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ORIGINAL ARTICLE

Hepatotoxicity of intra-arterial combination chemotherapy in patients with liver cirrhosis and advanced hepatocellular carcinoma

Hidenari Nagai · Teppei Matsui · Masahiro Kanayama · Kouichi Momiyama · Kazue Shizawa · Noritaka Wakui · Mie Shinohara · Manabu Watanabe · Kazunari Iida · Koji Ishii · Yoshinori Igarashi · Yasukiyo Sumino

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Abstract
Purpose We have previously reported that 24-h intra-arterial combination chemotherapy (IACC) prolongs the survival of patients with advanced hepatocellular carcinoma (aHCC). However, it has also been reported that 5-fluorouracil (5-FU) exacerbates liver damage in patients with liver cirrhosis (LC). The aim of this study was to clarify the hepatotoxicity of IACC in LC patients with aHCC.
Methods Twenty-one adult Japanese patients (20 men and 1 woman) with aHCC and LC underwent IACC between 2004 and 2007 at our hospital. These patients showed multiple partial responses or stable disease, except for five patients who showed no response and three patients with tumors more than 30 mm in diameter. All patients had inoperable disease on the basis of computed tomography (CT) findings. IACC (leucovorin at 12 mg/h, cisplatin at 10 mg/h, and 5-FU at 250 mg/22 h) was delivered via the proper hepatic artery every 5 days for 4 weeks.
Results Twelve patients were in Child-Pugh class A (group A), and nine were in class B (group B). The Child-Pugh score was significantly increased after chemotherapy compared with before chemotherapy in both groups. Serum albumin was significantly decreased after chemotherapy, and the number of patients with ascites also increased after chemotherapy. Serum type IV collagen and N-terminal propeptide of type III procollagen were significantly increased after chemotherapy, although there was no significant change in serum aminotransferases.
Conclusions IACC might cause hepatotoxicity that induces fibrosis without releasing aminotransferases.

Keywords 5-FU · HCC · Liver cirrhosis · Intra-arterial chemotherapy · Hepatotoxicity · Liver fibrosis · 7S domain of type IV collagen · Hyaluronic acid · N-terminal propeptide of type III procollagen

H. Nagai (✉) · T. Matsui · M. Kanayama · K. Momiyama · K. Shizawa · N. Wakui · M. Shinohara · M. Watanabe · K. Iida · K. Ishii · Y. Igarashi · Y. Sumino
Division of Gastroenterology and Hepatology, Department of Internal Medicine (Omori), School of Medicine, Faculty of Medicine, Toho University, 6-11-1, Omorinishi, Ota-ku, Tokyo 143-8541, Japan
e-mail: hidenari@aol.com

Introduction

There are a considerable number of patients with advanced hepatocellular carcinoma (aHCC) for whom intra-arterial combination chemotherapy (IACC) is one of the few remaining options. We have shown that intra-arterial treatment with a combination of low-dose 5-fluorouracil (5-FU), cisplatin (CDDP), and leucovorin (LV) prolongs the survival of patients with aHCC [1]. We have also reported that continuous intra-arterial infusion for 24 h was more effective compared with 6-h infusion in patients who had liver cirrhosis (LC) and aHCC due to HCV infection, although 24-h infusion was associated with stronger hematologic toxicity [2]. 5-FU has been reported to have two main anticancer mechanisms. (1) It inhibits deoxyribonucleic acid (DNA) synthesis through inactivation of thymidylate synthase (TS) by forming a complex between methylenetetrahydrofolate (CH_2FH_4) and 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP), which is synthesized from 5-FU. (2) It interferes with ribonucleic acid (RNA) metabolism via the uptake of phosphated 5-fluorouridine 5′-triphosphate into RNA [3]. It has been reported that a single dose of 5-FU is more effective for causing RNA damage,

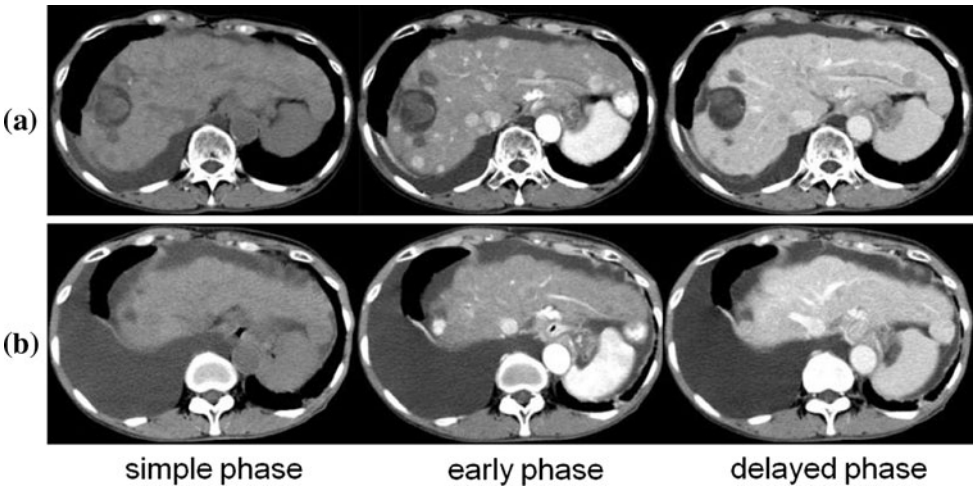

Fig. 1 **a**, **b** Enhanced CT scans of the liver from patient with advanced hepatocellular carcinoma before and after treatment. Ascites appears after chemotherapy, although the tumors show a reduction in size

while continuous infusion causes more DNA damage [4]. With regard to hepatic effects, it was reported that long-term oral administration of Tegafur-Uracil (UFT^R), a 5-FU derivative preparation that contains tegafur and uracil, induces fibrosis of the normal liver without elevation of serum aminotransferase levels or necroinflammation [5]. Indeed, we often succeed in reducing the size aHCC by IACC, but at the same time we often find that patients show a decrease of liver function (Fig. 1).

7S collagen is the major cross-linking domain of type IV collagen and is not cleaved during assembly of the type IV collagen molecule. The serum level of 7S collagen is a valuable marker for assessing the extent of liver fibrosis in patients with chronic viral and alcoholic liver disease [6, 7], and it reflects enhanced metabolism (mainly synthesis) of type IV collagen in chronic liver disease [8]. N-terminal propeptide of type III procollagen (P-III-P) is released during the conversion of type III procollagen into collagen and enters the circulation, so the serum level of P-III-P is considered to mainly reflect inflammatory activity and active fibrogenesis [9]. Hyaluronic acid is synthesized by mesenchymal cells, such as fibroblasts and hepatic stellate cells, and is carried by the lymph to the systemic circulation [10].

Because it is unclear whether IACC causes hepatotoxicity in LC patients with aHCC, the present retrospective study was performed to assess the hepatotoxicity of IACC in these patients by using the Child-Pugh score and various fibrosis markers, such as serum type IV collagen, P-III-P, and hyaluronic acid.

Methods

Patients

Twenty-one adult Japanese LC patients admitted to Toho University Medical Center Omori Hospital were diagnosed as having aHCC between 2004 and 2007. These patients were not eligible for surgical resection or for other interventions, such as transcatheter arterial embolization, percutaneous ethanol injection, microwave coagulation therapy, or radiofrequency ablation, because they had multiple tumors in both lobes of the liver. All of the patients received IACC. These patients showed multiple partial responses or stable disease, except for five patients who showed no response and three patients with tumors more than 30 mm in diameter (because of liver damage caused by tumor progression). Early morning blood samples were collected from the patients before and after chemotherapy.

Drug delivery system

In all patients, an intra-arterial catheter was inserted via the femoral artery and was attached to a subcutaneously implanted reservoir [11]. In principle, the gastroduodenal artery and the right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the anticancer agents (Fig. 2). Written informed consent was obtained from all of the patients.

Chemotherapy regimen

All patients were treated by IACC (LV at 12 mg/h, CDDP at 10 mg/h, and 5-FU at 250 mg/m^2/22 h) via the proper hepatic artery at 5-day intervals for 4 weeks using a catheter connected to a subcutaneously implanted drug delivery system. Doses of the chemotherapy agents were set according to a previous report [12].

Evaluation of efficacy

Using CT scans obtained after 4 weeks of treatment, the product of the two longest perpendicular diameters of the largest tumor was calculated. A complete response (CR)

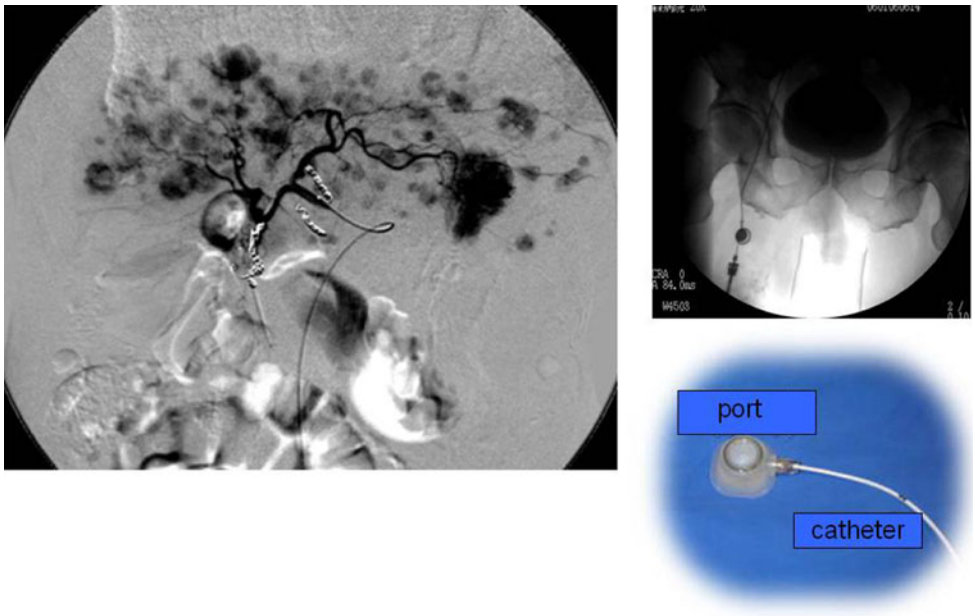

Fig. 2 Subcutaneous implanted drug delivery system. An intra-arterial catheter is inserted via the femoral artery and is attached to a subcutaneously implanted reservoir. In principle, the gastroduodenal artery and right gastric artery should be occluded with steel coils to prevent gastroduodenal damage by anticancer agents

was defined as a 100% reduction in the product, while a partial response (PR) was defined as reduction in the product by more than 50%. An increase of more than 25% was defined as progressive disease (PD), and smaller changes between the PR and PD limits were defined as stable disease (SD).

Assays

The serum concentration of the 7S domain of type IV collagen (type IV collagen) was determined with a commercial RIA kit (Nippon DPC Corporation, Tokyo, Japan) that contained a polyclonal antibody targeting type IV collagen [8]. The normal level of type IV collagen was defined as less than 6 ng/ml. Serum P-III-P was also measured with a commercial RIA kit (Nippon DPC Corporation, Tokyo, Japan) [13]. Serum hyaluronic acid was measured by using a sandwich enzyme-binding assay (Chugai Seiyaku, Tokyo, Japan) [14]. The normal hyaluronic acid level was defined as less than 50 ng/ml.

Endpoints

The primary endpoint of the study was to assess the response rate according to the World Health Organization (WHO) criteria. The secondary endpoint was the toxicity of chemotherapy at the dosages given.

Statistical analysis

Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS version 11.0; SPSS, Chicago, IL, USA). Data are expressed as the mean ± standard deviation (SD). Wilcoxon's signed rank sum test was used to compare patient characteristics within the same group. A probability of less than 0.05 was considered to indicate statistical significance.

Results

Twenty-one adult Japanese patients (20 men and 1 woman) who had aHCC and LC were treated with IACC at our hospital between 2004 and 2007. Twelve patients were in Child-Pugh class A (group A) and nine were in class B (group B). Group A comprised a total of 11 men and 1 woman aged 54–83 (mean ± SD: 69.8 ± 8), while group B comprised 9 men aged 62–72 (mean ± SD: 67.0 ± 5). There were no significant differences in background factors between the two groups. In group A, 1 patient had HBV infection, 10 patients had HCV infection, and 1 patient had alcoholic cirrhosis, while the respective numbers were 1, 5, and 3 in group B. There were four patients with stage III disease, five patients with stage IVA disease, and three patients with stage IVB disease in group A, while the respective numbers were 2, 5, and 2 in group B. None of the patients in group A had tumor thrombi involving major portal vein branches (Vp4), while two patients had such thrombi in group B. There were no patients with tumor thrombi involving the first portal vein branches (Vp3) in group A versus 1 patient in group B. One patient in each group had tumor invasion of the inferior the vena cava (vv3). Although no patient had tumor invasion of the right hepatic vein (vv2) in group A, one patient had such invasion in group B (Table 1).

Table 1 Clinical characteristics of the 21 patients with advanced HCC and cirrhosis

	Child A	Child A	*P*
Mean age	69.8 ± 8 years	67.0 ± 5 years	0.158
Gender	Male: 11, female: 1	Male: 9, female: 0	0.386
Etiology of cirrhosis	HBV: 1, HCV: 10, alcohol: 1	HBV: 1, HCV: 5, alcohol: 3	0.324
Stage of tumor	III: 4, IVA: 5, IVB: 3 (Vp4: 0, Vp3: 0, vv3: 1, vv2: 0)	III: 2, IVA: 5, IVB: 2 (Vp4: 2, Vp3: 1, vv3: 1, vv2: 1)	0.788

Data show the number of patients

Groups were compered by the Mann–Whitney *U* test

Table 2 Objective response stratified by Child-Pugh class Objective response rate: 30.8% (8/26, except for 3 patients with tumors more than 30 min in diameter)

	CR	PR	SD	PD	Response rate (%)
Child A	0	6	6	2	42.9
Child B	0	2	7	3	16.7

CR:PR:SD:PD = 0:8:13:5

Data show the number of patients

Response of growth were compered by the χ^2 test ($P = 0.121$)

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Response stratified by Child-Pugh class

Six of the 12 patients in group A (42.9%) and two of the 9 patients in group B (16.7%) achieved PR. Six of the 12 patients in group A (42.9%) had SD, as did 7 of the 9 patients in group B (58.3%) (Table 2).

Serum aminotransferases

Figure 2 summarizes the changes in serum aminotransferases in each group. There were no significant changes in serum alanine aminotransferase (ALT) after chemotherapy in group A (before: 59.1 ± 39.9 IU/l, after: 69.4 ± 42.2 IU/l) or group B (before: 33.9 ± 18.0 IU/l, after: 24.1 ± 13.8 IU/l). There were also no significant changes in serum aspartate aminotransferase (AST) in group A (before: 73.8 ± 49.0 IU/l, after: 82.3 ± 48.5 IU/l) or group B (before: 52.5 ± 16.9 IU/l, after: 40.7 ± 16.1 IU/l) (Fig. 3).

Serum albumin

Figure 3 summarizes the changes in serum albumin in each group. In group B, serum albumin was significantly decreased after chemotherapy (2.50 ± 0.6 g/dl) compared with before chemotherapy (2.64 ± 0.5 g/dl) ($P = 0.038$), although there was no significant difference in albumin level between before (3.30 ± 0.5 g/dl) and after (3.35 ± 0.4 g/dl) chemotherapy in group A (Fig. 4).

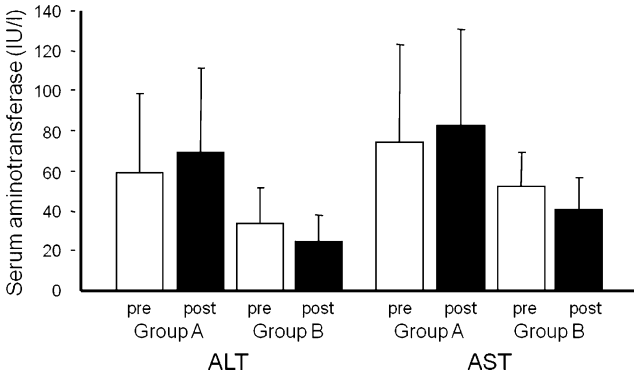

Fig. 3 Serum aminotransferases before and after treatment. There were no significant differences in serum alanine aminotransferase (*ALT*) or serum aspartate aminotransferase (*AST*) after chemotherapy compared with before chemotherapy in either group

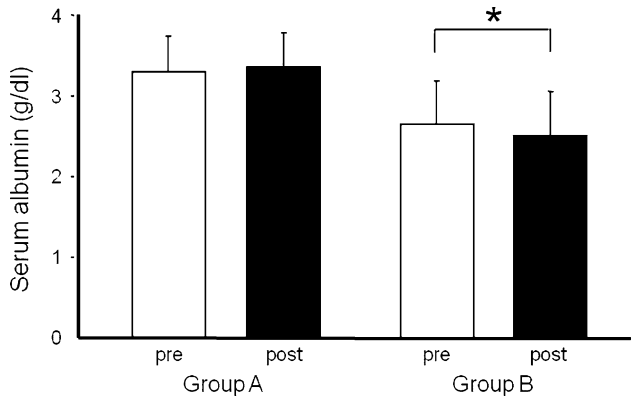

Fig. 4 Serum albumin levels before and after treatment. In group B, serum albumin was significantly decreased after chemotherapy compared with before chemotherapy ($P = 0.038$, Wilcoxon's signed rank sum test), although there was no significant change in albumin in group A

Child-Pugh scores

Figure 4 compares the Child-Pugh scores of each group. The Child-Pugh score was significantly increased after chemotherapy (group A: 6.42 ± 1.0, group B: 8.43 ± 1.1) compared with before chemotherapy (group A: 5.83 ± 0.4, group B: 7.71 ± 0.8 points) in both groups ($P \leq 0.046$) (Fig. 5).

In group A, four patients developed ascites after chemotherapy, while there were no patients with ascites before chemotherapy. In group B, three patients had ascites after chemotherapy versus two patients before chemotherapy (data not shown). There were no significant differences in

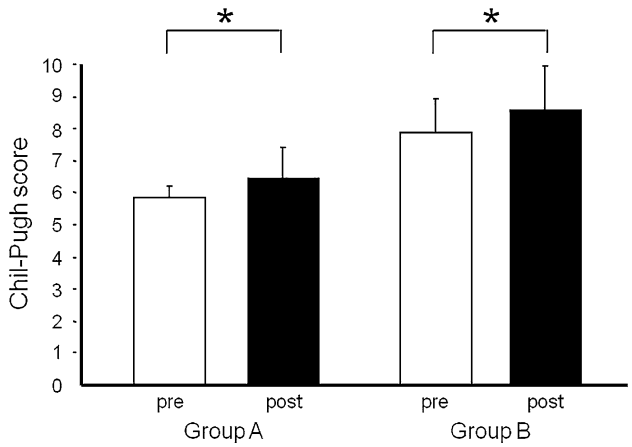

Fig. 5 Child-Pugh score before and after treatment. The Child-Pugh score showed a significant increase after chemotherapy compared with before chemotherapy in both groups ($P = 0.046$, Wilcoxon's signed rank sum test)

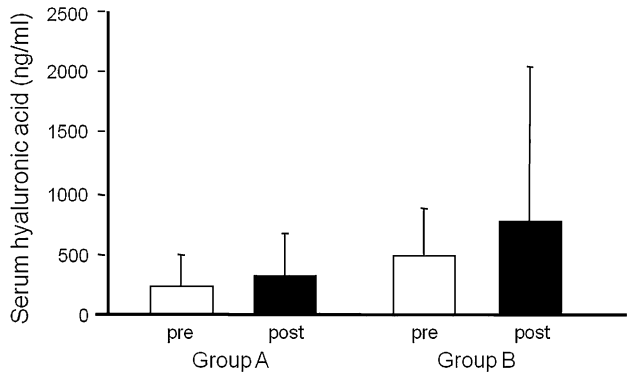

Fig. 6 Serum hyaluronic acid levels before and after treatment. There was no significant difference in the serum hyaluronic acid level between before and after chemotherapy in either group

the serum total bilirubin level or the prothrombin time between before and after chemotherapy in each group (data not shown).

Serum hyaluronic acid

Figure 5 shows the serum hyaluronic acid levels in both groups. There were no significant differences in hyaluronic acid between before (group A: 223.1 ± 280.2 ng/ml, group B: 215.6 ± 244.9 ng/ml) and after (group A: 372.0 ± 390.1 ng/ml, group B: 377.4 ± 559.8 ng/ml) chemotherapy in both groups (Fig. 6).

Serum type IV collagen

Figure 6 summarizes the changes in the serum type IV collagen level in each group. Type IV collagen was significantly increased after chemotherapy compared with before chemotherapy in group B (11.32 ± 1.2 vs.

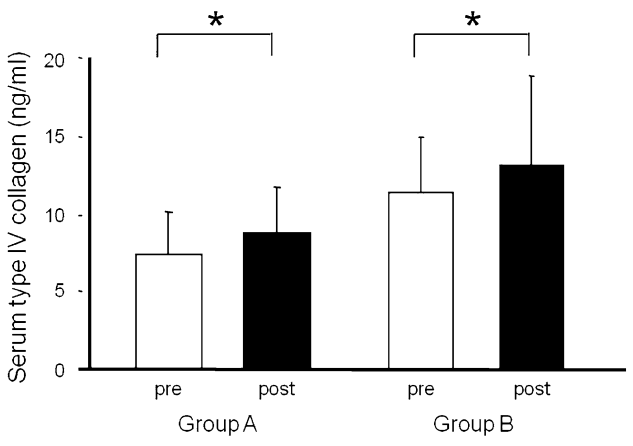

Fig. 7 Serum 7S domain of type IV collagen (type IV collagen) levels before and after treatment. Serum type IV collagen was significantly increased after chemotherapy compared with before chemotherapy in both groups (group A: $P = 0.028$, group B: $P = 0.042$, Wilcoxon's signed rank sum test)

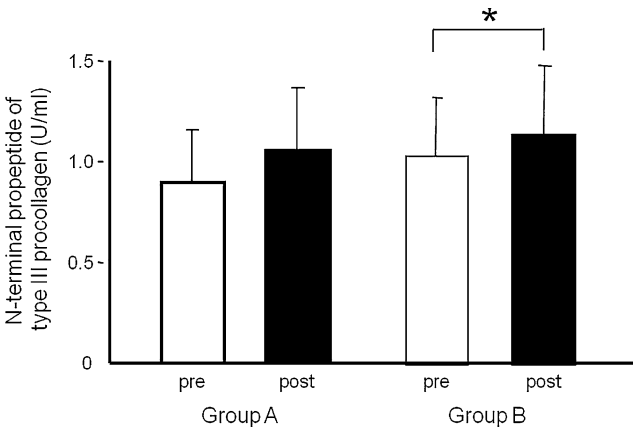

Fig. 8 Serum N-terminal propeptide of type III procollagen (P-III-P) levels before and after treatment. In group B, serum P-III-P was significantly increased after chemotherapy compared with before chemotherapy ($P = 0.043$, Wilcoxon's signed rank sum test), although there was no significant changes in serum P-III-P in group A

9.84 ± 1.3 ng/ml, $P = 0.042$), and there was also a significant increase in type IV collagen level after chemotherapy in group A and after (7.79 ± 2.6 vs. 6.61 ± 2.3 ng/ml, $P = 0.028$) (Fig. 7).

N-terminal propeptide of type III procollagen

Figure 7 summarizes the P-III-P levels in each group. P-III-P was significantly increased after chemotherapy compared with before chemotherapy in group B (1.12 ± 0.4 vs. 1.02 ± 0.3 ng/ml, $P = 0.043$), although there was no significant difference in serum P-III-P between before (0.88 ± 0.3 U/ml) and after (1.05 ± 0.3 U/ml) chemotherapy in group A (Fig. 8).

Discussion

It was reported that the majority of patients with aHCC do not survive for longer than 6 months from the time of diagnosis [15], and other reports have indicated an average survival period of 4 months from the onset of symptoms or 2 months from admission [16]. We previously reported that continuous intra-arterial chemotherapy for 24 h was more effective than 6-h infusion in patients with LC and aHCC due to HCV infection, although 24-h infusion was associated with stronger hematologic toxicity [2]. However, it is unclear whether IACC causes hepatotoxicity in aHCC patients underlying LC. Therefore, the present study was performed to retrospectively assess the hepatotoxicity of IACC in such patients. We found that the serum albumin level decreased significantly after chemotherapy compared with before chemotherapy in group B, although there was no significant change in albumin after chemotherapy in group A. Moreover, there was an increase of patients with ascites after chemotherapy in both groups compared with before chemotherapy. These changes resulted in an increase in the Child-Pugh score after chemotherapy in both groups, although there was no change in the Child-Pugh class. The National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC ver. 2.0) has been widely used for development and evaluation of chemotherapeutic agents. However, hepatotoxicities of IACC could not evaluate by NCI-CTC ver. 2.0 or the Seattle criteria, because patients had aHCC underlying LC which already sustained liver injury. In aHCC patients underlying LC, the prediction of hepatotoxicities of IACC was very difficult. Our results might indicate that evaluations of the Child-Pugh score before and after chemotherapy were important for assessments of hepatotoxicities of IACC.

In present study, the serum type IV collagen level and P-III-P level were significantly increased after chemotherapy compared with before chemotherapy in group B, although there was no significant change in hyaluronic acid after chemotherapy in either group. More than 95% of hyaluronic acid entering the blood is taken up and degraded by hepatic sinusoidal endothelial cells [17, 18], so the serum hyaluronic acid level is thought to reflect both endothelial cell function and hepatic fibrosis [14, 19, 20]. There might have been no significant change in serum hyaluronic acid after chemotherapy because the alterations of serum hyaluronic acid reflected endothelial cell inflammation due to IACC. It has been reported that nucleotides of 5-FU are responsible for both its antitumor activity and toxicity [21] and that 5-FU is rapidly degraded by hepatic dihydropyrimidine dehydrogenase (DPD) [22, 23]. It has been reported that long-term oral administration of UFT^{R}, a preparation that contains tegafur and uracil, induces fibrosis in the normal liver without causing elevation of serum aminotransferase levels or necroinflammation, and comparison with liver biopsy specimens that type IV collagen and P-III-P is useful marker for early detection of UFT^{R}-induced hepatic fibrosis [5]. In this report, patients received oral administration for about 20 months. The present study suggested that hepatotoxicity due to IACC induced fibrosis without elevation of serum aminotransferase levels in LC patients with aHCC, although there was an increase of the Child-Pugh score. The hepatic fibrosis inducing IACC might be caused in short-term because patients had aHCC underlying LC and patients were directly injected 5-FU to hepatic artery via a subcutaneously implanted drug delivery system. These findings might indicate that 5-FU suppresses aminotransferase synthesis and induces fibrosis because its decomposition is delayed in patients with LC, or that leukocytes or Kupffer cells release mediators that accelerate the activation of hepatic stellate cell without causing morphologic evidence of necroinflammation [24]. In rats, clearance of 5-FU was relatively well maintained even in the presence of liver damage accompanied by an increase in plasma bilirubin and reduction in CYP2B [25]. However, HCC can spread within the portal venous system, and this may lead to the creation of intrahepatic arteriovenous shunts [26, 27]. In the present study, IACC showed stronger hepatotoxicity in Child-Pugh class B patients compared with those in Child-Pugh class A, which may have been related to arteriovenous shunting, reduction in DPD, or CYP2B. When aHCC patients underlying LC receive IACC, we will be not able to timely detect a fibrosis change, and repeated measurements of various fibrosis markers will cost a great deal. It might be important for early prediction of hepatic fibrosis in LC patients with aHCC receiving IACC that we constantly perform the assessment of the Child-Pugh score.

In conclusion, IACC might induce hepatic fibrosis without releasing aminotransferases, although we could not confirm this by liver biopsy because our LC patients with aHCC were unable to undergo any operations. It might be important to prevent hepatotoxicity due to IACC in order to prolong the survival of patients with aHCC, so we might need to reduce the 5-FU dosage when IACC is done for patients in Child-Pugh class B. Further studies will be needed to assess the optimum dosage of 5-FU in relation to the Child-Pugh class of patients receiving IACC.

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