Korean J Hepatobiliary Pancreat Surg 2016;20:8-11
http://dx.doi.org/10.14701/kjhbps.2016.20.1.8

Original Article

High-dose tenofovir is not effective in suppressing hepatitis B virus replication in patients with hepatocellular carcinoma progression: a preliminary result

Shin Hwang[1], Gi-Won Song[1], Dong-Hwan Jung[1], Young-In Yoon[1], Hyun Ju Yoo[2], and Eunyoung Tak[2]

[1]Department of Surgery and [2]Asan Institute for Life Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Backgrounds/Aims: Nucleos(t)ide analogues (NUCs) effectively suppress hepatitis B virus (HBV) replication, but hepatocellular carcinoma (HCC) recurrence often leads to HBV replication despite NUC therapy. The aim of this study was to determine whether high-dose tenofovir (TNF) therapy can suppresses HCC recurrence-associated HBV replication. **Methods:** We performed a single-arm prospective study to assess the clinical feasibility of high-dose TNF (hdTNF). We recruited 10 patients during September 2015 and followed up for 3 months or early drop-out. **Results:** All 10 patients had HCC of advanced stages due to HCC recurrence and gradual progression. The average age of patients was 51.2±4.7 years and 9 were male. Three patients did not tolerate the increased TNF dosage and were dropped out early. The other 7 patients were relatively tolerable to the increased dosage of TNF 5 tablets per day. One patient had mild gastrointestinal symptoms and another patient complained of insomnia. Increased HBV replication and HCC progression was observed despite hdTNF for 4-8 weeks. All 7 patients showed tumor progression during the 3 month follow-up. In these patients, blood HBV DNA before hdTNF was 50-200 copies/ml; and 4-8 weeks after hdTNF, the HBV replication status was not improved with blood HBV DNA of 50-300 copies/ml. This clinical study was terminated early after these negative results were confirmed. **Conclusions:** The results of this study indicated that high dose of TNF up to 5-fold the recommended dosage is not tolerated by a considerable proportion of patients and also ineffective in suppressing HCC progression-associated HBV replication. **(Korean J Hepatobiliary Pancreat Surg 2016;20:8-11)**

Key Words: Nucleoside analogues; Entecavir; Covalently closed circular DNA; HBV X protein; Recurrence

INTRODUCTION

It is well recognized that hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC) development are closely associated. Inhibition of HBV replication through nucleos(t)ides (NUCs) administration effectively prevents *de novo* HCC development and HCC recurrence in addition to blockage of fibrosis progression.[1-5] NUC with a high genetic barrier to resistance such as entecavir and tenofovir (TNF) can effectively suppress replication of HBV for a long time, but it is generally regarded that these agents do not control the covalently closed circular DNA (cccDNA) or integrated HBV DNA in the hepatocytes.[6]

Active replication of HBV in HCC cells acting as a viral reservoir is well recognized as the clinical sequence of HCC recurrence following resection or liver transplantation.[7] It is generally accepted that HCC recurrence is a risk factor for posttransplant HBV recurrence.[7-9] Similarly, the blood HBV DNA can be converted to detectable status after HCC recurrence following NUC maintenance, despite negative status for a long time after HCC treatment.

The aim of the study was to determine whether high-dose TNF therapy (hdTNF) could suppress HCC recurrence-associated HBV replication. We accordingly performed a clinical study and presented the preliminary result.

Received: January 9, 2016; **Revised:** January 20, 2016; **Accepted:** January 25, 2016
Corresponding author: Shin Hwang
Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-3930, Fax: +82-2-3010-6701, E-mail: shwang@amc.seoul.kr

MATERIALS AND METHODS

This study was a single-arm prospective study to assess the clinical applicability of hdTNF. The primary purpose of this study was to assess whether hdTNF is tolerable to the adult patients and can suppress HCC recurrence-associated HBV replication. The secondary purpose was to determine whether it could have any oncological influence on HCC.

The patient selection criteria was as follows: HBV patients who were administered entecavir or TNF for more than 1 year; HCC recurrence and progression to the advanced stage according to Barcelona Clinic Liver Cancer (BCLC)/American Association for the Study of Liver Diseases (AASLD) guideline update in 2014;[10] Child class A and Eastern Cooperative Oncology Group (ECOG) 0 or 1; and normal renal function.

We recruited 10 patients who met the selection criteria during one month of September 2015. TNF (tenofovir disoproxil fumarate 300 mg: VIREAD, Gilead Sciences) was selected as the test NUC because it is the most potent NUC against HBV. The protocol of hdTNF included 2 tablets of TNF per day for first 2 days; if tolerable, the dosage was increased to 3 or 4 tablets per day for the next 2-4 days; and finally 5 tablets per day and if tolerable, 5 tablets were maintained for 4 weeks or more. All patients were followed up every week for first 3 weeks and then every other week until termination of hdTNF therapy. If any side-effect beyond mild gastrointestinal symptoms occurred, the patient was dropped out immediately.

The patients were followed up with liver function test, blood HBV polymerase chain reaction (PCR), and computed tomography/magnetic resonance imaging studies on HCC status. This study protocol was approved by the Institutional Review Board of our institution.

RESULTS

All 10 patients had HCC of advanced stage due to HCC recurrence and gradual progression. The average age of the patients was 51.2±4.7 years (range: 35-64) and 9 were male. Their initial treatments for HCC were liver resection in 8, liver transplantation in 1, and non-surgical treatment in 1. Despite their advanced tumor, the general condition was relatively good, with ECOG 0 or 1 in all patients.

Of these patients, 3 absolutely did not tolerate the increased dosage of TNF, even 2 tablets per day, because of serious gastrointestinal symptoms such as nausea and anorexia. Thus, these 3 patients were dropped out early after only 3-5 days of TNF medication. Two of them were administered TNF previously, thus they were reverted to TNF 1 tablet per day with loss of gastrointestinal symptoms. One patient who was administered entecavir did not tolerate even TNF 1 tablet per day, and was reverted to entecavir 0.5 mg per day.

The other 7 patients were relatively tolerable to the increased dosage of TNF 5 tablets per day. One patient had mild gastrointestinal symptoms and another patient complained of insomnia. They maintained on hdTNF for 4-8 weeks until overt demonstration of no beneficial effect on both HBV replication and HCC progression. One patient was administered sorafenib concurrently, but HCC slowly progressed during the 3-month follow-up. One liver transplant recipient received everolimus monotherapy for immunosuppression without use of calcineurin inhibitors or mycophenolate. Three patients received transcatheter arterial chemotherapy (TACE) and 1 patient underwent stereotactic beam radiotherapy (SBRT) during the 3-month follow-up. All 7 patients showed tumor progression during the 3-month tumor marker study and computed tomography follow-up. All 7 patients showed detectable blood HBV DNA (50-200 copies/ml) before hdTNF and their HBV replication status was not improved, showing detectable blood HBV DNA of 50-300 copies/ml after 4-8 weeks of hdTNF therapy.

This clinical study was terminated early after confirming these negative results.

DISCUSSION

The preliminary results of this study indicated that hdTNF is not well tolerated by half of the adult patients, as well as ineffective in suppressing viral replication during HCC progression. Antitumor effect was also not expected in patients who were administered hdTNF over 1-2 months. The study was terminated early because we confirmed that further treatment with hdTNF could not lead to other conclusions.

Based on the results of this study, the recommended dosage of TNF 300 mg/day for adult patients appears to

be maximally adjusted by the relatively high occurrence of side effects such as gastrointestinal symptoms. Since TNF can be administered at any time during the day, patients can take the oral TNF medication after food intake; but such gastrointestinal symptoms were often unimproved, as compared with TNF taken before food ingestion. Thus, double-dose TNF is not recommended, unlike entercavir double-dose therapy (1 mg per day). The recommended daily NUC dosage differs considerably between entecavir and TNF. The steady-state maximal and trough plasma concentrations are 4.2 ng/ml and 0.3 ng/ml for entecavir 0.5 mg and 0.3 μg/ml and 0.07 μg/ml for TNF 300 mg. Interestingly, the molecular weights of the these two NUCs are very similar (277.3 g/mol for entecavir and 287.2 g/mol for TNF).

Interestingly, we found that a very high-dose TNF can suppress both cccDNA load and HBx expression in a cell line study (unpublished data). In our preceding studies, we presumed that the NUCs effects are mediated by several cascade mechanisms. NUC administration in the clinical setting decreased HBV DNA load effectively in blood and liver tissues. HBV cccDNA load in the liver tissue was also decreased along with the decreased HBV DNA load in the liver tissue, but HBV eradication was not achieved. HBV-associated HCC cells presented definitely higher expression of HBV cccDNA and HBx than the adjacent non-tumor liver tissues, suggesting that such high rises are closely associated with hepatocarcinogenesis. Very high-concentration NUCs induced the downregulation of HBx transcription and apoptosis in cccDNA-positive HCC cell lines, which was dependent on NUC concentration and treatment duration. Briefly, the doses of NUCs within the *in vivo* therapeutic range reduce viral loads in both liver tissues and blood; but only very high-concentration TNF can decrease the HBV cccDNA load and HBx production in HCC cells with induction of HCC cell apoptosis. This direct antitumor effect on pre-existing or replicating cccDNA-containing HCC cells themselves has not yet been reported, probably because the clinical therapeutic TNF concentration was too low to induce such an antitumor effect. The results of the current study indicated that the 5-fold dosage of TNF does not have any antitumor effect.

Active replication of HBV in HCC cells acting as a viral reservoir has been well recognized as the clinical sequence of HCC recurrence and treatment after LT.[7] A previous report on 1 patient who presented with both HBV and HCC recurrences, indicated that HBV surface antigen disappeared after resection of the adrenal metastasis but reappeared after the second extrahepatic tumor recurrence. In this patient, HBV DNA was detected in the adrenal metastasis but not in the liver graft. In another patient who presented with HBV and HCC recurrences, the HBV DNA sequences simultaneously obtained from both the recurring tumor and serum were identical and exhibited a marked alteration within the major hydrophilic region when compared with the pretransplant sequences. It is generally accepted that HCC recurrence is a risk factor for HBV recurrence or replication following liver transplantation or resection.[7-9]

In conclusion, the result of our study indicated that hdTNF using up to 5-folds of the recommended dosage is not only intolerable to a considerable proportion of patients but also ineffective to suppress HCC progression-associated HBV replication.

REFERENCES

1. Wu CY, Lin JT, Ho HJ, Su CW, Lee TY, Wang SY, et al. Association of nucleos(t)ide analogue therapy with reduced risk of hepatocellular carcinoma in patients with chronic hepatitis B: a nationwide cohort study. Gastroenterology 2014;147:143-151.e5.
2. Sung JJ, Tsoi KK, Wong VW, Li KC, Chan HL. Meta-analysis: Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. Aliment Pharmacol Ther 2008;28:1067-1077.
3. Kumada T, Toyoda H, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, et al. Effect of nucleos(t)ide analogue therapy on hepatocarcinogenesis in chronic hepatitis B patients: a propensity score analysis. J Hepatol 2013;58:427-433.
4. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology 2013;58:98-107.
5. Wu CY, Chen YJ, Ho HJ, Hsu YC, Kuo KN, Wu MS, et al. Association between nucleoside analogues and risk of hepatitis B virus–related hepatocellular carcinoma recurrence following liver resection. JAMA 2012;308:1906-1914.
6. Baran B. Nucleos(t)ide analogs in the prevention of hepatitis B virus related hepatocellular carcinoma. World J Hepatol 2015;7:1742-1754.
7. Faria LC, Gigou M, Roque-Afonso AM, Sebagh M, Roche B, Fallot G, et al. Hepatocellular carcinoma is associated with an increased risk of hepatitis B virus recurrence after liver transplantation. Gastroenterology 2008;134:1890-1899.
8. Hwang S, Ahn CS, Song GW, Kim KH, Moon DB, Oh HB, et al. Posttransplantation prophylaxis with primary high-dose hepatitis B immunoglobulin monotherapy and complementary preemptive antiviral add-on. Liver Transpl 2011;17:456-465.
9. Chun J, Kim W, Kim BG, Lee KL, Suh KS, Yi NJ, et al. High

viremia, prolonged Lamivudine therapy and recurrent hepatocellular carcinoma predict posttransplant hepatitis B recurrence. Am J Transplant 2010;10:1649-1659.

10. Bruix J, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology 2011;53:1020-1022.