薬剤の同定は困難であった．

表1　急性肝障害の診断基準

	略号	診断
A型肝炎	HAV	血清 IgM 型 HA 抗体陽性
B型肝炎	HBV	血清 HBs 抗原陽性，かつ血清 IgM 型 HBc 抗体陽性
C型肝炎	HCV	血清 HCV 抗体陰性，かつ血清 HCV RNA 陽性
E型肝炎	HEV	血清 HEV RNA 陽性
サイトメガロウイルス肝炎	CMV	血清 IgM 型 CMV 抗体陽性
EB ウイルス肝炎	EBV	血清 IgM 型 EBV 抗体陽性
薬剤性肝障害	Drug	ウイルス性肝炎否定，かつ薬剤服用歴と薬剤中止による改善
アルコール性肝障害	Alchol	ウイルス性肝炎否定，かつ飲酒歴と飲酒中止による改善
原因不明肝障害	Unknown	上記いずれにも該当しない
重症肝炎		肝炎発症後にプロトロンビン活性40%以下
劇症肝炎		プロトロンビン活性40%以下かつ肝性脳症Ⅱ度以上

表2　年次別，原因別急性肝障害症例のうちわけ

	HAV	HBV	HCV	HEV	CMV	EBV	Drug	Alchol	Unknown	計
2000	0	1	0	0	1	3	1	0	1	7
2001	0	7	0	0	0	1	3	1	6	18
2002	0	0	1	0	0	1	0	0	1	3
2003	3	2	1	0	0	0	0	0	6	12
2004	2	3	0	1	0	1	1	0	5	13
2005	1	4	2	0	1	0	2	0	4	14
2006	2	8	0	0	0	0	4	1	4	19
2007	0	6	0	0	1	0	2	0	4	13
2008	0	3	0	0	0	0	1	0	0	4
2009	0	2	0	0	0	0	4	2	1	9
計	8	36	4	1	3	6	18	4	32	112

HAV：A型肝炎
HBV：B型肝炎
HCV：C型肝炎
HEV：E型肝炎
CMV：サイトメガロウイルス肝炎
EBV：EB ウイルス肝炎
Drug：薬剤性肝障害
Alchol：アルコール性肝障害
Unknown：原因不明肝障害

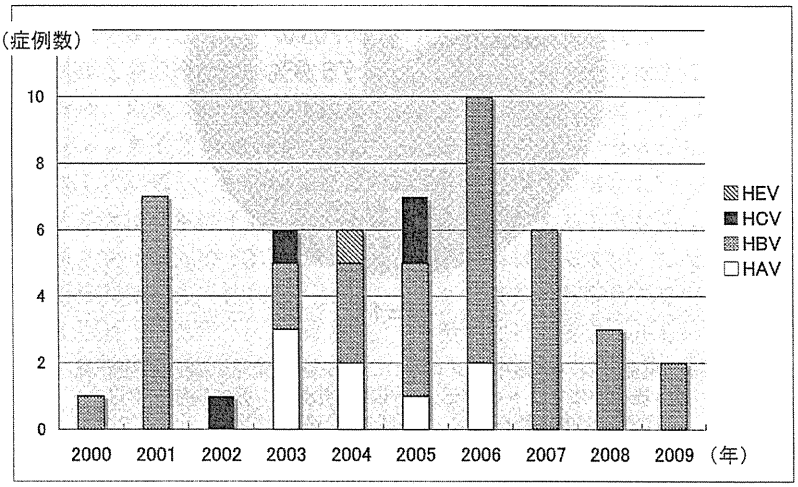

図1　年次別，ウイルス性肝炎の症例数を示す．最も頻度の高い疾患は，B型急性肝炎（38例）であった．
HAV：A型肝炎，HBV：B型肝炎，HCV：C型肝炎，HEV：E型肝炎

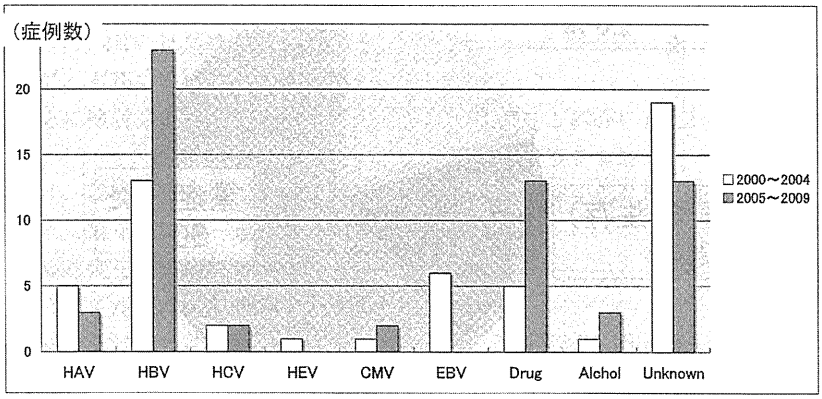

図2　2000年～2004年の前期と2005年～2009年の後期の2群での各疾患の患者数を示す．B型肝炎と薬剤性肝障害が，後期に増加していた．
HAV：A型肝炎，HBV：B型肝炎，HCV：C型肝炎，HEV：E型肝炎，CMV：サイトメガロウイルス肝炎，EBV：EB ウイルス肝炎，Drug：薬剤性肝障害，Alchol：アルコール性肝障害，Unknown：原因不明肝障害

全112例中，重症肝炎は8例，劇症肝炎が5例であった．重症肝炎8例の内訳は，B型肝炎6例，薬剤性肝炎1例．原因不明が1例であった．劇症肝炎の5例中，死亡症例は3例であった．劇症肝炎の原因は，B型肝炎が4例であり，そのうち2例は死亡している．死亡例の残り1例は，原因不明肝障害であった．重症肝炎・劇症肝炎の疾患別割合を図3に示す．重症肝炎とは肝炎発症後にプロトロンビン活性40%以下に低下した症例，劇症肝炎はプロトロンビン活性40%以下となり，かつ肝性脳症Ⅱ度以上が出現した症例である（表1）．13例中10例（76.9%）がB型肝炎であった．次に，B型肝炎による重症肝炎・劇症肝炎の症例内訳を図4に示す．B型肝炎は重症化すると40%が劇症化し，劇症化すると50%が死亡していた．

考　　察

当科に入院となった急性肝障害患者のこの10年の検討では，B型急性肝炎の頻度が最も高いことが明らかとなった．現在HBVキャリアの母親から出産した新生児

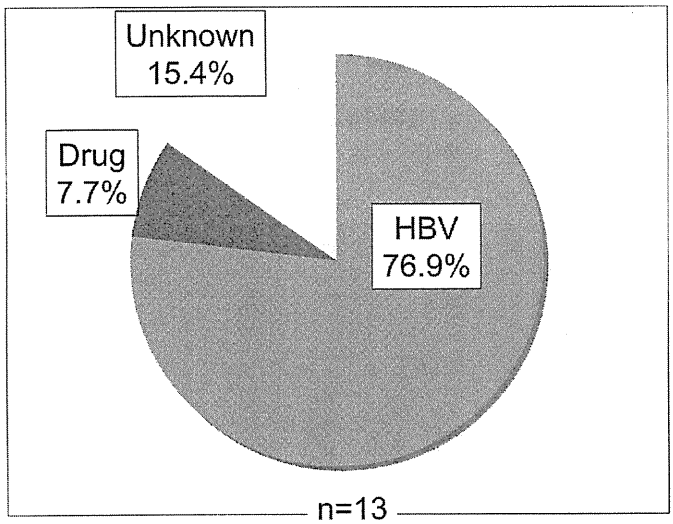

図3　重症肝炎（経過中にプロトロンビン活性40%未満）・劇症肝炎（経過中にプロトロンビン活性40%未満かつ肝性脳症Ⅱ度以上）症例の疾患分類を示す．B型肝炎が10例（76.9%）と最多であった．
HBV：B型肝炎，Drug：薬剤性肝障害，Unknown：原因不明肝障害

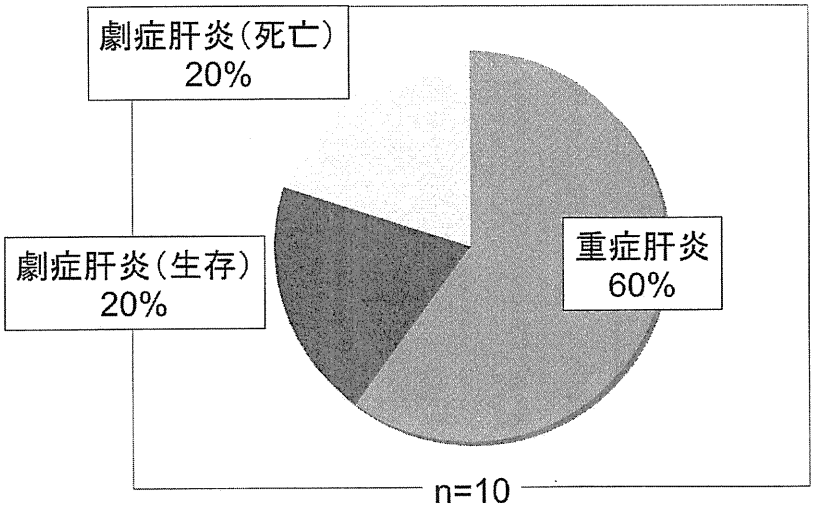

図4　B型重症肝炎，B型劇症肝炎の頻度を示す．B型肝炎は重症化すると40%が劇症肝炎となり，劇症肝炎を発症すると50%が死亡していた．

には，抗 HBs 抗体含有γ－グロブリン製剤を投与し，その後，HBV ワクチンを接種することで出産時の経産道的感染を防止している．このように垂直感染の防止策が取られており，将来，本邦の HBV キャリアの撲滅が期待されている．しかしながら，当科の10年間の検討では，成人のＢ型急性肝炎は平均3.6例/年で発生している．したがって，成人の水平感染は，決して減少していないと考えられた．成人のＢ型急性肝炎の感染経路は，ほとんどが性交渉である[1]．本邦では，HBV に暴露する危険性が高いと考えられる医療従事者や医学部学生には，HBV のワクチン接種が行われている．しかし，一般住民へのＢ型肝炎ワクチン摂取はほとんど行われていない．今回の我々の検討でも，前期の５年と比較して最近の５年では，Ｂ型肝炎による入院患者数が増加している．さらには，Ｂ型肝炎は重症化すると高率に（40%）に劇症化し，劇症化すると50%が死亡している．Ｂ型肝炎ウイルスによる重症肝炎・劇症肝炎の治療には，抗ウイルス薬である核酸アナログと免疫抑制療法が導入されているが，現在においてもＢ型急性肝炎は死亡率の高い疾患であると考えられた[2][3]．国際化がすすみ，以前では，本邦には発生していなかった欧米型のジェノタイプＡの HBV によるＢ型急性肝炎の症例も増加している[4]．近年の調査では，すでに，この欧米型ウイルスは日本に土着しているものと考えられている．ジェノタイプＡの HBV は，成人への初感染であっても，急性肝炎の後，約10%～20%が慢性肝炎へ移行すると考えられている[5][6]．このため，WHO，UNICEF は全世界的に全新生児および青少年を対象としたＢ型肝炎の universal vaccination を行うように勧告しており，世界193カ国中の約80%で水平感染防止のために導入されている[7]．これにより，全世界的にＢ型肝炎ウイルスの排除を目指している．しかしながら，本邦では，一部の限られた対象群だけにワクチン接種が限定されている．成人に感染しても，慢性化する危険性があるジェノタイプＡによるＢ型急性肝炎も散発していることから，今後さらに HBV は蔓延していくことが予想される，したがって，本邦においても，ユニバーサルワクチンの導入を早急に検討する必要があると考えられた．

原因不明の急性肝障害は，1980年以来の国立病院共同研究で示されているように，毎年，ほぼ一定の割合で発生している．発熱や感冒様症状を伴う症例が多いことから，原因については未知のウイルスが考えられている．しかしながら，いわゆる非Ａ非Ｂ非Ｃ非Ｅ型急性肝炎の多くは，一過性の肝障害で慢性化することはない．当科の原因不明急性肝障害患者も ALT のピークが過ぎれば，その後は，急速に ALT は低下し，慢性化する症例はなかった．また，このように一峰性の ALT の動きをとる患者では，全症例が治癒している．当科での原因不明急性肝障害のうち，１例のみ劇症肝炎で死亡しているが，この症例は入院後，ALT の低下と再上昇を繰り返していた．原因不明急性肝障害では，ALT が多峰性の動き示す際には，重症化に注意が必要であると考えられた．

Ｅ型急性肝炎は，これまでは，輸入感染症と考えられていたが，本邦にも土着ウイルスがいることが判明した．猪，鹿，豚の生肉摂取により感染が成立する[5][8]．北海道や東北地方では散発的に発症しているが，九州地区ではまれであり，当科でもこの10年で４例と少なかった．Ｅ型急性肝炎は北部九州地区では頻度が少ないと考えられた．

今回の我々の検討では，薬剤性肝障害はＢ型肝炎と同様に，前期５年の発生数より後期５年の発症数が多かった．これは，近年の健康志向とそれに乗じた数多くの健康食品の発売によって，健康食品の摂取率が高くなっている現在の状況を反映していると考えられた．実際に，当科の症例でも医師の処方した薬剤による肝障害ばかりではなく，健康食品，サプリメントによる肝障害も散見された．神代らの報告によると，消化器疾患を有する患者の451人中304名（72.3%）が，なんらかの健康食品を摂取しており，そのうち，64.5%は主治医に相談すること無く摂取していた[9]．現状では，多くの患者が，主治医に相談することなく，健康食品を摂取しているものと考えねばならない．したがって，今後も，健康食品を含めた薬剤性肝障害の発生は増加すると考えられるため，我々には，患者へ正しい情報提供を行う必要がある．

結　　論

この10年間に，当科に入院した急性肝障害の検討では，Ｂ型肝炎ウイルスと薬剤による肝障害が増加していた．近年においても，Ｂ型肝炎による重症肝炎例では劇症肝炎発症率・死亡率が高率である．

謝　　辞

症例をご紹介いただいた先生方，および肝障害回復後に経過観察をお願いした際に快くお引き受けいただいた先生方に深く御礼申し上げます．

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Delayed-Onset Caspase-Dependent Massive Hepatocyte Apoptosis upon Fas Activation in Bak/Bax-Deficient Mice

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The proapoptotic Bcl-2 family proteins Bak and Bax serve as an essential gateway to the mitochondrial pathway of apoptosis. When activated by BH3-only proteins, Bak/Bax triggers mitochondrial outer membrane permeabilization leading to release of cytochrome c followed by activation of initiator and then effector caspases to dismantle the cells. Hepatocytes are generally considered to be type II cells because, upon Fas stimulation, they are reported to require the BH3-only protein Bid to undergo apoptosis. However, the significance of Bak and Bax in the liver is unclear. To address this issue, we generated hepatocyte-specific Bak/Bax double knockout mice and administered Jo2 agonistic anti-Fas antibody or recombinant Fas ligand to them. Fas-induced rapid fulminant hepatocyte apoptosis was partially ameliorated in Bak knockout mice but not in Bax knockout mice, and was completely abolished in double knockout mice 3 hours after Jo2 injection. Importantly, at 6 hours, double knockout mice displayed severe liver injury associated with repression of XIAP, activation of caspase-3/7 and oligonucleosomal DNA breaks in the liver, without evidence of mitochondrial disruption or cytochrome c–dependent caspase-9 activation. This liver injury was not ameliorated in a cyclophilin D knockout background nor by administration of necrostatin-1, but was completely inhibited by administration of a caspase inhibitor after Bid cleavage. Conclusion: Whereas either Bak or Bax is critically required for rapid execution of Fas-mediated massive apoptosis in the liver, delayed onset of mitochondria-independent, caspase-dependent apoptosis develops even in the absence of both. The present study unveils an extrinsic pathway of apoptosis, like that in type I cells, which serves as a backup system even in type II cells. (HEPATOLOGY 2011;54:240-251)

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Fas, also called APO-1 and CD95, is one of the death receptors that are potent inducers of apoptosis and constitutively expressed by every cell type in the liver.[1] Dysregulation of Fas-mediated apoptosis is involved in several liver diseases.[2] In the liver of patients with chronic hepatitis C, Fas is overexpressed in correlation with the degree of hepatitis, and Fas ligand can be detected in liver-infiltrating mononuclear cells.[3,4] Fas is also strongly expressed in the livers of patients with chronic hepatitis B, autoimmune hepatitis, and nonalcoholic steatohepatitis.[4,5] Moreover, in the liver of patients with fulminant hepatitis, Fas is up-regulated with strong detection of Fas ligand.[6] In mice, injection of Jo2 agonistic anti-Fas antibody leads

Abbreviations: ALT, alanine aminotransferase; CypD, cyclophilin D; DISC, death-inducing signaling complex; DKO, double knockout; DMSO, dimethylsulfoxide; IAP, inhibition of apoptosis protein; KO, knockout; PARP, poly(adenosine diphosphate ribose) polymerase; RIP, receptor-interacting protein; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling; WT, wild-type.

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**These authors contributed equally to this work.*

to massive hepatocyte apoptosis and lethality, suggesting that the hepatocyte is one of the most sensitive cell types to Fas stimulation.[7] This model is considered to at least partly mimic human fulminant liver failure.

Fas, upon ligation by Fas ligand, activates caspase-8 through the recruitment of Fas-associated protein with a death domain and formation of the death-inducing signaling complex (DISC).[1,2] Whereas activated caspase-8 directly activates effector caspases such as caspase-3 and caspase-7 through the so-called extrinsic pathway, leading to apoptosis in type I cells, it activates caspase-3/7 through the mitochondrial pathway in type II cells. In type II cells, activated caspase-8 cleaves the BH3-only protein Bid into its truncated form, which in turn directly or indirectly activates and homo-oligomerizes Bak and/or Bax to form pores at the mitochondrial outer membrane, leading to the release of cytochrome c. After being released, cytochrome c assembles with Apaf-1 to form apoptosomes which promote self-cleavage of procaspase-9 followed by activation of caspase-3/7 to cleave a variety of cellular substrates such as poly(adenosine diphosphate ribose) polymerase (PARP) and finally to execute apoptosis.[8,9] Hepatocytes are considered to be typical type II cells, because Bid knockout (KO) mice were reported to be resistant to hepatocyte apoptosis upon Fas activation.[10,11] Although Bak and Bax are crucial gateways to apoptosis of the mitochondrial pathway, little information is available about their significance in hepatocyte apoptosis because most traditional Bak/Bax double knockout (DKO) mice ($bak^{-/-}$ $bax^{-/-}$) die perinatally.[12]

In the present study, we tried to address this issue by generating hepatocyte-specific Bak/Bax DKO mice. We demonstrate that either Bak or Bax is required and sufficient to induce Fas-mediated early-onset hepatocyte apoptosis and lethal liver injury. Importantly, even if deficient in both Bak and Bax, Bak/Bax DKO mice still develop delayed-onset caspase-dependent massive hepatocyte apoptosis, suggesting that the mitochondria-independent pathway of apoptosis, as observed in type I cells, works as a backup system when the mitochondrial pathway of apoptosis in the liver is absent. This study is the first to demonstrate the significant but limited role of Bak and Bax in executing Fas-induced apoptosis in the liver.

Materials and Methods

Mice. Heterozygous Alb-Cre transgenic mice expressing Cre recombinase gene under the promoter of the albumin gene were described.[13] We purchased Bak KO mice ($bak^{-/-}$), Bax KO mice ($bax^{-/-}$), and Bak KO mice carrying the *bax* gene flanked by 2 *lox*P sites ($bak^{-/-}$ $bax^{flox/flox}$) from the Jackson Laboratory (Bar Harbor, ME). Traditional cyclophilin D (CypD) KO mice have been described.[14] All mice strains that we used were created from a mixed background (C57BL/6 and 129). We generated hepatocyte-specific Bak/Bax DKO mice ($bak^{-/-}$ $bax^{flox/flox}$ *Alb-Cre*) or hepatocyte-specific CypD/Bak/Bax triple KO mice ($cypd^{-/-}$ $bak^{-/-}$ $bax^{flox/flox}$ *Alb-Cre*) by mating the strains. Mice were injected intraperitoneally with 1.5 or 0.5 mg/kg Jo2 anti-Fas antibody (BD Bioscience, Franklin Lakes, NJ) or intravenously with 0.25 mg/kg recombinant Fas ligand (Alexis Biochemicals, San Diego, CA) cross-linked with 0.5 mg/kg anti-Flag M2 antibody (Sigma-Aldrich, St. Louis, MO) to induce apoptosis. In some experiments, mice were intraperitoneally injected with 2 mg/kg necrostatin-1 (Sigma-Aldrich) or 40 mg/kg Q-VD-Oph (R&D Systems, Minneapolis, MN). They were maintained in a specific pathogen-free facility and treated with humane care with approval from the Animal Care and Use Committee of Osaka University Medical School.

Apoptosis Assay. Measurement of serum alanine aminotransferase (ALT) levels, hematoxylin and eosin staining, and terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL) of liver sections have been described.[15] Analysis of cytochrome c release from isolated mitochondria have also been described.[16] To detect DNA fragmentation, 1.5 μg DNA extracted from 30 mg liver tissue by Maxwell16 (Promega, Madison, WI) was incubated with 0.5 μg RNase A (Qiagen, Tokyo, Japan) and separated by way of electrophoresis on a 1.5% agarose gel.

Western Blot Analysis. For western immunoblotting, the following antibodies were used: anti–full-length Bid, anti–Cox IV, anti–cleaved caspase-3, anti–caspase-7, anti–caspase-8, anti–caspase-9, anti-PARP, anti-Bax, anti-cIAP1, and anti-XIAP antibodies were

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Additional Supporting Information may be found in the online version of this article.

obtained from Cell Signaling Technology (Beverly, MA); anti-Bax and anti-cIAP2 antibodies were obtained from Millipore (Billerica, MA); anti-Bid antibody, which detects truncated Bid, was generously provided by Xiao-Ming Yin (Indiana University School of Medicine, Indianapolis, IN)[17]; and anti–β-actin antibody was obtained from Sigma-Aldrich. For isolation of the mitochondria-rich fraction, a Mitochondrial Isolation Kit (Thermo Scientific, Rockford, IL) was used. The isolation of hepatocytes from whole liver has been described.[13]

Detection of Bax Oligomerization. Liver tissue was lysed with HCN buffer (25 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, 300 mM NaCl, 2% CHAPS, protease inhibitor cocktail, phosphatase inhibitor cocktail, 100 μM BOC-Asp(OMe)CH2F [MP Biomedicals, Solon, OH]; pH 7.5). After the liver lysate was sonicated and centrifuged, the supernatant was collected and the concentration was adjusted. For cross-linking, 100 μL of the lysate was incubated with 5 μL 100 mM bis(maleimido)hexane (Thermo Scientific) and 5 μL 100 mM BS^3 (Thermo Scientific) for 30 minutes at room temperature as described.[18] After quenching the cross-linkers by way of incubation with 12 μL 1 M Tris-HCl (pH 7.5) for 15 minutes at room temperature, the lysate was boiled with sample buffer followed by western blot analysis for Bax.

Electron Microscopy. Livers were fixed by perfusion of phosphate-buffered saline with 2.5% glutaraldehyde solution buffered at pH 7.4 with 0.1 M Millonig's phosphate, postfixed in 1% osmium tetroxide solution at 4°C for 1 hour, dehydrated in graded concentrations of ethanol, and embedded in Quetol 812 epoxy resin (Nisshin EM, Tokyo, Japan). Ultrathin sections (80 nm) cut on ultramicrotome were stained with uranyl acetate and lead citrate and examined with an H-7650 electron microscope (Hitachi Ltd., Tokyo, Japan) at 80 kV.

Statistical Analysis. Data are presented as the mean $\pm$ SE. Differences between two groups were determined using the Mann-Whitney U test for unpaired observations. The survival curves were estimated using the Kaplan-Meier method and were tested by way of log-rank test. $P < 0.05$ was considered statistically significant.

Results

Bak Deficiency Partially Ameliorates Fas-Induced Hepatocellular Apoptosis but Fails to Prevent Animal Death. First, to examine the significance of Bak in hepatocellular apoptosis induced by Fas stimulation, Bak KO mice ($bak^{-/-}$) and wild-type (WT) littermates ($bak^{+/+}$) were intraperitoneally injected with 1.5 mg/kg Jo2 anti-Fas antibody and analyzed 3 hours later. Consistent with previous reports,[10,19] WT mice showed severe elevation of serum ALT levels with massive hepatocellular apoptosis (Fig. 1A,B). Bak KO mice also developed liver injury, but the levels of serum ALT and the number of TUNEL-positive hepatocytes were significantly lower in Bak KO mice than in WT mice (Fig. 1A-C). Western blotting for cleaved caspase-3, caspase-7, and PARP revealed that activation of effector caspases were partially inhibited in KO livers compared with WT livers (Fig. 1D). Cleavage of procaspase-9, which is initiated by mitochondrial release of cytochrome c, was also suppressed in Bak KO livers compared with WT liver (Fig. 1D). The cleaved form of caspase-8, a direct downstream target of Fas activation, was detected in both mice, but its levels were reduced in Bak KO mice compared with WT mice (Fig. 1D). This reduction may be explained by the lesser activation of caspase-3/7, because it has been reported that caspase-3/7 could activate caspase-8 through an amplification loop during apoptosis.[20] Collectively, these findings demonstrated that Bak deficiency partially ameliorated Fas-induced hepatocellular apoptosis associated with reduced cleavage of caspase-9, caspase-3/7, and PARP. We then compared survival of mice after Jo2 injection but found that Bak KO mice also rapidly died with kinetics similar to those of WT mice, suggesting that partial amelioration of hepatocellular apoptosis induced by Bak deficiency did not lead to survival benefit under our experimental conditions (Fig. 1E). Because Bax residing in the cytosol moves to the mitochondria upon activation, where it undergoes oligomerization,[21] we analyzed its translocation and oligomerization in the liver at 3 hours after Jo2 injection. Western blot analysis revealed that the levels of Bax expression clearly increased in the mitochondrial fraction in both WT livers and Bak KO livers (Fig. 1F). Signals for the Bax dimer were also detected in both livers (Fig. 1F). These findings indicate that Bax is also activated after Fas stimulation, raising the possibility of its involvement in hepatocellular apoptosis.

Bax Deficiency Fails to Ameliorate Fas-Induced Hepatocellular Apoptosis. Next, to examine the significance of Bax in hepatocellular apoptosis induced by Fas stimulation, Bax KO mice ($bax^{-/-}$) and WT littermates ($bax^{+/+}$) were injected with Jo2 and examined 3 hours later. There was no significant difference in the levels of serum ALT or the number of TUNEL-positive hepatocytes between the two groups (Fig. 2A-C), which is consistent with a previous report.[22] The levels of the cleaved forms of caspase-8, -9, -3, -7, and

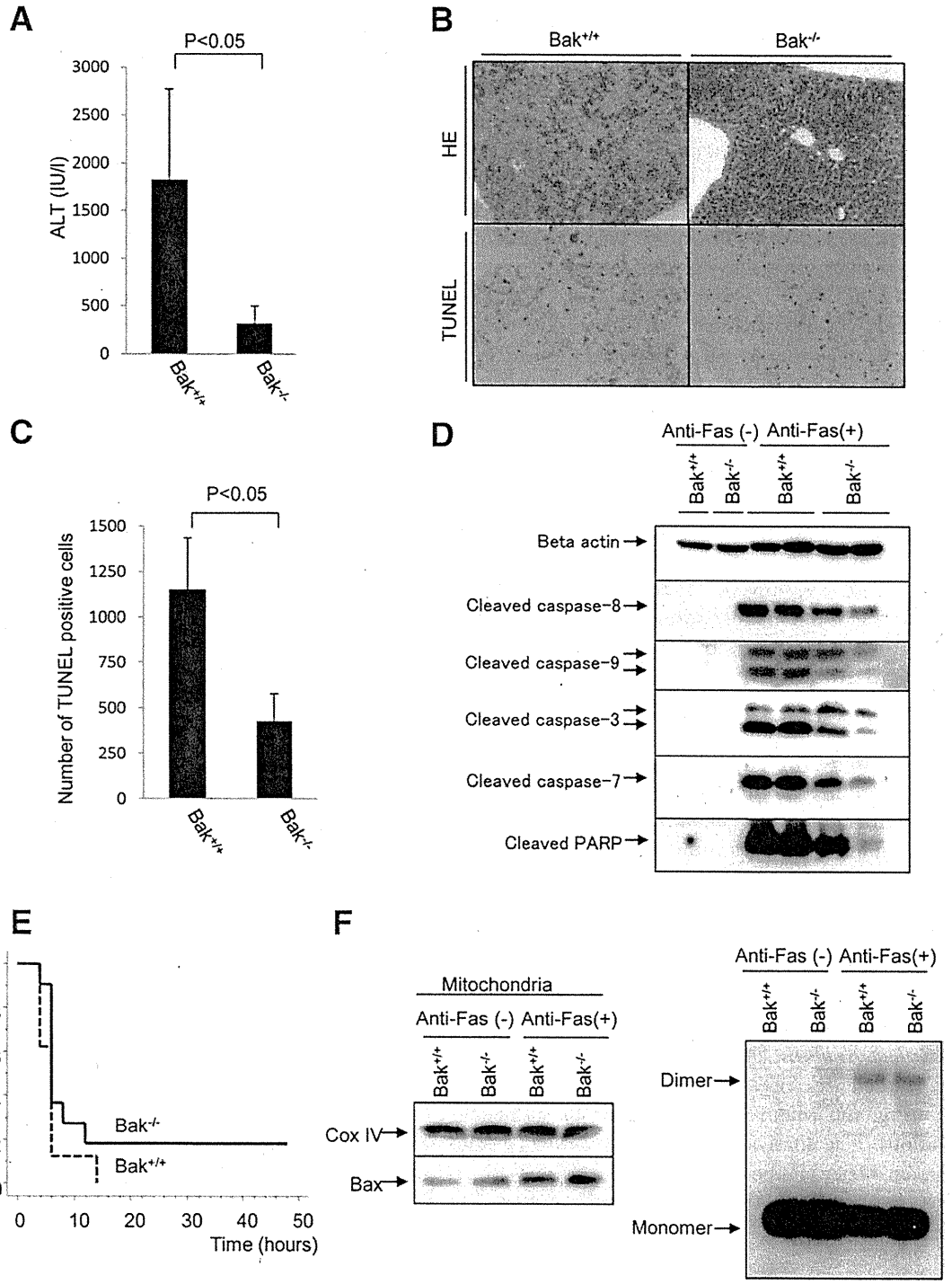

Fig. 1. Bak KO mice are partially resistant to Fas-induced hepatocellular apoptosis. Bak KO mice ($Bak^{-/-}$) or control WT littermates ($Bak^{+/+}$) were analyzed at 3 hours after intraperitoneal injection of 1.5 mg/kg Jo2 anti-Fas antibody. (A) Serum ALT levels (n = 10 or 11, respectively). (B) Hematoxylin and eosin (HE) and TUNEL staining of the liver sections. (C) Number of TUNEL-positive cells (n = 8 or 9, respectively). (D) Western blot analysis for the expressions of cleaved caspase-8, 9, -3, -7 and PARP. (E) Bak KO mice or control WT littermates were intraperitoneally injected with 1.5 mg/kg Jo2 anti-Fas antibody (n = 8 or 11, respectively). Survival rates after Jo2 injection are shown. (F) Bak KO mice or control WT littermates were analyzed 3 hours after intraperitoneal injection of Jo2 anti-Fas antibody (1.5 mg/kg) or vehicle. Left: Western blot analysis of the mitochondrial fraction of the liver for the expression of Bax. Right: Western blot analysis for the expression of Bax monomer and dimer in the liver.