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Liver Injury and Tumor-Inhibiting Effect of Sequential Transcatheter Arterial Chemoembolization and Portal Venous Embolization on Rabbit VX2 Liver Carcinoma

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The concepts of sequential transcatheter arterial chemoembolization (TACE) and portal venous embolization (PVE) were proposed to prevent the detrimental tumor growth-inducing effect of PVE and to facilitate growth of further future liver remnant (FLR). This study aimed to investigate the effect of sequential TACE and PVE on liver damage and the therapeutic effect in a rabbit VX2 liver tumor model.

Material/Methods: Rabbits bearing VX2 liver tumors were randomly divided into TACE+PVE, TACE, PVE, and Sham groups. Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), and alkaline phosphatase (ALP) at 6 h, 24 h, 3 days, and 7 days were measured by ELISA assay. Tumor diameter on day 7 was measured and the tumor sections with cleaved caspase-3 was stained and observed.

Results: Plasma ALT, AST, and ALP levels were significantly increased at the first hours after the interventions. The TACE group had higher increases than the TACE+PVE and PVE alone groups. ALT, AST, and ALP levels decreased on day 7 and presented a trend to return to the baseline level. The TACE+PVE group showed stronger tumor-inhibiting effect than the TACE and PVE alone groups and also induced the highest level of tumor cell apoptosis.

Conclusions: The liver damage caused by TACE+PVE is mild and recoverable. TACE+PVE showed stronger tumor-inhibiting effect than in the TACE and PVE group and also induced the highest level of tumor cell apoptosis.

MeSH Keywords: Carcinoma, Hepatocellular • Chemoembolization, Therapeutic • Liver Regeneration

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Background

Metastatic hepatic carcinoma (MHC), especially the metastases from colorectal carcinomas, are the most common secondary liver tumors [1]. Currently, only complete resection offers the potential for curative treatment of these metastases [2,3]. However, if the FLR is too small to meet the needs of liver function and volume, these patients are considered as unresectable [4]. Portal vein embolization (PVE) is a common strategy currently used to increase the FLR before major liver resection [5]. However, a significant drawback of PVE is the induction of tumor growth after the intervention, which increases the potential risk of secondary unresectability [5].

To prevent this risk, transcatheter arterial chemoembolization (TACE) before PVE is recommended for major hepatectomy. Recent studies suggest that sequential TACE and PVE (TACE+PVE) could significantly inhibit tumor growth and promote the increase of FLR volume, which are beneficial following tumor resection [6,7]. However, few animal studies have investigated the exact effect of TACE in preventing the detrimental tumor growth-inducing effect of PVE. Our previous study found that in a rabbit VX2 liver tumor model, TACE+PVE could significantly inhibit tumor growth and also induce a significantly higher level of liver regeneration than TACE or PVE alone via inducing higher levels of IL-6, TNF-α, and HGF secretion [8]. Therefore, this combination shows a great potential in liver carcinoma surgery. A previous study suggests that one of the disadvantages of TACE is its subsequent damage of liver function [9]. The level of liver damage caused by the sequential TACE and PVE are still not well understood. However, this is a prerequisite to ensure the combination is safe and tolerable in clinical practice.

This study aimed to investigate the effect of sequential TACE and PVE on liver damage and the therapeutic effect in a rabbit VX2 liver tumor model.

Material and Methods

Establishment of the VX2 tumor model

The study protocol was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University. Adult New Zealand White rabbits weighing 2–2.5 kg were purchased from the Experimental Animal Center of Southern Medical University.

Tumor implantation was performed according to the method introduced in a previous study [10]. In brief, VX2 tumor cell suspension were injected into thigh muscles of a carrier rabbit. Three weeks later, the solid tumor was harvested from the donor rabbit. Then, 3 tumor fragments about 0.5 mm^3 in volume were injected superficially in the subcapsular area of the left medial liver lobe using a 16-gauge angiocatheter. At 18 days after implantation, the tumor sizes were measured by CT scan and the rabbits carrying tumors of 15–30 mm in diameter were used for subsequent experiments. The images of gross specimens of VX2 liver tumors were captured after rabbits were killed.

Experimental design

Forty rabbits with VX2 tumors were divided into 4 groups: TACE+PVE, TACE, PVE, and Sham groups (n=10 in each group). In the PVE and TACE groups, PVE or TACE was performed on day 0. In the TACE+PVE groups, TACE was performed on day –7, while PVE was performed on day 0. The Sham group received sterile physiological saline as placebo embolization material. On day 7, the rest of the rabbits were killed to obtain tumors for measurement of diameter and immunofluorescent staining.

TACE and PVE interventions

TACE was performed by infusion of a suspension of 1 mg/kg 10-hydroxycamptothecin and 0.4 mL iodized oil into the left hepatic artery, and subsequent embolization was performed with PVA particles 150–250 μm in diameter. PVE was performed with polyvinyl alcohol (PVA) particles 90–180 μm in combination with 300–500 μm particles and 3 or 4 platinum coils.

Digital silhouette angiography (DSA)

DSA for hepatic artery and portal vein was performed on tumor-bearing rabbits according to the methods introduced in a previous study [11] by using a C-arm unit (PowerMobil; Siemens, Erlangen, Germany).

Immunofluorescent staining of active caspase-3

The carcinoma tissues collected the groups were fixed, embedded into paraffin, and cut into 4-μm-thick sections. The sections were transferred onto cover slips. The sections were dewaxed and rehydrated in Trilogy buffer (Cell Marque, Rocklin, CA) in a pressure cooker for 15 min, blocked in 5% serum for 30 min, and then incubated with antibodies against cleaved caspase-3 at Asp175 (1: 500, #P42574, Cell Signaling, Danvers, MA) at 4°C overnight. After washing, the sections were incubated in a secondary Alexa Fluor-555-labeled goat anti-rabbit IgG (#4413, Cell Signaling) for 30 min at room temperature in the dark. Cover slips were mounted with mounting media containing DAPI to stain the nuclei. The number of cleaved caspase-3-positive cells was scored by counting 3 sets of at least 100 tumor cells each under the microscope.

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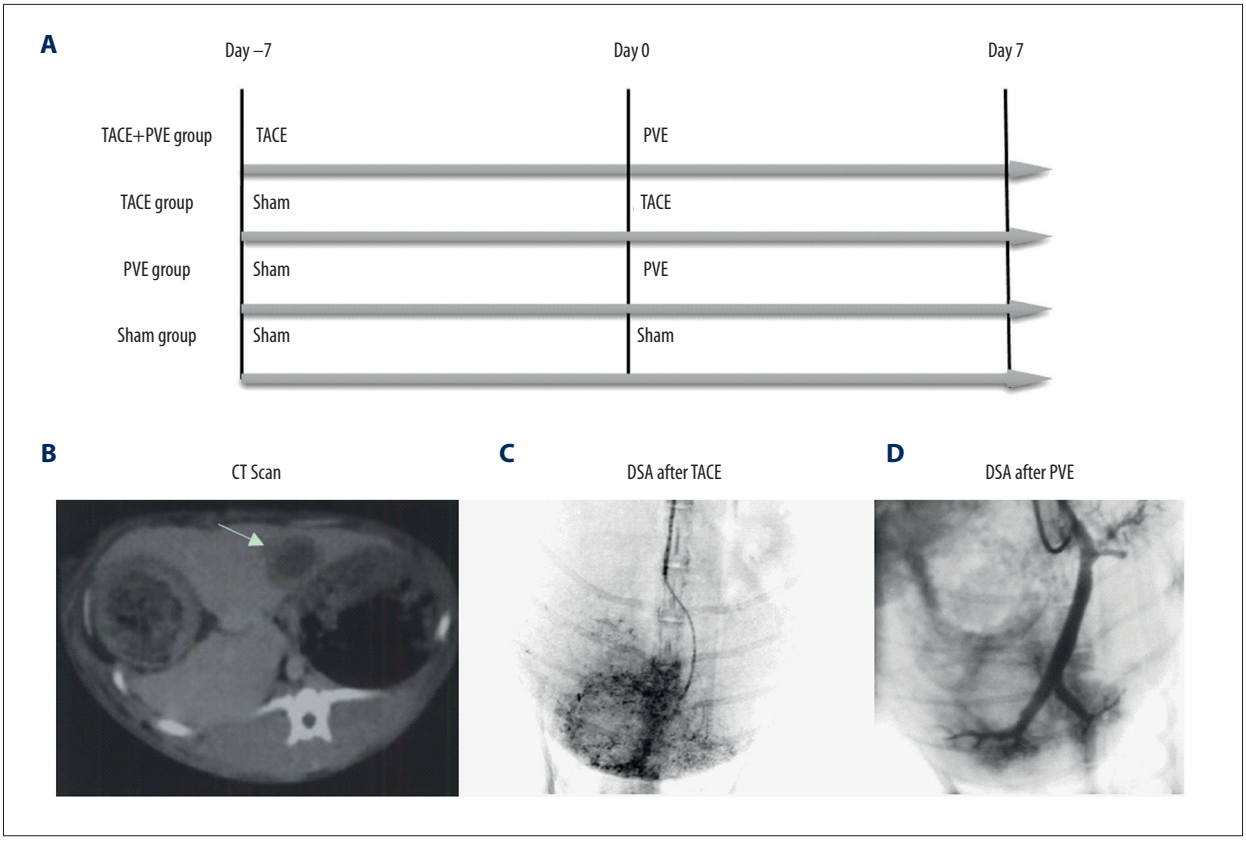

Figure 1. Therapeutic interventions. (**A**) Experimental design of TACE+PVE, TACE, PVE, and Sham groups. (**B**) CT images of the VX2 tumor. (**C, D**) DSA image after TACE (**C**) or PVE (**D**).

ELISA assay

Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), and alkaline phosphatase (ALP) were measured at 6 h, 24 h, 3 days, 7 days using ELISA assay kits purchased from Boster (Wuhan, China), according to the manufacturer's instructions.

Statistical analysis

Data are reported as means ± standard deviation (SD). Comparison between groups was performed using unpaired *t* test in GraphPad Prism 5.0. A *p* value of <0.05 was considered as statistically significant.

Results

Therapeutic interventions

After the VX2 tumor model was established, the rabbits carrying tumors were randomly divided into TACE+PVE, TACE, PVE, and Sham groups (n=10 in each group). In the PVE and TACE groups, PVE or TACE was performed on day 0. In the TACE+PVE groups, TACE was performed on day –7, while PVE was performed on day 0 (Figure 1A). The sham group received sterile physiological saline as placebo embolization material (Figure 1A). CT scans helped to confirm the tumor position (Figure 1B, white arrow) before interventions. DSA images showed that there was obvious iodized oil accumulation within the tumor after TACE (Figure 1C), and PVE also was performed successfully (Figure 1D).

Assessment of the liver damage caused by TACE+PVE, TACE, or PVE

We compared the extent of liver damage caused by TACE+PVE, TACE, or PVE. The results showed that ALT, AST, and ALP levels were significantly increased in the 3 groups starting in the first hours after the interventions (Figure 2A–2C). The TACE groups had higher increases than the TACE+PVE and PVE alone groups (Figure 2A–2C). ALT, AST, and ALP levels decreased on day 7 and presented a trend to return the baseline level (Figure 2A–2C). No obvious change of TBIL was observed in the different groups (Figure 2D).

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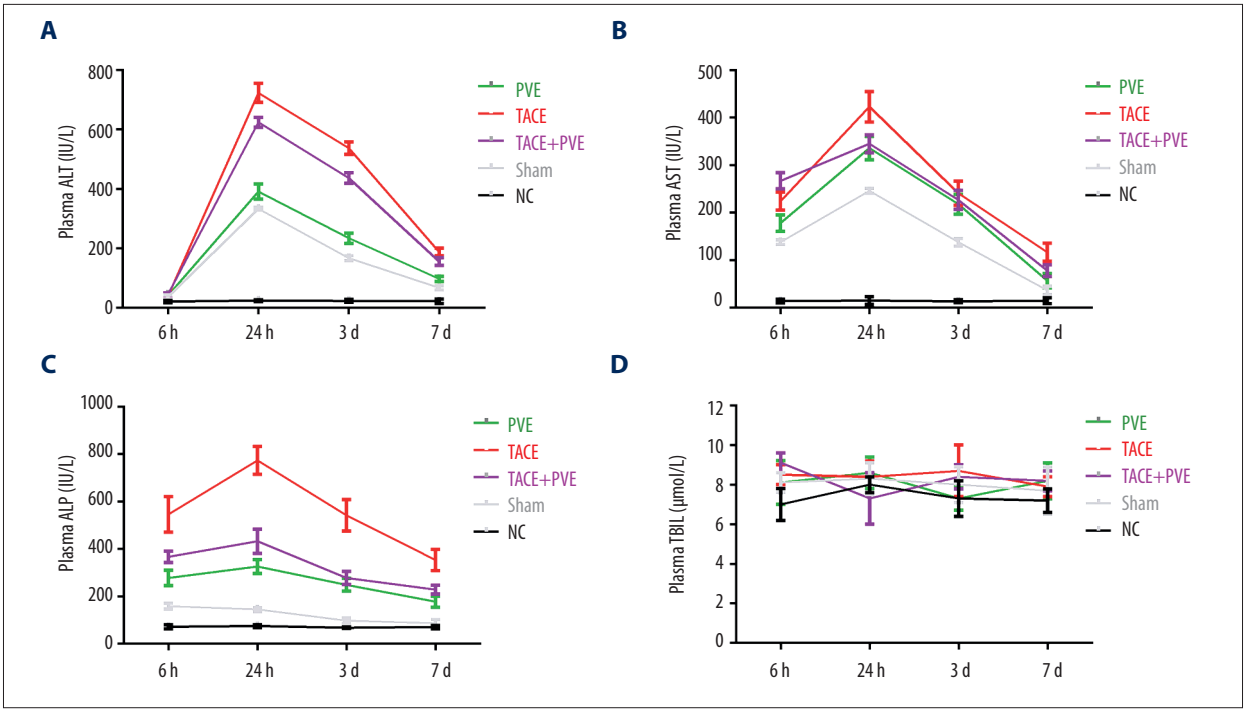

Figure 2. Assessment of the liver damage caused by TACE+PVE, TACE or PVE. (**A–D**) The serum level of ALT (**A**), AST (**B**), ALP (**C**), and TBIL (**D**) in the 4 groups at 6 h, 24 h, 3 days, and 7 days.

The therapeutic effect of the interventions on the VX2 liver carcinoma

On day 7, the rabbits in the 4 intervention groups were all killed and the tumor size was measured in terms of tumor diameter (Figure 3A). The results showed that only TACE+PVE and TACE significantly inhibited tumor growth (Figure 3B, 3C). The TACE+PVE group showed stronger tumor-inhibiting effect than in the TACE group (Figure 3C). To further examine tumor cell apoptosis caused by the interventions, the tumor sections were stained with anti-cleaved caspase-3. The results of immunofluorescent staining showed that the tumor sections from the TACE+PVE, TACE, and PVE groups all had obvious cleaved caspase-3 staining (Figure 3D, 3E), while the TACE group had the highest ratio of staining (Figure 3D, 3E), which suggests the highest level of cell apoptosis.

Discussion

PVE can redirect portal vein flow toward specific hepatic segments and is a method currently used for FLR increase before surgical resection [12,13]. However, previous research also indicated that PVE was associated with poor prognosis due to higher disease recurrence and tumor growth acceleration in both embolized and non-embolized liver lobes [14–16]. TACE has been considered as a therapeutic option in multiple situations. It can be used in the palliative setting for patients with post-resection intrahepatic recurrent hepatocellular carcinoma (HCC), as an adjuvant therapy for preventing postoperative recurrence, and as a primary treatment modality in ruptured HCC [17]. A previous meta-analysis showed that TACE significantly reduced the overall 2-year mortality rate in patients with unresectable HCC [18].

Recently, the concept of sequential TACE and PVE was proposed to prevent the detrimental tumor growth-inducing effect of PVE and to facilitate further FLR. Several recent studies evaluated the safety and efficacy of sequential TACE and PVE before major hepatectomy for patients with HCC and obtained some positive findings to support this concept. One study found that the FLR after sequential TACE and PVE increased from 32.3% to 71.4% (mean 55.4%). In addition, no patients had intra- or extrahepatic metastasis[19]. A Korean study compared the prognostic impact of preoperative sequential TACE and PVE or PVE alone in patients who underwent right liver resection for solitary HCC [7]. The results showed that the 1-, 3-, 5-, and 10-year overall survival rates in the TACE+PVE group were 96.3%, 83.4%, 83.4%, and 47.6%, respectively, and in the PVE alone group were 84.6%, 76.9%, 57.7%, and 19.2%, respectively [7]. A recent retrospective study based on 116 Chinese patients with primary HCC showed that the 1-, 3-, and 5-year overall survival rates for the TACE and TACE + PVE groups were 39/64, 16/64, 0/64, and 42/52, 19/52, 6/52, respectively (P=0.015, 0.046, and 0.002, respectively) [20]. Our previous study also indicated that, in rabbits bearing VX2 liver

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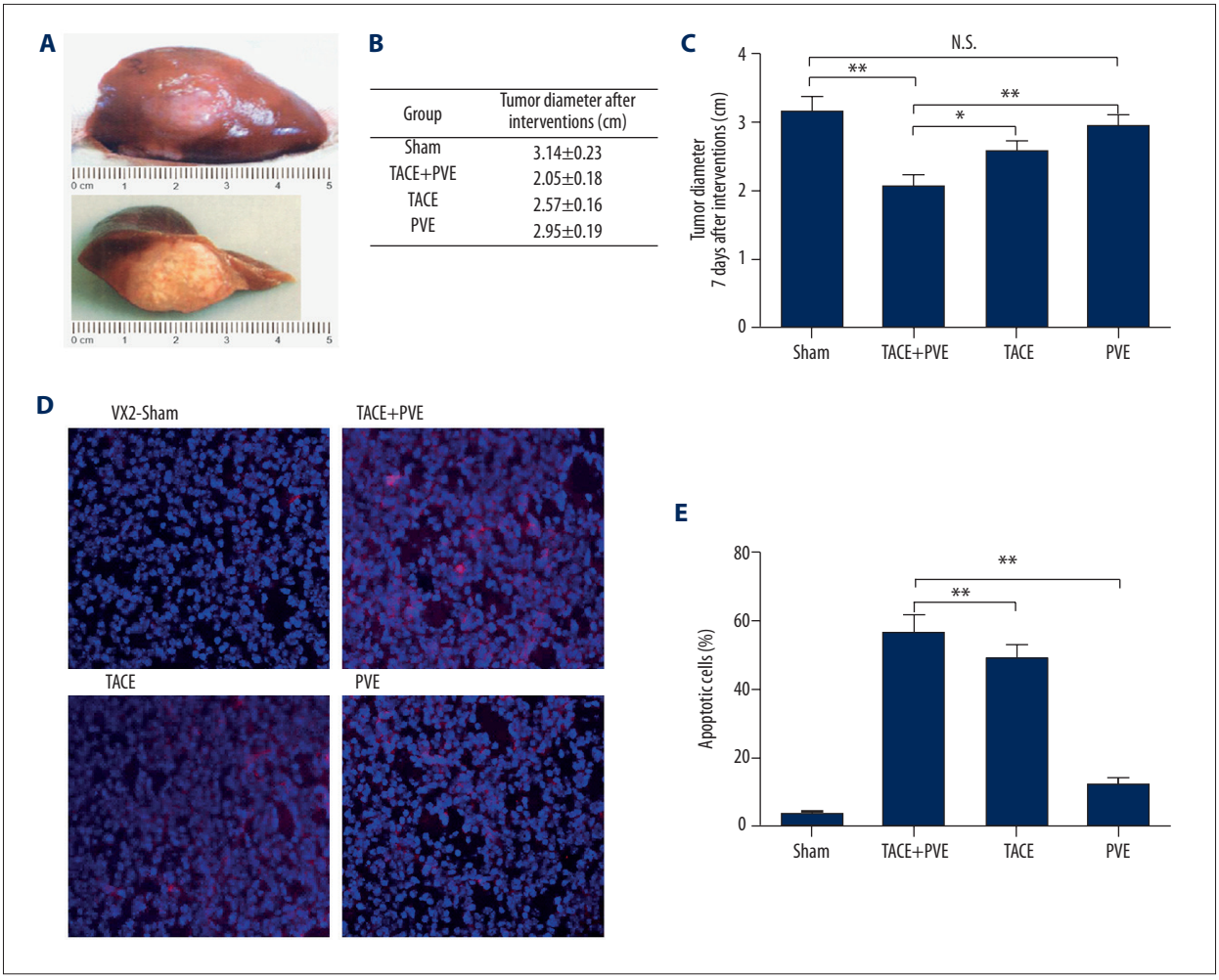

Figure 3. Therapeutic effect of interventions on VX2 liver carcinoma. (**A–C**) The gross specimen and cross-section of rabbit VX2 liver tumor (**A**), quantitation (**B**) and bar chart (**C**) of tumor diameter in the 4 groups on day 7.)(**D, E**) Representative images (**D**) and quantitation (**E**) of cells with positive staining of cleaved caspase-3 in the tumor sections from the 4 groups on day 7. * $p<0.05$; ** $p<0.01$. N.S. – not significant

tumor model, TACE+PVE significantly inhibited tumor growth and also induced a significantly higher level of liver regeneration than TACE or PVE alone via inducing higher levels of IL-6, TNF-α, and HGF secretion [8]. These studies suggest that TACE+PVE might be a useful strategy to inhibit tumor growth and induce FLR at the same time.

However, although TACE is an interventional strategy with low invasiveness and injury to liver, it still may cause considerable damage to liver function [9,21] . In this study, we further assessed and compared liver damage caused by TACE+PVE, TACE, or PVE alone. The results showed that the liver damage was even lower in the TACE+PVE group than in the TACE group in terms of plasma AST, ALT, and ALP levels. This might be a result of the 1-week interval between TACE and PVE, which enables the liver to recover before undergoing another intervention. In addition, the plasma AST, ALT, and ALP levels all presented a trend to return to baseline level by 7 days after the intervention, suggesting that the liver damage was mild and recoverable. To further verify and compare the tumor-suppressive effect of the interventions, we compared tumor diameter and assessed the expression of cleaved caspase-3 in each group. The results confirmed that the TACE+PVE group showed a stronger tumor-inhibiting effect than in the TACE and PVE groups, and also induced the highest level of tumor cell apoptosis.

Conclusions

The liver damage caused by TACE+PVE is mild and recoverable. TACE+PVE showed stronger tumor-inhibiting effect than in the TACE and PVE groups, and also induced the highest level of tumor cell apoptosis.

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