Send Orders for Reprints to reprints@benthamscience.ae

112 *Clinical Cancer Drugs,* 2015, *2,* 112-118

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

Koichi Momiyama, Hidenari Nagai*, Yu Ogino, Takanori Mukouzu, Daigo Matsui, Michio Kogame, Teppei Matsui, Noritaka Wakui, Mie Shinohara, Yoshinori Igarashi and Yasukiyo 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

K. Momiyama

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 reccurence 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 infuser 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].

*Address correspondence to this author at the 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; Tel: 81-3-3762-4151; Fax: 81-3-3763-8542; E-mail: hidenari@aol.com

2212-6988/15 $58.00+.00

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 Desment.

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/m^2 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 (Amplicor 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**).

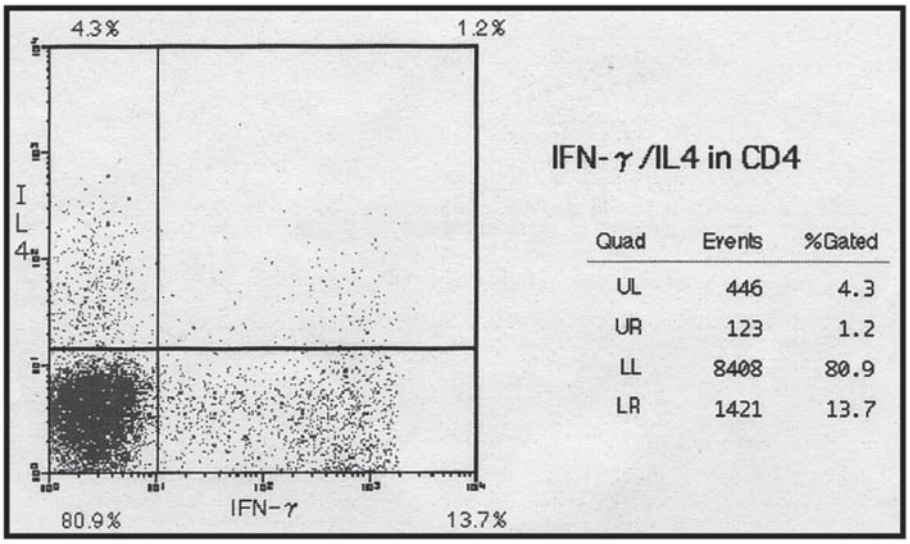

Fig. (1). Flow cytometric detection of interferon (IFN)-γ and interleukin (IL)-4 in CD4-positive T cells. *Upper left* INF-γ negative and IL-4 positive cells (Th2), *lower right* IFN-γ positive and IL-4 negative cells (Th1), *upper right* IFN-γ positive and IL-4 positive cells (Th0).

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 (III/IVA/IVB)	0/4/4	1/4/4
	(Vv3: 0, Vv4: 1)	(Vv3: 2, Vv4: 1)
	(Vp3: 2, Vp4: 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 rest), 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	Group LAM
	(n = 8)	(n = 9)
1 year	25.0	33.3
2 years	12.5	11.1

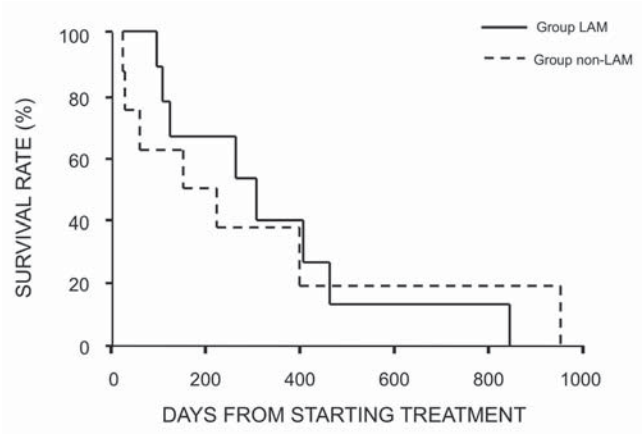

Fig. (2). Survival curves plotted by the Kaplan-Meier method. Among patients with HBV-related LC and aHCC, those treated with lamivudine showed better survival than those without lamivudine, although there was no significant (p=0.06, by Kaplan-Meier method and log-rank test). The median survival time was 310 and 157 days, respectively.

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

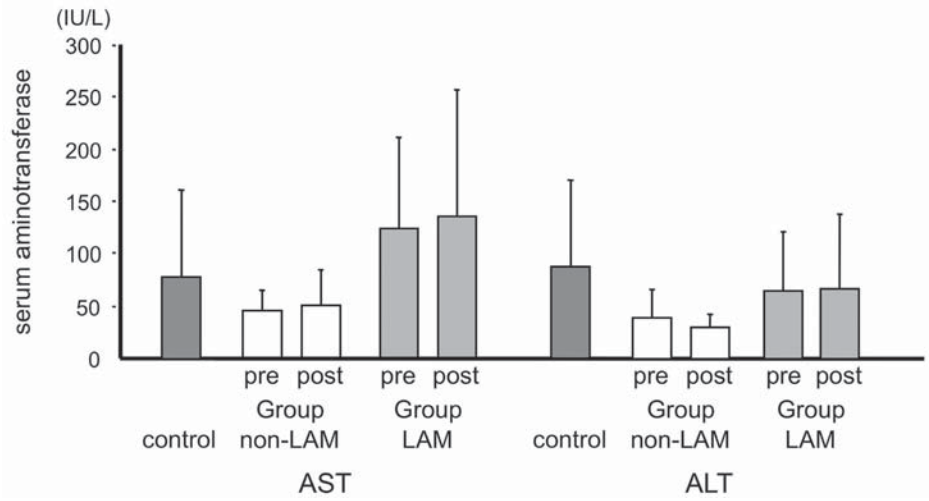

Fig. (3). Comparison of serum aminotransferases before and after IACC in the non-LAM and the LAM groups. There were no significant differences.

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.

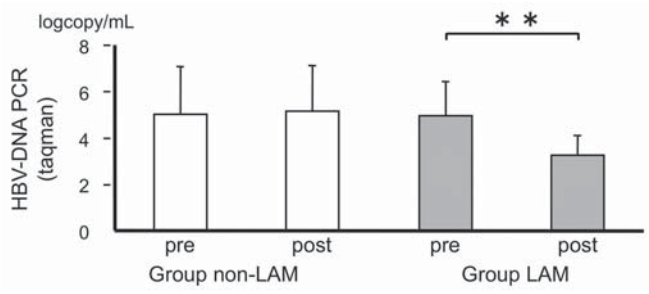

Fig. (4). Serum HBV-DNA levels before and after treatment in the non-LAM and LAM groups. HBV-DNA levels were lower after treatment than before treatment in the LAM group ($p < 0.01$, Wilcoxon's signed rank sum test).

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-

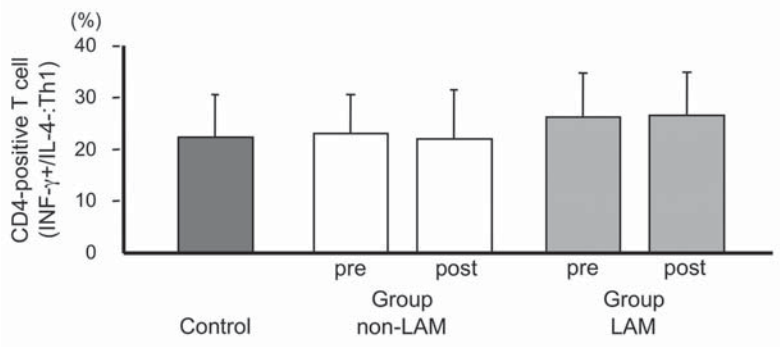

Fig. (5). Comparison of IFN-γ positive and IL-4 negative CD4-positive T cells (Th1 cells) before and after chemotherapy in the non-LAM and LAM groups. There was no significant difference.

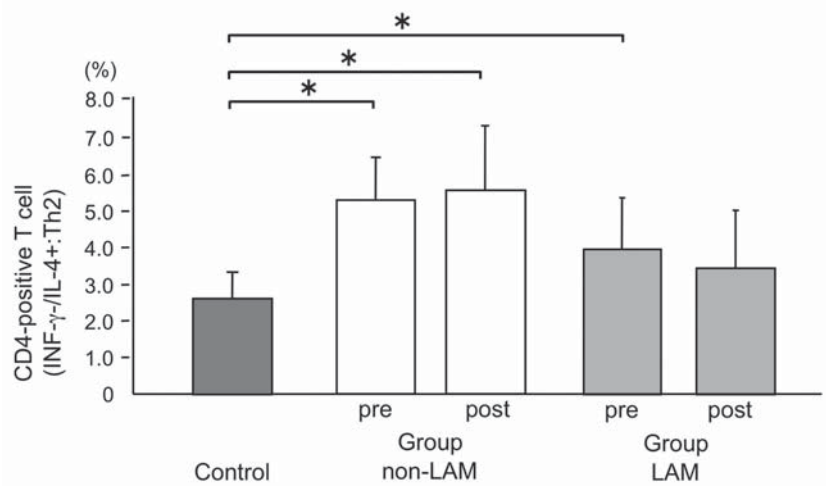

Fig. (6). Comparison of IFN-γ negative and IL-4 positive CD4-positive T cells (Th2 cells) before and after chemotherapy in the non-LAM and LAM groups. The percentage of Th2 cells was singnificantly higher before and after chemotherapy in the non-LAM group than in the control group. However, the percentage 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).

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.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Kumada T, Nakano S, Takeda I, *et al.* Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. Hepatology 1997; 25: 87-92.

[2] Ou DP, Yang LY, Huang GW, Tao YM, Ding X, Chang ZG. Clinical analysis of the risk factors for recurrence of HCC and its relationship with HBV. World J Gastroenterol 2005; 11: 2061-2066.

[3] Lin SM, Lin CJ, Hsu CW, *et al.* Prospective randomized controlled study of interferon-alpha in preventing hepatocellular carcinoma recurrence after medical ablation therapy for primary tumors. Cancer 2004; 100: 376-382.

[4] Okuda K, Ohtsuki T, Obata H, *et al.* Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer 1985; 56: 918-928.

[5] Nagasue N, Yukaya H, Hamada T, Hirosue S, Kanashima R, Inokuchi K. The natural history of hepatocellular carcinoma. A study of 100 untreated cases. *Cancer* 1984; 54: 1461-1465.

[6] Toyoda H, Nakano S, Kumada T, *et al.* The efficacy of continuous local arterial infusion of 5-fluorouracil and cisplatin through an implanted reservoir for severe advanced hepatocellular carcinoma. Oncology 1995; 52: 295-299.

[7] Murata K, Shiraki K, Kawakita T, *et al.* Low-dose chemotherapy of cisplatin and 5-fluorouracil or doxorubicin via implanted fusion port for unresectable hepatocellular carcinoma. Anticancer Res 2003; 23: 1719-1722.

[8] Okuda K, Tanaka M, Shibata J, *et al.* Hepatic arterial infusion chemotherapy with continuous low dose administration of cisplatin and 5-fluorouracil for multiple recurrence of hepatocellular carcinoma after surgical treatment. Oncol Rep 1999; 6: 587-591.

[9] Nagai H, Sumino Y. Therapeutic strategy of advanced hepatocellular carcinoma by using combined intra-arterial chemotherapy. Recent Pat Anticancer Drug Discov 2008; 3: 220-226.

[10] Nagai H, Kanayama M, Higami K, *et al.* Twenty-four hour intra-arterial infusion of 5-fluorouracil, cisplatin, and leucovorin is more effective than 6-hour infusion for advanced hepatocellular carcinoma. World J Gastroenterol 2007; 13(2): 280-284.

[11] Kanayama M, Nagai H, Sumino Y. Influence of the etiology of liver cirrhosis on the response to combined intra-arterial chemotherapy in patients with advanced hepatocellular carcinoma. Cancer Chemother Pharmacol 2009; 64: 109-114.

[12] Papatheodoridis GV, Dimou E, Dimakopoulos K, *et al.* Outcome of hepatitis B e antigen-negative chronic hepatitis B on long-term nucleos(t)ide analog therapy starting with lamivudine. Hepatology 2005; 42: 121-129.

[13] Dienstag JL, Goldin RD, Heathcote EJ, *et al.* Histological outcome during long-term lamivudine therapy. Gastroenterology 2003; 124: 105-117.

[14] Yao FY, Terrault NA, Freise C, Maslow L, Bass NM. Lamivudine treatment is beneficial in patients with severely decompensated cirrhosis and actively replicating hepatitis B infection awaiting liver transplantation: a comparative study using a matched, untreated cohort. Hepatology 2001; 34: 411-416.

[15] Villeneuve JP, Condreay LD, Willems B, *et al.* Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. Hepatology 2000; 31: 207-210.

[16] Liaw YF, Sung JJ, Chow WC, *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004; 351: 1521-1531.

[17] Lok AS. Prevention of hepatitis B virus-related hepatocellular carcinoma. Gastroenterology 2004; 127: S303-309.

[18] Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999; 29: 971-975.

[19] van Zonneveld M, Honkoop P, Hansen BE, *et al.* Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004; 39: 804-810.

[20] Iwamiya T, Sawada S, Ohta Y. Repeated arterial infusion chemotherapy for inoperable hepatocellular carcinoma using an implantable drug delivery system. Cancer Chemother Pharmacol 1994; 33(Suppl.): S134-138.

[21] Shinohara M, Ishii K, Takamura N, *et al.* Long-term changes of peripheral blood CD4-positive T cell subsets (Th1, Th2) in chronic hepatitis C patients with a sustained response no response to IFN. Hepatol Res 2003; 27: 260-265.

[22] Jung T, Schauer U, Heusser C, Neumann C, Rieger C. Detection of intracellular cytokines by flow cytometry. J Immunol Methods 1993; 159: 197-207.

[23] Kudo M, Chung H, Osaki Y, *et al.* Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for new staging system, the Japan Integrated Staging score (JIS score). J Gastroenterol 2003; 38: 207-215.

[24] Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001; 34: 1225-12241.

[25] Kobayashi M, Kobayashi H, Polland RB, *et al.* A pathogenic role of Th2 cells and their cytokine products on the pulmonary metastasis of murine B16 melanoma. J Immunol 1998; 160: 5869-73.

[26] Zitvogel L, Mayordomo JI, Tjandrawan T, *et al.* Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, and T helper cell 1-associated cytokines. J Exp Med 1996; 183: 87-97.

[27] Tsung K, Meko JB, Peplinski GR, *et al.* IL-12 induces T helper 1-directed antitumor response. J Immunol 1997; 158: 3359-65.

[28] Weiner GJ, Liu HM, Wooldridge JE, *et al.* Immunostimulatory oligodeoxynucleotides containing the CpG motif are effective as immune adjuvants in tumor antigen immunization. Proc Natl Acad Sci USA 1997; 94: 10833-7.

[29] Hu H-M, Urba WJ, Fox BA. Gene-modified tumor vaccine with therapeutic potential shifts tumor-specific T cell response from Type 2 to a type 1 cytokine profile. J Immunol 1998; 161: 3033.

[30] Momiyama K, Nagai H, Sumino Y. Changes of host immunity in relation to efficacy in liver cirrhosis patients with advanced hepatocellular carcinoma treated by intra-arterial chemotherapy. Cancer Chemother Pharmacol 2009; 64: 271-277.

[31] Popper H, Shafritz DA, Hoofnagle JH. Relation of the hepatitis B

virus carrier state to hepatocellular carcinoma. Hepatology 1987; 7: 764-772.

[32] Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 1991; 351: 317-320.

[33] Yu DY, Moon HB, Son JK, *et al.* Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. J Hepatolo 1999; 31: 123-132.

[34] Koike K, Moriya K, Iino S, *et al.* High-level expression of hepatitis B virus HBx gene and hepatocarcinogenesis in transgenic mice. Hepatology 1994; 19: 810-819.

[35] Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004; 22: 531-562.

[36] Unitt E, Rushbrook SM, Marshall A, *et al.* Compromised lymphocytes infiltrate hepatocellular carcinoma: the role of T-regulatory cells. Hepatology 2005; 41: 722-730.

[37] Ormandy LA, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. Cancer 2005; 65: 2457-2464.

[38] Yang XH, Yamagiwa S, Ichida T, *et al.* Increase of CD4+ CD25+ regulatory T cells in the liver of patients with hepatocellular carcinoma. J Hepatol 2006; 45: 254-262.

[39] Gabrilovich D. Mechanisms and functional significance of tumor-induced dendritic-cell defects. Nat Rev Immunol 2004; 4: 941-952.

[40] Hoffmann TK, Muller-Berghaus J, Ferris RL, Johnson JT, Storkus WJ, Whiteside TL. Alterations in the frequency of dendritic cell subsets in the peripheral circulation of patients with squamous cell carcinoma of the head and neck. Clin Cancer Res 2002; 8: 1787-1793.

[41] Della Bella S, Gennaro M, Ferraris C, *et al.* Altered maturation of peripheral blood dendritic cells in patients with breast cancer. Br J Cancer 2003; 89: 1463-1472.

[42] Gabrilovich DI, Corak J, Ciernik IF, Kavanaugh D, Carbone DP. Decreased antigen presentation by dendritic cells in patients with breast cancer. Clin Cancer Res 1997; 3: 483-490.

[43] Ishida T, Oyama T, Carbone DP, Gabrilovich DI. Defective function of langerhans cells in tumor-bearing animals is the results of defective maturation from hemopoietic progenitors. J Immunol 1998; 161: 4842-4851.

[44] Ormandy LA, Farber A, Cantz T, *et al.* Direct ex vivo analysis of dendritic cells in patients with hepatocellular carcinoma. World J Gastroenterol 2006; 12: 3275-3282.

[45] Gonzalez-Carmona MA, Lukacs-Kornek V, Timmerman A, *et al.* CD40Ligand-expressing dendritic cells induce regression of hepatocellular carcinoma by activating innate and acquired immunity *in vivo*. Hepatology 2008; 48: 157-168.

Received: December 24, 2014 **Revised: April 08, 2015** **Accepted: June 02, 2015**