

Experimental evaluation of different locoregional therapies in an animal model of hepatocellular carcinoma: combination of transarterial chemoembolization and different therapies
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or: Combination of transarterial chemoembolization and different locoregional therapies for hepatocellular carcinoma: Experimental study in rat

Experimental

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ABSTRACT

PURPOSE: To compare the effects of different locoregional therapies including immunotherapy, antiangiogenesis therapy and Chinese medicinal therapy combined with transarterial chemoembolization (TACE) vs. TACE alone in an animal model of hepatocellular carcinoma (HCC).

MATERIALS AND METHODS: Subcapsular implantation of a solid Morris Hepatoma 3924A, hepatocellular carcinoma (2 mm^3) in the livers was carried out in 70 male ACI rats. 13 days after implantation, the tumor volume (V_1) was measured by magnetic resonance imaging (MRI). After laparotomy and retrograde placement of a catheter into the gastroduodenal artery (14 days after implantation), the following protocols of interventional procedure were performed: (A) Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + $\text{TNF}\alpha$ (7.5 μg) + IL-2 (5×10^3 IU) (n=10); (B) Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + OK-432 (0.05 mg) + IL-2 (5×10^3 IU) (n=10); (C) Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + TNP-470 (5.0 mg) (n=10); (D) Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + Endostatin (0.8 mg) (n=10); (E) Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + Bletilla striata microspheres (a Chinese medicinal and embolic agent, 50 μm) (1.0 mg) (n=10); (F) Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + Bletilla striata (1.0 mg) + ligation of hepatic artery (n=10); (G) Mitomycin (0.1 mg) + Lipiodol (0.1 ml) (control group, n=10). 13 days after these therapies the tumor volumes (V_2) was measured by MRI again, and the tumor growth ratios (V_2/V_1) were calculated.

RESULTS: The mean tumor growth ratio of V_2/V_1 was 7.2993 in group A, 6.5280 in group B, 4.0134 in group C, 7.7796 in group D, 6.2791 in group E, 1.5324 in group F and 9.1382 in group G, respectively. Compared to the control group (TACE alone), groups B (OK-432 + TACE), C (TNP-470 + TACE), E (Bletilla striata + TACE) and F (Bletilla striata + TACE + ligation) showed significant reduction of tumor growth rate ($P<0.05$), while groups A ($\text{TNF}\alpha$ + TACE) and D (Endostatin + TACE) failed to do so ($P>0.05$) by multivariate analysis (ANOVA, Dunnett's t Test). There were significant differences between group F or group C and any other groups by Student-Newman-Keuls Test ($P<0.05$). The best therapeutic effect could be obtained in group F.

CONCLUSION: The results suggested that most combined locoregional therapies with TACE were more effective than TACE alone in rats with HCC.

Index terms: Liver neoplasms, chemotherapeutic embolization, interventional procedure

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world, responsible for an estimated one million deaths annually and carrying a poor prognosis due to its rapid infiltrating growth and complicating liver cirrhosis^[1, 2].

To date, surgical approaches including liver resection, liver transplantation and cryosurgery are regarded as potentially curative treatment for HCC, particularly in patients with small and non-invasive tumors^[3]. However, only a small minority of patients are suitable for surgical therapy due to multicentric tumors, extrahepatic metastasis, early vascular invasion, limitation of donor organs, high complication rate and comorbidities^[4, 5, 6].

Local methods for tumor ablation, which include transarterial chemoembolization (TACE), percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), microwave coagulation therapy (MCT), laser-induced thermotherapy (LITT), are promising extensions of tumor therapy, especially in patients with limited liver function, unresectable or multifocal tumors^[7]. Since TACE was introduced as a palliative treatment in patients with unresectable HCC, it has become one of the most common forms of interventional therapies^[8, 9, 10]. TACE has been showed to reduce systemic toxicity and increase local effects and thus improve the therapeutic results^[9]. However, its perceived benefit for survival has not been substantiated in randomized trials, presumably because its anticancer effect is offset by its adverse effect on liver function. Its therapeutic effect is also limited by the lack of appropriate and reliable embolic agents and when the tumor is infiltrative in nature or is hypovascular, too large or too small^[11, 12].

In the past years, locoregional immunotherapy, antiangiogenesis therapy and Chinese medicinal therapy for treating unresectable HCC have been reported with encouraging results in clinic, especially for inhibiting of intrahepatic metastasis and the recurrence of HCC^[13, 14, 15]. Such adjuvant treatments have the potential in conjunction with TACE in attempt to enhance the therapeutic effect of TACE alone. However, it fails randomized experimental study to assess the value of these combined therapies in an animal model of HCC up to now.

The purpose of this study was to evaluate the value of combination of these different locoregional therapies plus TACE vs. TACE alone by MRT in the rat model of HCC.

MATERIALS AND METHODS

Tumor

Morris hepatoma 3924A, a rapidly growing, poorly differentiated hepatocellular carcinoma, was used in ACI rats in this study. The hepatoma specimens were obtained from the German Cancer Research Center (DKFZ; Heidelberg, Germany).

Animal

70 inbred male ACI-rats (Harlan Winkelmann; Borchon, Germany) weighing 220 to 260g were used. The animals were kept under conventional conditions with a temperature of $22\pm 2^{\circ}\text{C}$, a relative humidity of $55\pm 10\%$, a dark-light rhythm of 12 hr, and were fed standard laboratory chow and tap water ad libitum. All of the experiments on animals were approved by the German government.

Agents

TNF α , IL-2 were purchased from Sigma-Aldrich chemical GmbH (Munich, Germany). Endostatin, Lipiodol and Mitomycin were purchased from Calbiochem company (Darmstadt, Germany), Gulden (Konstanz, Germany) and Medac company (Wedel, Germany), respectively. OK-432, TNP-470 and Bletilla striata microspheres were kindly provided by Chugai pharmaceutical company (Tokyo, Japan), Takeda chemical company (Osaka, Japan) and Tongji Medical University (Wuhan, China), respectively.

TNF α (7.5 μg), OK-432 (0.05 mg), IL-2 (5×10^3 IU) were dissolved in 0.5 ml 0.9% NaCl solution respectively 20 minutes before application. TNP-470 (5.0 mg). Endostatin (0.8 mg) and Bletilla striata microspheres (50 μm , 1.0 mg) were suspended in 0.5 ml 0.9% NaCl solution plus 0.05 ml 10% ethanol, 1.0 ml citrate-phosphate buffer (pH 6.2) and 0.5 ml 0.9% NaCl, respectively 10 minutes before administration.

Anesthesia

The animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (Ketanest, Parke-Davis, Germany; $100 \text{ mg}\cdot\text{kg}^{-1}$), Xylazinehydrochloride (Rompun, Bayer, Germany; $15 \text{ mg}\cdot\text{kg}^{-1}$) and atropine sulfate (Atropinsulfat Braun, Braun, Germany; $0.1 \text{ mg}\cdot\text{kg}^{-1}$) in all interventional and imaging procedures.

Tumor Implantation (At day 1)

The technique for tumor implantation was basically similar to that described by Yang et al^[16] with minor modifications^[17]. The Morris Hepatoma 3924A tumor tissue, recovered from the passaged animals 2 weeks after subcutaneous implantation (corresponding to 5×10^6 tumor cells), was cut into small cubes about 2 mm^3 .

A small subcapsular incision on the left lateral lobe of the liver was made in the recipient ACI-rats under anesthesia. The tumor fragment was gently placed into the pocket with a small cotton swab on the liver surface as hemostasis and the abdominal wall was then closed.

Interventional therapy (At day 14)

A PE-10 polyethylene catheter (inner diameter 0.28 mm, outer diameter 0.61 mm; Wenzel; Heidelberg, Germany) was used for catheterization under a second laparotomy. By using a binocular operative microscope (M651, Leica; Wetzler, Germany), the catheter was retrograde inserted into the gastroduodenal artery. After slightly drawing the thin rope around the common hepatic artery, different agents were injected through the catheter to the hepatic artery by sandwich technique in 20 minutes. 70 rats were randomly assigned into one of seven following groups:

Group A (n=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + TNF α (7.5 mg)+ IL-2 (5×10^3 IU)

Group B (n=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + OK-432 (0.05 mg) + IL-2 (5×10^3 IU)

Group C (n=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + TNP-470 (5.0 mg)

Group D (n=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + Endostatin (0.8 mg)

Group E (n= 0): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + Bletilla striata (1.0 mg)

Group F (n=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + Bletilla striata (1.0 mg) + ligation of A. hepatica propria

Group G (control group, n=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml)

MR Imaging and Analysis (At day 13 and 27)

All MR imaging were performed with a 1.5 Tesla Sonata superconducting system (Siemens; Erlangen, Germany) supplemented by a wrist coil before and after therapy (At day 13 and 27). T1-weighted (SE: TR/TE, 460/15 ms) and T2-weighted (TSE: TR/TE, 3170/99 ms) transverse

images with a section thickness of 2 mm and 184× 256 matrix were acquired. There was no gap between sections. No contrast medium was administered. The tumor volume was determined and evaluated in T2-weighted image according to the formula: Tumor volume (mm³)= Length (mm) × Width² (mm)²/2^[18]

The mean tumor growth ratio (V2/V1) was analyzed by using Dunnett's t Test for comparing the effect of each therapeutic group with control group respectively, and by using Student-Newman-Keuls Test for comparing the effect of each two therapeutic groups. A P-value of less than 0.05 was considered to indicate a significant difference.

RESULTS

The rate of tumor implantation reached 100% in all the rats. None of the animals died during implantation or operation. A total of 70 individual HCC tumors were seen with unenhanced MR imaging in the liver of 70 rats (100%) before treatment. Intrahepatic metastasis occurred in one of 10 rats (10%) in group D and two of 10 rats (20%) in group G, respectively. Intratumoral necrosis occurred in two of 10 rats (20%) in group C, one of 10 rats (10%) in group F and one of 10 rats (10%) in group G, respectively.

The tumor volume ratio (V_2/V_1) in different groups ($n=70$) were shown in Table 1.

Table 1. The tumor volume rate (V_2/V_1) in different groups ($n=70$)

Rat No.	Group A (TNF)	Group B (OK-432)	Group C (TNP-470)	Group D (Endostatin)	Group E (BS)	Group F (BS+Lig.)	Group G (control)
1	5,9792	8,7896	3,5532	8,2162	6,4810	0,8556	5,6284
2	9,8397	7,3346	4,3816	7,7182	5,7038	1,4565	9,5091
3	7,9724	5,2397	5,6096**	7,1319	6,2490	1,6469	10,5063**
4	6,7697	5,5863	3,0130	5,4892	7,8920	1,3920	7,7416
5	9,9536	6,0568	3,8746	6,2878	7,8023	1,6577	8,6378
6	5,3795	7,8123	5,7461**	9,6705	7,4781	1,6911	8,2029
7	8,4878	5,3728	3,6657	8,9791	5,5685	0,9025	8,3670
8	6,0243	5,4676	3,7174	10,0050*	6,8346	1,9530	8,5399*
9	7,3052	4,7810	3,2756	6,8738	5,5800	1,9636**	11,5310
10	5,2817	8,8391	3,2971	7,4239	3,2015	1,8054	12,7182*

BS: *Bletilla striata*

Lig.: Ligation of hepatic artery

*: tumor with intrahepatic metastasis

** : intratumoral necrosis

The mean ratio of V_2/V_1 was 7.2993 in group A, 6.5280 in group B, 4.0134 in group C, 7.7796 in group D, 6.2791 in group E, 1.5324 in group F and 9.1382 in group G, respectively.

Compared to the control group (TACE alone), groups B (OK-432 + TACE), C (TNP-470 + TACE), E (Bletilla striata + TACE) and F (Bletilla striata + TACE + ligation) showed significant reduction of tumor growth ratio ($P < 0.05$), while groups A (TNF α + TACE) and D (Endostatin + TACE) did not ($P > 0.05$) by multivariate analysis (Dunnett's t Test). There was significant difference between group F or group C and any other each group by Student-Newman-Keuls Test ($P < 0.05$). The best therapeutic effect was obtained in group F.

The following histogram illustrates the mean tumor growth ratio (V2/V1) in different groups (Fig 1).

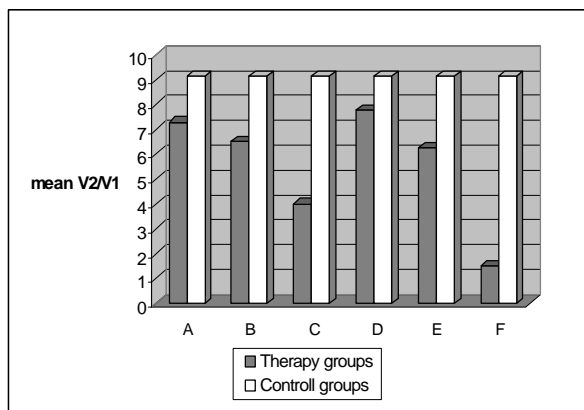


Figure 1. Histogram illustrates the comparison of the mean tumor growth rate (V2/V1) in different groups and in control group. Grey and white bar represent different therapeutic groups and control group, respectively. Groups A-F represent the therapeutic group of TNF α + TACE, OK-432 + TACE, TNP-470 + TACE, Endostatin + TACE, Bletilla striata + TACE, Bletilla striata + TACE + ligation, respectively. Compared to the control group, groups B, C, E and F shows significant reduction of tumor growth rate ($P < 0.05$), while groups A and D did not (ANOVA, $P > 0.05$). There are significant differences between group F or group C and any other groups (ANOVA, $P < 0.05$). The best therapeutic effect could be obtained in group F.

The tumors showed homogeneously hypointense on the T1-weighted images and hypertense on the T2-weighted images. T2-weighted SE sequences provided significantly higher tumor-

liver contrast than T1-weighted sequences, and improved the detectability of intrahepatic metastasis and intratumoral necrosis.

In group C (TNP-470), intratumoral necrosis with unregular homogeneous hyperintense pattern on the T1-weighted image and hypointense pattern on the T2-weighted image were seen in two rats (Fig. 3).

In group D (Endostatin), there was a intrahepatic metastasis with unhomogeneous hypointense pattern on the T1-weighted images and hypertense pattern on the T2-weighted images surrounding the larger tumor lesions in one rat (Fig. 2).

In group F (*Bletilla striata* with ligation), relative small tumors with the size of $0.52 \times 0.37 \text{ mm}^2$ and $0.44 \times 0.38 \text{ mm}^2$ in diameter were shown in two treated rats, respectively, indicating minimal response but no tumor growth after therapy compared with that before therapy. (Fig. 4).

In group G (control group), the tumor volume was generally markedly increased in treated rats than before therapy. Two rats appeared to be accompanied with intrahepatic metastasis (Fig. 5).

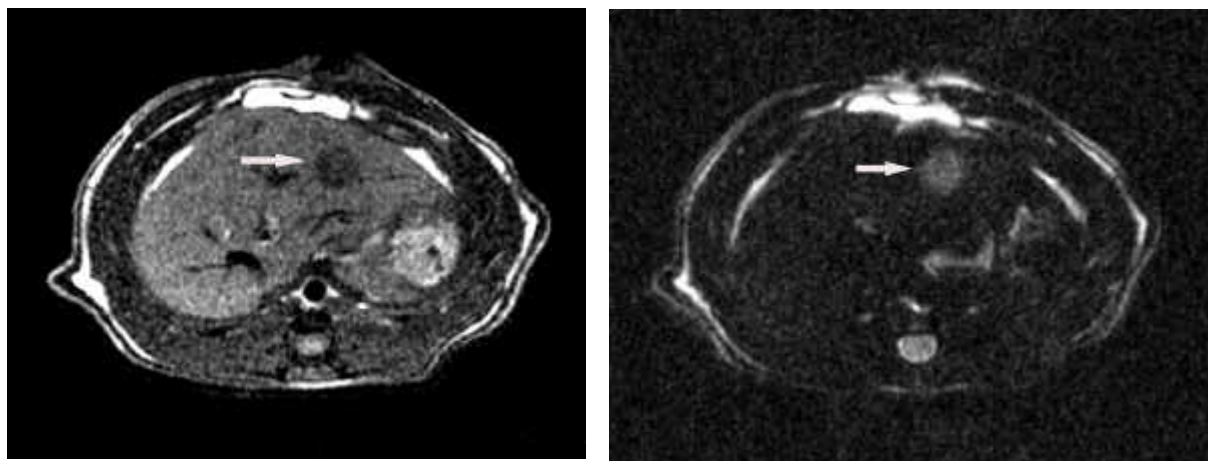


Figure 2a. Images in a ACI rat with a solid HCC in group C. (a) Pretherapeutic unenhanced T1-weighted MR imaging with SE sequence (460/15). It shows a small hypointense tumor (arrow) in the left lateral lobe of liver; (b) Pretherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). The hyperintense lesion with a size of $0.41 \times 0.40 \text{ mm}^2$ (arrow) is well discernible from the surrounding liver tissue.

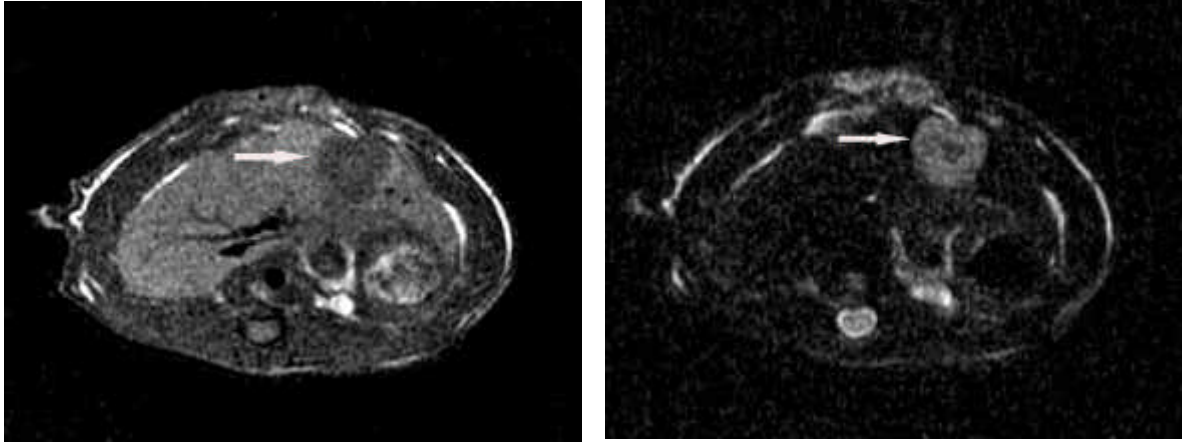


Figure 2b. Images in a ACI rat with a solid HCC in group C. Posttherapeutic unenhanced T1-weighted MR imaging with SE sequence (460/15). It shows the hypotense tumor (arrow) in the left lateral lobe of liver; (b) Posttherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). It shows the hyperintense lesion ($0.73 \times 0.71 \text{ mm}^2$) with central hypointense area (arrow) corresponding to the intratumoral necrosis.

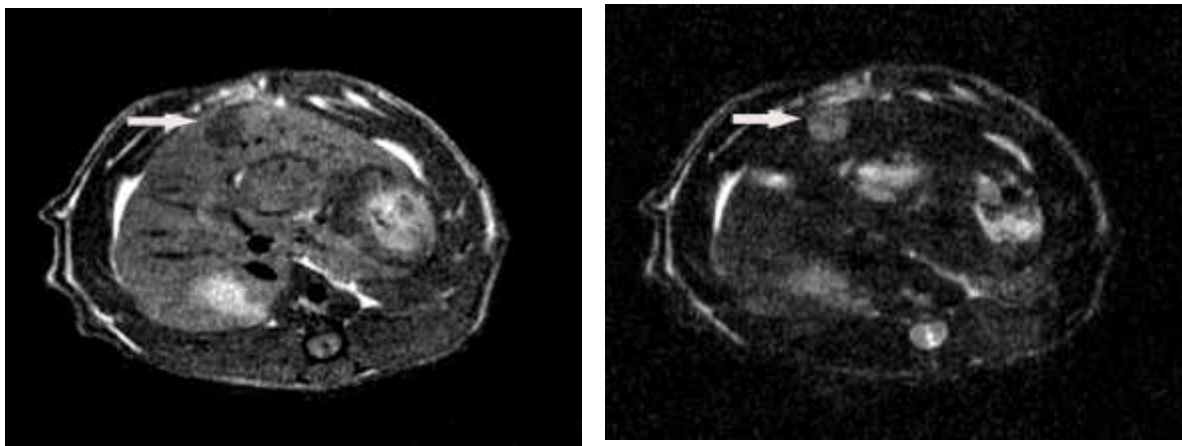


Figure 3a. Images in a ACI rat with a solid HCC in group D. (a) Pretherapeutic unenhanced T1-weighted MR imaging with SE (460/15). It shows a small hypointense tumor (arrow) in the left lateral lobe of liver; (b) Pretherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). The hyperintense lesion with a size of $0.46 \times 0.43 \text{ mm}^2$ (arrow) is well discernible from the surrounding liver tissue.

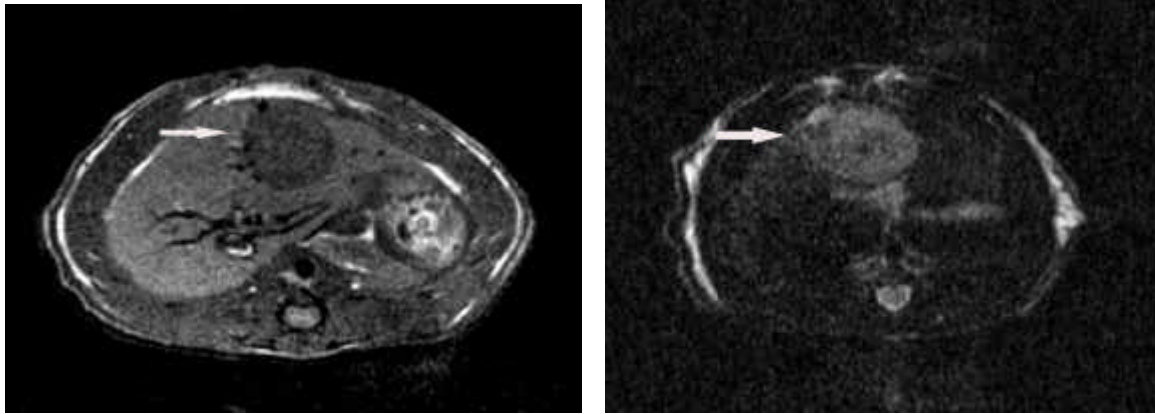


Figure 3b. Images in a ACI rat with a solid HCC in group D. (a) Posttherapeutic unenhanced T1-weighted MR imaging with SE sequence (460/15). It shows that the tumor (arrow) increases rapidly after therapy; (b) Posttherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). It shows that the hypertense lesion with a size of $1.05 \times 0.90 \text{ mm}^2$ is surrounded by unhomogeneous hyperintense area (arrow) corresponding to the intrahepatic metastasis.

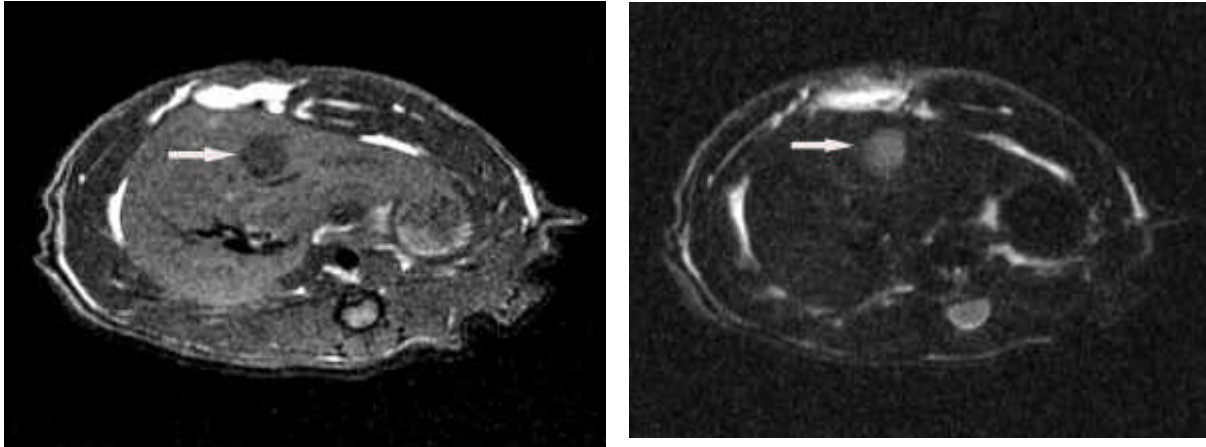


Figure 4a. Images in a ACI rat with a solid HCC in group F. (a) Pretherapeutic unenhanced T1-weighted MR imaging with SE sequence (460/15). It shows a small hypointense tumor (arrow) in the left lateral lobe of liver; (b) Pretherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). The hyperintense lesion with a size of $0.44 \times 0.40 \text{ mm}^2$ (arrow) is well discernible from the surrounding liver tissue.

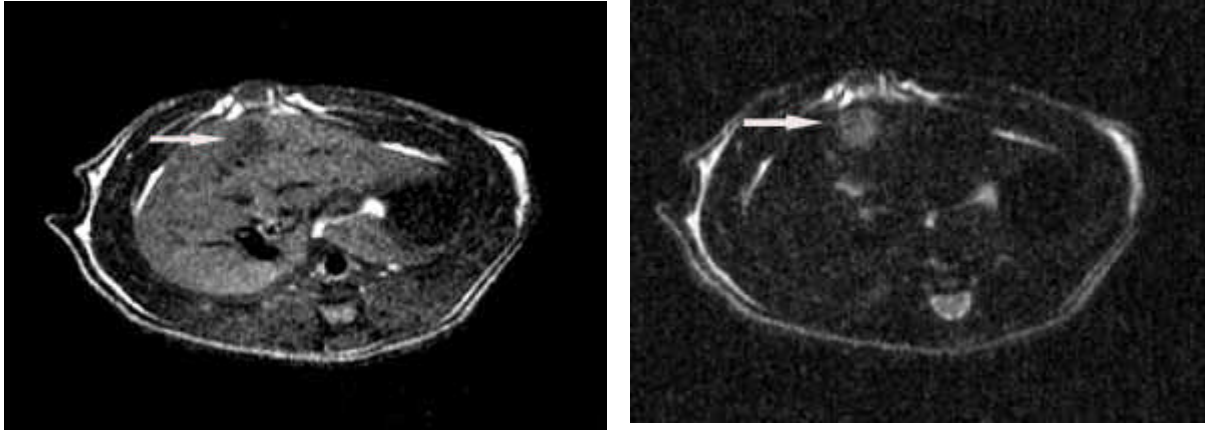


Figure 4b. Images in a ACI rat with a solid HCC in group F. Posttherapeutic unenhanced T1-weighted MR imaging with SE sequence (460/15). It shows a small hypointense tumor (arrow) in the left lateral lobe of liver; (b) Posttherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). It shows the unhomogeneous hyperintense lesion with a size of $0.44 \times 0.38 \text{ mm}^2$ (arrow) and demonstrates that there is no difference between the tumor volume before and after therapy.

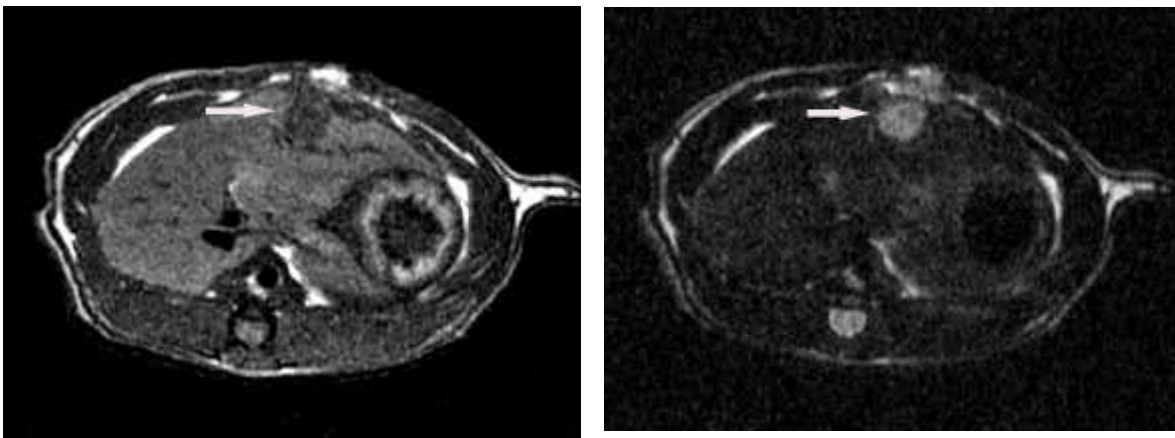


Figure 5a. Images in a ACI rat with a solid HCC in group G. (a) Pretherapeutic unenhanced T1-weighted MR imaging with SE sequence (460/15). It shows a small hypointense tumor (arrow) in the left lateral lobe of liver; (b) Pretherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). The hyperintense lesion with a size of $0.49 \times 0.46 \text{ mm}^2$ (arrow) is well discernible from the surrounding liver tissue.

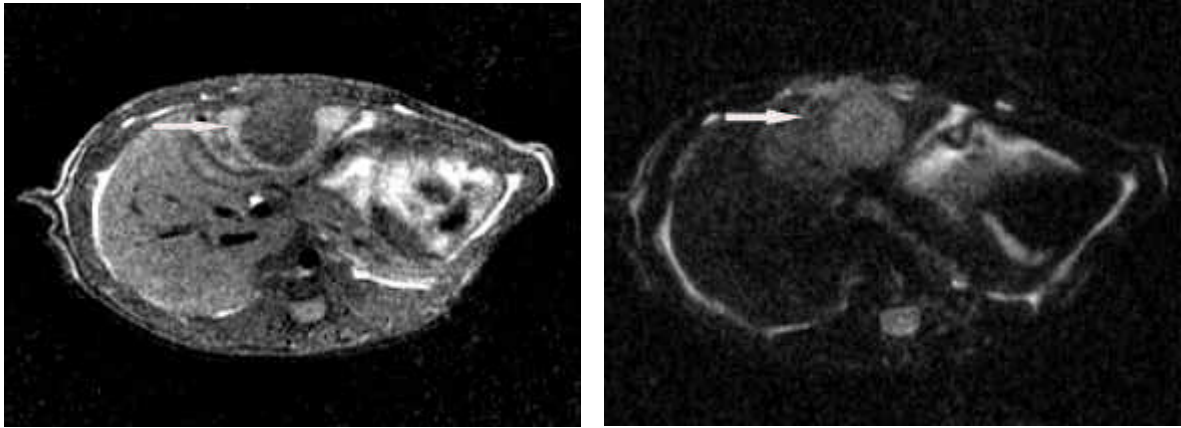


Figure 5b. Images in a ACI rat with a solid HCC in group G. Posttherapeutic unenhanced T1-weighted MR imaging with SE sequence (460/15). It shows a large hypointense tumor (arrow) in the left lateral lobe of liver. The lesion is surrounded by unregular hypointense area. (b) Posttherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). The tumor with a size of $0.96 \times 0.96 \text{ mm}^2$ has a rapid growth compared with that before therapy. It shows also the unhomogeneous hyperintense area (arrow) corresponding to the intrahepatic metastasis.

Discussion

Since TACE was introduced as a palliative treatment in patients with unresectable HCC, it has become one of the most common forms of interventional therapies^[8, 9, 10]. TACE with iodized oil has been shown to result in regression of HCC and reduction of systemic toxicity, thus improve the therapeutic effects^[9]. However, the prolongation of overall survival of patients remains questionable^[19].

It is well known that improvement of the overall therapeutic effects of liver malignancies depends on the combined therapies. In the past years, combined interventional therapies including TACE plus percutaneous ethanol injection (PEI), radiofrequency thermal ablation (RFA) or laser-induced thermotherapy (LITT) have been demonstrated with promising results for patients under specified conditions^[20, 21, 22]. However, there were only few experimental or clinic reports about the combination of TACE with biotherapy and others. Therefore our present study was to determine the value of combination of TACE with targeting locoregional immunotherapy, antiangiogenesis therapy or Chinese medicinal therapy vs. TACE alone by MRI in the rat model of Morris Hepatoma 3924A, which was demonstrated as similar as hepatocellular carcinoma in human, and was superior than tumor model of Walker-256 or VX-2^[23]. Our results showed that most combined locoregional therapies including biotherapy, Antiangiogenesis therapy and Chinese medicinal therapy with TACE provide obvious reduction of tumor growth compared with TACE alone in ACI rats with HCC.

OK-432, which is a biological response modifier (BRM) derived from the weakly virulent Su strain of *Streptococcus pyogenes* and was used in our study, has been previously applied in combination with locoregional chemotherapy or transarterial embolization (TAE) for treating HCC in clinic. OK-432 can augment the antitumor effect of mitomycin, because OK-432 itself has a direct cytotoxic and cytostatic activity against tumor cells and inhibits DNA and RNA synthesis in tumor cells. Chemotherapy can also increase the susceptibility of tumor cells to cytotoxic effector cells including lymphocytes, macrophages and neutrophils activated by OK-432 through direct damage or modulation of surface antigens by chemotherapy^[24]. In addition, mitomycin can eliminate the suppressor cells or suppressor factor in the blood or effusion, resulting in augmented anticancer activity of OK-432-activated immunopotentiating cells, especially T-cells^[25, 26]. It was also proved by histologic examination that transarterial immunoembolization (TIE) seems to be more effective than conventional TAE against extracapsular invasion and intrahepatic metastasis in clinic. It was

suggested that embolization of the hepatic artery provides a major ischaemic attack on liver tumors, and regional infusion of immunostimulants includes an inflammatory response in the tumor and the surrounding tissues. Furthermore, local stimulation of the immune system may result in development of a systemic immune response against tumor cells, which suppresses the growth of extrahepatic metastasis^[13]. Data for disease-free survival and recurrence site suggest TIE may be a useful preoperative treatment^[13, 27]. Okuno et al found that hepatic infusion of IL-2 can increase the permeability of the hepatic endothelium, resulting in increase of hepatic extraction of antitumor agents^[28]. This effect may improve the delivery of antitumor drugs to the liver, thereby reducing the systemic drug-associated toxicity^[29]. Our approach of immuno-chemoembolization was based on above-mentioned reports showing the value of TIE and locoregional immunochemotherapy. The results showed a significant reduction of tumor growth rate by OK-432 plus TACE compared with TACE alone (Fig. 1). No intrahepatic metastasis was observed. OK-432 seems to exert its more potent antitumor effects in TACE in our experiments. The comparative study about the therapeutic effects of immuno-chemoembolization and TIE or locoregional immunochemotherapy with OK-432 is needed in the future.

Another BRM, which was used in TACE in our study is Tumor necrosis factor (TNF). TNF is a cytokine derived from activated monocytes/macrophages and has a variety of biological activities in vitro such as cytotoxic activity to cancer cells, damage to endothelial cells and enhancement of fibroblast proliferation^[30]. Watanabe et al found that the intraarterial infusion of TNF/lipiodol emulsion may produce prominent antitumor effects in rabbits, possibly due to the higher concentration and prolonged retention of TNF in the tumors, which causes damage to the endothelium of the tumor vessels^[30]. In the experimental study of Yang et al, it was indicated that tumor growth is significantly retarded by continuous hepatic infusion of TNF α plus ligation of hepatic artery in ACI rats^[31]. Placement of catheter in the hepatic artery for relative longer time could herein be regarded as central embolization for tumor vessel. Our study design was limited by the long-term placement of catheter in the artery. hepatica propria, and for this reason, a lower total dosis of TNF was transarterial injected to liver tumor compared with the study of Yang et al. It is possible the reason that the single combined therapy of TNF+TACE did not induce significant reduction of tumor growth and did not bring any benefit for treating HCC in our study.

Development of tumor angiogenesis-targeting agents is often referred to as a new concept in anticancer therapy. It was demonstrated that antiangiogenic agents have the characteristics: 1. they may overcome drug resistance in solid tumors; 2. identification of the angiogenic factors in serum or microvessels in tumors can allow the efficacy of the new agents to be quantified; 3. antiangiogenic agents have low toxicity due to their selective effect on tumor vasculature; 4. their combination with anticancer agents may potentate their anticancer effects^[32, 33, 34]. For the best clinical results, antiangiogenic therapy should be used in combination with another adjuvant therapies^[35, 36, 37].

TNP-470 is the first angio-inhibitor which has reached phase III clinical trials. TNP-470 (AGM-1470) is a fumagillin analogue which inhibits proliferation and migration of endothelial cells and capillary tube formation at cytostatic but not cytotoxic concentrations. It is believed that ischaemic hypoxia and necrosis induced by TACE stimulates angiogenesis in the residual viable HCC^[38]. TNP-470 can inhibit the proliferation of new microvascular channels and consequently inhibit the development of multiple arterial collaterals^[39]. Charnsangavej et al suggested that TNP-470 may be particularly effective in inhibiting the extrahepatic collaterals and may make it possible to perform TAE repeatedly^[40]. Combination treatment in animals showed that TNP-470 potentates the anticancer effects of some cytotoxic and biological agents^[41], but the terminal plasma half-life of TNP-470 is short and the drug is rapidly cleared from the circulation after a single 1-hour infusion^[42]. The use of embolic substances can prolong retention of the anticancer drug at the tumor site, and augment the efficacy of the anticancer therapy^[43]. It was reported that TAE combined with TNP-470 may enhance the anticancer effect of TAE alone in the treatment of HCC without severe side effects on the liver or body weight gain^[44]. Our results indicated that the tumor growth ratio is markedly reduced by combined transarterial administration of TNP-470 and TACE compared with TACE alone ($P < 0.05$). Its antitumor effect is stronger than OK-432+TACE and TNF+TACE (Fig. 1). Intratumoral necrosis were shown in two rats (20%) (Fig. 2), and no metastasis occurred in this group.

Endostatin, which was used as angiogenesis inhibitor in our study is a new kind of potent antiangiogenic factor consisting of 184 amino acids in C-terminal fragment of endogenous collagen 18a^[45]. Endostatin was shown to inhibit VEGF-induced endothelial cell migration in vitro and to have antitumor activity in vivo, without any apparent sign of toxicity^[46]. Its antitumor activity is now being evaluated in phase I trials for a variety of solid tumors. It has

also been reported that endostatin can inhibit the growth of transplantable HCC in nude mice by 52.2%^[47], and the addition of endostatin to conventional chemotherapy enhances antitumor effect in a murine model of early colorectal liver metastasis^[48]. Our present results indicated that the selective delivery therapy of endostatin in conjunction with TACE has no significant advantage against tumor growth comparing with TACE alone (Fig. 1). Intrahepatic metastasis (10%) occurred in this group (Fig. 3). We hypothesized that the availability of this therapy relate to the higher dosis of citrate-phosphate buffer solution (1.0 ml), in which 0.8 mg endostatin can be dissolved. As a result, more Mitomycin and Lipiodol could perhaps be washed out from the tumor and thus the antitumor effect was influenced. Further more histoimmunologic examinations for explanation of this phenomenon are required.

In the past years, Chinese medicinal therapy has gained wide acceptance as a safe, palliative and effective treatment even in patients with large HCC and cirrhosis in China. Bletilla Striata (BS) is a common Chinese medicinal herb and is usually used as an embolic material in TACE for HCC. Its compositions are mucilage, starch, and a little volatile oil^[13]. The mechanisms of embolization by BS are attributable to following factors: non-absorbent property, mechanical obstruction; effects on coagulative and anticoagulative systems and secondary obstruction resulted from the injury to wall of blood vessels^[49]. Zheng et al have confirmed that the BS has an adherent function and can expand slowly in blood flow, leading to mechanical blockade of vessels. It was also hypothesized that BS can slowly diffuse into the liver parenchyma around the tumor as colloidal form, leading to prolonged anticancer effect and inhibiting the collateralisation and metastasis of tumor^[50]. Compared with gelfoam embolus, BS has the following characteristics: 1. it can produce extensive and permanent vascular embolization, while it can not be absorbed by body tissue. After embolization, tumor necrosis and shrinkage are significant with less collateral circulation that forms later; 2. the mucilage component of BS is a wide-spectrum anticancer element that may inhibit tumor occurrence and development^[51]. The 1, 2 and 3 year survival rates were 44.9%, 33.6% and 33.6% in BS group while the rates were 48.9%, 31.1% and 16.0% in gelfoam group, suggesting that BS is superior to gelfoam as an embolic agent, and the transarterial administration of BS may provide a beneficial therapeutic modality for HCC^[52]. In our experimental study, the best therapeutic effect has been shown in combined therapy of BS+TACE+ligation of hepatic artery. There is almost no significant difference between the tumor volume before and after therapy (Fig. 4). Intratumoral necrosis were seen in one rats (10%) and no intrahepatic metastasis was observed in this group. Its dramatic antitumor effect

is stronger than any other treated group including BS+TACE (Fig. 1). This approach of central- and peripheral chemoembolization is able to increase tumor treatment more completely, resulting in local control of tumor growth in rats and has bright prospects for treating patients with HCC in the future.

In summary, by combining most different locoregional therapies with TACE for treating HCC in rats, the encouraging results can be obtained compared with TACE alone. However, the detailed therapeutic mechanisms, therapeutic indications, optimal strategy for the use, monitoring, and validation of these combined therapies remains unclear and required more randomized experimental and clinic studies

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