The objective of the present study was to investigate the tumor-associated vascular changes in hepatocellular carcinoma (HCC) following treatment with transarterial chemoembolization (TACE) combined with sorafenib. The data of 20 patients were retrospectively analyzed. Patients underwent treatment depending on their chosen regimens (orally administered sorafenib was recommended, however the cost prevented some study participants from selecting this course). Based on this, the patients were divided into TACE combined with sorafenib (TS) (n=10) and TACE-only treatment groups (n=10). Digital subtraction angiography images of all patients were analyzed by 2 radiologists who were blind to the type of treatment administered. The diameters of the hepatic and proper hepatic arteries, and hepatic artery branches (tumor-associated arteries), the splenic, left gastric and gastroduodenal arteries or portal veins (non-tumor-associated arteries) and the number of microvascular vessels were compared prior to and following sorafenib treatment in the TS group, between the first and second sessions of TACE in the TACE-only group and between the TS and TACE-only groups. In the TS group, the diameters of the hepatic and proper hepatic arteries, their branches and the number of microvascular vessels were significantly decreased following sorafenib treatment (P<0.05), while the diameters of the splenic, gastroduodenal and left gastric arteries were not significantly altered (P>0.05). In the TACE-only group, the diameters of the hepatic, proper hepatic, splenic, left gastric and gastroduodenal arteries were not significantly different between the first and second TACE sessions (P>0.05), while the diameters of the hepatic artery branches and the number of microvascular vessels were significantly altered (P<0.05). TACE combined with sorafenib significantly decreased the diameters of the tumor-associated arteries and the number of tumor microvascular vessels when compared with TACE treatment alone (P<0.05). No significant difference in the diameters of the portal vein and its branches between the two groups was observed (P>0.05). Treatment with TACE combined with sorafenib may significantly affect the tumor-associated vasculature compared with treatment with TACE alone in HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most commonly diagnosed types of cancer worldwide (1). While there are specific differences in its etiology associated with different regional effects for example, in western countries, alcoholic hepatitis is the main reason for HCC. However, in China, a large number of HCC cases arise as a consequence of Hepatitis B virus (HBV) infection (2). HCC is a complicated disease and represents a significant cause of morbidity and mortality in China (2,3).

Previous advances in treatment methods for HCC have improved the clinical outcomes of patients with HCC. Liver transplantation, curative surgical resection, percutaneous ethanol injection and radiofrequency ablation are considered curative treatments, while transarterial treatments, including transarterial chemoembolization (TACE) in particular, are currently recognized as treatments that may prolong survival time (3).

TACE has been suggested to treat Barcelona Clinic Liver Cancer (BCLC) B- or C-stage HCC through 2 potential
methods (4). Firstly, arterial embolization may stop blood supply to the HCC tissue and inhibit tumor growth until neovascularization occurs (5). Secondly, the controlled release of chemotherapeutic agents allows the delivery of a stable concentration of drug to the lesions, and decreases the severity of systemic side effects (6). However, a previous study demonstrated that TACE may trigger cancer angiogenesis by upregulating the expression of certain cytokines and growth factors, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α (7). Furthermore, additional studies have demonstrated that the expression levels of these cytokines and growth factors were markedly decreased in patients treated with surgery alone compared with those that underwent pre-surgical TACE (8).

Sorafenib is an orally-administered small molecule drug (9) that inhibits multiple protein kinases, including VEGF receptors 2 and 3 and Platelet-derived growth factor (PDGF) receptor B (9), which are involved in tumor angiogenesis, and the family of Raf kinases, which facilitates the proliferation of cancer cells (10). Sorafenib is globally accepted and widely used as the first-line treatment for advanced-stage HCC (11). However, the effects of treatment with sorafenib alone on overall survival (OS) have not been satisfactory in patients with advanced HCC (12).

Novel therapies are urgently required in order to improve the OS of patients with advanced HCC. At present, sorafenib is the only approved systemic drug for patients with advanced-stage disease (BCLC C-stage) (13). TACE is suggested as the primary treatment method for BCLC B-stage HCC in the BCLC guidelines (14). Physicians have demonstrated that TACE was also effective for patients with BCLC C-stage HCC with vascular invasion or metastases (14,15). Furthermore, previous data have indicated that combined therapy, including sorafenib combined with TACE, may achieve significant improvements in the treatment of advanced HCC compared with single TACE treatment (16).

At present, clinical studies generally use the modified Response Evaluation Criteria In Solid Tumors (mRECIST) to evaluate the effect of sorafenib and TACE treatment on HCC (17). However, hemodynamic digital subtraction angiography (DSA) images and the structure of the vascular network, which reflect the hemodynamic features of liver cancer, have been proposed as priority concerns by interventional radiology physicians for analyzing the effects of treatments including TACE prior and subsequent to initiation of treatment (18). To the best of our knowledge, few studies have been performed investigating the arterial characteristics of HCC following treatment with sorafenib combined with TACE. Therefore, the aim of the present study was to compare the changes in the tumor vasculature of HCC following the use of TACE alone compared with its use in combination with sorafenib. The results of the present study may provide insight into this combination treatment method as a therapy for advanced HCC.

Patients and methods

In the present study, 20 patients (5 females and 15 males; median age 51 years, age range, 37-74 years) with HBV-associated HCC admitted to Zhongshan Hospital Affiliated to Xiamen University (Xiamen, China) our hospital between January 2011 and August 2016 were reviewed. All patients exhibited advanced HCC according to the BCLC staging system (BCLC C-stage) (19), confirmed by abdominal enhanced computed tomography (CT) or magnetic resonance imaging (MRI). None of the patients had been previously treated for HCC. A total of 10 patients were included in the TACE combined with sorafenib treatment (TS) group. All of these patients were initially treated with TACE, followed by treatment with sorafenib (200 mg orally; Bayer AG, Leverkusen, Germany) at a dose of 400 mg twice daily, for 1 week. After 6 weeks, the patients in the TS group were treated with a second session of TACE. The first angiography was performed prior to each session of TACE and prior to sorafenib treatment; the angiography was then repeated during the second session of TACE and following sorafenib treatment. The remaining 10 patients were treated with TACE only, with at least two sessions of TACE. The data from the first TACE session were then compared with those from the second TACE session using the angiography data collected. The angiography was performed following each TACE session in the TACE-only group, then the first angiography was compared with the second angiography.

In the present study, grade ≤3 hepatic artery branches were defined as microvascular vessels. The patient inclusion criteria are summarized in Table I and exclusion criteria are described in Table II. The baseline information of patients is summarized in Table III. The present study was approved by the Ethics Committee of Zhongshan Hospital Affiliated to Xiamen University (Xiamen, China). Patients provided written informed consent to participate prior to treatment, and for publication. TACE was performed according to a standardized approach.

The TACE procedure was performed. Firstly, a catheter was implanted into the celiac artery using the Seldinger technique, and then a hepatic angiography was performed via a common femoral approach using a 4 Fr angiographic catheter (Terumo Medical Co., Tokyo, Japan). Subsequent to locating the lesion by selective arteriography using a 2.0-2.7 Fr microcatheter (Terumo Medical Co.), an emulsion consisting of lipiodol (Guerbet, Roissy, France), epirubicin (Pfizer, Inc., New York, NY, USA) and oxaliplatin (Jiangsu Aosaikang Pharmaceutical Industry Ltd., Co., Nanjing, China) was infused via the feeding arteries.

The angiography was then performed to observe the tumor and to identify all the feeding arteries of the tumor. The nonionic contrast iopromide (Bayer AG) was injected into each hepatic artery at 5 ml/sec using an automated power injector. The duration of the arterial injection was 11 sec. Subsequently, the diameters of the branches of the celiac trunk, including the hepatic and proper hepatic arteries, hepatic arterial branches, splenic, gastroduodenal and left gastric arteries and the microvessels within tumors, were evaluated and analyzed. The tumor lesions in all 20 patients were supplied with nutrients and connected via the proper hepatic arteries and their branches, but not the left gastric, splenic or gastroduodenal arteries. Therefore, in the present study, the proper hepatic arteries and their branches were considered tumor-associated arteries, while the left gastric, splenic and gastroduodenal arteries were considered non-tumor-associated arteries. The diameters of the portal vein and its branches were evaluated using a contrast-enhanced liver CT scan. Then, the
Statistical analysis. The data are presented as the mean ± standard deviation, and differences were compared using paired Student's t-tests. P<0.05 was considered to indicate a statistically significant difference. Analysis was performed using SPSS software (version 19.0; IBM Corp., Armonk, NY, USA) and GraphPad Prism software (version 5.01; GraphPad Software, Inc., La Jolla, CA, USA).

Table I. Patient inclusion criteria.

| Criteria                  | Cut-off |
|---------------------------|---------|
| BCLC grade                | C       |
| Liver function            | Child-Pugh class A or B (39) |
| Total bilirubin, µmol/l   | ≤34.2 (3.4-17.1) |
| Alanine transaminase level, U | <100 (7-40) |
| Aspartate aminotransferase level, U | <90 (13-35) |
| Prothrombin time activity, sec | <22 (9-13) |
| Serum creatinine level, µmol/l | 1.5-fold the normal value (53-97) |
| Neutrophil count, /l      | ≤4·10^9·(4-10) |
| Hemoglobin level, g/l     | ≥90 (115-150) |
| Cardiopulmonary function  | NA      |
| Cerebral function         | NA      |
| Estimated life expectancy, weeks | >12      |

BCLC, Barcelona Clinic Liver Cancer.

Table II. Patients exclusion criteria.

| Criteria                                   | Cut-off |
|--------------------------------------------|---------|
| Tumor volume/total liver volume, %         | >70     |
| Portal vein patency                        | Main portal vein/right branch of portal vein completely obstructed |
| Previous treatment (surgical resection, ablation or TAI) | Yes |
| TAI, transhepatic artery-infusion chemotherapy. |

Results

TACE combined with sorafenib decreases the diameters of tumor-associated arteries. In the TS group, the diameters of tumor-associated arteries prior to sorafenib administration were significantly increased compared with those measured following sorafenib treatment, including the diameters of the hepatic artery (0.218±0.0423 vs. 0.158±0.0321 cm; P<0.05), the proper hepatic artery (0.350±0.042 vs. 0.314±0.040 cm; P<0.05) and the hepatic artery branches (0.218±0.042 vs. 0.158±0.032 cm; P<0.05; Fig. 1).

TACE combined with sorafenib does not decrease the diameters of non-tumor-associated arteries. In the TS group, the diameters of the non-tumor-associated arteries prior to the administration of sorafenib were not significantly different compared with those measured following sorafenib treatment, including the diameters of the splenic artery (0.454±0.063 vs. 0.418±0.055 cm; P>0.05), the gastroduodenal artery (0.294±0.036 vs. 0.29±0.053 cm; P>0.05) and the left gastric artery (0.310±0.012 vs. 0.296±0.022 cm; P>0.05; Fig. 1).

TACE alone does not cause a decrease in diameter of the tumor-associated arteries, with the exception of the hepatic artery branches. In the TACE group, the diameters of the tumor-associated arteries following the first session were not significantly different compared with those following the second session of TACE, including the diameters of the splenic artery (0.454±0.063 vs. 0.418±0.055 cm; P>0.05), the gastroduodenal artery (0.294±0.036 vs. 0.29±0.053 cm; P>0.05) and the left gastric artery (0.310±0.012 vs. 0.296±0.022 cm; P>0.05; Fig. 1).

TACE alone does not cause a decrease in diameter of the tumor-associated arteries. In the TACE group, the diameters of non-tumor-associated arteries following the first session of TACE were not significantly different compared with those measured following the second session of TACE, including the diameters of the left gastric artery (0.304±0.027 vs. 0.30±0.032 cm; P>0.05), the proper hepatic artery (0.450±0.037 vs. 0.407±0.0419 cm; P>0.05). However, the diameters of the hepatic artery branches were significantly decreased subsequent to the second session of TACE compared with the measurements taken following the first session of TACE (0.110±0.010 vs. 0.087±0.003 cm; P<0.05; Fig. 2).

TACE combined with sorafenib decreases the diameters of tumor microvascular vessels. In the TS group, the number of tumor microvessels prior to the administration of sorafenib was significantly increased compared with the number following the sorafenib treatment (8.60±1.07 vs. 4.6±0.980 cm; P<0.05). In the TACE-alone group, the number of microvessels within the tumor following the first session of TACE was also significantly increased compared with that measured following the second session of TACE (6.00±0.745 vs. 3.80±1.14 cm; P<0.05; Fig. 3).
TACE combined with sorafenib and TACE-alone treatments do not affect the diameters of the portal vein and its branches. In the TS group, the diameters of the portal vein and its branches prior to sorafenib treatment were not significantly different compared with those measured following sorafenib treatment (portal vein, 1.44±0.146 vs. 1.426±0.138 cm; portal vein branches, 0.710±0.0491 vs. 0.718±0.0591 cm; both P>0.05). In the TACE group,
the diameters of the portal vein and its branches following the first session of TACE were not significantly different compared with those measured following the second session of TACE (portal vein, 1.304±0.069 vs. 1.289±0.060 cm; portal vein branches, 0.879±0.063 vs. 0.859±0.055 cm; both P>0.05; Fig. 4).

**Discussion**

The results of the present study demonstrated that TACE combined with sorafenib significantly decreased the diameter of tumor-associated hepatic arteries and their branches, notably following the oral administration of sorafenib, while TACE alone did not alter the diameter of the tumor-associated arteries (20). Furthermore, the number of microvascular vessels in the tumor, as detected by hepatic arteriography, was markedly decreased following combination treatment with
sorafenib and TACE. Compared with the first angiography, TACE alone also significantly decreased the number of microvascular vessels within the HCC tumor tissues (21). TACE combined with sorafenib significantly reduced the diameter of tumor-associated arteries and tumor microvascular vessels when compared with TACE alone.

Previous studies have indicated that VEGF expression in patients with HCC is associated with radiological features (21,22). Previous data have also indicated that the degree of enhancement in hepatic arteriography in HCC was closely associated with the pro-angiogenic cytokine level, and that VEGF contributed to the development of dense vascular networks in cancer lesions (23,24). In the present study, following combinatorial therapy with TACE and sorafenib, the number of tumor microvascular vessels was significantly decreased. Previous studies have also demonstrated that

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**Figure 3.** Microvascular vessels. (A) TS group. "***P<0.05. (B) TACE-alone group. "*P<0.05. TACE, transarterial chemoembolization; TS group, TACE combined with sorafenib.

**Figure 4.** TS and TACE-only treatments do not decrease the diameter of portal vein and its branches. (A) Portal vein and (B) portal vein branches in the TS group. (C) Portal vein and (D) portal vein branches in the TACE group. (E-G) MRI prior to administration of sorafenib. (H-J) MRI subsequent to administration of sorafenib. E, H, F, I, G and J represent the same patient prior and after administration of sorafenib respectively. TACE, transarterial chemoembolization; TS group, TACE combined with sorafenib. The magnification for all images in Figure 4 is 1:4.
Following infusion through the portal vein, and also indicated concentrations were increased 10-fold compared with those was infused via the hepatic artery, the intratumoral contrast was obtained 80% of its blood supply from the portal vein. Previous studies have demonstrated that when contrast agent obtaining 80% of its blood supply from the portal vein, the development of unpaired arteries are among the multiple steps involved in HCC carcinogenesis. Ultimately, the increase in neo-vascularized arteries, with the assistance of pro-angiogenic cytokine secretion, arterial blood flow becomes dominant.

Recent advances in imaging have enabled clinicians to evaluate the hemodynamics of hepatocellular lesions, in particular dysplastic nodules or HCC, using CT or MRI hepatic arteriography and CT or MRI during arterial portography. As a tumor with a typically good blood supply, HCC exhibits hyper-enhancement on DSA in the majority of cases; more specifically, it demonstrates marked hyper-enhancement during the arterial phase, with such enhancement gradually decreasing during the venous phase and disappearing during the delayed phase. To the best of our knowledge, few studies have been performed to investigate the hemodynamic characteristics of HCC treated with sorafenib. In addition, the hemodynamic changes following combination treatment with sorafenib and TACE have not been well-evaluated radiologically. The present study compared the diameter of different hepatic arteries in patients treated sorafenib combined with TACE or TACE alone (36). Previously, the response of HCC to treatment has been primarily evaluated using the mRECIST criteria. However, the staining of DSA images and the structure of the vascular network prior to and following interventional therapy are priority concerns for interventional radiologists. In the present retrospective study, DSA provided a useful evaluation tool for assessing the response of HCC to combination treatment with TACE and sorafenib.

In conclusion, TACE and sorafenib are used individually in the treatment of HCC at different stages, and the two treatments have an anti-angiogenic effect, with sorafenib exhibiting broader effects due to its capacity for tyrosine kinase inhibition.
The results of the present study indicated improved hepatic hemodynamic outcomes when TACE and sorafenib were used in combination for the treatment of advanced-stage HCC, compared with TACE alone.

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Availability of data and materials

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

YFZ, HZ, HP, QL, XZ, WW, YL, MZ and JW collected and analyzed the data. YLZ, LC and JZ designed the research and wrote the manuscript. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Zhongshan Hospital Xiamen University. Patients provided written informed consent prior to treatment.

Consent for publication

Patients provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

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