



Expression of MMP-2 in residual VX2 liver tumor after transcatheter arterial embolization combined with portal venous embolization in an animal model

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ABSTRACT

Objective: This study aimed to analyze the effects of transcatheter arterial embolization (TAE) combined with portal venous embolization (PVE) on the expression of MMP-2 in residual VX2 liver tumor tissues, liver function and non-embolic lobe regeneration.

Methods: A total of 72 rabbits were randomly divided into Sham, TAE, PVE and TAE + PVE groups (n = 18/group). The tissue samples from each group were taken at 6 h, 3 days and 7 days after interventional operation, respectively. MMP-2 expression was detected by immunohistochemistry, Real-time PCR, and Western-blotting. The main indicators (such as AST, ATL, and TBIL) of liver function and the volume of non-embolized hepatic lobes were measured in each group after operation. One-way ANOVA and Kruskal-wallis method were used for statistical analysis.

Results: The expression of MMP-2 mRNA and protein remained the highest in the Sham group, and the expression of MMP-2 mRNA and protein in TAE, PVE and TAE + PVE groups were successively increased, and the expression of MMP-2 in TAE + PVE group was always significantly higher than TAE group. The AST and ALT levels in each group on day 7 after operation showed a significant declination, and all groups have recovered to the preoperative baseline level and TBIL has a slight fluctuation in each group after operation with no statistical difference. On day 7 after operation, the increasing volume of non-embolized liver lobes in TAE + PVE group showed a more significant effect than those in PVE group, but there was no statistical significance (37.62 ± 1.54 ml VS 36.18 ± 1.15 ml, $P = 0.881$), and its volume was significantly higher than those in the sham group (27.03 ± 1.11 ml).

Conclusion: TAE + PVE is considered to be an efficient and safe approach for treating rabbit VX2 liver transplantation tumor, but the expression of MMP-2 increased fastest after TAE + PVE, which might promote tumor cell invasion and metastasis.

Introduction

In recent years, the incidence of both metastatic hepatic cancer (MHC) and primary hepatic cancer (PHC) has been similar in China.¹ Tumor resection and liver transplantation are still recommended as primary methods for treating hepatic tumors potentially.^{2–4} However, the resection rate of liver cancer remains less than 20%.⁵ Transcatheter arterial embolization/chemoembolization (TAE/TACE) and portal venous embolization (PVE) are complementary methods for treating advanced liver cancer.^{6,7} Theoretically, there are anastomotic branches between hepatic artery and portal venous, and the TAE combines with PVE to completely block the blood supply of the tumor area, producing a

more hypoxic environment than TAE alone.⁸ Therefore, the effect of ischemic and hypoxic environment produced by TAE when combined with PVE on tumor cell invasion needs further elucidation. In clinical practice, the serum level of Matrix metalloproteinase (MMP)-2 has long been an important reference for judging the prognosis of liver cancer.⁹ Rabbit VX2 liver cancer can also express a variety of MMPs. So, it is considered as a suitable animal model for studying the expression of MMPs.¹⁰

Based on the above background, in order to further understand the invasiveness of tumor cells after TAE + PVE, this study established a VX2 liver tumor model to explore the effect of local hypoxic environment caused due to TAE + PVE on the expression of MMP-2 in residual cancer

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tissues. Future liver remnant (FLR) and liver function were also observed.

Materials and methods

Animals models

New Zealand white rabbits weighing 2.5–3.0 kg (regardless of sex) were purchased from the Experimental Animal Center of Anhui Provincial Hospital. After induction of general anesthesia with an intravenous injection of 2.0 ml/kg of chloral hydrate, a longitudinal incision under the xiphoid process was made to expose the left inner lobe of the liver. Firstly, an 18-gauge puncture needle (Hakko, Nagano, Japan) was used to puncture the liver lobe with a depth of 10 mm. The needle core was then used to push into the three VX2 tumor fragments (Hongshun Biotechnology Co., Ltd. Shanghai, China) with a volume of 0.5 mm³. Next, a gelatin sponge strip (Alicon, Hang zhou, China) with a size of 2 mm*2 mm*5 mm was used to block the puncture passage. Finally, the incision was sutured. On day 14 after implantation, intrahepatic tumors were identified by 3.0T magnetic resonance imaging (Magnetom Avanto, Siemens Medical Solution, Erlangen, Germany). Animal models with a tumor diameter of 1–2 cm, single lesion, with rich blood supply and no extrahepatic metastases were selected for the study.

Grouping design

A total of 72 rabbits with VX2 liver tumor were divided into 4 groups of Sham, TAE, PVE and TAE + PVE groups, with 18 in each group. On day 15 after implantation, TAE and PVE were performed. In the Sham and TAE groups, normal saline solution was used as embolic material in PVE operation, and these are regarded as placebo. In the Sham and PVE groups, normal saline solution was also used during operation by TAE. In the TAE + PVE group, the specific operation conducted was described in the following paragraph. Animals in each group were euthanized at 6 h, 3 days and 7 days after interventional operation. The regions of residual tumor tissues sampling were located in the adjacent area of VX2 tumor margin and normal liver tissues, which were harvested for further analysis.

Magnetic resonance Imaging

The self-made plastic device was used for supine fixation after general anesthesia. The scanning mode included T1WI and T2WI sequences, spin echo (SE) sequence was used in T1WI, and the parameters were as follows: repetition time/echo time (TR/TE) = 540 MS/7.1 MS, layer thickness was 4.0 mm, spacing was 1.0 mm, visual field was 14 cm × 14 cm, matrix was 352 × 192, NEX was 4, imaging time was 90 s. Fast spin echo (FSE) sequence was used in T2WI, and the parameters were as follows: TR/TE = 3180 MS/86.7 MS, layer thickness was 4.0 mm, spacing was 1.0 mm, visual field was 14 × 14 cm, matrix was 320 × 256, NEX was 4, imaging time was 90s. After adjusting the parameters, a DWI scan was performed. The echo-planar imaging (EPI) sequence was used in diffusion-weighted imaging (DWI). The B value was 0 and 300 s/mm², TR/TE = 3000 ms/76.6 MS, field of view (FOV) was 20 × 15, excitation times was 8, and scan time was 45 s. gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA, 1 ml/kg) was used as a contrast agent for enhanced scanning. The scanning was initiated 15 s after intravenous injection of Gd-DTPA. T1WI, T2WI and DWI images were obtained directly after the inspection, and ADC images were obtained after post-processing by workstation software.

TAE and PVE procedures

TAE and PVE were performed under the guidance of digital subtraction angiography (DSA). For TAE, the rabbits were fixed on the operating table in supine position after inducing general anesthesia, and the skin at the right femoral artery was cut open to bluntly separate the right

femoral artery, followed by introduction of a 4F-Cobra catheter (Boston Scientific, USA). After celiac arteriography with 4F-Cobra catheter, a 2.6F coaxial catheter system (Terumo, Tokyo, Japan) was advanced into the left hepatic artery. Next, for defining the position of the catheter, superliquid iodine-oil (Laboratoire Andre Guerbet, France) was slowly injected at a dose of tumor's maximum diameter (cm) multiplied by 0.2 ml. The end point of embolization was the left hepatic artery contrast agent stagnation without iodized oil reflux. For PVE, the preoperative preparation was similar as described above, and the hilum of the liver was exposed through a subxiphoid incision. A self-made puncture needle was used to directly puncture the external segment of portal vein and introduced a 2.6 F coaxial catheter system (Terumo, Tokyo, Japan) into the left branch of portal vein. After portal portography with a micro-catheter, the portal vein branches of the hepatic lobe in which the tumor was located were embolized with 150–350µm PVA particles (Hang Zhou, Alicon, Pharm SCI&TEC, Co., Ltd., Hang zhou, China). The end point of embolization was the disappearance of portal vein branches during repeat angiography. The contrast agent used in the operation was iodoxanol injection (Heng Rui, Pharmaceutical, Co., Ltd., Lian yungang, China).

Immunohistochemistry

After the rabbits were euthanized by anesthetic overdose, both the residual tumor tissues and the adjacent normal liver tissues were harvested, immobilized in neutral formalin and then embedded in paraffin. The paraffin wax blocks were cut into 4 µm thick pieces, and then antigen repair was performed. After 0.01 mol/L sodium citrate buffer (pH6.0) was heated in a microwave oven and brought to boil, the tissue chip was placed in it. Next, immunohistochemical staining of paraffin sections was performed. Fresh 3% H₂O₂ was prepared with distilled water or PBS. It was sealed at room temperature for 5–10 min and washed thrice with distilled water to eliminate the activity of endogenous peroxidase. A suitable proportion of diluted anti-MMP-2 (Bioss) was added to the tissue sections and incubated at 4 °C overnight. The sections were then incubated with secondary antibody at 37 °C for 10–30 min. Finally, Streptomyces ovalbumin labeled with horseradish enzyme was dripped and incubated at 37 °C for 10–30 min. After washing with PBS, DAB (Regal) substrate was used to observe the binding reactions.

MMP-2 immunohistochemical staining was evaluated by two pathologists in a blinded manner under a light microscope (EVOS). Five high-power fields (× 400) were randomly selected to count the positive cells, in which 0 indicates no positive cells or only a few cells; 1 indicates number of positive cells was less than 25%; 2 indicates number of positive cells was less than 50%; 3 indicates number of positive cells was less than 75% and 4 indicates number of positive cells was more than 75%; and 0 point for no coloring, 1 point for light yellow, 2 points for tan, and 3 points for dark brown. The final point was obtained by multiplying the result of the first point with the second point, and the results were classified into 4 grades: negative (points ≤2); weakly positive (2 < points ≤4); positive (4 < point ≤6); and strongly positive (point > 6).

Real-time PCR

Real-time PCR (Thermo, PIKOREAL 96) was performed to measure the expression of MMP-2 mRNA. The total RNA was isolated from the tissues using TRIzol (Invitrogen) reagent, and then cDNA was used for reverse transcription. Fluorescence quantitative PCR reaction was performed in accordance with the conventional amplification process by referring to the kit (QuantiFast SyBr Green PCR kit, Qiagen). The forward primer of MMP-2 was 5'-ATG ACA TCA AGG GCA TTC AA-3', and the reverse primer was 5'-ACG ATG TCC TGT GTG CAG AT-3'. The forward primer of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was 5'-CAC CCA CTC CTC TAC CTT CG -3', and the reverse primer was 5'-TGC TGT AGC CAA ATT CGT TG -3'. The experimental procedures were carried out according to the instructions of the kit. The Ct values of the

target gene and the caregiver gene were obtained through real-time fluorescence curve. The expression of the MMP-2 mRNA was quantified using $2^{-\Delta\Delta C_t}$ method relatively.

Western-blotting

Western blot analysis was performed to measure the relative expression of MMP-2 at the protein level. A total of 100 mg of tumor tissue from each group was grinded and 1 ml of RIPA cell lysate was added and centrifuged at 12,000 RPM for 10min. After that, equal amounts of protein were separated by SDS-PAGE, transferred, and then blocked using 5% BCA. Next, the membrane was incubated with anti-MMP-2 antibody (Bioss, dilution:1/300) for overnight at 4 °C. Next, the membranes were incubated with Horseradish peroxidase (HPR) and conjugated with secondary antibodies for 2 h at room temperature. The ECL western blotting substrate (Promega, Madison, WI, USA) was used to visualize the protein band signals and Image J software (Beijing Kechuang Ruixin bio-gel imaging system) was used to quantify the relative gray-scale value.

Blood tests

The blood samples were collected from the ear veins and stored in anticoagulant tubes. The plasma levels of alanine aminotransferase

(ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) were measured for 1 h before operation, 6 h, 3d and 7d after operation by the laboratory department of Anhui Provincial Hospital.

Volume of nonembolized liver lobes

For determining the levels of liver hypertrophy, the volume of non-embolized liver lobes (liver right lobes and caudate lobe) were measured after euthanasia by water displacement method.¹¹ The liver samples were placed in a beaker containing normal saline at 37 °C, and the volume of the liver was calculated by the height difference of water surface.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 (SPSS Inc. Chicago, IL, USA), and GraphPad Prism 6 (GraphPad software, Inc., La Jolla, CA, USA). Continuous variables were presented as mean ± standard deviation (SD). Qualitative data were expressed as the frequencies and percentages. One-way ANOVA method was used for comparison of continuous variables, and Kruskal-wallis method was used for qualitative data. P values of less than 0.05 were considered to be statistically significant.

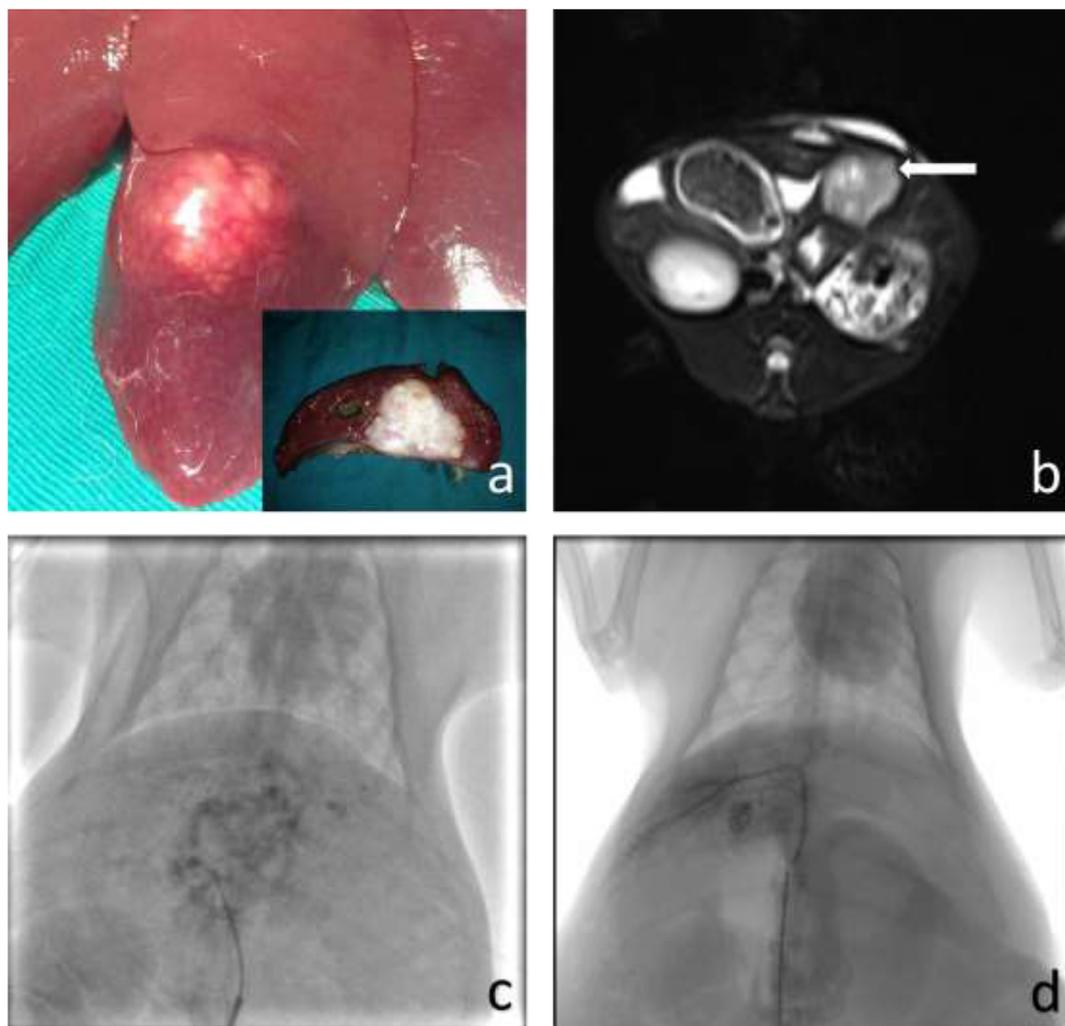


Fig. 1. (a) The general features of rabbit VX2 liver tumor and tumor specimen section. (b) The T2WI axial fat suppressed imaging. DSA features of rabbit VX2 liver tumor after TAE (c) and PVE (d).

Results

Establishment of animal model and interventional operation

A total of 72 rabbits were randomly selected by successfully establishing the animal models from the experiment. After day 14 of tumor implantation, all the tumors were located in the left inner lobe of the liver, and are spherical in shape (Fig. 1a). The part that protrudes from the liver surface appeared gray-white with transparent capsule and hard texture. The yellow-white necrotic tissue was seen in the center of the section, which was surrounded with a vigorous and uneven thickness of fish-like tissue (Fig. 1a). MRI confirmed the tumor characteristics before operation, and the results showed that the liver signal was decreased and the tumor was still in the middle, whereas high signal in T2WI fat suppressed imaging (Fig. 1b, white arrow). DSA showed that the left hepatic artery was thicker than that of the right hepatic artery, the tumor nourishing vessels were irregular and distorted, and the parenchymal tumor was stained obviously with uneven internal staining. Some models showed “blood pool” signs. Obvious tumor staining was observed after slow injection of super liquid iodine oil into the target vessels (Fig. 1c). The portal vein angiography showed clear and complete secondary to tertiary branches, and were in the shape of inverted roots. The signs of portal vein compression deformation were visible in the liver lobe at the tumor location, which was more obscure than the opposite branches. After PVE, the branch ends of the portal vein were truncated, and the blood flow of the non-embolized branch remained normal (Fig. 1d).

Immunohistochemical analysis of MMP-2 in postoperative residual tumor area

Immunohistochemical staining showed that MMP-2 was widely expressed in the cytoplasm of residual tumor, in the extracellular space of the tumor and in the adjacent extracellular space of normal liver, while the expression of MMP-2 was rare in the distant normal liver tissues and in the center of tumor necrosis (Fig. 2a). Compared with sham group, the positive expression rates of TAE, PVE, TAE + PVE in different groups at the same time window were not completely equal (Table 1). The P values of statistical analysis were less than 0.05 (p = 0.032 in 6 h group, p = 0.045 in 3d group and p = 0.030 in 7d group). The positive expression

rates of PVE and TAE + PVE groups were significantly higher than those of TAE group.

Detection of the relative expression of MMP-2 mRNA in each group by RT-PCR

The MMP-2 mRNA expression level of the sham group remained the highest at all time points, and that of TAE, PVE and TAE + PVE groups was increased successively in a time-dependent manner. The relative expression of MMP-2 mRNA in TAE + PVE group was significantly higher than that of TAE group at different postoperative time points with statistical significance (Fig. 2b).

Detection of relative expression of MMP-2 protein in each group by Western-blotting

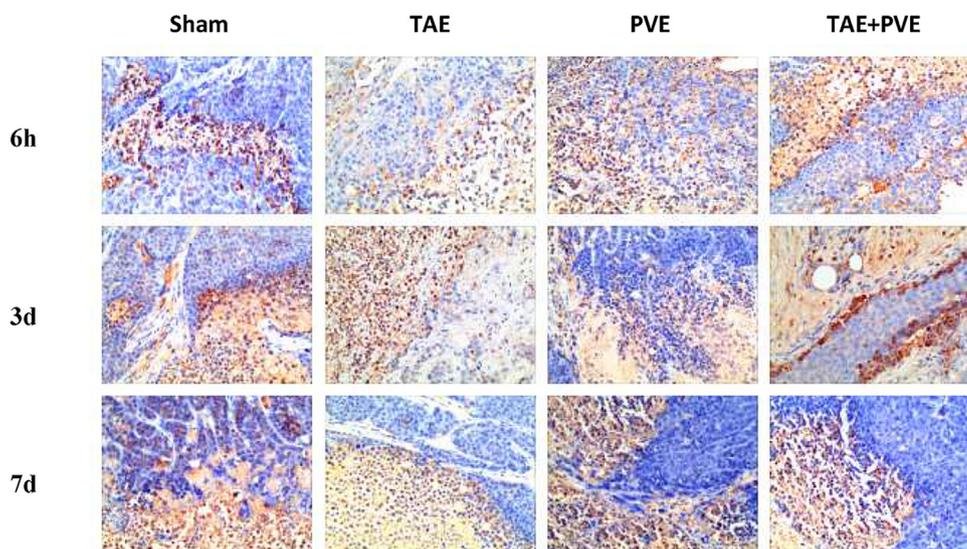
At any time point, the MMP-2 protein expression level of the sham group demonstrated to be the highest, and that of TAE, PVE and TAE + PVE groups was successively increased in a time-dependent manner. Electrophoretic bands for some groups were shown in Fig. 2c. The relative expression of MMP-2 protein in TAE + PVE group was significantly higher than that of TAE group at all time points (Fig. 2d). After operation, the relative expression of MMP-2 protein in TAE group was significantly decreased, but the difference between TAE group and TAE + PVE group was gradually narrowed with prolonged time period, and there was a tendency to return to the level of the sham group.

The effect of operation on the physiological state of the liver

There was no statistical difference in the main indexes of liver function (AST, ALT, and TBIL) in each group before operation. In the sham

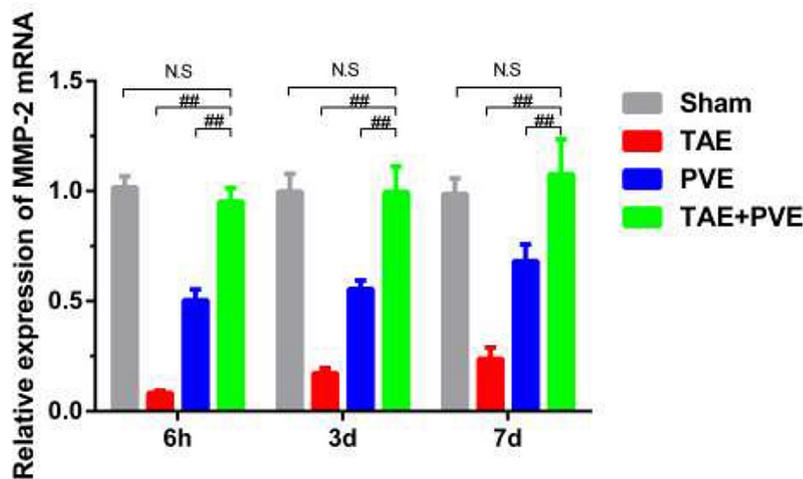
Table 1
The positive and strong positive number of MMP-2 immunohistochemistry in each group.

Time	Sham (18)	TAE (18)	PVE (18)	TAE + PVE (18)
6 h	6 (100%)	1 (16.7%)	4 (66.7%)	4 (66.7%)
3d	6 (100%)	3 (50.0%)	5 (83.3%)	5 (83.3%)
7d	6 (100%)	4 (66.7%)	5 (83.3%)	6 (100%)



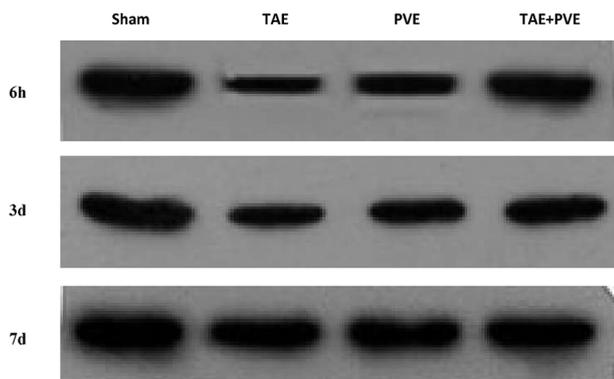
a. MMP-2 immunohistochemical pathological picture after operation (×200).

Fig. 2a. MMP-2 immunohistochemical pathological picture after operation (×200).



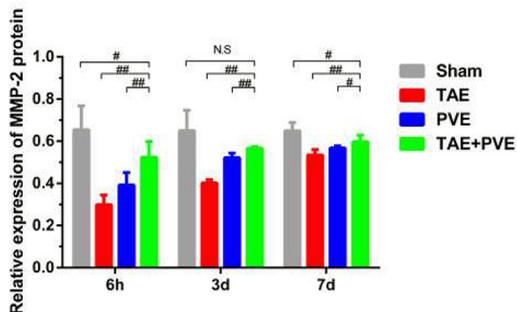
b. The relative expression of MMP-2 mRNA at three time points (##-P<0.01; N.S-not significant).

Fig. 2b. The relative expression of MMP-2 mRNA at three time points (##-P<0.01; N.S-not significant).



c. MMP-2 protein electrophoresis bands.

Fig. 2c. MMP-2 protein electrophoresis bands.



d. The relative expression of MMP-2 protein at three time points (#-P<0.05; ##-P<0.01; N.S-not significant).

Fig. 2d. The relative expression of MMP-2 protein at three time points (#-P<0.05; ##-P<0.01; N.S-not significant).

group, there was no significant fluctuation in the indexes of liver function at each time point. Six hours after operation, the AST and ALT levels were significantly increased in each group (Fig. 3a and Fig. 3b), and the indexes of transaminase in TAE + PVE group and PVE group were significantly higher than those in TAE and sham groups (P < 0.05). With

prolongation of time, the transaminase index in each group was decreased gradually, and the transaminase level was significantly dropped on the day 7 after operation. There was no significant fluctuation in the serum bilirubin level in each group after operation (Fig. 3c). After selective hepatic artery and selective portal vein embolization, the left lobe of the liver was completely embolized, while the right lobe and caudate lobe of the liver were non-embolized. The volume of non-embolized liver lobe was increased rapidly in TAE + PVE group and PVE group, while no significant change was observed in TAE group and sham group (Fig. 3d). On day 3 after operation, TAE + PVE group and PVE group had statistical significance when compared with TAE group, while TAE + PVE group demonstrated no statistical significance when compared with PVE group. On day 7 after operation, TAE + PVE group and PVE group still maintained a high growth rate of non-embolized liver lobe, and the TAE + PVE group was slightly higher than the PVE group (P < 0.05).

Discussion

TAE/TACE can selectively block the tumor nourishing artery to killing the tumor cells.^{12,13} PVE can compensate hyperplasia of non-embolized liver lobes and provide sufficient liver function reserve for surgical resection.¹⁴ Previous studies have suggested that liver cancer receive dual blood supply, in which the hepatic arterial blood supply acts as the main source of tumors, while portal vein mainly supplies the surrounding tissues of the tumors.^{15,16} Early animal studies also showed that with the growth of liver tumors, blood supply from the hepatic artery increases and that from the portal vein decreases, but the portal vein still exists around the tumor.¹⁷ Based on the complex hemodynamic characteristics of liver tumors, both TAE and PVE are regarded as inadequate when used alone. Because of the balance between hepatic artery blood flow and portal vein blood flow, the portal vein blood flow can be increased by feedback after TAE alone, and this might be the main source of nutrition for growth of residual cancer cells.¹⁸ Compared with hepatic artery, portal vein contains more pro-cell growth substances, such as hepatocyte growth factor (HGF), epidermal growth factor (EGF), insulin, etc., which in turn can guide normal hepatocyte proliferation and also increase the risk of progression of potential tumor cells in the non-embolized liver lobes.^{19–21} Overall, TAE + PVE can maximize the cutting off of tumor blood supply and reduce the possibility of tumor spread via the portal vein theoretically.

Indeed, clinical studies have shown that TAE/TACE combined with

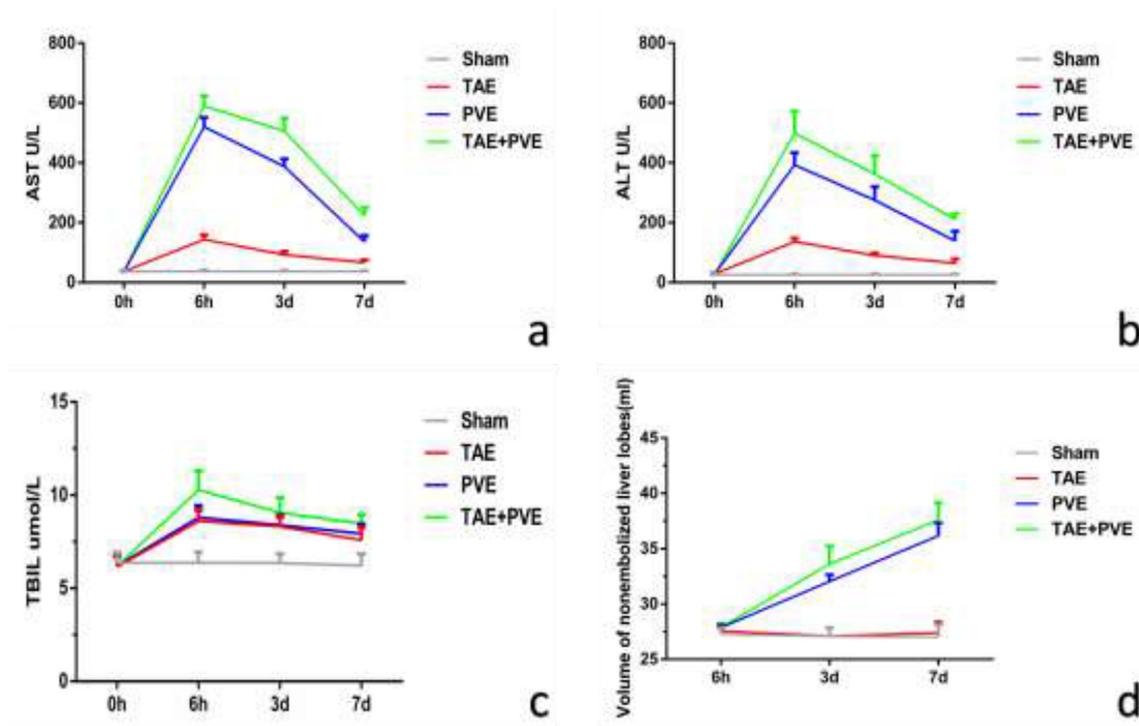


Fig. 3. The trend of AST(a), ALT (b) and TBIL(c) in each group after operation. (d)The trend of nonembolized liver lobes volume increase in each group after operation.

PVE demonstrated a high survival rate than TAE/TACE alone or PVE combined with surgical resection for HCC.^{22,23} However, some clinical reports have confirmed that liver tumor recurrence and metastasis can still be observed after TAE/TACE + PVE.^{5,24,25} To investigate the changes of invasion of residual cancer cells after TAE combined with PVE, MMP-2 was used as a measurement index and a preliminary experimental study was conducted by establishing a rabbit VX2 liver transplantation tumor model. MMP-2 is secreted in zymogen form, and is activated to type IV collagenase form. This in turn degrades the extracellular matrix and basement membrane, providing the tumor cells to infiltrate the surrounding tissues through basement membrane penetration, ultimately leading to cells invasion and migration.²⁶ Ottino P believes that hypoxic microenvironment caused by rapid growth of malignant tumors might induce the expression of MMP-2.²⁷ Recent study also found that MMP-2 is believed to activate ERK and JNK signaling pathways and promote tumor cell migration and invasion.²⁸ This study found that the expression of MMP-2 was significantly lower in each group after TAE at different time points than in the sham group. This concluded that most of the proliferating cells were killed after TAE, and the number of relatively active tumor cells was reduced, resulting in decreased macro-MMP-2 expression. The expression of MMP-2 in PVE group was also lower than those of the control group, but the effect remained weaker than that of TAE group. The reason for this might be that most of the parenchymal areas of the tumors were supplied by hepatic artery, and PVE alone could not kill the tumor cells completely. Compared with TAE + PVE group, the expression of MMP-2 in TAE + PVE group was lower than that in sham group, but was significantly higher than that in TAE group. RT-PCR showed that the relative expression of TAE + PVE group was always higher than that in TAE group. Although the difference between the two groups was decreased with time, there still remained a significant difference between the two groups until 7 days after operation (1.077 ± 0.065 VS 0.238 ± 0.052 , $P < 0.01$). Western-blotting results showed that the protein synthesis of MMP-2 was similar to that of mRNA. On day 7 after operation, the protein synthesis of TAE + PVE group was close to that of sham group (0.598 ± 0.031 VS 0.650 ± 0.039 , $P < 0.05$). It can be seen that the harsh hypoxic environment after combined embolization

promotes significant expression of MMP-2 in VX2 residual cancer cells.

In addition, PVE group and TAE + PVE group showed obvious advantages of promoting compensatory proliferation of non-embolized liver lobes. The results showed that FLR increased rapidly in PVE group and TAE + PVE group after operation. There was no significant difference in volume change between the two groups on day 3 after operation, but it was significantly higher than that of TAE group and sham group. On day 7 after operation, combined embolization showed a more significant effect than simple portal vein embolization, but no statistical significance was observed (37.62 ± 1.54 ml VS 36.18 ± 1.15 ml, $P = 0.881$), and its volume was significantly higher than that observed in the sham group (27.03 ± 1.11 ml). Previous studies have reported^{29,30} that TACE combined with PVE better regulates the expression of liver cytokines than PVE alone, promoting the regeneration of liver cells. The results of this study might be related to this mechanism.

In terms of safety, except the sham group, the transaminases in other groups were significantly increased after operation, especially in TAE + PVE group and PVE group. The possible reason for this was that the normal liver tissues were supplied by portal vein. The liver function damage after portal vein embolization was more serious than that after simple hepatic artery embolization, and the extreme phenomenon of complete necrosis of liver tissues was observed in combined embolization. Therefore, the AST and ALT levels were increased more than ten times at 6 h after operation. However, the transaminase levels in each group on day 7 after surgery showed a significant declination, and the levels were recovered to the preoperative baseline levels. In terms of TBIL, there was a slight fluctuation in each group after surgery, but no statistical difference was found, and generally remained stable. This experiment ensured a high survival rate of rabbits after operation without using hepatic protective agents, indicating that the combined embolization is reliable and safe.

This study did not use chemotherapy drugs, and the main purpose of this study is to exclude the interference of tumor cells caused by chemotherapy drugs, and only observe the interference of ischemia and hypoxia on tumor cell physiology. In further research, the use of chemotherapeutic drugs and even targeted anti-angiogenesis drugs

should be planned in the treatment, as closer clinical treatment methods of combined embolization controls the progression of tumors, promotes the balance between hepatic lobe proliferation and provides inspiration for the choice of interventional therapy to a certain extent.

Conclusions

TAE + PVE is an efficient and safe choice for treating VX2 liver transplantation tumor in rabbits, but the expression of MMP-2 increased fastest after TAE + PVE, and it quickly recovered and even exceeded the expression level before operation, which might promote tumor cell invasion and metastasis. In addition, TAE + PVE is not worse than PVE alone in increasing the FLR.

Author contribution

N.Wei: writing the article and conduct experiments.
Z.Q.Wu: writing the article and conduct experiments.
D.Lu: conduct experiments.
J.K.Xiao: analysis and interpretation.
C.Z.Zhou: conception and design.
S.L.Chu: data collection.
W.F.Lv: critical revision of the article and obtaining funding

Ethical approval

The study protocol was approved by the Ethics Committee of Anhui Medical University (LLSC 20190745). All clinical practices and observations were conducted in accordance with the Declaration of Helsinki.

Declaration of competing interest

The authors declare that we have no conflicts of interests to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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References

- Wang S, Shen ZL. Considerations on the therapy of colorectal cancer liver metastasis in China. *Zhonghua Wei Chang Wai Ke Za Zhi*. 2012;15(10):993–996.
- Mahipal A, Tella SH, Kommalapati A, et al. Immunotherapy in hepatocellular carcinoma: is there a light at the end of the tunnel? *Cancers*. 2019;11(8):1078.
- Hoekstra LT, van Lienden KP, Verheij J, et al. Enhanced tumor growth after portal vein embolization in a rabbit tumor model. *J Surg Res*. 2013;180(1):89–96.
- Sapisochin G, Bruix J. Liver transplantation for hepatocellular carcinoma: outcomes and novel surgical approaches. *Nat Rev Gastroenterol Hepatol*. 2017;14(4):203–217.
- Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol*. 2008;48(Suppl 1):S20–S37.
- Fornier A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet*. 2018;391:1301–1314.
- Revel-Mouroz P, Otal P, Jaffro M, et al. Other non-surgical treatments for liver cancer. *Rep Practical Oncol Radiother*. 2017;22(2):181–192.
- Choi JH, Hwang S, Lee YJ, et al. Prognostic effect of preoperative sequential transcatheter arterial chemoembolization and portal vein embolization for right hepatectomy in patients with solitary hepatocellular carcinoma. *Korean J Hepatobiliary Pancreat Surg*. 2015;19(2):59–65.
- Kuyvenhoven JP, van Hoek B, Blom E, et al. Assessment of the clinical significance of serum matrix metalloproteinases MMP-2 and MMP-9 in patients with various chronic liver disease and hepatocellular carcinoma. *Thromb Haemostasis*. 2003;89(4):718–725.
- Mandic R, Dünne AA, Eikelkamp N, et al. Expression of MMP-3, MMP-13, TIMP-2 and TIMP-3 in the VX2 carcinoma of the New Zealand white rabbit. *Anticancer Res*. 2002;22(6A):3281–3284.
- Zou RH, Li AH, Han F, et al. Liver hypertrophy and accelerated growth of implanted tumors in nonembolized liver of rabbit after left portal vein embolization. *J Surg Res*. 2012;178(1):255–263.
- Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet*. 2003;362(9399):1907–1917.
- Gruber-Rouh T, Marko C, Thalhammer A. Current strategies in interventional oncology of colorectal liver metastases. *Br J Radiol*. 2016;26:20151060.
- Sugawara Y, Yamamoto J, Higashi H, et al. Preoperative portal embolization in patients with hepatocellular carcinoma. *World J Surg*. 2002;26(1):105–110.
- Voboril R. Blood supply of metastatic liver tumors: an experimental study. *Int Surg*. 2005;90(2):71–77.
- Liu CL, Fan ST. Nonresectional therapies for hepatocellular carcinoma. *Am J Surg*. 1997;173(4):358–365.
- Ackerman NB. Experimental studies on the role of the portal circulation in hepatic tumor vascularity. *Cancer*. 1986;58(8):1653–1657.
- Liu H, Li D, Yang S, et al. Changes of blood supply from portal system after transcatheter arterial chemoembolization in huge hepatocellular carcinoma. *Hunan Yi Ke Da Xue Xue Bao*. 1998;23(3):295–298.
- Hayashi S, Baba Y, Ueno K, et al. Acceleration of primary liver tumor growth rate in embolized hepatic lobe after portal vein embolization. *Acta Radiol*. 2007;48(7):721–727.
- Pamecha V, Levene A, Grillo F, et al. Effect of portal vein embolisation on the growth rate of colorectal liver metastases. *Br J Canc*. 2009;100(4):617–622.
- Seo DD, Lee HC, Jang MK, et al. Preoperative portal vein embolization and surgical resection in patients with hepatocellular carcinoma and small future liver remnant volume: comparison with transarterial chemoembolization. *Ann Surg Oncol*. 2007;14(12):3501–3509.
- Choi JH, Hwang S, Lee YJ, et al. Prognostic effect of preoperative sequential transcatheter arterial chemoembolization and portal vein embolization for right hepatectomy in patients with solitary hepatocellular carcinoma. *Korean J Hepatobiliary Pancreat Surg*. 2015;19(2):59–65.
- Tan X, Xie P, Liu J, et al. Therapeutic value of transcatheter arterial chemoembolization combined with portal vein embolization for primary hepatocellular carcinoma with portal vein tumor thrombus: a pilot study. *Asia Pac J Clin Oncol*. 2015;11(3):e6–e12.
- Tustumi F, Ermani L, Coelho FF, et al. Preoperative strategies to improve resectability for hepatocellular carcinoma: a systematic review and meta-analysis. *HPB*. 2018;20(12):1109–1118.
- Zhang Q, Teng G. Interventional therapy of colorectal liver metastasis. *Zhonghua Wei Chang Wai Ke Za Zhi*. 2017;20(6):621–624.
- Daniele A, Divella R, Quaranta M, et al. Clinical and prognostic role of circulating MMP-2 and its inhibitor TIMP-2 in HCC patients prior to and after trans-hepatic arterial chemo-embolization. *Clin Biochem*. 2014;47(3):184–190.
- Ottino P, Finley J, Rojo E, et al. Hypoxia activates matrix metalloproteinase expression and the VEGF system in monkey choroid-retinal endothelial cells: involvement of cytosolic phospholipase A2 activity. *Mol Vis*. 2004;10:341–350.
- Huang CF, Teng YH, Lu FJ, et al. β -mangostin suppresses human hepatocellular carcinoma cell invasion through inhibition of MMP-2 and MMP-9 expression and activating the ERK and JNK pathways. *Environ Toxicol*. 2017;32(11):2360–2370.
- Guo WC, He XF, Li YH, et al. The effect of sequential transcatheter arterial chemoembolization (TACE) and portal venous embolizations (PVE) vs. TACE or PVE alone on rabbit VX2 liver carcinoma and on liver regeneration. *Eur Rev Med Pharmacol Sci*. 2016;20(15):3186–3193.
- De Graaf W, Van den Esschert JW, Van Lienden KP, et al. Induction of tumor growth after preoperative portal vein embolization: is it a real problem? *Ann Surg Oncol*. 2009;16(2):423–430.