Table 4. The prevalence of the 9 drug-resistant mutations detected by ultra-deep sequencing derived from liver tissue.

	M204V/I		L180M		T184S/A/I/ L/G/C/M		S202C/G/I		I169T	
Drugs	**LAM/ETV**		**LAM/ETV**		**ETV**		**ETV**		**ETV**	
Chronic-naive										
Liver #1	**27/5421**	**(0.5%)**	2/3694	(-)	9/3886	(-)	5/5613	(-)	5/3784	(-)
Liver #2	**35/5344**	**(0.7%)**	0/538	(-)	1/563	(-)	17/6340	(-)	0/512	(-)
Liver #3	**13/1363**	**(1.0%)**	0/304	(-)	1/358	(-)	1/1379	(-)	0/264	(-)
Liver #4	11/5113	(-)	0/556	(-)	**2/547**	**(0.4%)**	11/5133	(-)	0/639	(-)
Liver #5	**2/117**	**(1.1%)**	0/409	(-)	1/380	(-)	1/189	(-)	1/474	(-)
Liver #6	12/8451	(-)	0/309	(-)	0/328	(-)	22/8457	(-)	0/334	(-)
Liver #7	**10/3098**	**(0.3%)**	1/1547	(-)	3/1477	(-)	8/3161	(-)	0/1621	(-)
Liver #8	**13/2442**	**(0.5%)**	1/2378	(-)	6/2312	(-)	1/2564	(-)	1/2507	(-)
Liver #9	**67/13879**	**(0.5%)**	2/5443	(-)	2/5107	(-)	6/13804	(-)	0/5650	(-)
Liver #10	16/7400	(-)	0/3524	(-)	3/3283	(-)	5/7113	(-)	0/3492	(-)
Liver #11	0/412	(-)	1/1328	(-)	**1/295**	**(0.3%)**	0/425	(-)	3/4729	(-)
Liver #12	**4/1098**	**(0.4%)**	1/1389	(-)	0/1272	(-)	2/1102	(-)	0/1544	(-)
Liver #13	**8/2476**	**(0.3%)**	1/2192	(-)	3/2085	(-)	4/2529	(-)	4/5029	(-)
Liver #14	5/3713	(-)	0/2009	(-)	4/1925	(-)	2/3820	(-)	5/3784	(-)
Chronic-NA										
Liver #15	0/339	(-)	0/49	(-)	0/49	(-)	0/338	(-)	0/40	(-)
Liver #16	**28/7278**	**(0.4%)**	0/4403	(-)	6/4053	(-)	14/7556	(-)	6/6084	(-)
Liver #17	**177/945**	**(18.7%)**	0/1059	(-)	0/1009	(-)	0/945	(-)	0/1051	(-)
Liver #18	**13/2655**	**(0.5%)**	0/1239	(-)	0/1185	(-)	**10/2708**	**(0.4%)**	0/1332	(-)
Liver #19	**80/6795**	**(1.2%)**	0/3168	(-)	2/2971	(-)	3/6734	(-)	0/3384	(-)

	M250V/I		A181T/V		N236T		P237H	
Drugs	**ETV**		**ADV**		**ADV**		**ADV**	
Chronic-naive								
Liver #1	**23/2719**	**(0.9%)**	10/3755	(-)	4/4210	(-)	2/4139	(-)
Liver #2	**9/2079**	**(0.4%)**	**2/549**	**(0.4%)**	1/1144	(-)	1/1188	(-)
Liver #3	**10/1699**	**(0.6%)**	**1/298**	**(0.3%)**	3/1636	(-)	1/1666	(-)
Liver #4	**3/388**	**(0.8%)**	**3/549**	**(0.5%)**	0/560	(-)	0/533	(-)
Liver #5	**2/91**	**(2.2%)**	1/409	(-)	0/55	(-)	0/60	(-)
Liver #6	0/214	(-)	**6/305**	**(2.0%)**	**1/294**	**(0.3%)**	0/257	(-)
Liver #7	**7/1289**	**(0.5%)**	4/1531	(-)	**24/2738**	**(0.9%)**	1/2692	(-)
Liver #8	2/1117	(-)	**689/2336**	**(29.5%)**	2/1713	(-)	0/1639	(-)
Liver #9	**27/7325**	**(0.4%)**	**38/5334**	**(0.7%)**	1/6607	(-)	4/6702	(-)
Liver #10	**12/3815**	**(0.3%)**	0/3454	(-)	**13/3245**	**(0.4%)**	2/3272	(-)
Liver #11	**1/199**	**(0.5%)**	1/972	(-)	0/251	(-)	0/251	(-)
Liver #12	**2/672**	**(0.3%)**	**408/1362**	**(30.0%)**	0/598	(-)	0/597	(-)
Liver #13	1/947	(-)	2/2160	(-)	0/1406	(-)	1/1374	(-)
Liver #14	**23/2719**	**(0.9%)**	10/3755	(-)	4/4210	(-)	2/4139	(-)
Chronic-NA								
Liver #15	**1/303**	**(0.3%)**	**2/49**	**(4.1%)**	0/377	(-)	0/384	(-)
Liver #16	1/922	(-)	0/4403	(-)	1/1597	(-)	3/1572	(-)
Liver #17	0/755	(-)	1/1050	(-)	0/698	(-)	**145/698**	**(20.8%)**
Liver #18	1/1464	(-)	2/1206	(-)	0/3156	(-)	0/3107	(-)
Liver #19	8/3834	(-)	**16/3128**	**(0.5%)**	0/3372	(-)	0/3428	(-)

(-): mutant clones less than 0.3% among total clones at each nucleotide sites.
LAM: lamivudine, ADV: adefovir, ETV: entecavir.
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Table 5. The prevalence of M204VI mutation at YMDD site in patients before and after entecavir administration.

	Entecavir treatment				
	Before		After		
	Prevalence of the mutated clones		Prevalence of the mutated clones		Period of NA treatment
Serum #3	222/32,238	(0.7%)	2,284/23,791	(9.6%)	2w
Serum #2	401/34,041	(1.2%)	266/25,301	(1.1%)	24w
Serum #5	521/48,723	(1.1%)	245/25,521	(1.0%)	56w
Serum #8	748/65,573	(1.1%)	336/28,702	(1.2%)	48w
Serum #9	312/30,599	(1.0%)	169/14,172	(1.2%)	56w
Serum #1	9/22,843	(-)	2,839/34,162	(8.3%)	8w
Serum #7	26/65,564	(-)	923/66,458	(1.4%)	4w
Serum #12	91/65,616	(-)	258/27,958	(0.9%)	24w
Serum #13	11/23,209	(-)	206/64,747	(0.3%)	32w
Serum #4	3/7,923	(-)	39/65,575	(-)	12w
Serum #6	52/65,582	(-)	77/55,273	(-)	16w
Serum #10	38/22,522	(-)	8/21,053	(-)	8w
Serum #11	47/43,853	(-)	5/16,520	(-)	16w
Serum #14	42/42,784	(-)	40/36,668	(-)	12w

Mutation frequency (%): the ratio of total mutant clones to total aligned coverage at each nucleotide sites.
(-): mutant clones less than 0.3% among total clones at each nucleotide sites.
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and the elucidation of other unknown mutations involved in HBe seroconversion are necessary for a better understanding of the underlying mechanisms of HBe seroconversion.

One thing to be noted is that the majority of the chronic-NA cases had extremely low levels of the G1896A pre-C mutant in their liver tissues, even though those cases were serologically positive for anti-HBe and negative for HBeAg. Moreover, entecavir administration significantly reduced the proportion of the G1896A pre-C mutant in the serum of the majority of patients irrespective of their HBeAg serostatus, while the G1896A pre-C mutant clones were detectable in a substantial proportion before treatment in all cases. These findings suggest that the G1896A pre-C mutant have higher sensitivity to NA than the wild-type viruses. Consistent with this hypothesis, several previous studies reported that NA is effective against acute or fulminant hepatitis caused by possible infection with the G1896A pre-C mutant [34,35]. Based on these findings, early administration of NA might be an effective strategy for treating patients with active hepatitis infected predominantly with the G1896A pre-C mutant.

Ultra-deep sequencing has a relatively higher sensitivity than conventional direct population sequencing and is thus useful for detecting drug-resistant mutations not detected by standard sequencing [20,21]. Recently, we revealed that drug-resistant mutants were widely present in treatment-naïve HCV-infected patients, suggesting a putative risk for the expansion of resistant clones to anti-viral therapy [19]. Here, we demonstrated that various drug-resistant HBV variants are present in a proportion of chronically HBV-infected, NA-naïve patients. Several studies using ultra-deep sequencing provided evidence that naturally-occurring drug-resistant mutations are detectable in treatment-naïve individuals with human immunodeficiency virus-1 infection [30,36,37]. Consistent with the cases of human immunodeficiency virus-1 infection, a few studies detected minor variants resistant to NA in the plasma of treatment-naïve patients with chronic HBV infection [20,21]. It remains unclear, however, whether these minor drug-resistant mutations have clinical significance. Our observation of the relative expansion of viral clones with the M204VI mutation during entecavir therapy in some cases indicates the possibility that preexisting minor mutants might provide resistance against NA through the selection of dominant mutant clones. Future studies with a larger cohort size are required to clarify the clinical implications of the latently existing low-abundant drug-resistant mutations.

The current ultra-deep parallel sequencing technology has limitations in the analyses of viral quasispecies. First, because the massively-parallel ultra-deep sequencing platform is based on a multitude of short reads, it is difficult to evaluate the association between nucleotide sites mapped to different genome regions in a single viral clone. Indeed, potential mutational linkages between the pre-C and reverse transcriptase regions were difficult to elucidate due to the short read length of the shotgun sequencing approach. Second, accurate analysis of highly polymorphic viral clones by ultra-deep sequencing is also difficult because the identification of mutations depends strongly on the mapping to the reference genome sequences.

In conclusion, we demonstrated that the majority of patients positive for anti-HBe and negative for HBeAg lacked the predominant infection of the G1896A pre-C mutant in the presence of NA treatment, suggesting that the G1896A pre-C mutant have increased sensitivity to NA therapy compared with wild-type HBV. We also revealed that drug-resistant mutants are widely present, even in the liver of treatment-naïve HBV-infected patients, suggesting that the preexisting low-abundant mutant clones might provide the opportunity to develop drug resistance against NA through the selection of dominant mutations. Further analyses utilizing both novel and conventional sequencing technologies are necessary to understand the significance and clinical relevance of the viral mutations in the pathophysiology of various clinical settings in association with HBV infection.

Supporting Information

Figure S1 Comparison of the viral complexity between the liver and serum of the same individual. Shannon entropy values throughout the whole viral genome of the liver and serum of the representative two cases are shown. (upper two panels, case #11; lower two panels, case #14). preC-C: pre-core~core, preS: pre-surface, P: polymerase.
(TIF)

Table S1 The oligonucleotide primers for amplifying HBV sequences in each clinical specimen.
(DOCX)

Table S2 Error frequency of Ultra-deep sequencing for the expression plasmid encoding wild-type genotype C HBV genome sequences by the three control experiments.
(DOCX)

Table S3 The sensitivity and accuracy of detecting the low abundant minor clones in association with the different coverage numbers.
(DOCX)

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Author Contributions

Conceived and designed the experiments: NN HM. Performed the experiments: NN HM. Analyzed the data: NN HM YU AN TF ST KS TC. Contributed reagents/materials/analysis tools: NN HM YU YO TK SY SU. Wrote the paper: NN HM YU KT TC.

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BRIEF ARTICLE

Branched-chain amino acid treatment before transcatheter arterial chemoembolization for hepatocellular carcinoma

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Abstract

AIM: To examine the significance of branched-chain amino acid (BCAA) treatment before transcatheter arterial chemoembolization (TACE) for hepatocellular carcinoma (HCC).

METHODS: This study included 99 patients who underwent TACE therapy for HCC at our hospital and were followed up without treatment for at least 6 mo between January 2004 and January 2010. They were divided into 2 groups: those receiving BCAA granules (n = 40) or regular diet (n = 59, control). Data obtained were retrospectively analyzed (prior to TACE, and 1 wk, 1, 3, and 6 mo after TACE) in terms of nutritional condition and clinical laboratory parameters (serum albumin level and Child-Pugh score), both of which are determinants of hepatic functional reserve.

RESULTS: The BCAA group comprised 27 males and 13 females with a mean age of 69.9 ± 8.8 years. The patients of the BCAA group were classified as follows: Child-Pugh A/B/C in 22/15/3 patients, and Stage Ⅱ/Ⅲ/ⅣA HCC in 12/23/5 patients, respectively. The control group comprised 32 males and 27 females with a mean age of 73.2 ± 10.1 years. In the control group, 9 patients had chronic hepatitis, Child-Pugh A/B/C in 39/10/1 patients, and Stage Ⅰ/Ⅱ/Ⅲ/ⅣA HCC in 1/11/35/12 patients, respectively. Overall, both serum albumin level and Child-Pugh score improved significantly in the BCAA group as compared with the control 3 and 6 mo after TACE ($P < 0.05$). Further analysis was performed by the following categorization: (1) child-Pugh classification; (2) liver cirrhosis subgroup with a serum albumin level > 3.5 g/dL; and (3) epirubicin dose. A similar trend indicating a significant improvement of all variables in the BCAA group was noted ($P < 0.05$).

CONCLUSION: Treatment with BCAA granules in patients who have undergone TACE for HCC is considered useful to maintain their hepatic functional reserve.

Key words: Hepatocellular carcinoma; Branched-chain amino acid granules; Transcatheter arterial chemoembolization; Liver function; Improvement; Cirrhosis; Protein-energy malnutrition

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common

carcinoma worldwide[1]. Treatment for HCC varies depending on the disease stage and liver function, and includes radiofrequency ablation, percutaneous ethanol injection therapy, hepatic resection, liver transplantation, transcatheter arterial chemoembolization (TACE), and molecular target therapy[2-4].

TACE is a procedure whereby an embolizing agent is injected into the hepatic artery to deprive the tumor of its major nutrient source *via* embolization of the nutrient artery, resulting in ischemic necrosis of the tumor. Hepatic arterial embolization, which had been used until early in the 1990s, is divided into two treatment methods: injection of an embolizing agent after intra-arterial injection of an anticancer drug and intra-arterial injection of a mixture of an embolizing agent and an anticancer drug[5,6]. Subsequently, it was revealed that an oil contrast medium or iodized oil (Lipiodol) accumulates within the tumor after injection. This led to introduction of TACE, in which an embolizing agent is injected after injection of a mixture of Lipiodol and an anticancer drug (Lipiodol emulsion)[7,8]. Until the middle of the 1990s TACE had been performed in a large majority of patients with unresectable HCC. With the subsequent introduction of local treatment, however, TACE is now mainly indicated for treatment of an HCC measuring 3 to 5 cm in diameter or treatment of 4 or more HCCs less than 3 cm in diameter that are both unresectable and not indicated for local treatment.

Takayasu *et al*[9] reported that independent prognostic factors in relation to survival in patients who underwent TACE include (1) degree of hepatic damage; (2) tumor staging; and (3) serum α-fetoprotein level, and recommended TACE, which can sufficiently maintain the volume ratio of a chemoembolized tumorous liver to the entire tumor-free liver as well as of residual hepatic functional reserve, while emphasizing the importance of maintenance of hepatic functional reserve in these patients.

Branched-chain amino acids (BCAAs) are three amino acids possessing branched side chains (i.e., valine, leucine, and isoleucine). Patients with liver cirrhosis are known to have decreased plasma BCAA levels, which can lead to protein-energy malnutrition (PEM). PEM is associated with a high morbidity and mortality due to an increased risk of life-threatening complications, resulting in poor survival and quality of life (QoL)[10].

A considerable proportion of patients with HCC have concurrent liver cirrhosis. In those patients with underlying PEM, interventional therapy such as TACE may further worsen their nutritional condition and even occasionally cause development of ascites and jaundice, resulting in an irreversible outcome[11].

Supplementation with BCAAs in patients with liver disorder has been attracting attention. BCAA treatment can correct malnutrition associated with liver cirrhosis in animals and humans[12-14], and long-term nutritional BCAA supplementation may also be useful for prevention of hepatic failure while it also improves surrogate markers in patients with advanced cirrhosis[15,16]. BCAA supplementation is also effective in down-regulating protein metabolism in liver cirrhosis patients by reducing ammonia (NH_3) level, thus improving the nitrogen balance and resulting in better clinical outcomes[17,18]. The mechanism underlying these beneficial effects of BCAAs might be mediated by stimulation of hepatocyte growth factor activity that induces liver regeneration[19]. Therefore, nutritional support may play an important role in management of liver cirrhosis in patients with unresectable HCC. Studies dealing with the effect of treatment with BCAA granules before TACE in patients with HCC, nevertheless, are few as yet to our knowledge. This study was thus performed to investigate the significance of BCAA treatment in HCC patients who had undergone TACE.

MATERIALS AND METHODS

Patients

This retrospective study included 99 patients who underwent TACE alone for treatment of HCC at our hospital and were followed up thereafter without treatment for at least 6 mo between January 2004 and January 2010. Patients were divided into two groups: those receiving BCAA treatment (n = 40) or regular diet (n = 59, control). BCAA therapy had been started at least one month before the day TACE was performed, and treatment compliance was good in all patients receiving BCAAs.

Diagnosis of hepatocellular carcinoma

Dynamic computed tomography (CT) and abdominal echography were performed in all patients. A lesion visualized as a tumor blush in the early phase scan and as a defect area in the late phase scan on dynamic CT was diagnosed as HCC. It has been verified that such lesions appear as blushes on CT hepatic angiography and as defect areas on CT arterial portography during TACE. Two radiologists proficient in diagnostic imaging of the liver made a diagnosis of HCC. No pathological examination was conducted.

Branched-chain amino acid granules

BCAA granules, containing 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine per sachet, were orally administered to subjects at a dose of one sachet three times daily after meals. The control patients received no such treatment.

Transcatheter arterial chemoembolization procedure

Written informed consent was obtained from each patient prior to TACE. The protocol for TACE was approved by the independent ethics committee of the hospital. TACE for HCC was performed in conformity with Japanese guidelines for this therapy[20] and consisted of catheterization *via* the femoral artery with super-selective cannulation to the hepatic artery feeding the target HCC. Farmorubicin (epirubicin hydrochloride, Pfizer) emulsion was infused at 10 to 60 mg, and Lipiodol (iodine addition products of ethyl esters of fatty acids obtained from pop-

Table 1 Baseline characteristics of study groups (mean ± SD)

	BCAA group (n = 40)	Control group (n = 59)	P value
Gender			
Male	27	32	0.215
Female	13	27	
Age (yr)	69.9 ± 8.8	73.2 ± 10.1	0.092
Etiology of liver disease			
Chronic hepatitis C	28	43	0.287
Chronic hepatitis B	2	8	
Non B non C	10	10	
Child-Pugh classification			
Chronic hepatitis	0	9	0.006
Child-Pugh A	22	39	
Child-Pugh B	15	10	
Child-Pugh C	3	1	
WBC ($\times 10^3/\mu L$)	38.2 ± 10.8	44.7 ± 16.0	0.082
Hb (g/dL)	11.9 ± 1.8	12.5 ± 1.7	0.091
Platelet ($\times 10^4/mm^3$)	10.2 ± 9.4	11.4 ± 4.9	0.431
Alb (g/dL)	3.32 ± 0.50	3.74 ± 0.51	< 0.001
T-Bil (mg/dL)	1.28 ± 0.81	1.05 ± 0.63	0.123
PT (%)	77.5 ± 14.1	85.9 ± 17.3	0.012
AST (IU/L)	65.8 ± 39.6	73.8 ± 56.4	0.445
ALT (IU/L)	48.0 ± 38.8	54.2 ± 39.0	0.438
AFP (ng/mL)	626.1 ± 2009.8	1109.2 ± 2652.5	0.331
PIVKAII (mAU/mL)	1471.7 ± 5033.5	3421.5 ± 8211.2	0.183
HCC Stage			
Stage I	0	1	0.412
Stage II	12	11	
Stage III	23	35	
Stage IVa	5	12	
Max tumor size (cm)	3.34 ± 1.67	3.59 ± 1.47	0.422
Epirubicin dose (mg)	34.8 ± 10.4	39.5 ± 9.2	0.024

WBC: White blood cell; Hb: Hemoglobin; Alb: Albumin; T-Bil: Total bilirubin; PT: Prothrombin time; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AFP: Alpha-fetoprotein; PIVKA II: Protein induced vitamin K absence or antagonist II; HCC: Hepatocellular carcinoma; BCAA: Branched-chain amino acids.

py seed oil; Mitsui, Japan) was also injected at 2 to 10 mL according to the tumor size and tumor number. This was followed by embolization with gelatin (Spongel; Yamanouchi, Japan), which was injected slowly to prevent reflux into untreated segments. The sites of injection of the embolizing agents were segmental or subsegmental in all patients.

Follow-up after transcatheter arterial chemoembolization

At 1 wk and 1, 3 and 6 mo after TACE, patients underwent hematological and blood biochemical tests and were assessed for their hepatic functional reserve and development of any adverse events. Dynamic CT was carried out to assess for any ascites or recurrence of HCC at 1, 3 and 6 mo after TACE.

Statistical analysis

Student t test, χ^2 test and Fisher's exact test were used to compare data between BCAA patients and the control. Serum albumin level and Child-Pugh score constituted parameters for assessment of hepatic functional reserve. Absolute changes in serum albumin level observed at 1 wk and 1, 3 and 6 mo after TACE were compared between the two groups and evaluated using Student t test, and the absolute change was defined as the difference found at each assessment time point from the baseline (pre-TACE level). Changes in Child-Pugh score were also evaluated similarly using Student t test at 1, 3 and 6 mo after TACE.

Data were analyzed using SPSS software, version 9.0 (SPSS Inc., Chicago, IL, United States) for Microsoft Windows. Data are expressed as mean ± SD. Values of $P < 0.05$ were considered to be statistically significant.

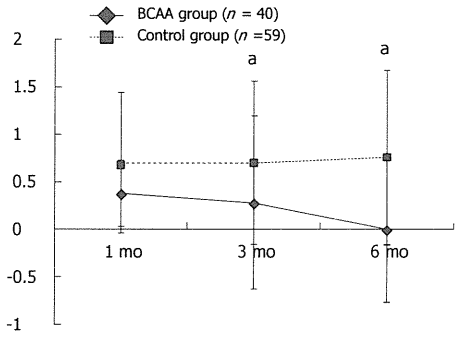

Figure 1 Overall comparison of changes in Child-Pugh score between the branched-chain amino acids group and the control group over time. There was a significant difference in changes in Child-Pugh score 3 and 6 mo after transcatheter arterial chemoembolization. $^aP < 0.05$ *vs* control group. BCAA: Branched-chain amino acid.

RESULTS

Patient demographic characteristics are summarized in Table 1. Significant differences were noted for the following parameters: Child-Pugh score, serum albumin level, prothrombin time, and dose of epirubicin at the time of TACE. A patient in the control group had stage I HCC, for which percutaneous therapy is indicated, but TACE alone was performed because the patient refused percutaneous therapy.

Overall comparison of hepatic functional reserve between the branched-chain amino acid group and the control group over time

A significant difference in serum albumin level was observed at all assessment time points ($P < 0.05$). Also, there was a significant difference in Child-Pugh score 3 and 6 mo after TACE ($P < 0.05$) (Table 1, and Figure 1).

The categorized analysis results are presented below.

Comparison of hepatic functional reserve in Child A patients

There were 22 Child A patients in the BCAA group and 39 in the control group. A significant difference was noted in serum albumin level 1, 3 and 6 mo after TACE and in Child-Pugh score 3 and 6 mo after TACE ($P < 0.05$) (Table 1, and Figure 2A).

Comparison of hepatic functional reserve in Child B patients

There were 15 Child B patients in the BCAA group and 10 in the control group. A significant difference was

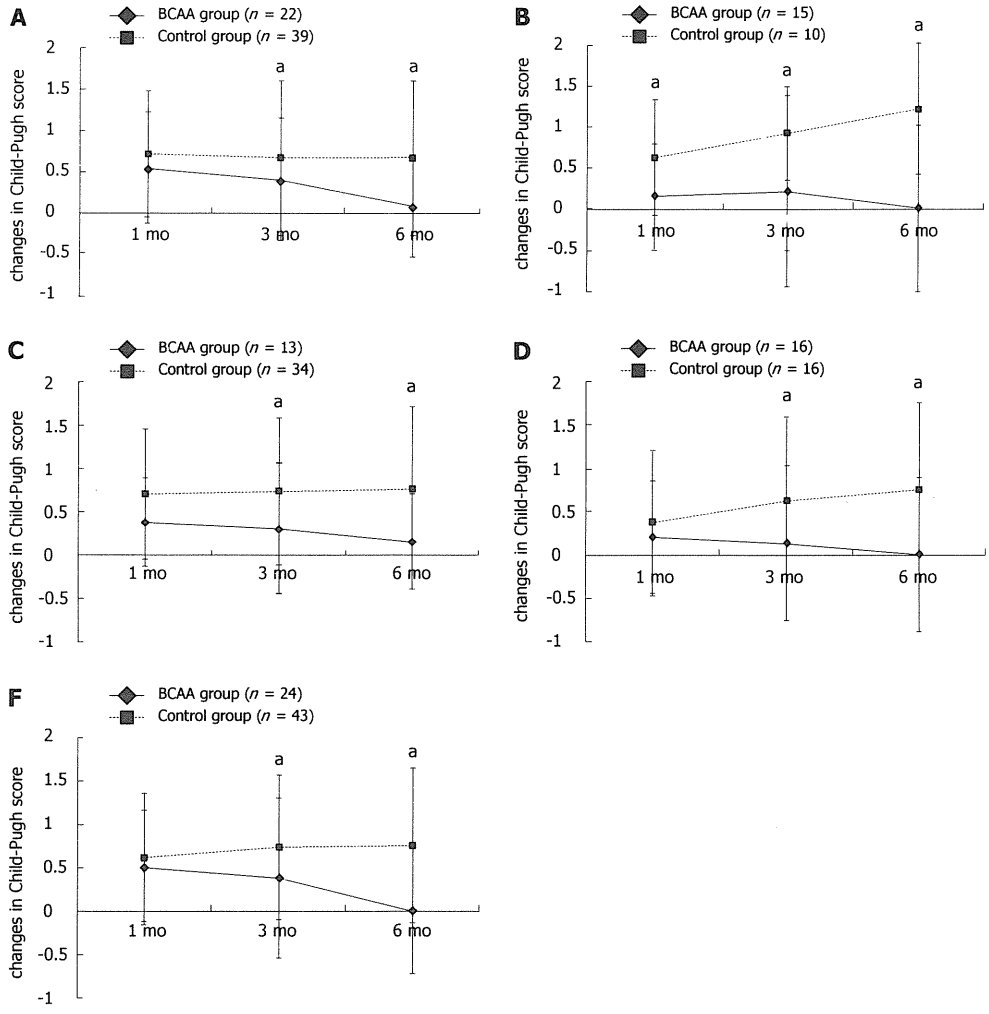

Figure 2 Comparison of changes in Child-Pugh score in Child A and Child B patients: patients with a serum albumin level of 3.5 g/dL or more, low-dose epirubicin subgroups, high-dose epirubicin subgroups. A: A significant difference was noted in changes in Child-Pugh score 3 and 6 mo after transcatheter arterial chemoembolization; B: A significant difference was noted in changes in Child-Pugh score 1, 3 and 6 mo after transcatheter arterial chemoembolization; C: A significant difference was observed in changes in Child-Pugh score 3 and 6 mo after TACE; D: A significant difference was noted in changes in Child-Pugh score 3 and 6 mo after TACE; E: A significant difference was noted in changes in Child-Pugh score 3 and 6 mo after TACE. BCAA: Branched-chain amino acids; TACE: Transcatheter arterial chemoembolization. $^{a}P < 0.05$ *vs* control group.

noted in serum albumin level 3 and 6 mo after TACE and in Child-Pugh score 1, 3 and 6 mo after TACE ($P < 0.05$) (Table 1 and Figure 2B).

Comparison in patients with a serum albumin level of 3.5 g/dL or more

There were 13 and 34 patients who fell in this category in the BCAA group and the control group, respectively. A significant difference was observed in both serum albumin level and Child-Pugh score 3 and 6 mo after TACE ($P < 0.05$) (Table 1, and Figure 2C).

As it is thought that antineoplastic agents used during TACE may cause hepatic impairment in a dose-dependent fashion, the data were further evaluated in patients classified into two subgroups: those treated with low-dose epirubicin (less than 40 mg) or a high-dose epirubicin (40 mg or more).

Comparison in low-dose epirubicin subgroups

Sixteen patients each received low-dose epirubicin in the BCAA group and the control group. Serum albumin level was significantly different 1, 3 and 6 mo after TACE and Child-Pugh score 3 and 6 mo after TACE ($P < 0.05$) (Table 1 and Figure 2D).

Comparison in high-dose epirubicin subgroups

Twenty-four and 43 patients received high-dose epirubicin in the BCAA group and the control group, respectively. A significant difference was noted in serum albumin level at all assessment time points and in Child-Pugh score 3 and 6 mo after TACE ($P < 0.05$) (Table 1 and Figure 2E).

DISCUSSION

PEM occurs frequently in patients with liver cirrhosis and represents an important predictive factor for the prognosis of liver cirrhosis patients with HCC [18,21]. Supplementation with BCAA formula is reportedly useful for improving PEM and QoL in these patients. However, few studies have assessed the importance of such nutritional intervention in patients with HCC who underwent nonsurgical therapies such as TACE. The purpose of the present study was to investigate to what extent BCAA treatment can contribute to maintaining hepatic functional reserve in HCC patients after TACE.

A significant difference was observed in the overall patient population in terms of change in serum albumin level at all assessment time points. As seen in Table 1, hepatic functional reserve was relatively well maintained in the control group; therefore, anticancer chemotherapy was given at relatively high doses (60% of patients treated with BCAA received epirubicin at 40 mg or more whereas the corresponding percentage for the control group was 72.9%). Patients receiving high-dose anticancer chemotherapy are often unable to sufficiently ingest food over several weeks after TACE. This may account for lower serum albumin levels observed in the control group compared with the BCAA group. Other possible causes of decreased serum albumin levels after TACE include (1) impaired ability of the liver to synthesize serum albumin due to decreased hepatocyte count; (2) inhibition of the synthesis of albumin by inflammatory cytokines; and (3) leakage of albumin due to inflammation of the cauterized areas[22,23].

The assessments in Child-Pugh A patients revealed a significant difference in serum albumin level 1, 3 and 6 mo after TACE and in Child-Pugh score 3 and 6 mo after TACE. TACE is best indicated for Child-Pugh A HCC. In patients undergoing TACE, caution should be exercised to minimize depression of hepatic functional reserve in preparation for the next treatment session. The above results thus suggest the usefulness of BCAA treatment in this regard.

The assessments in the Child-Pugh B subgroup showed a significant difference in Child-Pugh score 1, 3 and 6 mo after TACE. Once hepatic functional reserve has worsened from Child-Pugh B to Child-Pugh C following TACE, the next TACE cannot be performed according to the Barcelona Clinic Liver Cancer guidelines[24]. Therefore, particular caution should be exercised in maintaining hepatic functional reserve at the time of TACE in patients with Child-Pugh B HCC, indicating the indispensability of BCAA therapy.

In Japan, BCAA granules are indicated for the treatment of liver cirrhosis in patients with a serum albumin level of 3.5 g/dL or less. However, conversely, the present study demonstrated similar results between patients with a serum albumin level of more than 3.5 g/dL and those in other categories of serum albumin level. Therefore, treatment with BCAA proved to improve hepatic functional reserve even in cirrhotic patients with HCC whose serum albumin level exceeds 3.5 g/dL. It is thus recommended to actively provide BCAA treatment in such patients.

There was a conspicuous difference between the BCAA and control groups in respect of response to BCAA therapy when assessed in patients receiving high-dose epirubicin compared to those treated with low-dose epirubicin. TACE may cause a marked damage to the liver in HCC patients, eventually leading to a considerable impact on their hepatic functional reserve[9]. BCAA treatment is thus recommended at sufficient doses prior to TACE in patients with advanced HCC in whom high-dose anticancer chemotherapy is anticipated.

TACE is often repeated because a single session of therapy seldom provides complete necrosis of a tumor. The procedure is commonly repeated once every 2 to 3 mo[25-27]. In the present study, however, many patients failed to attain recovery of hepatic functional reserve to a pre-TACE level, particularly in the control group, within 2 to 3 mo of TACE. It is thus estimated that every repeated session of TACE may worsen hepatic functional reserve and thereby shorten the prognosis for survival. Treatment with BCAA would therefore be essential in order to allow for providing TACE periodically while securely maintaining hepatic functional reserve.

One of the findings commonly noted in regard to all the variables assessed in this study is that a significantly greater improvement was noted in both serum albumin level and Child-Pugh score for the BCAA group 6 mo after TACE in comparison to the control group. What is suggested by this fact is simply the usefulness of long-term BCAA treatment prior to TACE. It is also important that patients should be fully instructed on the use of BCAA granules to maintain their treatment compliance.

The present study has several limitations. Firstly, it is a retrospective study. Furthermore, there was a bias in patient demographic characteristics between the BCAA and control groups since BCAA is usually used for patients showing low serum albumin levels. Therefore, pertinent data were evaluated for improvement or exacerbation using absolute serum albumin change as a parameter. The present study did not include assessment for the prognosis for survival, which should be addressed by a prospective study using comparable demographic characteristics among patients.

In conclusion, treatment with BCAAs before TACE in HCC patients is extremely useful in maintaining their hepatic functional reserve.

COMMENTS

Background

Patients with hepatocellular carcinoma (HCC) due to liver cirrhosis are known to have decreased plasma branched-chain amino acid (BCAA) levels, which can lead to protein-energy malnutrition (PEM). BCAA treatment can correct malnutrition associated with liver cirrhosis.

Research frontiers

Studies dealing with the effect of treatment with BCAA granules before transcatheter arterial chemoembolization (TACE) in patients with HCC are few as

yet. In this study, the authors analyzed the effect of BCAA treatment before TACE for HCC patients.

Innovations and breakthroughs

Recent studies imply that by BCAA supplementation, malnutrition associated with liver cirrhosis is corrected and liver function improves. The present study shows that in HCC patients who underwent TACE, liver function was maintained by BCAA supplementation.

Applications

This study emphasizes the importance of BCAA treatment before TACE for HCC patients with regard to maintaining liver function.

Peer review

This is a very good and novel study in which authors analyze the effect of BCAA treatment before TACE for HCC patients. The results are interesting and suggest the usefulness of BCAA treatment before TACE in HCC patients in maintaining their hepatic functional reserve.

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Review

Hepatic Arterial Infusion Chemotherapy for Advanced Hepatocellular Carcinoma in Japan

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Abstract: Transcatheter methods such as transcatheter arterial chemoembolization (TACE) and hepatic arterial infusion chemotherapy (HAIC) have an important role in the treatment for advanced hepatocellular carcinoma (HCC). Recently, sorafenib, an inhibitor of tyrosine kinases, has been found to obtain survival benefits in patients with HCC, leading to major advances in the treatment of advanced HCC. However, it is associated with a low tumor response rate, minimal survival advantage, and high rates of adverse events. On the other hand, high rates of objective treatment response with HAIC for advanced HCC have been reported, although convincing evidence of it contributing to overall survival in HAIC has been lacking. In Japan, HAIC still tends to be the preferred method for the treatment of advanced HCC, even in patients with poor liver function. However, the choice of chemotherapeutic agents in TACE/HAIC for HCC varies between institutions. In this review, based on studies reported to date in the literature, we refer to current knowledge regarding the chemotherapeutic agents used for TACE/HAIC for HCC in Japan and consider the future perspectives for HAIC for this cancer.

Keywords: advanced hepatocellular carcinoma; chemotherapy; hepatic arterial infusion chemotherapy; sorafenib; response rate

1. Introduction

Hepatocellular carcinoma (HCC) is a problem worldwide, especially in Asian countries [1–3] Unlike most solid cancers, the incidence and mortality rates for HCC are projected to increase substantially in many countries over the next 20 years, mostly as a result of viral infections with hepatitis C and hepatitis B [4]. It has become possible to identify a group of patients with chronic liver disease who are at a high risk of developing HCC. In addition, improvements in diagnostic imaging have allowed early diagnosis of HCC. However, unfortunately, most HCC patients are first seen when the disease has reached an advanced stage at which curative treatment is no longer possible [4].

Potentially curative treatments for HCC include hepatic resection and liver transplantation [5,6]. Several non-surgical treatment options, including percutaneous ethanol injection, microwave coagulation, and radiofrequency ablation (RFA) have also been developed and are widely used for the treatment of HCC [4]. However, in general, these modalities are not indicated for patients with multiple tumors, invasion or thrombosis of major vessels, extrahepatic metastases, or poor liver function. Various anticancer agents have been employed for transcatheter arterial chemoembolization (TACE) and hepatic arterial infusion chemotherapy (HAIC) in these patients with advanced HCC, in whom effective chemotherapy is required.

Regarding systemic chemotherapy for advanced HCC, no agent has reproducibly shown a high response rate, and few chemotherapeutic regimens have a meaningful impact on patient survival, although numerous clinical trials have been performed to establish effective systemic treatment for patients with advanced HCC [7–11].

Sorafenib, an inhibitor of tyrosine kinases, was the first systemic chemotherapeutic agent found to improve the survival time of patients with advanced HCC, in the SHARP trial [12]. However, it is associated with a low tumor response rate, minimal survival advantage, and high rates of adverse events [12]. Moreover, because most patients enrolled in this study had Child–Pugh A cirrhosis with well-preserved liver function, the benefits and safety profile of sorafenib in advanced HCC patients with poor liver function such as Child–Pugh B/C cirrhosis or other poor prognostic factors remain unclear. Alternative approaches to the treatment of advanced HCC are needed, especially in patients with poor liver function.

Transcatheter arterial iodized oil chemoembolization has become established as an effective treatment for unresectable HCC. However, it is generally suitable only for patients with well-preserved liver function and multiple tumors without major vascular invasion [13,14]. HAIC is one of the few remaining options for advanced HCC patients with poor liver function [15]. In general, patients with HCC are less tolerant to chemotherapy than patients with other malignancies owing to poor liver function. In most HCC patients, pancytopenia may already be present because of concomitant liver cirrhosis, and myelosuppression and a bleeding tendency are likely to occur when performing chemotherapy. Owing to these clinical features of cirrhotic patients, HAIC is not commonly used for HCC in North America and Europe. However, in cases in which extrahepatic spread is minimal and local control of liver tumors is considered more important, HAIC is useful and may offer survival benefits, even when there are extrahepatic metastases. HAIC has often been used in these cases in Japan [16,17].

Compared with systemic chemotherapy, HAIC requires several technical procedures, including catheterization, and is associated with a risk of vascular disorders related to catheter placement and reservoir management [17]. However, it is a useful therapeutic modality because higher concentrations of the anticancer agent are obtained in the targeted tumor. The agent is administered directly into the liver via the hepatic artery, increasing the antitumor effect while being associated with a lower rate of incidence of systemic adverse events, and numerous clinical studies have reported that HAIC has moderate chemotherapeutic effect with favorable toxicity profiles in selected patients with advanced HCC [17]. However, there have been no large randomized studies and convincing clinical evidence is lacking for HAIC in advanced HCC. Therefore, HAIC is not yet a well-established treatment for advanced HCC, and its further investigation is required.

Several intra-arterial chemotherapy regimens, using doxorubicin, epirubicin, mitomycin C, 5-fluorouracil (5-FU), zinostatin stimalamer (SMANCS), cisplatin, miriplatin and oxaliplatin administered singly or in combination, have been reported as treatments for HCC. However, the optimal regimen for intra-arterial chemotherapy for HCC remains unknown. In this review, based primarily on clinical studies reported to date in the literature regarding transcatheter chemotherapeutic agents in HAIC for advanced HCC, we refer to current knowledge regarding the chemotherapeutic agents used in Japan for HCC and consider the future perspectives for hepatic arterial chemotherapy for this cancer.

2. Chemotherapeutic Agents

2.1. Doxorubicin

Doxorubicin (Adriacin; Kyowa Hakko Kogyo, Tokyo, Japan) is an anthracycline-based anticancer drug that had been conventionally used as a first-line therapy for HCC. In 1982, Hirose *et al.* reported that one shot of high-dose doxorubicin intra-arterial infusion therapy showed useful chemotherapeutic effects against HCC [18]. In 1994, Yasui *et al.* reported that of 86 patients with unresectable or recurrent HCC who underwent doxorubicin containing HAIC, 21 (34.4%) showed an objective response, and responders to doxorubicin containing HAIC achieved longer survival than non-responders [19]. In 1999, Tzoracoleftherakis *et al.* reported a randomized comparative study between HAIC with doxorubicin and systemic chemotherapy with doxorubicin involving 72 patients with unresectable (Stage IVA) HCC; patients who received HAIC with doxorubicin had a higher rate of objective and subjective remission and greater Karnofsky performance status improvement than did those in the systemic chemotherapy with doxorubicin group [20]. However, previous studies of systemic chemotherapy with doxorubicin had confirmed the minimal efficacy of this anticancer drug in HCC [9–11]. In Japan, doxorubicin is used for both HAIC and TACE in the treatment of advanced HCC [21].

2.2. Epirubicin

Epirubicin (Farmorubicin; Nihon Kayaku, Tokyo, Japan) is the 4-epimer of doxorubicin. It has a more favorable toxicity profile with less myelosuppression and cardiotoxicity than doxorubicin, and is routinely used for HCC. Because epirubicin easily undergoes glucuronidation, it is less toxic than

doxorubicin. In 1986, the Japan Epirubicin Study Group for Hepatocellular Carcinoma reported that of 53 HCC patients who received HAIC with epirubicin, eight patients (15.1%) showed an objective response (complete response [CR] or partial response [PR]) [22]. Furthermore, a retrospective comparison with intra-arterial administration of doxorubicin showed that epirubicin was more effective than doxorubicin in terms of survival rate. Since then, epirubicin has been routinely used in HAIC for HCC in Japan [22]. Additional clinical trials have been performed in several faculties. Epirubicin alone or in combination with other chemotherapeutic agents such as mitomycin C or 5-FU has been used in HAIC for HCC in various Asian countries including Japan, with objective response rates ranging from 5% to 78% [22–29]. Reports of HAIC with anthracycline-based chemotherapeutic agents are listed in Table 1.

Table 1. Reports of HAIC with anthracycline-based chemotherapeutic agents for advanced hepatocellular carcinoma.

Authors (year) [ref.]	Country	Number of patients	Agents	Characteristics	Response rate (%)
Nagasue *et al.* (1986) [22]	Japan	53	Epirubicin	Unresectable	15.1
Yoshikawa *et al.* (1994) [23]	Japan	17	Epirubicin	Unresectable	12
Yasui *et al.* (1994) [19]	Japan	86	Doxorubicin, MMC, 5-FU	Unresectable	34.4
Tzoracoleftherakis *et al.* (1999) [20]	Greece	72	Doxorubicin	Unresectable	60
Hwang *et al.* (2005) [24]	Korea	18	Epirubicin, MMC, 5-FU	Unresectable	38.9
Ikeda *et al.* (2007) [25]	Japan	45	Epirubicin	PV invasion	9
Tanaka *et al.* (2008) [26]	Japan	20	Epirubicin	TAE refractory	5
Ikushima *et al.* (2009) [27]	Japan	18	Epirubicin	Unresectable	77.8
Kim *et al.* (2010) [30]	Korea	36	Doxorubicin	Unresectable	16.7

HAIC: hepatic arterial infusion chemotherapy; MMC: mitomycin C; 5-FU: 5-fluorouracil; PV: portal vein; TAE: transcatheter arterial embolization.

2.3. Mitomycin C

Mitomycin C is an antineoplastic antibiotic isolated from the culture fluid of *Streptomyces caespitosus*. The objective response rate of intra-arterial chemotherapy with mitomycin C alone has been reported as 25% (9/36) [31]. However, this agent has recently been used in HAIC as a combination therapeutic agent. In Japan, mitomycin C alone has never been used in HAIC [21,24,32].

2.4. 5-FU

The pyrimidine antimetabolite 5-FU was the first reported chemotherapeutic agent used in the treatment of HCC. Monotherapy with intra-arterial 5-FU has a relatively low objective response rate, ranging from 13% to 22%, with a median survival of only 3.5 to 14 months [33,34]; therefore, 5-FU is often used in combination in HAIC for HCC.

Combined therapy comprising intra-arterial infusion of 5-FU and systemic interferon-α (IFN-α) (FAIT) has been reported to be useful as a palliative treatment for HCC patients with major vascular

invasion, although monotherapy with IFN-α had minimal objective response rate against HCC [35–37]. In 2002, Sakon *et al.* reported that of 11 advanced HCC patients with portal vein tumor thrombus (PVTT), eight patients (73%) showed an objective response to FAIT [38]. In 2006, Obi *et al.* reported that in 116 advanced HCC patients with PVTT who received FAIT, 19 (16%) showed a CR and another 42 (36%) showed a PR; adverse events were limited to nausea and appetite loss, and the overall survival rates at 1 and 2 years were 34% and 18%, respectively [39]. In 2011, Nagano *et al.* reported that of 102 HCC patients with PVTT, 40 (39.2%) showed an objective response to FAIT, and no major treatment-related complications were noted [40]. In 2006, Kondo *et al.* reported that a combination of 5-FU and IFN-α strongly inhibited the growth of human HCC cells, and suggested that the effects of this combination therapy may be attributable to changes in the induction of apoptosis through IFN-α/β receptors [41]. In 2009, Kasai *et al.* reported that intra-arterial 5-FU and systemic pegylated (PEG)-IFN-α2b combination therapy for advanced HCC had an objective response rate of 71.4% [42]. In 1999, Yano *et al.* compared the *in vivo* antitumor effects of PEG-IFN-α2b and IFN-α in nude mice injected with cultured HCC cells, and found that PEG-IFN-α2b induced apoptosis more strongly than IFN-α [43]. Intra-arterial 5-FU infusion and systemic PEG-IFN-α2b combination therapy appears to be highly promising, although further prospective studies are required [42,44]. Reports of the use of HAIC with 5-FU combined with systemic IFN in advanced HCC are listed in Table 2.

Table 2. Reports of hepatic arterial infusion chemotherapy with 5-FU combined with systemic interferon for advanced hepatocellular carcinoma.

Authors (year) [ref.]	Country	Number of patients	Characteristics	IFN	Response rate (%)
Sakon *et al.* (2002) [38]	Japan	8	PV invasion	IFN-α	62.5
Enjoji *et al.* (2005) [45]	Japan	28	PV invasion or unresectable	IFN-α	21.5
Ota *et al.* (2005) [46]	Japan	55	PV invasion	IFN-α	43.6
Obi *et al.* (2006) [39]	Japan	116	PV invasion	IFN-α	52.5
Uka *et al.* (2007) [47]	Japan	31	PV invasion	IFN-α	29.1
Kuroda *et al.* (2007) [48]	Japan	10	PV invasion	IFN-α	10
Katamura *et al.* (2009) [49]	Japan	16	PV invasion	IFN-α	25
Kasai *et al.* (2009) [44]	Japan	9	PV invasion	Peg-IFN α2b	77.8
Kasai *et al.* (2011) [42]	Japan	21	PV invasion	Peg-IFN α2b	71.4
Nagano *et al.* (2011) [40]	Japan	102	PV invasion	IFN-α	39.2

IFN: interferon; PV: portal vein; Peg-IFN: pegylated interferon.

Low-dose cisplatin (CDDP) combined with 5-FU (low-dose FP therapy) has synergistic effects. This combination is often used in the treatment of gastrointestinal tract malignancies. In combination with 5-FU, cisplatin is a modulator rather than an effector, and increases the antitumor efficacy of 5-FU by increasing the intracellular concentration of reduced folate [37,50]. In 2002, Ando *et al.* reported that of 48 HCC patients with PVTT, 23 (48.0%) showed an objective response to low-dose FP therapy and concluded that HAIC using low-dose FP might be a useful therapeutic option for patients with advanced HCC with PVTT [51]. In 2010, Ueshima *et al.* reported that of 52 advanced HCC patients, 20 (38.5%) showed an objective response to low-dose FP therapy [52]. However, there

are few reports of favorable objective response rates with the use of high-dose CDDP combined with 5-FU therapy for the treatment of advanced HCC [53–55]. Reports of intra-arterial 5-FU and CDDP combination therapy in HAIC are listed in Table 3.

Table 3. Reports of hepatic arterial infusion chemotherapy with 5-FU and CDDP for advanced hepatocellular carcinoma.

Author (year) [ref.]	Country	Number of patients	Characteristics	Dose	Response rate (%)
Ando *et al.* (2002) [51]	Japan	48	PV invasion	Low	48
Itamoto *et al.* (2002) [56]	Japan	7	PV invasion	Low	33
Lai *et al.* (2003) [57]	China	18	PV invasion	Low	33
Sumie *et al.* (2003) [58]	Japan	16	PV invasion	Low	56.3
Yamasaki *et al.* (2005) [59]	Japan	15	Unresectable	Low	20
Park *et al.* (2007) [54]	Korea	41	Unresectable	High	22
Kim *et al.* (2010) [53]	Korea	36	Unresectable	High	16.7
Ueshima *et al.* (2010) [52]	Japan	52	PV invasion or unresectable	Low	38.5
Woo *et al.* (2010) [55]	Korea	32	PV invasion	Low	0
Woo *et al.* (2010) [55]	Korea	36	PV invasion	High	16.7

PV: portal vein.

2.5. CDDP

Clinical investigation of cisplatin (*cis*-diamminedichloroplatinum; CDDP) had been started in 1972. The usefulness of CDDP as an anticancer agent was first confirmed in the treatment of urinary tract cancers. Currently, CDDP is a key chemotherapeutic agent for the treatment of various cancers, including tumors of the respiratory, genitourinary, and digestive systems [60]. The anticancer effect of CDDP is characterized by both time-dependent and concentration-dependent features.

The response rate of CDDP monotherapy administered by HAIC for advanced HCC ranges from 14% to 42% [61–64]. Reported objective response rates to arterial infusion regimens containing CDDP, such as low-dose FP therapy, range from 0% to 56%, although high efficacy of systemic chemotherapy with CDDP has not been reported in HCC [17,51–53,65–71].

Microfine powder CDDP preparations (DDP-H) (IA-call; Nippon Kayaku Co., Ltd., Japan) for arterial infusion were approved for its use in Japan in 2004. Recently in Japan, favorable results have been obtained using DDP-H in the treatment of advanced HCC patients. In 2008, Yoshikawa *et al.* conducted a phase II study in advanced HCC patients, reporting that in 80 patients who received HAIC with DDP-H, the overall response rate was 33.8%, the 1-year and the 2-year survival rates were 67.5% and 50.8%; respectively, they concluded that DDP-H has higher antitumor effect than other anticancer drugs when administered by HAIC [62]. The usefulness of DDP-H as a second-line treatment for advanced HCC refractory to TACE using an epirubicin-lipiodol emulsion has also been reported [72,73].

Systemic combination therapy with S-1 and CDDP is a promising treatment for advanced HCC. In 2010, Katamura *et al.* reported that of 16 HCC patients with extrahepatic metastases who received S-1 and CDDP combination therapy, two (13%), none (0%), five (31%), and nine (56%) showed CR,

PR, stable disease, and progressive disease, respectively, with an overall objective response rate of 13% (2/16) and an overall survival rate at 1 year of 77% [74].

There is one report that RFA with sequential HAIC using cisplatin contributed to a longer disease-free interval [16]. HAIC using cisplatin before RFA might prevent an increase in the size of pre-existing microscopic tumor foci.

2.6. SMANCS

SMANCS is a lipophilic intra-arterial chemotherapeutic agent for HCC. In 1983, Konno *et al.* reported a reduction in tumor size and tumor markers in 13 of 14 patients with HCC following arterial infusion of SMANCS-lipiodol emulsion [75]. A subsequent study of 124 patients with HCC or metastatic liver tumors showed significantly higher survival rate following infusion of a SMANCS-lipiodol emulsion [76].

In 2002, Okusaka *et al.* reported a phase II study of TACE using SMANCS in which the overall response rate was 32% (16/50); they concluded that TACE using SMANCS, which was well tolerated, might be a useful treatment for advanced HCC [77]. However, hepatic arterial infusion with SMANCS caused severe vascular endothelial damage and loss of the hepatic artery for infusion [77,78]. SMANCS is thus unsuitable for repeated TACE/HAIC in HCC and is not widely used for this purpose in Japan.

2.7. Miriplatin

Miriplatin (Miripla; Dainippon Sumitomo Co., Ltd., Tokyo, Japan), a cisplatin derivative, is a novel chemotherapeutic agent designed for use in transarterial infusion chemotherapy for HCC [79]. Miriplatin: (1) inhibits cell proliferation in a similar manner to cisplatin and has superior solubility in ethyl esters of iodized fatty acids derived from poppy seed oil; (2) releases its platinum constituent continuously, together with the ethyl esters (sustained release), by remaining at the site of the tumor; and (3) has fewer adverse effects, because of its sustained release and its minimal presence in the general circulation [80–83]. In Japan, miriplatin was approved for use in October 2009.

In 2004, Okusaka *et al.* conducted a phase II study of miriplatin to assess its antitumor effect and toxicity in treatment-naive patients with HCC. They reported that the CR rate was 56% (9/16) and that none of the patients exhibited grade 4 toxicity or episodes of renal dysfunction, and concluded that miriplatin was well tolerated and showed promising antitumor effect in patients with HCC [84]. In 2011, Okusaka *et al.* reported their phase II comparative study of miriplatin and SMANCS, in which HAIC with miriplatin had a similar efficacy to HAIC with SMANCS, and repeated dosing with miriplatin was possible without hepatic vascular injury in cases of relapse, whereas HAIC with SMANCS caused hepatic vascular injury [78]. In 2011, Imai *et al.* reported that in 162 unresectable HCC patients who underwent transcatheter arterial chemotherapy using miriplatin with or without embolization, the objective response rates to HAIC with miriplatin and TACE with miriplatin were 33% (13/40) and 57% (70/120), respectively; they concluded that an objective response was achieved in a significantly higher number of patients treated with TACE with miriplatin than with HAIC with miriplatin [85].

It is well known that HAIC with CDDP causes renal dysfunction [86]. However, in 2011, Imai *et al.* reported that HAIC with miriplatin could be used safely in HCC patients with chronic renal failure, probably owing to its minimal presence in the general circulation [87]. In terms of renal toxicity, HAIC with miriplatin is considered safer than HAIC with CDDP.

In an animal experiment comparing the antitumor effects of two platinum agents (miriplatin and DDP-H), Watanabe *et al.* reported no significant difference between miriplatin-lipiodol emulsion and DDP-H-lipiodol emulsion after 7 days post-administration [88]. To date, however, there is no convincing clinical evidence regarding the antitumor effects of these drugs. In Japan, several clinical studies comparing the antitumor effects of miriplatin and other chemotherapeutic agents such as CDDP and epirubicin for advanced HCC are ongoing. If the results for miriplatin are positive in these studies, it will become a key chemotherapeutic drug in the treatment of unresectable HCC.

2.8. Oxaliplatin

The platinum-based chemotherapeutic agent oxaliplatin (L-OHP) displays a wide range of antitumor activities [89]. Oxaliplatin has often been used for the treatment of advanced colorectal cancer, in which its effectiveness has been confirmed in many clinical studies [90]. To date, however, few detailed clinical data are available concerning the effects of HAIC with oxaliplatin in advanced HCC. In Japan, oxaliplatin has not been used for the treatment of advanced HCC. In 2010, Rathore *et al.* reported that in their phase I trial, HAIC with oxaliplatin was feasible, well tolerated, and had demonstrated activity in patients with advanced HCC [91]. A phase II prospective randomized study of the effectiveness of HAIC with oxaliplatin in unresectable HCC is underway. If positive results are obtained, oxaliplatin could become a key chemotherapeutic agent in the treatment of advanced HCC.

3. Discussion

There are four main reasons why HAIC may be well suitable for the treatment of advanced HCC compared with systemic chemotherapy. First, because HCC derives almost all of its blood supply from the hepatic artery, high anticancer drug concentrations can be obtained while hepatic perfusion is maintained via the portal vein [17,92,93]. Second, normal hepatic tissue metabolizes various agents, such that first pass metabolism leads to higher local drug concentrations in the liver, reducing systemic adverse effects [17,93]. Third, prolonged drug exposure, which may increase the antitumor effect, is easily obtained with HAIC [17,93]. Fourth, unlike other locoregional therapies for HCC, HAIC is not limited by tumor size, tumor number, or proximity to major vascular structures, all of which preclude surgical resection or RFA in general.

There are two methods for administration of HAIC: one-shot hepatic arterial infusion and continuous hepatic arterial infusion. In one-shot hepatic arterial infusion, concentration-dependent agents such as anthracycline-based agents, mitomycin C, CDDP, and miriplatin are suitable for HAIC. In continuous hepatic arterial infusion, 5-FU, which exerts its antitumor effects time-dependently, anthracycline-based agents, mitomycin C, and intermittent administration of CDDP based on the concept of biochemical modulation are suitable for HAIC. In continuous hepatic arterial infusion, an implantable port system is required. Several recent studies indicate that probably owing to technical improvements of angiographic procedure, only 0–4% of patients develop catheter-related complications

such as breakage of the reservoir device, catheter dislocation, artery dissection, artery occlusion, subcutaneous hematoma, and infection [38,56]. Therefore, HAIC using an implantable port system can be performed safely.

In general, HCC responds poorly to chemotherapy. The possible explanations are tumor heterogeneity, inducible overexpression of the multidrug resistance gene, and/or inherent resistance due to an unknown mechanism [94–97]. Combination therapy is therefore considered to be more effective than monotherapy. Synergistic cooperative effects have been observed in experiments on HCC cell lines [96].

In sorafenib treatment for advanced HCC, as mentioned above, because most patients enrolled in the SHARP trial had Child–Pugh A cirrhosis with well-preserved liver function, the benefits and safety profile of sorafenib in advanced HCC patients with Child–Pugh B/C or other poor prognostic factors remains unclear [12]. However, even patients with poor performance status or reduced hepatic functional reserve could be eligible for HAIC. Indeed, as shown in Tables 2 and 3, many studies of HAIC have focused on HCC patients with PVTT. Moreover, the objective response rate of sorafenib treatment is low in general. In the SHARP trial, it was very low (CR and PR were 0% and 2%, respectively) [12]. In Japan, however, there have been many reports of high rates of response to HAIC in advanced HCC, although most of these studies were not randomized and few demonstrated any survival benefit using controls. The clinical response reflects the survival benefits [14,37–39,52,98,99]. Probably, chemosensitive subgroups of patients with advanced HCC are present, and it is therefore important to identify optimal candidates for HAIC as well as to investigate novel therapeutic strategies. A novel method using gene expression profiling has recently been reported for the prediction of treatment response in advanced HCC [100]. In practice, however, responders and non-responders should be distinguishable after the first session of HAIC by evaluating tumor size using imaging modalities and the levels of tumor markers. In early non-responders, HAIC should not be continued; instead, different therapeutic options, including sorafenib, should be explored. We have experience of a case of advanced HCC with PVTT refractory to epirubicin that showed a marked decrease in tumor markers after HAIC with miriplatin [101].

4. Future Perspectives

The favorable objective response rate reported for HAIC in advanced HCC in Japan provides a sound basis for its clinical use. In the era of molecular targeted anticancer therapies such as sorafenib, incorporating such agents into HAIC regimens may obtain further improvement of treatment effect. However, the effects of combination therapy of sorafenib and HAIC are unclear. According to a subgroup analysis of the SHARP trial, it is speculated that sorafenib in combination with resection, ablation, TACE or HAIC will prolong overall survival in various stages of HCC [12,102]. There are several case reports showing a favorable response of advanced HCC to combination therapy with sorafenib and HAIC [103,104], although in 2011 Kemeny *et al.* reported that in 22 primary liver cancer patients, addition of systemic bevacizumab to HAI floxuridine/dexamethasone appeared to increase biliary toxicity without any clear improvement in outcome [105]. To elucidate this issue, a randomized clinical trial of sorafenib alone versus sorafenib combined with maintenance TACE/HAIC

and/or HAIC in intermediate and advanced HCC was initiated in Japan in 2009 [102]. If favorable results are obtained by these trials, the treatment strategy for HCC will be drastically changed.

5. Conclusions

As shown in Tables 1, 2, and 3, many clinical studies with favorable response rates for HAIC in advanced HCC have been reported in Japan, and the improvement of survival can be ascribed to treatment-related effects. We believe that HAIC as well as sorafenib should be considered an effective treatment for advanced HCC.

Conflict of Interests

The authors declare that they have no conflicts of interest. In addition, none of the authors had any financial relationship (within the past 12 months) with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has any interest in the subject matter, materials, or processes discussed in the manuscript.

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