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Arsenic trioxide-eluting Callispheres beads is more effective and equally tolerant compared with arsenic trioxide/lipiodol emulsion in the transcatheter arterial chemoembolization treatment for unresectable hepatocellular carcinoma patients

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Abstract. – OBJECTIVE: This study aimed to investigate the efficacy and safety of transcatheter arterial chemoembolization (TACE) using CalliSpheres beads loading with arsenic trioxide (ATO) (CBATO) in unresectable hepatocellular carcinoma (HCC) patients.

PATIENTS AND METHODS: Eighty-six unresectable HCC patients about to receive TACE with CBATO or conventional TACE (cTACE) with ATO were consecutively enrolled and divided into CBATO group (N=38) or cTACE group (N=48), respectively. Treatment response at 3 months (M3) and 6 months (M6) after the first treatment, and the progression-free survival (PFS) and overall survival (OS) were evaluated. Also, the biochemical indexes were documented before treatment, at 7 days, M3, and M6 after the first treatment.

RESULTS: The 3-month complete response (CR), overall response rate (ORR), and the 6-month CR, ORR, as well as the disease control rate (DCR) were increased in CBATO group compared with the cTACE group. Also, the TACE with CBATO was an independent predicting factor for lower stable disease+ progressive disease (non-ORR). Besides, PFS and OS were longer in CBATO group compared with cTACE group. Referring to biochemical indexes (including liver function indexes, kidney function indexes, and blood routine indexes), no difference between the two groups was found. As for adverse events, the prevalence of nausea and vomiting was decreased, while the prevalence of other adverse events were similar in CBATO group compared to cTACE group.

CONCLUSIONS: TACE with CBATO is more effective and equally tolerant compared with cTACE in treating unresectable HCC patients.

Key Words:
Hepatocellular carcinoma, Arsenic trioxide, Drug-eluting beads, Transcatheter arterial chemoembolization, Efficacy.

Introduction

Liver cancer, one of the most common cancers worldwide, ranks 7th among the most prevalent cancers and is the 2nd leading cause of cancer-related death[1,2]. According to a recent epidemiology survey, the standard incidence of liver cancer is 10.1 per 100,000 people (15.3 in males and 5.3 in females), and the standardized mortality rate for liver cancer is 9.5 per 100,000 people (14.3 in males and 5.1 in females)[2] . Hepatocellular carcinoma (HCC) is the main subtype of liver cancer and about 75%-85% of total liver cancer cases are HCC[3-5]. For the current treatment options for HCC, surgery is usually utilized in early-stage HCC patients, radiofrequency ablation exhibits high recurrence rate, systemic chemotherapy is limited to treat advanced-stage HCC patients, as for target therapy and immunotherapy, their applications are restrained, partly due to the extremely high cost[6-9]. Therefore, additional treatment options should be explored to improve the long-term outcome for HCC patients.

Transcatheter arterial chemoembolization (TACE), a locoregional therapy, serves as a first choice for unresectable HCC patients, and due to

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that it is able to increase the inter-tumor concentration of chemotherapeutic agents and decrease the circulating concentration of chemotherapeutic agents[10-12]. Whereas conventional TACE (cTACE) utilizes lipiodol or gelatin sponge as embolic agents/drug carriers, which frequently fail to embolize feeding arteries accurately and often cause injury to normal liver tissue[11-13]. During the last decades, drug-eluting bead-TACE (DEB-TACE) that utilizes microspheres as embolic agents/drug carriers has been more and more popular due to the more completed embolization and more sustained drug release of microspheres over conventional embolic agents/drug carriers (such as lipiodol and gelatin sponge)[14,15]. Accumulating evidence[11,14,15] reveals that DEB-TACE is superior to cTACE in treating HCC patients regarding treatment efficacy and safety. Therefore, DEB-TACE has a considerable application prospect in clinical practices.

Arsenic trioxide (ATO), a cytotoxic drug, has been approved for the treatment of leukemia in the USA[16,17]. Recently, ATO is also discovered to exhibit superb anti-cancer activity in several other cancers (including lung cancer, pancreatic cancer, and osteosarcoma), and has been regarded as a palliative treatment for late-staged HCC patients in China[18-22]. However, systemic ATO treatment brings in severe adverse events (including ventricular arrhythmia, gastrointestinal bleeding, and renal failure), which pronouncedly restrain its application[23,24]. Considering that ATO presents with great anti-cancer activity while hyper toxicity, and that DEB-TACE is able to reduce the systemic concentrations of chemotherapeutic agents, we hypothesized that ATO-eluting bead-TACE might maximize the efficacy of ATO while minimizing its adverse events. However, limited information could be obtained regarding the effectiveness and safety of ATO-eluting bead-TACE in HCC patients. Hence, we conducted this present study to evaluate the efficacy and safety of TACE treatment using CalliSpheres Beads loading with ATO (CBATO) in unresectable HCC patients.

Patients and Methods

Patients

A total of 86 unresectable HCC patients about to receive CBATO or cTACE therapy at our hospital between January 2017 and September 2018 were enrolled and analyzed in this prospective study. Inclusion criteria: (1) diagnosed as unresectable HCC according to the American Association for the Study of the Liver Diseases (AASLD) guideline based on imaging techniques and/or biopsy[25]; (2) unable to undergo palliative surgery or radiotherapy, and there was a measurable lesion and intention to undergo CBATO or cTACE therapy; (3) did not receive any treatment after the diagnosis of HCC was established (before recruitment), including liver transplantation, surgical resection, TACE, radiofrequency, microwave or chemical ablation, argon-helium knife, ultrasound knife, radiotherapy, etc.; (4) Barcelona Clinic Liver Cancer (BCLC) tumor stage B or C, and Child-Pugh stage A or B; (5) the diameter of single tumor more than 5 cm or the sum of diameter of 2-3 tumors more than 5 cm; (6) Eastern Cooperative Oncology Group (ECOG) performance score 0-1 within 1 week before enrollment; (7) aged 18-75 years; (8) normal renal function and normal coagulation function (if not, it was required to be correctable by appropriate treatment); (9) life expectancy more than 12 weeks. The exclusion criteria included: (1) diffuse liver cancer; (2) severe coagulation dysfunction which cannot be corrected; (3) severe renal failure and cardiopulmonary failure; (4) the main portal vein was completely blocked by the tumor thrombus, and the collateral circulation was reduced or there was portal vein hypertension accompanied by the reverse blood flow; (5) radiofrequency or microwave ablation, seed implantation, and other interventional treatments were selected during the cTACE or DEB-TACE treatment; (6) allergic to arsenic or iodine oil; (7) pregnant or lactating women; (8) unlikely to be regularly followed up. This investigation was approved by the Ethics Committee of our hospital and registered in the Chinese Clinical Trial Registry with the Registration Number: ChiCTR-IOR-17012159. All patients signed informed consents before recruitment.

Baseline Characteristics Collection

After recruitment, the characteristics of these patients were recorded, including: (1) demographic information, including age and gender; (2) medical history including hepatitis B (HB), hepatitis C (HC), and liver cirrhosis; (3) clinical features including Child-Pugh stage, BCLC stage, ECOG score, tumor number, tumor size, and portal vein invasion; (4) biochemical indexes including red blood cell (RBC), hemoglobin, platelet, white blood cell (WBC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total bilirubin (TBIL), alkaline phosphatase (ALP), total protein (TP), creatinine, urea, and alpha fetoprotein (AFP).

Grouping and Therapy

Based on clinical needs and personal willingness, patients who selected the CBATO therapy were included in the CBATO group (N=38), and those who received the cTACE treatment were included in the cTACE group (N=48). Patients in the CBATO group were treated with DEB-TACE using CalliSpheres® Beads (Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China) loading with ATO, and the patients in the cTACE group were treated with cTACE using iodine oil-ATO emulsion.

Preparation of CalliSpheres® Beads

The ATO of 60 mg was dissolved in solution of 6 ml 5% glucose and extracted into 10 ml injector to prepare the solution of 10 mg/ml ATO. One bottle of CalliSpheres® Beads containing 1 g beads with a diameter of 100 μm-300 μm was extracted into a 20 ml injector, which then stood at room temperature for 5 min, and the liquid supernatant was pushed out and thus left the beads in the injector. Then, the ATO solution was mixed with the beads using a tee joint by the repeated push and pull. Subsequently, the mixed solution was extracted into the 20 ml injector followed by shaking up and vertical placement for loading 45 min. Next, the supernatant was further pushed out. Finally, the contrast agent was added to the mixed solution as a ratio of 1:1.

TACE Procedure for CBATO Group

After routine disinfection and local anesthesia, femoral artery puncture was performed using Seldinger technology. Then, 5F vascular sheath and RH catheter were placed into the femoral artery. Under the digital subtraction angiography (DSA) perspective, the catheter was inserted to celiac trunk artery, superior mesenteric artery, and splenic artery to perform high-pressure angiography. When the supplying artery and location of the tumor were identified, the tumor supplying artery was catheterized by microcatheter, then, the embolization was initiated. In CBATO group, the CBATO mixture was infused into the tumor supplying artery until the staining of the tumor was disappeared. If there was still tumor staining after the CalliSpheres® Beads were exhausted, embospheres with a diameter of 300 μm-500 μm (Merit Medical Systems, South Jordan, UT, USA) were added until the blood flow of the tumor supplying artery was almost stagnated. After completion of embolization, the vascular sheath and microcatheter were pulled out, the hemostasis by compression was conducted, the punctured wound was bound up, and the symptomatic and supportive treatments, such as liver protection, relieving pain, and vomiting, acid suppression and so on, were performed as appropriate. For instance, a 51-year-old man presenting with preoperative AFP of 43581 ng/ml was diagnosed as unresectable HCC. Preoperative magnetic resonance imaging (MRI) displayed that there was a lesion with the size of 172×130 mm on the right lobe of the liver, significant enhancement in arterial phase, and involvement of the right inferior branch of the portal vein (Figure 1A). During the TACE, hepatic angiography revealed the staining of huge tumor in the right lobe of the liver (Figure 1B), and then, the supplying artery of the tumor was embolized using CalliSpheres® Beads (100 μm-300 μm) loading with ATO (≈15 mg) until the supplying artery of the tumor was presented as "withered branches" (Figure 1C). At the first month after the first therapy of CBATO, hepatic arteriography disclosed that there was still staining in periphery of huge tumor in the right lobe of the liver (Figure 1D), consequently, the tumor was embolized again using CalliSpheres® Beads (100 μm-300 μm) loading with ATO until the blood flow of the tumor supplying artery was stagnated (Figure 1E). At the sixth month after the first therapy of CBATO, AFP of patient decreased to 29.2 ng/ml, and MRI revealed that there was no tumor staining in the liver (Figure 1F). The treatment outcome was assessed as complete response (CR).

TACE Procedure for cTACE Group

In the cTACE group, the detection procedure of the supplying artery and location of the tumor was performed as in the CBATO group. The iodine oil-ATO emulsion which was prepared by use of 20 mg ATO mixed with 10-20 ml of 48% Lipiodol® (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was infused into the tumor supplying artery until the tumor staining was disappeared or the deposition of iodine oil occurred in the subbranches of the portal vein. If there was still tumor staining after the iodine oil-ATO emulsion was used up, gelfoam-particle embolic agent with a diameter of 350 μm-560 μm (Hangzhou Alikang Pharmaceutical Technology Co., Ltd., Hangzhou, Zhejiang, China) was added until the tumor staining was disappeared. After completion of embolization, the vascular sheath and microcatheter were pulled out, the hemostasis by compression was conducted, the punctured wound was bound up, and the symptomatic and supportive treatments, such as liver protection, relieving pain, and vomiting, acid suppression and so on, were performed as appropriate.

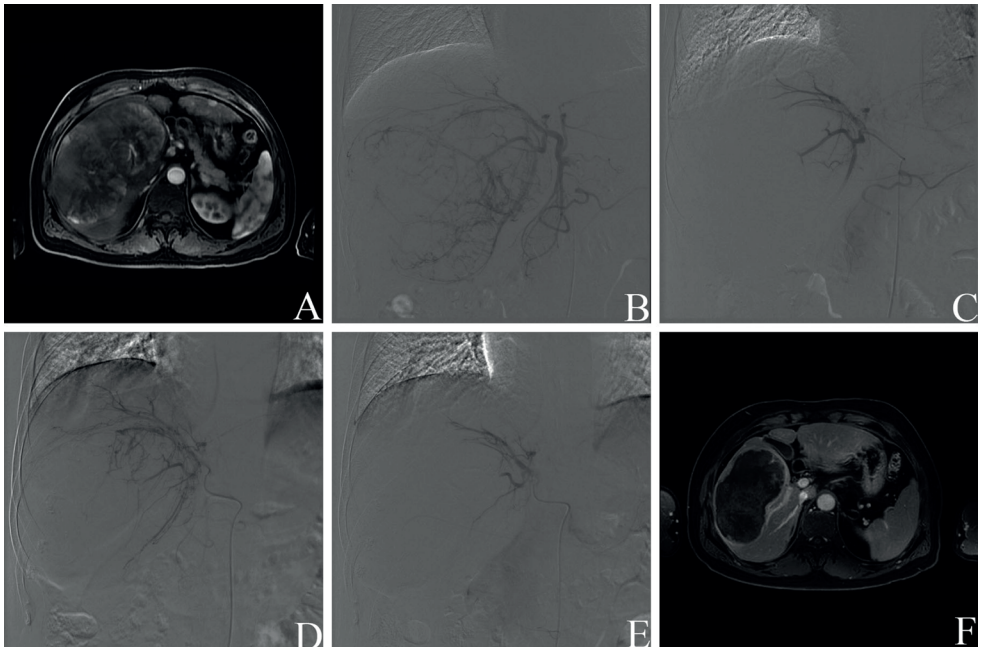

Figure 1. A classic case of HCC patients underwent TACE with CBATO treatment. The MRI images before TACE with CBATO treatment (**A**). The hepatic angiography during the first TACE with CBATO treatment (**B**). The hepatic angiography at the end of the first TACE with CBATO treatment (**C**). The hepatic arteriography at the first month after the first TACE with CBATO treatment (**D**). The hepatic arteriography at the end of the second TACE with CBATO treatment (**E**). The MRI images at the sixth month after the first TACE with CBATO treatment (**F**). HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; CBATO, CalliSpheres beads loading with arsenic trioxide.

Follow–Up and Assessment

After patients were discharged from hospital, they were prescribed with apatinib of 0.25 g once daily, orally (Jiangsu Hengrui Medicine Co. Ltd., Lianyungang, Jiangsu, China). All patients were required to undergo a review of blood and iconography every 4-6 weeks, in which, if the disease progression was discovered, TACE was repeated according to the grouping. According to the computerized tomography (CT) or magnetic resonance imaging (MRI) examination, therapy response at 3 months (M3) and 6 months (M6) after first TACE treatment was assessed according to the new response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1)[26], including CR, partial response (PR), stable disease (SD), and progressive disease (PD). The overall response rate (ORR) was defined as CR+PR, and the disease control rate (DCR) was defined as CR+PR+SD. In addition, the biochemical indexes at 7 days, 3 months (*3-month*), and 6 months (*6-month*) post first TACE were documented (such as ALT, AST, ALB, RBC, hemoglobin, platelet, WBC, creatinine, and urea). Adverse events (such as pain, fever, ascites, nausea and vomiting, and gastrointestinal hemorrhage) occurred after first TACE were also recorded and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Moreover, all patients were followed up until death, loss of follow-up, or withdrawal from the study, with the last follow-up date of 2019/3/31. The progression-free survival (PFS) was defined as the duration from the start of first TACE treatment date to the date of first disease progression, death from any cause, or censored at the date of the last contact. The overall survival (OS) was defined as the duration from the first TACE treatment date to the date of death from any cause or censored at the date of last contact.

Statistical Analysis

Statistical analysis was carried out by SPSS 21.0 statistical software (IBM Corp., Armonk, NY, USA). Data were displayed as mean and standard deviation (SD) or count (percentage), and comparison between two groups was determined by the Student's *t*-test or Chi-square test. PFS and OS curves were plotted using the Kaplan-Meier methods, and the difference of PFS and OS between the

two groups was determined by the Log-rank test. Factors related to the SD+PD were assessed by the stepwise forward multivariable logistic regression model analysis, and the factors affecting the PFS and OS were evaluated by the stepwise forward multivariable Cox's proportional hazard regression model analyses. All tests were two-sided. *p*-value <0.5 was considered as statistically significant.

Results

Study Flow

Totally 127 HCC patients about to receive CBATO or cTACE therapy were invited, among whom 21 patients were excluded since they disagreed to participate. After that, the remaining 106 patients were screened, while 20 patients were excluded due to the following reasons: 13 patients unmet the criteria, and 7 patients disagreed to sign informed consents. After that, 86 patients were recruited and classified into CBATO group (N=38) and cTACE group (N=48) according to their treatment scheme. During the subsequent experiment period, no patient lost to follow up or withdrew from the study. Thus, all the 38 patients in CBATO group and 48 patients in cTACE group were included in the final analysis (Figure 2).

Comparison of Baseline Characteristics Between CBATO Group and cTACE Group

There was no difference in demographic and clinical characteristics between CBATO group and cTACE group (all $p > 0.05$, Table I). The mean values of age were 56.5±10.0 years and 53.7±11.6 years in CBATO group and cTACE group, the number of male and female patients was 37 (97.4%) and 1 (2.6%) respectively in CBATO group and was 46 (95.8%) and 2 (4.2%), respectively in cTACE group. For Child-Pugh stage, the number of patients in stage A and stage B was 25 (65.8%) and 13 (34.2%), respectively in CBATO group and was 35 (72.9%) and 13 (27.1%), respectively in cTACE group. For BCLC stage, the number of patients in stage B and stage C was 20 (52.6%) and 18 (47.4%), respectively in CBATO group and was 25 (52.1%) and 23 (47.9%), respectively in cTACE group. For

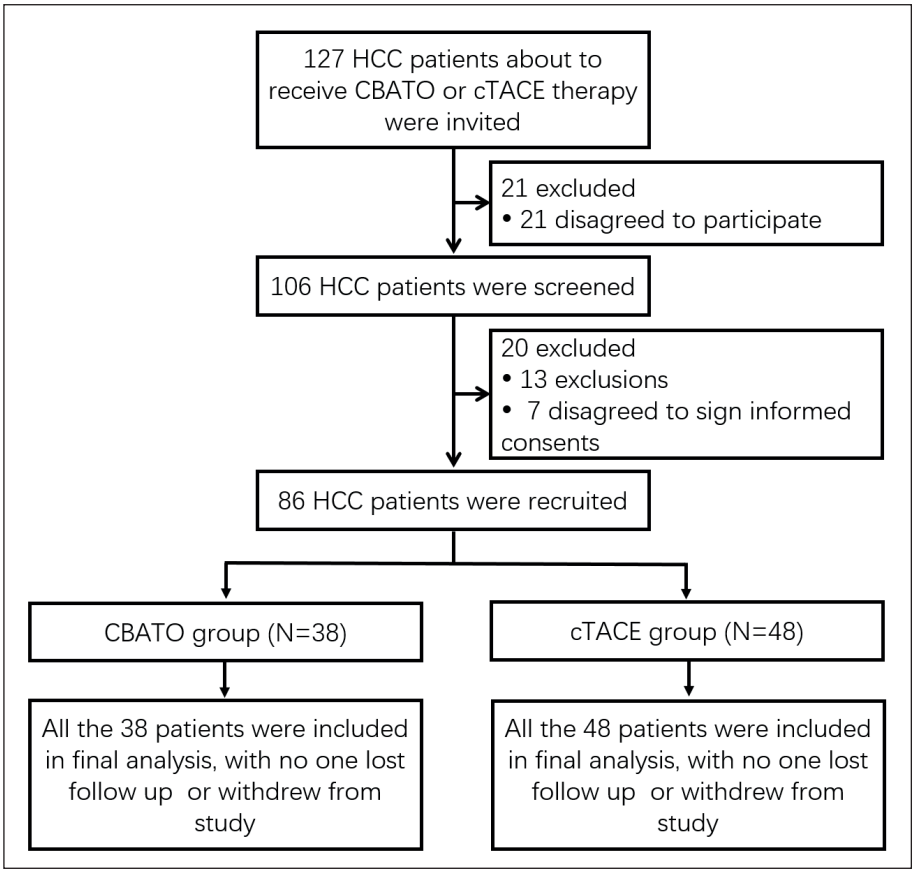

Figure 2. Study flow.

ECOG score, the number of patients in score 0 and score 1 was 20 (52.6%) and 18 (47.4%), respectively in CBATO group and was 20 (41.7%) and 28 (58.3%), respectively in cTACE group. As for AFP expression, the number of patients with AF*P* < 200, 200-400 and > 400 was 20 (52.6%), 0 (0.0%), and 18 (47.4%), respectively in CBATO group, and was 18 (37.5%), 3 (6.2%), and 27 (56.3%), respectively in cTACE group. Other detailed baseline characteristics of HCC patients were listed in Table I.

Table I. Baseline characteristics of HCC patients.

Items	CBATO group (N=38)	cTACE group (N=48)	p-value
Age (years), mean±SD	56.5±10.0	53.7±11.6	0.241
Gender, No. (%)			0.700
Male	37 (97.4)	46 (95.8)	
Female	1 (2.6)	2 (4.2)	
History of hepatitis, No. (%)			0.652
No	1 (2.6)	1 (2.1)	
HB	33 (86.9)	40 (83.3)	
HC	4 (10.5)	37 (77.1)	
History of cirrhosis, No. (%)			0.248
No	5 (13.1)	11 (22.9)	
Yes	33 (86.9)	37 (77.1)	
Child-Pugh stage, No. (%)			0.475
A	25 (65.8)	35 (72.9)	
B	13 (34.2)	13 (27.1)	
BCLC stage, No. (%)			0.960
B	20 (52.6)	25 (52.1)	
C	18 (47.4)	23 (47.9)	
ECOG score, No. (%)			0.311
0	20 (52.6)	20 (41.7)	
1	18 (47.4)	28 (58.3)	
Tumor number, No. (%)			0.159
Unifocal	14 (36.8)	25 (52.1)	
Multifocal	24 (63.2)	23 (47.9)	
Tumor size (cm), mean±SD	9.4±4.3	9.9±4.2	0.589
Portal vein invasion, No. (%)			0.738
No	20 (52.6)	27 (56.3)	
Yes	18 (47.4)	21 (43.8)	
RBC (*1012/L), mean±SD	4.2±0.6	4.3±0.8	0.524
Hemoglobin (g/L), mean±SD	129.3±19.4	128.1±25.0	0.808
Platelet (*109/L), mean±SD	161.8±97.8	160.9±84.8	0.964
WBC (*109/L), mean±SD	5.9±2.6	5.4±1.9	0.306
ALT (U/L), mean±SD	47.4±47.2	49.2±69.1	0.891
AST (U/L), mean±SD	75.6±88.3	62.2±68.8	0.431
ALB (g/L), mean±SD	35.8±4.8	37.7±4.9	0.075
TBIL (μmmol/L), mean±SD	17.1±10.5	14.2±7.7	0.143
ALP (U/L), mean±SD	166.7±115.8	143.8±81.2	0.285
TP (g/L), mean±SD	65.7±6.0	67.9±5.7	0.086
Creatinine (μmmol/L), mean±SD	66.0±10.8	66.1±11.7	0.968
Urea (mmol/L), mean±SD	5.1±1.7	4.8±1.5	0.388
AFP (ng/mL), No. (%)			0.271
<200	20 (52.6)	18 (37.5)	
200-400	0 (0.0)	3 (6.2)	
>400	18 (47.4)	27 (56.3)	

Comparison was determined by Student's *t*-test or Chi-square test. HCC, hepatocellular carcinoma; CBATO, CalliSpheres beads loading with arsenic trioxide; cTACE, conventional transarterial chemo-embolization; SD, standard deviation; HB, hepatitis b; HC, hepatic c; BCLC, Barcelona Clinic Liver Cancer; ECOG, Eastern Cooperative Oncology Group; RBC, red blood cell; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin; ALP, alkaline phosphatase; TP, total protein; AFP, alpha fetoprotein.

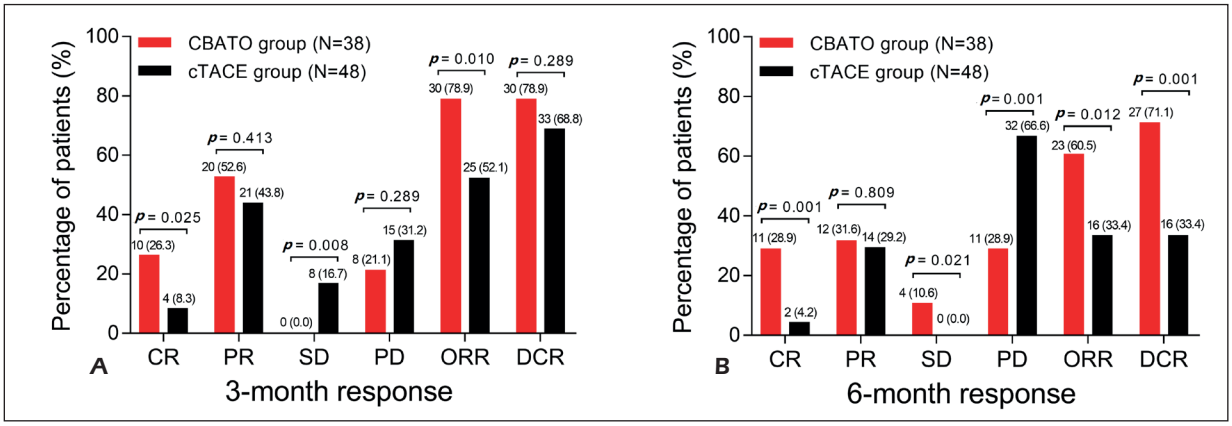

Figure 3. The 3-month and 6-month treatment response in CBATO group and cTACE group. The comparison of 3-month treatment response between CBATO group and cTACE group (**A**). The comparison of 6-month treatment response between CBATO group and cTACE group (**B**). Comparison between two groups was determined by Chi-square test. $p<0.05$ was considered as significant. CB, CalliSpheres® Beads; ATO, arsenic trioxide; cTACE, conventional transarterial chemoembolization; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, overall response rate; DCR, disease control rate.

Comparison of 3-Month and 6-Month Treatment Response Between CBATO Group and cTACE Group

The 3-month CR (p = 0.025) and ORR (p = 0.010) were increased, while DCR (p = 0.289) showed no difference in CBATO group compared with cTACE group (Figure 3A); also, the 6-month CR (p = 0.001), ORR (p = 0.012) and DCR (p = 0.001) were elevated in CBATO group compared to cTACE group (Figure 3B). These analyses revealed that TACE with CBATO exhibited better treatment response compared with cTACE in HCC patients.

Analysis of Factors Predicting SD+PD (non-ORR) at 6 Months

Factors affecting SD+PD were analyzed by the multivariate logistic regression model, which revealed that cTACE *vs.* CBATO [p = 0.025, OR=3.123 (95% CI: 1.151-8.473)] was an independent factor for predicting increased SD+PD in HCC patients (Table II). Besides, the higher ECOG score [p = 0.001, OR=5.669 (95% CI: 2.064-15.571)] and the higher AFP [p = 0.046, OR=1.683 (95% CI: 1.010-2.807)] were also independent factors for predicting elevated SD+PD in HCC patients. These data further suggested that TACE with CBATO provided more favorable treatment response compared with cTACE in HCC patients.

Comparison of PFS and OS Between CBATO Group and cTACE Group

The medium values of PFS and OS were 308 days (95% CI: 157-469 days) and 548 days (95% CI: 341-755 days) in CBATO group and were 148 days (95% CI: 78-217 days) and 404 days (95% CI: 137-671 days) in cTACE group. Log-rank test re-

Table II. Multivariate logistic regression model analysis of factors related to SD+PD at 6 months.

Items	Forward stepwise multivariate logistic regression				
	β	SE (β)	Wald χ^2	*p*-value	OR (95% CI)
cTACE *vs.* CBATO	1.139	0.509	5.000	0.025	3.123 (1.151-8.473)
Higher ECOG score	1.735	0.516	11.326	0.001	5.669 (2.064-15.571)
Higher AFP	0.521	0.261	3.990	0.046	1.683 (1.010-2.807)

Factors included in multivariate logistic regression model were as follows: group (cTACE *vs.* CBATO), age (years), gender (male vs. female), history of hepatitis (no/HC *vs.* HB), history of cirrhosis (no *vs.* yes), Child-Pugh stage (B *vs.* A), BCLC stage (C *vs.* B), ECOG score (1 *vs.* 0), tumor number (multifocal *vs.* unifocal), tumor size (cm) and AFP (<200 ng/mL=1, 200-400 ng/mL=2, >400 ng/mL=3). SD, stable disease; PD, progressive disease; TACE, transarterial chemoembolization; OR, odds ratio; CI, confidence interval; CBATO, CalliSpheres beads loading with arsenic trioxide; ECOG, Eastern Cooperative Oncology Group; AFP, alpha fetoprotein.

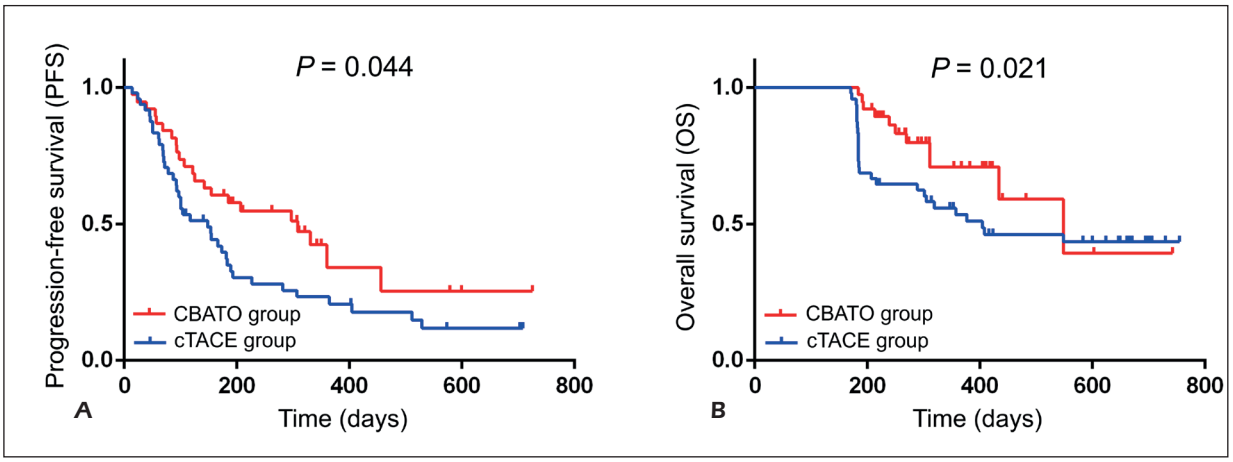

Figure 4. The PFS and OS in CBATO group and cTACE group. The comparison of PFS between CBATO group and cTACE group (**A**). The comparison of OS between CBATO group and cTACE group (**B**). PFS and OS curves were plotted using Kaplan-Meier methods, and difference of PFS and OS between two groups was determined by the Log-rank test. $p<0.05$ was considered as significant. PFS, progression-free survival; OS, overall survival; CB, CalliSpheres® Beads; ATO, arsenic trioxide; cTACE, conventional transarterial chemoembolization.

vealed that both the PFS ($p = 0.044$; Figure 4A) and OS ($p = 0.021$; Figure 4B) were higher in CBATO group than that in cTACE group, indicating that the TACE with CBATO provided more survival benefits compared to cTACE in HCC patients.

Analyses of Factors Predicting PFS and OS

Factors predicting PFS and OS were analyzed by multivariate Cox's proportional hazards regression model, which disclosed that the higher ECOG score [$p = 0.016$, HR=1.914 (95% CI: 1.131-3.240)] and the higher AFP [$p = 0.047$, HR=1.315 (95% CI: 1.003-1.722)] were independent factors for worse PFS (Table III); meanwhile, the higher ECOG score [$p = 0.003$, HR=3.041 (95% CI: 1.461-6.328)] and the higher AFP [$p = 0.002$, HR=1.829 (95% CI: 1.248-2.681)] were also independent factors for worse OS (Table IV).

Comparison of Liver Function Between CBATO Group and cTACE Group Before and After TACE

There was no difference in ALT, AST or ALB between the two groups at baseline, 7-day after TACE, 3-month after TACE or 6-month after TACE (all $p > 0.05$, Table V). These analyses implied that TACE with CBATO did not deteriorate liver function of HCC patients compared with cTACE.

Comparison of Kidney Function and Blood Routine Between CBATO Group and cTACE Group Before and After TACE

There was no difference of creatinine, urea, RBC, hemoglobin, platelet or WBC between the two groups at baseline, 7-day after TACE, 3-month after TACE or 6-month after TACE (all $p > 0.05$, Ta-

Table III. Multivariate Cox's proportional hazards regression model analysis of factors related to PFS.

Items	Forward stepwise multivariate logistic regression				
	β	SE (β)	Wald χ^2	*p*-value	OR (95% CI)
Higher ECOG score	0.649	0.269	5.843	0.016	1.914 (1.131-3.240)
Higher AFP	0.274	0.138	3.939	0.047	1.315 (1.003-1.722)

Factors included in multivariate Cox's proportional hazards regression model were as follows: (cTACE *vs.* CBATO), age (years), gender (male *vs.* female), history of hepatitis (no/HC *vs.* HB), history of cirrhosis (no *vs.* yes), Child-Pugh stage (B *vs.* A), BCLC stage (C *vs.* B), ECOG score (1 *vs.* 0), tumor number (multifocal vs. unifocal), tumor size (cm) and AFP (<200 ng/mL=1, 200-400 ng/mL=2, >400 ng/mL=3). PFS, progression-free survival; HR, hazards ratio; CI, confidence interval; CBATO, CalliSpheres beads loading with arsenic trioxide; ECOG, Eastern Cooperative Oncology Group; AFP, alpha fetoprotein.

Table IV. Multivariate Cox's proportional hazards regression model analysis of factors related to OS.

Items	Forward stepwise multivariate logistic regression				
	β	SE (β)	Wald χ^2	*p*-value	OR (95% CI)
Higher ECOG score	1.112	0.374	8.846	0.003	3.041 (1.461-6.328)
Higher AFP	0.604	0.195	9.574	0.002	1.829 (1.248-2.681)

Factors included in multivariate Cox's proportional hazards regression model were as follows: (cTACE *vs.* CBATO), age (years), gender (male *vs.* female), history of hepatitis (no/HC *vs.* HB), history of cirrhosis (no *vs.* yes), Child-Pugh stage (B *vs.* A), BCLC stage (C *vs.* B), ECOG score (1 *vs.* 0), tumor number (multifocal *vs.* unifocal), tumor size (cm) and AFP (<200 ng/mL=1, 200-400 ng/mL=2, >400 ng/mL=3). OS, overall survival; HR, hazards ratio; CI, confidence interval; CBATO, CalliSpheres beads loading with arsenic trioxide; ECOG, Eastern Cooperative Oncology Group; AFP, alpha fetoprotein.

ble VI). These data implied that TACE with CBATO did not affect the kidney function or blood routine indexes of HCC patients compared to cTACE.

Comparison of Adverse Events Between CBATO Group and cTACE Group

To further compare the safety between CBATO and cTACE, adverse events were recorded and compared. The data showed that the percentage of patients with nausea and vomiting (grade 1) was higher in cTACE group compared with CBATO group ($p = 0.017$), while for other adverse events including pain ($p = 0.669$), fever ($p = 0.451$), ascites ($p = 0.234$) or gastrointestinal hemorrhage ($p = 0.999$), there was no difference between the two groups (Table VII). These analyses indicated that TACE with CBATO was relatively safer compared with cTACE in HCC patients.

Discussion

TACE is a well-known interventional therapy which provides substantial survival benefits for unresectable HCC patients[10]. Compared with systemic chemotherapy, the inter-tumor concentration of chemotherapeutic agent is strikingly higher, while its concentration in normal tissues and plasma is pronouncedly lower by TACE[10,11,15]. Currently, there are mainly two types of TACE (including cTACE and DEB-TACE). Accumulating evidence[14,15] reveals that DEB-TACE is more effective and safer compared to cTACE in treating HCC patients, and it has attracted increasing attention in clinical practices.

ATO is a traditional Chinese medicine with an application history of over two thousand years[27,28]. During the last decades, ATO has been discovered to exhibit anti-cancer activity to leukemia

Table V. Liver function before and after TACE.

Items	CBATO group (N=38)	cTACE group (N=48)	*p*-value
ALT (U/L), mean±SD			
Baseline	47.4±47.2	49.2±69.1	0.891
7-day after TACE	70.0±48.2	68.8±47.4	0.908
3-month after TACE	41.5±23.1	36.7±33.9	0.438
6-month after TACE	36.1±18.5	42.9±49.0	0.380
AST (U/L), mean±SD			
Baseline	75.6±88.3	62.2±68.8	0.431
7-day after TACE	63.6±36.5	61.1±37.2	0.756
3-month after TACE	54.1±26.2	54.3±54.7	0.982
6-month after TACE	56.4±75.1	55.1±48.7	0.927
ALB (g/L), mean±SD			
Baseline	35.8±4.8	37.7±4.9	0.075
7-day after TACE	32.9±5.5	33.9±5.0	0.381
3-month after TACE	37.4±5.5	37.8±4.8	0.720
6-month after TACE	38.5±5.1	37.9±4.5	0.564

Comparison between groups was determined by Student's *t*-test. TACE, transarterial chemoembolization; CBATO, CalliSpheres beads loading with arsenic trioxide; cTACE, conventional transarterial chemoembolization; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin.

Table VI. Kidney function and blood routine before and after TACE.

Items	CBATO group (N=38)	cTACE group (N=48)	*p*-value
Creatinine (μmmol/L), mean±SD			
Baseline	66.0±10.8	66.1±11.7	0.968
7-day after TACE	64.0±14.8	67.7±16.6	0.285
3-month after TACE	63.7±14.7	63.6±15.2	0.976
6-month after TACE	68.5±24.8	63.3±9.0	0.225
Urea (mmol/L), mean±SD			
Baseline	5.1±1.7	4.8±1.5	0.388
7-day after TACE	4.8±2.3	4.7±1.2	0.809
3-month after TACE	4.8±1.6	4.9±1.5	0.741
6-month after TACE	5.1±1.5	4.7±1.3	0.140
RBC (*1012/L), mean±SD			
Baseline	4.2±0.6	4.3±0.8	0.524
7-day after TACE	4.0±0.6	4.2±0.9	0.221
3-month after TACE	4.0±0.7	4.2±0.8	0.227
6-month after TACE	3.9±0.7	4.2±0.7	0.052
Hemoglobin (g/L), mean±SD			
Baseline	129.3±19.4	128.1±25.0	0.808
7-day after TACE	124.3±18.8	124.0±23.8	0.946
3-month after TACE	125.4±19.9	125.4±22.5	1.000
6-month after TACE	123.2±20.9	129.5±18.1	0.144
Platelet (*109/L), mean±SD			
Baseline	161.8±97.8	160.9±84.8	0.964
7-day after TACE	145.7±92.6	152.8±82.3	0.708
3-month after TACE	132.2±91.8	159.1±74.6	0.138
6-month after TACE	169.8±118.2	150.2±93.7	0.393
WBC (*109/L), mean±SD			
Baseline	5.9±2.6	5.4±1.9	0.306
7-day after TACE	7.7±3.0	7.3±2.4	0.494
3-month after TACE	5.4±3.3	5.1±2.0	0.624
6-month after TACE	5.4±2.4	4.9±1.7	0.262

Comparison between groups was determined by Student's *t*-test. TACE, transarterial chemoembolization; CBATO, CalliSpheres beads loading with arsenic trioxide; cTACE, conventional transarterial chemoembolization; RBC, red blood cell; WBC, white blood cell.

and a few other solid tumors (including HCC, lung cancer, pancreatic cancer, and osteosarcoma) through multiple mechanisms[19,29-31]. For example, an *in vivo* study observes that ATO disturbs the morphological development of tumor vessels and suppresses the protein expressions of delta-like canonical Notch ligand 4 (Dll4), Notch1 and Hes1 in an animal model of small-cell lung cancer (SCLC), which indicates that ATO inhibits tumor growth of SCLC by antiangiogenesis and Notch signaling blockage[29,30]. In another *in vitro* experiment, ATO is found to reduce cell proliferation while promoting cell apoptosis of HCC cells (HepG2 cell line) in a dose-dependent manner by inducing oxidative stress and activating mitochondrial or intrinsic pathway of apoptosis[30]. Due to the good anti-tumor activity of ATO, it has been approved for treating refractory leukemia and late-staged HCC. However, due to its severe adverse events, the application of ATO in clinical practices remains restrained.

Considering the great anti-cancer activity while the hyper toxicity of ATO, and the locoregional therapy characteristics of TACE, we hypothesized that TACE using ATO might be a good treatment strategy to enhance the treatment efficacy of ATO while diminishing its systemic adverse events in HCC patients. However, only a few previous studies[32,33] investigate the treatment efficacy and the safety of cTACE using ATO, which disclose that cTACE using ATO is effective and safe in treating unresectable HCC patients. For the treatment efficacy of DEB-TACE using ATO in treating HCC patients, no studies have been explored to date. Therefore, we enrolled 86 unresectable HCC patients and evaluated the treatment efficacy in CBATO group and cTACE group, and we discovered that both groups achieved a relatively favorable

Table VII. Adverse events.

Items	CBATO group (N=38)	cTACE group (N=48)	*p*-value
Pain, No. (%)	21 (55.2)	28 (58.3)	0.669
Grade 1	7 (18.4)	10 (20.8)	
Grade 2	10 (26.3)	9 (18.8)	
Grade 3	4 (10.5)	9 (18.8)	
Fever, No. (%)	21 (55.2)	27 (56.3)	0.451
Grade 1	16 (42.1)	14 (29.1)	
Grade 2	5 (13.1)	12 (25.0)	
Grade 3	0 (0.0)	1 (2.1)	
Ascites, No. (%)	13 (34.2)	20 (41.7)	0.234
Grade 1	10 (26.3)	8 (16.7)	
Grade 2	2 (5.3)	6 (12.5)	
Grade 3	1 (2.6)	6 (12.5)	
Nausea and vomiting, No. (%)	8 (21.1)	22 (45.8)	0.017
Grade 1	8 (21.1)	22 (45.8)	
Gastrointestinal hemorrhage, No. (%)	0 (0.0)	1 (2.1)	0.999

Comparison between groups was determined by Student's *t*-test. TACE, transarterial chemoembolization; CBATO, CalliSpheres beads loading with arsenic trioxide; cTACE, conventional transarterial chemoembolization; RBC, red blood cell; WBC, white blood cell.

treatment response and survivals after TACE treatment. The possible reasons might be as follows: (1) ATO exerted powerful cytotoxicity to HCC cells via activating apoptosis-related pathways (such as Notch signaling pathways) to decrease the tumor burdens and staging of patients, thereby enhancing the treatment response and long-term outcomes for HCC patients[29,30]. (2) TACE enabled the infiltration of ATO in tumor tissue to directly cause the tumor necrosis on a large scale, thereby improving the treatment response and survivals of HCC patients. Of note, we discovered that patients who received TACE with CBATO had better treatment response and survivals compared with patients who received cTACE, and TACE with CBATO was an independent predicting factor for better treatment response, which suggested that TACE with CBATO improved the anti-cancer efficacy of ATO compared with cTACE in HCC patients. This better treatment efficacy of TACE with CBATO over cTACE might be explained by: (1) microspheres embolized cancer feeding artery more completely and long-lastingly compared with lipiodol (lipiodol might escape from the feeding artery and flow through the blood), thereby causing the better necrotic effect of tumor tissue[11-15]. (2) Microspheres released ATO in a sustained manner, while lipiodol released ATO rapidly, thus microspheres prolonged the anti-HCC efficacy of ATO compared to lipiodol[12,16,18,20,30].

Generally, HCC patients who receive TACE would occur the abnormal liver functions transitorily due to the surgical trauma and the necrosis of liver tissue[34,35]. To evaluate the safety profile of TACE with CBATO, we first compared the liver function between the CBATO group and cTACE group. The data disclosed that all liver function indexes (including ALT, AST, and ALB) remained stable on the whole, and these liver function indexes were similar between the two groups at each visit, which suggested that TACE with CBATO was as safe as cTACE in treating HCC patients in terms of liver function. Subsequently, we assessed the kidney function and blood routine before and after TACE and found that both the kidney function (including creatinine and urea) and blood routine indexes (RBC, hemoglobin, platelet, and WBC) were similar between the CBATO group and cTACE group at each visit. These results might derive from the low concentration of ATO in plasma and normal tissues (including kidney) by DEB-TACE and cTACE (low ATO concentration in plasma and normal tissue indicated the mild toxicity). So as to more comprehensively investigate the safety profiles of TACE with CBATO, we further evaluated the adverse events in HCC patients during and after TACE treatment between the CBATO group and cTACE group. We found that the common adverse events were pain, fever, ascites, nausea, and vomiting, as well as gastrointestinal hemorrhage in both groups, while most of which were not life-threatening, which was in line with Liu et al[32] showing that the common adverse events are fever, vomiting, nausea, and headache caused by cTACE with ATO treatment. In addition, the percentage of patients

occurred nausea and vomiting was lower in CBATO group compared with cTACE group, and the occurrence of other adverse events were similar between the two groups, indicating the comparative (or relatively better) safety profiles of TACE with CBATO compared with cTACE. Overall, we revealed that the TACE with CBATO was equally safe (or even safer) compared to cTACE in HCC patients. However, these findings were preliminary results in a small cohort of patients. Thus, there is still a need to focus on the risk of toxicity due to arsenic use, and further study with larger sample size for validation is necessary.

There were some limitations in this study. To begin, the sample size was relatively small, which might decrease the statistical power of the study. In addition, we only compared the efficacy and safety between TACE with CBATO and cTACE, while the superiority and the shortcomings of TACE with CBATO in comparison with other treatment agents (such as CB loading with doxorubicin) or other treatment approaches (such as radiotherapy) remained unclear. Besides, this was a signal center study without randomization, which might bring in selection bias and assessment bias. Therefore, a large-sample-size, randomized, multicentric study should be conducted in future. Finally, the follow-up time was relatively short. Further studies exploring the long-term efficacy and safety of TACE with CBATO in HCC patients are needed.

Conclusions

The above results indicate that TACE with CBATO is more effective and equally tolerant compared with cTACE in treating unresectable HCC patients.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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