The Importance of Lamivudine Therapy in Liver Cirrhosis Patients Related HBV with Advanced Hepatocellular Carcinoma Receiving Hepatic Arterial Infusion Chemotherapy

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Abstract: Purpose: We have previously reported that continuous hepatic arterial infusion chemotherapy (HAIC) might be more effective for advanced hepatocellular carcinoma (aHCC) in patients with liver cirrhosis (LC) related to HCV infection (C-LC) or alcohol abuse (A-LC) than in patients who had LC related to HBV infection (B-LC). The aim of the present study was to retrospectively assess the efficacy of lamivudine therapy for B-LC patients with aHCC undergoing HAIC. Methods: Seventeen adult Japanese B-LC patients with aHCC were treated by HAIC with or without lamivudine (100 mg/day) between 2002 and 2008 at our hospital. Their tumors were inoperable according to computed tomography findings. HAIC (LV at 12 mg/hr, CDDP at 10 mg/hr, and 5-FU at 250 mg/22 hr) was given via the proper hepatic artery every 5 days for 4 weeks using a catheter connected to a subcutaneously implanted drug delivery system. Results: Nine of the 17 patients received lamivudine at a dose of 100 mg/day together with HAIC (LAM group), while 8 patients did not receive lamivudine and only had HAIC (non-LAM group). The response rate was 12.5 in the non-LAM group and 0.0 % in the LAM group. However, the survival of the LAM group was better than that of the non-LAM group, although there was no significant difference between them. The median survival time of the LAM and non-LAM groups was 310 and 157 days, respectively. HBV-DNA levels were significantly lower after chemotherapy compared with that before chemotherapy in the LAM group. In the non-LAM group, the percentage of Th2 cells before HAIC and after HAIC was significantly higher than in the control group. However, the percentage of Th2 cells in the LAM group after HAIC was not different from that in the control group, although it was significantly higher in the LAM group than in the control group before chemotherapy. Conclusions: These results indicate that lamivudine therapy may prolong the survival of B-LC patients receiving HAIC for aHCC by reducing HBV-DNA level and inhibiting the increase of Th2 cells in host immunity.

Keywords: HBV, lamivudine, host immunity, hepatocellular carcinoma, hepatic arterial infusion chemotherapy.

I. INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly aggressive cancer that shows rapid progression and characteristically has a high recurrence rate including intrahepatic and multicentric recurrence due to the underlying chronic liver disease even if treatment achieves a complete response [1-3]. Various therapeutic modalities, including surgery, percutaneous ethanol injection, transcatheter arterial embolization, microwave coagulation therapy, and radiofrequency ablation, are used to treat patients with small hepatocellular carcinoma (HCC). However, there are also a considerable number of patients with advanced HCC (aHCC), and intra-arterial chemotherapy is one of their few remaining options. It was reported that the majority of patients with aHCC do not survive for longer than 6 months from the day of diagnosis [4], while other authors have found an average survival period of 4 months from the onset of symptoms or 2 months from the time of admission [5]. Improvement of implanted drug delivery systems has made it possible to perform repeated hepatic arterial infusion of anticancer agents in patients with aHCC, and such hepatic arterial infusion therapy has been found to improve both survival and the quality of life [6]. Continuous local intra-arterial infusion of 5-fluorouracil (5-FU) and cisplatin (CDDP) via an infusion pump and an implanted reservoir has been reported to prolong the survival of patients with aHCC [6-8]. We have also reported that the combination of low-dose intra-arterial 5-FU, CDDP, and leucovorin (LV) prolongs the survival of aHCC patients [9], with continuous intra-arterial infusion for 24 hr being more effective than infusion for 6 hr in patients with aHCC and liver cirrhosis (LC) due to HCV infection, although 24-hr infusion is associated with stronger hematologic toxicity [10]. Furthermore, we have found that hepatic arterial infusion chemotherapy (HAIC) might be more effective for aHCC patients with alcoholic or HCV-related LC than for patients with HBV-related LC [11].
Lamivudine, a nucleoside analog that inhibits reverse transcription by viral DNA polymerase, has been reported to be useful for HBV-infected patients with chronic hepatitis as well as those with decompensated LC [12-15]. It has been reported that lamivudine reduces both the risk of hepatic decompensation and the risk of HCC in patients with chronic HBV infection and advanced hepatic fibrosis [16], and that antiviral therapy contributing reduce incidences of HCC [17-19]. However, the usefulness of its influence on host immunity is still unclear when lamivudine is given to patients with HBV-related LC who are receiving HAIC for aHCC.

When chemotherapy for aHCC is given to LC patients, we must consider the influence of tumor factors, the host immune system, and anticancer drugs. In the present study, we retrospectively investigated the efficacy of antiviral therapy with lamivudine in patients with HBV-related LC receiving HAIC for aHCC in order to clarify the significance of lamivudine therapy and its effect on host immunity.

II. METHODS

Patients

Seventeen adult Japanese patients who had aHCC and LC due to HBV infection were treated with the combination of low-dose intra-arterial 5-FU, CDDP, and LV at our hospital between 2005 and 2008. Of those 17 patients, 9 received lamivudine (Zeffix, Glaxo-Smith-Kline, UK) at a dose of 100 mg/day (LAM group) for as long as possible after starting HAIC at the same time. The remaining 8 patients did not receive lamivudine (non-LAM group). All 17 patients had tumors that were inoperable on the basis of computed tomography findings. Blood samples were collected from each patient in the early morning both before and after chemotherapy. The control group comprised 20 adult Japanese patients with chronic hepatitis C diagnosed by examination of liver biopsy specimens, who had stage 1 disease according to the fibrosis score of Desmet.

Chemotherapy

All patients received 24-hr intra-arterial chemotherapy (LV at 12 mg/hr; Wyeth, Tokyo; CDDP at 10 mg/hr: Yakult, Tokyo; and 5-FU at 250 mg/m² for 22 hr: Kyowa Hakko, Tokyo). Continuous infusion was performed via the proper hepatic artery every 5 days for 4 weeks using a catheter connected to a subcutaneously implanted drug delivery system [9, 10].

In each patient, an intra-arterial catheter was inserted via the femoral artery and was attached to a subcutaneous reservoir [20]. The gastroduodenal artery and the right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the infused anticancer agents. Written informed consent was obtained from all of the patients.

Evaluation of Response

The primary endpoint of the study was the response rate according to World Health Organization (WHO) criteria. The secondary endpoints were progression-free survival and the toxicity of chemotherapy.

Using data from CT scans obtained after 4 weeks of treatment, the product of the 2 longest perpendicular diameters of the largest liver tumor was calculated. A complete response (CR) was defined as disappearance of the tumor, while a partial response (PR) was defined as reduction of the product of the 2 longest diameters by > 50%. An increase of the product by > 25% was defined as progressive disease (PD), while changes between PD and PR were defined as stable disease (SD).

Biochemical Tests

Biochemical tests, including measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were performed by standard methods. HBV-DNA was quantified by PCR assay (AmpliCior HBV monitor assay, Roche Diagnostics, Manheim, Germany). The lower limit of this assay was 2.6 log copies/mL. Tests were done before and after 4 weeks of IACC.

Analysis of CD4-positive T Cell Subsets

CD4-positive T cell subsets in the peripheral blood were analyzed after nonspecific stimulation with phorbol 12-myristate 13-acetate (PMA), ionomycin, or brefeldin A (Sigma Chemical Co., St. Louis, MO, USA), according to the modified method of Jung et al. [21, 22].

Flow cytometry was used to detect IFN-γ and IL-4 expression in the cytoplasm of peripheral blood CD4-positive T cells after culture and staining, as reported previously [21]. The percentage of Th1 and Th2 cytokine-producing cells was investigated among the CD4-positive T cell population, with IFN-γ-positive/IL-4-negative cells being classed as Th1 cells and IFN-γ-negative/IL-4-positive cells as Th2 cells (Fig. 1).

Statistical Analysis

Survival was evaluated by the Kaplan-Meier method, and the significance of differences was determined by the log-rank test. Wilcoxon’s signed rank sum test was used to compare patient characteristics within the same group, while Dunnet’s test was employed to compare patient characteristics among groups. Results are expressed as the mean ± S.D. A probability value of less than 0.05 was considered to indicate statistical significance.

III. RESULTS

The control group was 12 men and 8 women aged 56 to 68 years (mean ± SD, 61.6 ± 4 years). There were 8 men aged from 32 to 72 years (mean ± SD, 57.3 ± 12 years) in the non-LAM group, while there were 8 men and 1 woman aged from 58 to 77 years (mean ± SD, 64.9 ± 8 years) in the LAM group. The Child-Pugh class was A for 2 patients in the non-LAM group and 3 patients in the LAM group, while it was B for 6 and 6 patients, respectively. There were no patients with stage III disease, 4 patients with stage IVA disease, and 4 patients with stage IVB disease in the non-LAM group, while the respective numbers were 1, 4, and 4 in the LAM group. Two patients had a Japan Integrated Staging (JIS) score [23] of 3 and 6 patients had a score of 4 in the non-LAM group, while the respective numbers were 4 and 5 in the LAM group (Table 1).
Table 1. Clinical characteristics of the 17 patients with liver cirrhosis related with HBV.

|                      | Group Non-LAM | Group LAM |
|----------------------|---------------|-----------|
| Number of patients   | 8             | 9         |
| Mean age (mean ± SD) | 57.3 ± 12     | 64.9 ± 8  |
| Gender (M/F)         | 8/0           | 8/1       |
| Child-Pugh classification (A/B/C) | 2/6/0       | 3/6/0    |
| Stage (IIA/IVA/IVB)  | 0/4/4         | 1/4/4     |
| (Vv3: 0, Vv4: 1)     | (Vp3: 2, Vp4: 2) |
| JIS score (2/3/4/5)  | 0/2/6/0       | 0/4/5/0   |

Response in Relation to Lamivudine Therapy

One of the 8 patients in the non-LAM group (12.5%) and none of the 9 patients in the LAM group (0%) achieved PR, while five patients from the non-LAM group (62.5%) and seven patients from the LAM group (77.8%) showed PD. Two of the 8 patients (25.0%) in the non-LAM group had SD, as did two of 9 patients in the LAM group (22.2%) (Table 2).

Survival in Relation to Lamivudine Therapy

The 1-year survival rate of the non-LAM group was 25.0%, while it was 33.3% for the LAM group. The 2-year survival rate of the non-LAM group was 12.5%, while it was 11.1% in the LAM group (Table 3).

The survival time of the LAM group was longer than that of the non-LAM group, with the median survival time being 310 and 157 days, respectively ($P = 0.06$ by Kaplan-Meier analysis with the log-rank test), although there was no significant difference in both groups (Fig. 2).

Serum Aminotransferases

(Fig. 3) displays a comparison of serum aminotransferase levels. There were no significant differences among the three groups with respect to serum ALT or serum AST, and there were also no significant changes of aminotransferases between before and after HAIC in each group (Fig. 3).

Changes of Serum HBV-DNA with Lamivudine Therapy

We examined the changes of the serum HBV-DNA level after HAIC. In the LAM group, the serum HBV-DNA level was significantly lower after HAIC ($3.3 ± 0.8$ logcopies/mL).

Table 2. Objective responses of patients with HBV-treated liver cirrhosis with or without lamivudine therapy.

|                      | CR | PR | SD | PD | Response Rate (%) |
|----------------------|----|----|----|----|-------------------|
| Group non-LAM (n = 8)| 0  | 1  | 2  | 5  | 12.5              |
| Group LAM (n = 9)   | 0  | 0  | 2  | 7  | 0.0               |

Data show the number of patients

CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease
compared with before HAIC (5.0 ± 1.5 logcopies/mL) (P < 0.01, Wilcoxon signed rank sum test), while there was no significant difference of serum HBV-DNA levels between before (5.1 ± 2.1 logcopies/mL) and after HAIC (5.2 ± 2.0 logcopies/mL) in the non-LAM group (Fig. 4).

Table 3. Survival in relation to lamivudine therapy.

| Survival Rate (%) | Group non-LAM (n = 8) | Group LAM (n = 9) |
|-------------------|-----------------------|-------------------|
| 1 year            | 25.0                  | 33.3              |
| 2 years           | 12.5                  | 11.1              |

Peripheral Blood Th1 and Th2 Cells

In the non-LAM group, there was no significant difference in the percentage of Th1 cells before HAIC (24.3 ± 6%) and after HAIC (21.2 ± 9%) compared with that in the control group (23.5 ± 6%). There was also no significant difference in the percentage of Th1 cells before HAIC (26.2 ± 8%) and after HAIC (26.5 ± 8%) in the LAM group compared with that in the control group (Fig. 5). In the non-LAM group, the percentage of Th2 cells before HAIC (5.3 ± 1%) and after HAIC (5.6 ± 2%) was significantly higher than in the control group (2.6 ± 0.8%) (p < 0.05, Dunnet’s test). However, the percentage of Th2 cells in the LAM group after HAIC (3.7 ± 1%) was not different from that in the control group, although it was significantly higher in the LAM group (3.9 ± 2%) than in the control group before chemotherapy (p < 0.05, Dunnet’s test) (Fig. 6).

IV. DISCUSSION

It is well known that lamivudine treatment can achieve biochemical and virological improvement in chronic hepatitis B patients without HCC [24]. However, the effect of antiviral therapy with lamivudine on aHCC in patients with HBV-related LC receiving HAIC is unclear. In the present study, lamivudine did not alter the response rate to HAIC in LC patients with aHCC and HBV infection, although it reduced the serum HBV-DNA level at 4 weeks after the start of treatment. However, lamivudine prolonged the median survival time of the patients, although there was no significant difference of survival between those with or without lamivudine therapy. When chemotherapy is given to LC patients with aHCC, we must consider the effects of tumor factors, the host immune system, and anticancer drugs. This study demonstrated a beneficial effect of lamivudine therapy on the immune system in patients with HBV-related LC receiving HAIC for aHCC. In the non-LAM group, there was no significant difference in the percentage of Th1 cells both before and after HAIC compared with that in the control group. There was also no significant difference in the percentage of Th1 cells before or after HAIC in the LAM group compared with that in the control group. In the non-LAM group, the percentage of Th2 cells before and after HAIC
was also significantly higher than that in the control group. However, the percentage of Th2 cells was after HAIC did not differ between the LAM group and the control group, although it was significantly higher in the LAM group than the control group before chemotherapy. These results might indicate that lamivudine therapy delays the progression of aHCC, although it does not affect the response to HAIC, and that lamivudine prolongs the survival of patients with aHCC and HBV-related LC by inhibiting the increase of Th2 cells.

Th1 and Th2 cells cross-regulate their own development. It has been reported that Th2 cytokines suppress antitumor immunity [25], while activation of a Th1 response promotes antitumor immunity [26-29]. We previously reported that the Th1/Th2 balance might be a useful indicator of the efficacy of HAIC in LC patients with aHCC and that the percentage of Th2 cells is significantly higher (i.e., loss of Th1 dominance) in patients with PD [30]. HCC occasionally develops in healthy HBV surface antigen carriers who have persistent HBV infection but have normal liver function and no necroinflammation [31]. Hepatitis B virus X (HBx) oncoprotein has been implicated in HBV-mediated hepatocarcinogenesis [32, 33], and persistently high expression of HBx in the livers of transgenic mice results in hyperplasia that leads to HCC without a preceding stage of inflammation [34]. Such reports may help to explain why lamivudine therapy did not alter the response rate of aHCC to IACC in LC patients with HBV infection, although it reduced the serum HBV-DNA level. Regulatory T (Treg) cells have an important role in maintaining self-tolerance and modulating the immune response under both physiological and pathological conditions [35]. It has been reported that Treg cells are increased in the peripheral blood and/or tumor tissue of HCC patients and that this increase suppresses CD4+ helper T cell responses and appears to promote the progression of HCC [36-38]. However, it is not known whether lamivudine ther-

Fig. (4). Serum HBV-DNA levels before and after treatment in the non-LAM and LAM groups. HBV-DNA levels were lower after treatment than before treatment in the LAM group (p < 0.01, Wilcoxon’s signed rank sum test).

Fig. (5). Comparison of IFN-γ positive and IL-4 negative CD4-positive T cells (Th1 cells) before and after chemotherapy in the non-LAM and LAM groups. There was no significant difference.

Fig. (6). Comparison of IFN-γ negative and IL-4 positive CD4-positive T cells (Th2 cells) before and after chemotherapy in the non-LAM and LAM groups. The percentage of Th2 cells was significantly higher before and after chemotherapy in the non-LAM group than in the control group. However, the percentage of Th2 cells in the LAM group after chemotherapy did not differ from that in the control group, although it was significantly higher in the LAM group than the control group before chemotherapy (p < 0.05, Dunnet’s test).
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apy reduce peripheral blood Treg cells in patients with HBV-related LC receiving IACC for aHCC. Dendritic cells (DCs) are the most potent antigen-presenting cells with respect to their ability to efficiently prime both CD4-positive and CD8-positive cytotoxic T cells. It has been reported that impaired DC function might be an important factor in allowing tumors to escape from surveillance [39], and that peripheral blood DCs are significantly decreased in cancer patients [40, 41]. It has also been reported that the DCs of cancer patients are mainly immature and thus cannot stimulate T cells [42-44]. Gonzalez-Carmona et al. reported that the intratumoral injection of DCs activates both acquired and innate immunity, inducing complete regression of established tumors and long-term prevention of recurrence, and they concluded that this treatment increases the antitumor activity of DCs [45]. However, it is unknown whether lamivudine therapy leads to differences in the number or function of DCs in LC patients with aHCC, and we could not examine these points in the present study.

In conclusion, our results indicated that lamivudine therapy could prolong the survival of patients with HBV-related LC and aHCC by inhibiting the increase of Th2 cells. Lamivudine therapy may also delay the progression of aHCC, although it does not affect the response to HAIC. Further studies are needed to assess the influence of lamivudine on Treg cells and DCs, as well as on the relation between host immunity and HBx, in patients with HBV-related LC receiving HAIC for aHCC.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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