Postoperative adjuvant arterial chemoembolization improves the survival of hepatitis B virus-related hepatocellular carcinoma: a retrospective control study

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Abstract

Background The survival benefit of postoperative adjuvant transcatheter arterial chemoembolization (TACE) remains controversial.

Aims We aim to investigate the survival effect of postoperative adjuvant TACE on the prognosis of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) patients (stage B, the Barcelona Clinic Liver Cancer staging).

Methods Sixty consecutive HBV-related HCC patients (stage B) from February 2006 to May 2009 undergoing surgical resection were included in this study. Of these 60 patients, 34 patients underwent surgery only (Group A) and 26 patients underwent surgery plus TACE (Group B). We followed-up until May 2013. Overall survival rates as well as prognostic factors were analyzed by the Kaplan–Meier method, the log-rank test or Cox’s proportional hazard model. All patients’ data were collected from the hospital medical records, which were described precisely after accurate clinical samples detection.

Results The 1-, 2-, and 3-year overall survival rates in surgery-only group were 58.8, 32.4 and 12.6 %, and the rates in surgery plus TACE group were 73.1, 61.5, and 48.9 %, respectively \( (P = 0.033) \). The median survival time of the two groups after surgery and surgery plus TACE was 15.0 months [95 % confidence interval (CI) 10.714–19.286] and 35.0 months (95 % CI 20.974–49.026). In multivariate analysis, hemoglobin, HBeAg, peripheral blood regulatory T cells and tumor size were independent prognostic elements for HBV-related HCC patients (stage B).

Conclusions Postoperative adjuvant TACE improves the survival of patients with HBV-related HCC (stage B) after curative resection compared to surgery only.

Keywords Hepatocellular carcinoma · Transcatheter arterial chemoembolization · Surgery · HBV related · Regulatory T cells

Introduction

Hepatocellular carcinoma (HCC) accounts for more than 80 % of all primary liver cancers. HCC is a complex condition because of multiple variables, such as liver function, performance status of the patient, and tumor conditions, that can affect the course of HCC and the response to treatment [1, 2]. Hepatitis B virus (HBV) infection is also an important risk factor for the development of HCC, and over 85 % of patients with HCC present with HBV infection in China [3]. Lai et al. [4] concluded that HBV was closely linked to HCC, because integrated HBV fragments were frequently detected in the tumors of patients with positive hepatitis B surface antigen (HBsAg). High HBV DNA load has often been reported to have a correlation with high postoperative recurrence [5–7], but its impact on postoperative survival remains uncertain [5, 8,
It is important to ensure a high validity of HBV DNA for the assessment for postoperative HCC prognosis to provide a strong foundation for the use of antiviral therapy in the prevention of HCC recurrence.

For patients with Barcelona Clinic Liver Cancer (BCLC) stage B HCC (single nodule >5 cm or multinodular tumors; Okuda stage 1–2; Child–Pugh A–B; performance scale 0) [10], hepatic resection for BCLC stage B HCC has been considered a contraindication with unfavorable prognosis [11]. However, some authors have demonstrated good results of hepatic resection for large (tumor >5 cm) or multinodular HCC [12, 13]. And also Lin et al. [14] reported that hepatic resection for BCLC stage B HCC had better survival rates than transcatheter arterial chemoembolization (TACE). The results revealed that hepatic resection may be a choice for BCLC stage B HCC patients. However, even after curative resection, the prognosis for these patients is still not we expected because of the high recurrence rate (59 %) due to microscopic tumor thrombosis [15]. Therefore, the prevention of recurrence constitutes one of the most important challenges in improving surgical efficacy. Systemic chemotherapy has been attempted, but a large number of controlled and uncontrolled studies have been performed with most classes of chemotherapeutic agents, no single or combination chemotherapy regimen is significantly effective in HCC [16, 17], only a few of them have shown improved response rates [18].

Recently, it has become possible to administer repeated arterial infusions of chemotherapeutic agents because of new implantable drug delivery systems [19]. TACE or hepatic arterial infusion chemotherapy has the advantages of increasing local drug concentrations while reducing systemic side effects, is minimally invasive, and shows better reproducibility as compared with traditional surgical approaches. Furthermore, TACE induces a marked antitumor effect in HCC. When hepatic reserves are sufficient, TACE can be administered for any type of HCC, regardless of tumor size, tumor location, or tumor number [20]. Some clinical studies and meta-analyses have reported that postoperative adjuvant TACE may improve survival outcomes and reduce the recurrence rate [21–24]. Therefore, they may have a tendency for improved survival in those treated with surgery plus adjuvant TACE. TACE as adjuvant therapy is not a standard treatment currently, although uncovering the selection criteria, such as a specific cancer stage, may make adjuvant TACE a standard therapy. The aim of this retrospective control study was to explore the effect of postoperative adjuvant TACE on survival of patients with HCC (stage B of BCLC) after curative resection and to analyze prognostic factors with a few years follow-up. The analysis of prognostic factors may be helpful in predicting the life expectancy of HBV-related HCC patients treated with surgery plus adjuvant TACE and in the design of future clinical trials on adjuvant TACE for HCC.

Patients and methods

Patients

In total, 60 patients with HBV-related HCC treated between February 2006 and May 2009 at the Tianjin Medical University Cancer Hospital and Institution, China, were included in this study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Tianjin Anti-Cancer Association. These 60 cases were selected according to the following inclusion criteria: (1) had not received any prior treatments for HCC except hepatic curative resection treatment, which did not include liver transplantation (LT); (2) classified as stage B according to the BCLC staging classification [10]; (3) showed good or moderate liver function (Child–Pugh stage A or B evaluated according to the score of hepatic encephalopathy, ascites, bilirubin, albumin, and prothrombin time); (4) were HBsAg positive; (5) no distant metastasis and no contraindication for laparotomy; (6) The diagnosis of HCC was confirmed by biopsy. Patients were excluded if they did not meet the criteria above or: (1) received systemic chemotherapy, p.o. chemotherapy, portal vein chemotherapy or immune treatment (e.g., thymosin, interferon, lamivudine); (2) did not use lipiodol or embolic agent; (3) received anti-virus therapy. All patients were initially assigned to hepatic resection or hepatic resection plus adjuvant TACE according to the patients’ choice after they were informed of the surgical risk and the present treatment guidelines for BCLC stage B. None of the patients received any other treatments for HCC during the follow-up period. All patients’ data were collected from the hospital medical records, which were described precisely after accurate clinical samples detection, and cases of incomplete information were excluded.

Treatment methods of surgery and transcatheter arterial chemoembolization

Surgery was carried out under general anesthesia via an L-shaped laparotomy or bilateral subcostal incision with midline extension intraoperative ultrasound (US), which was routinely used. Hepatic resection with a resection margin of at least 1 cm was accomplished with/without the Pringle Maneuver [25]. We used intermittent Pringle’s
mechanism of 20 min and a 5-min clamp-free interval to reduce blood loss. Two size 10 Jackson-Pratt drains were placed after surgery. Among these 60 cases, 34 (56.7 %) cases had received segmentectomy and 26 (43.3 %) had received subsegmentectomy or wedge resection.

TACE was performed (first treatment 1 month after hepatectomy and the time interval between the two TACE treatments was 4–6 weeks to protect liver function) by selectively introducing a catheter into the proper, right, or left hepatic artery or a segmental branch of the hepatic artery and injecting iodized oil (Lipiodol: Guerbet, Paris, France) and anti-cancer agents followed by 30–50 mg/kg epirubicin. Prior to TACE, all the patients underwent digital subtraction angiography of the celiac artery and superior mesenteric artery and computed tomography (CT) during hepatic arteriography and arterial portography to identify the nourishing artery and reconfirm the possible site of the microscopic foci of HCC. To preserve liver function, highly selective catheterization was performed using a 2.5-F microcatheter to achieve complete occlusion of the feeding arteries. Embolization was subsequently performed using a small amount of contrast medium under fluoroscopic guidance.

Follow-up assessments

Before therapy, all patients were evaluated with a baseline history and physical examination, serum laboratory tests, and appropriate imaging studies and pathological diagnosis. All patients were followed-up from the date of initial treatment up to May 2013, or up to the time of death, for survival analysis. The antitumor effects of TACE are paramount to determine the time interval for repeated treatments and to identify treatment failure. A median of three TACE treatments (range 1–14) were performed in group B patients during the follow-up period. CT was performed to assess tumor response every 4–6 weeks after TACE. Subsequent follow-up examinations included serum levels of alpha-fetoprotein (AFP), peripheral blood regulatory T cells (Tregs), peripheral blood HBV DNA load, and other biochemistry tests at the time of monitoring the tumor response by CT, so did the operation only group.

Factors analyzed

Pretreatment clinical variables including host-related, tumor-related, and treatment-related factors were investigated for their relationship with survival using univariate and multivariate analyses. The pretreatment variables were chosen on the basis of our own clinical experience or on the basis of possible effects on prognosis as indicated in previous studies [7, 9, 26, 27]. Host-related variables include age, gender (male or female), serum total bilirubin, serum alanine transaminase (ALT), serum aspartate aminotransferase (AST), serum AFP, serum hemoglobin, serum hepatitis B surface antigen (HBsAg) (positive or negative), peripheral blood Tregs, ascites (positive or negative), arteriovenous fistula, cirrhosis (positive or negative), and peripheral blood HBV DNA load. Tumor-related variables were tumor number (single or multiple) and tumor size. The treatment-related variable was the type of liver resection.

Serological test for Tregs, AFP, and HBV DNA load and liver tests

In the present study, CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells (PerCP anti-human CD4, PE/Cy7 anti-human CD25, PE anti-human Foxp3; BioLegend) were considered to be Tregs. The cells were analyzed on an LSR II (BD Biosciences) flow cytometer. Flow cytometry data were further analyzed on FlowJo (Treestar, Inc.). Serum AFP was determined using an immunoradiometric assay (IRMA: RIA-gnost AFP; Cis-Bio International, Schering, Switzerland) based on the principle of the sandwich assay with 125I-labeled anti-AFP monoclonal antibody. Serum HBV DNA tests were performed using the Digene hybrid capture assay (Roche Diagnostics, Branchburg, NJ) (lower limit of detection: 500 copies/mL). HBsAg was identified using radioimmunoassay (Abbott Laboratories, North Chicago, IL). Serum biochemistry assessments, including those for bilirubin, ALT, and AST were performed using a systemic multiautoanalyzer (Technicon SMAC; Technicon Instruments Corp., Tarrytown, NY).

Statistical analysis

Univariate and multivariate analyses were performed to evaluate the possible factors that related to the prognosis and survival outcomes. Survival time was defined as the time from the date of the first TACE to the date of death or the date of the last follow-up. Comparisons between the two groups were made with Chi square test for categorical data and the Student’s t test for continuous data. Survival curves and overall survival rates were estimated using the Kaplan–Meier method and compared using the log-rank test or Cox’s proportional hazard model. All variables considered in univariate analysis were entered in the multivariate model, and variable selection was not performed. Variables with $P < 0.05$ were defined as statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL).
Results

Symptoms in HCC patients receiving TACE

Many symptoms related to the primary disease are also common to TACE therapy. In this study, of the group B patients, common symptoms included nausea, vomiting caused by chemotherapy in 15.38 % (4/26) of patients, severe abdominal pain in 3.85 % (1/26), stomach discomfort due to acute gastric mucosal lesion in 7.69 % (2/26), and transient fever and abdominal discomfort in almost all patients. Complications included hemorrhage in 7.69 % (2/26), mild or moderate liver decompensation in 3.85 % (2/26), and ascites in 3.85 % (1/26) of the patients. Catheter-associated complications included obstruction in 3.85 % (1/26), infection in 7.69 % (2/26), and agent leaking in 3.85 % (1/26) of patients. There was no hematoma around the injection site or dislocation of the catheter tip. Remedial measures for the symptoms and complications were performed on time. No intraoperative death occurred and no patients died within 30 days postoperatively.

Clinical and demographic characteristics of patients at baseline

Patients’ clinical and demographic characteristics are shown in Table 1. There were no significant differences in their age, gender, weight, total bilirubin, Child–Pugh stage, ALT, AST, AFP level, HBeAg positive or negative, Tregs,
HBV DNA load, tumor number, tumor size, ascites, cirrhosis, arteriovenous fistula and type of liver resection between two groups. The mean survival time was significantly different between two groups ($P = 0.014$).

Univariate and multivariate analysis

Results of univariate analysis are shown in Table 2. Age $\geq 55$ years, Child–Pugh stage B, ALT $\geq 45$ IU/L, AST $\geq 47$ IU/L, serum AFP level $\geq 400$ ng/dL, hemoglobin $\geq 140$ g/L, Tregs $\geq 5.0 \%$, HBeAg positivity, HBV DNA $\geq 10^4$ IU/mL, tumor size $\geq 5$ cm and treatment modalities of surgery only were associated with short survival times. There was no substantial difference in survival times among patients receiving different types of liver resection.

Results of multivariate analyses are shown in Table 3. HBeAg positivity, hemoglobin $\geq 140$ g/L, Tregs $\geq 5.0 \%$, tumor size $\geq 5$ cm, treatment modalities of surgery only were associated with short survival times.

Overall survival of two groups

The 1-, 2-, and 3-year overall survival rates in surgery-only group were 58.8, 32.4 and 12.6 $\%$, and the rates in surgery plus TACE group were 73.1, 61.5, and 48.9 $\%$, respectively ($P = 0.033$) (Fig. 1). The median survival time of the two groups after surgery and surgery plus TACE were 15.0 months [95 $\%$ confidence interval (CI) 10.714–19.286] and 35.0 months [95 $\%$ CI 20.974–49.026]. At the end time of follow-up, 29 patients in Group A and 16 patients in Group B had died because of tumor progression and/or hepatic decompensation. The rate of death of the two groups after surgery and surgery plus TACE was 85.3 and 61.5 $\%$, respectively.

Discussion

Prior or occult HBV infection has been strongly implicated in the development of HCC. Chronic HBV infection, which is endemic in Asia, accounts for more than 80 $\%$ of HCC in the region [28]. HBV-related HCC is characterized by large and multifocal tumors against a background of cirrhosis, whereby curative treatment is often not feasible [29, 30]. Adjuvant therapies such as TACE have been used to prolong survival and prevent the recurrence of HCC postoperatively [21–24]. However, adjuvant TACE is not a standard treatment, and the associated survival benefit remains controversial currently. TACE has been known to damage remnant liver and deteriorate liver function and has even been associated with more frequent extrahepatic recurrences and a poor outcome [31]. These adverse effects are likely to affect long-term survival of patients with resectable HCC.

Here, in patients with HBV-related HCC, univariate analyses revealed that HBV DNA $\geq 10^4$ IU/mL (postsurgical before TACE) was associated with shorter survival times ($P = 0.003$). However, this factor was not significant in multivariate analysis ($P = 0.524$). Chen et al. [32] confirmed that HBV is an important predictor of HCC prognosis after surgical treatment. Even inactive HBV

### Table 3

<table>
<thead>
<tr>
<th>Variables</th>
<th>SE</th>
<th>$P$ value</th>
<th>Exp ($\beta$)</th>
<th>95 $%$ CI for Exp ($\beta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST: $&lt;$47 vs. $\geq 47$ IU/L</td>
<td>0.674</td>
<td>0.065</td>
<td>0.288</td>
<td>0.077–1.081</td>
</tr>
<tr>
<td>Hemoglobin: $&lt;$140 vs. $\geq 140$ g/L</td>
<td>0.615</td>
<td>$&lt;$0.001</td>
<td>0.961</td>
<td>2.715–30.241</td>
</tr>
<tr>
<td>HBeAg: positive vs. negative</td>
<td>0.451</td>
<td>0.023</td>
<td>2.798</td>
<td>1.156–6.771</td>
</tr>
<tr>
<td>Tregs: $&lt;$5 vs. $\geq 5$ %</td>
<td>0.474</td>
<td>0.045</td>
<td>0.387</td>
<td>0.153–0.979</td>
</tr>
<tr>
<td>Tumor number: single vs. multiple</td>
<td>0.568</td>
<td>0.073</td>
<td>2.765</td>
<td>0.909–8.413</td>
</tr>
<tr>
<td>Tumor size: $&lt;$5 vs. $\geq 5$ cm</td>
<td>0.532</td>
<td>0.004</td>
<td>0.219</td>
<td>0.077–0.621</td>
</tr>
<tr>
<td>Treatment modalities (Groups A vs. B)</td>
<td>0.583</td>
<td>0.033</td>
<td>2.461</td>
<td>1.075–5.639</td>
</tr>
</tbody>
</table>

### Notes

- **HBV** alpha-fetoprotein, **ALT** alanine transaminase, **AST** aspartate aminotransferase, **HBeAg** hepatitis B e antigen, **Tregs** regulatory T cells, **HBV** hepatitis B virus, **CI** confidence interval, **Group A** surgery only, **Group B** surgery plus TACE

**Fig. 1** Overall survival curves for the surgery-only group and surgery plus transarterial chemoembolization (TACE) group of patients with HBV-related HCC (stage B, BCLC)
posed a risk for hepatocellular carcinoma [33]. Ohkubo et al. [9] suggested that the level of serum HBV DNA was an independent and significant prognostic factor ($P = 0.0022$). Therefore, further studies should be focused on the development of a robust strategy, integrating the viral-related factors of complementary prognostic value, to ensure high validity of the assessment for postoperative HCC prognosis.

There might still be microscopic foci that were not detected before surgery, which then would be the source of recurrence, although the macroscopic tumor foci have been removed. To our knowledge, most of the blood supply to HCC is derived from the hepatic artery and TACE is a hepatic-directed therapy that takes advantage of the relatively selective hepatic arterial tumor vascularization. Ren et al. [34] found no significant difference in survival with adjuvant TACE use that for HCC patients. For patients with BCLC stage B HCC, TACE is clearly defined as the first-line therapy and hepatic resection is not considered as a suitable method because of the high risk of hepatic decompensation after surgical resection. However, the results of a meta-analysis and some studies revealed that hepatectomy plus adjuvant TACE was superior to hepatic resection only. We obtained good outcomes from postoperative adjuvant TACE in selected patients with HBV-related HCC (stage B) compared to surgery only. We obtained good outcomes from postoperative adjuvant TACE in selected patients with HBV-related HCC (stage B). Although there may exist some bias between the selection for surgery and TACE because of the single center study and the small sample data, e.g., the results still give us a new direction of the treatment of HCC (stage B).

Our data also suggested that the benefits of adjuvant TACE depend on the selection of patients. In future, prospective randomized clinical trials in a larger number of patients are required to determine selection criteria for adjuvant TACE. It is also essential to perform strict and detailed preoperative examinations to obtain an exact evaluation of the tumor and the patient’s condition. In this study, univariate analysis showed that Child–Pugh stage, ALT, serum AFP, HBeAg positivity, HBV DNA load, and tumor size were significant factors; multivariate analysis showed that HBeAg positivity, Tregs, and tumor size were independent prognostic elements, whereas serum AFP and HBV DNA load were not. Therefore, these findings may be helpful in predicting life expectancy in HCC patients treated using surgery plus adjuvant TACE and may provide more information for stratifying patients in the design of future adjuvant TACE trials. However, it is important to note that these results are only based on the analysis of small population patients in the present study; other parameters may emerge significant in a larger sample pool.

In addition, as shown here, peripheral blood Tregs $\geq 5.0 \%$ ($P = 0.045$) were associated with shorter survival times in both univariate and multivariate analyses, demonstrating significant survival benefit in patients with low levels of Tregs. Our results on the level of Tregs in patients with HBV-related HCC help to form the basis for new immunotherapeutic strategies directed toward Tregs. Moreover, a report indicating that vaccination of tumor-bearing mice expands Tregs, thereby blocking the execution of effector function in vitro and in vivo [36], suggests that strategies combining Tregs depletion with cancer vaccines may be an effective means of improving immunotherapeutic outcomes for patients with cancer.

In conclusion, postoperative adjuvant TACE improves the survival of patients with HBV-related HCC (stage B) after surgery compared to surgery only. Appropriate patient selection remains the most important factor able to influence the clinical outcome of postoperative adjuvant TACE.

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Conflict of interest We declare that we have no conflict of interest.

Ethical statement We declare that all study participants provided informed consent, and the study design was approved by the Tianjin Anti-Cancer Association and Tianjin Medical University Cancer Hospital and Institution Ethics Committee and have been performed in accordance with the ethical standards laid down in the 1975 Declaration of Helsinki.

References
