• LIVER CANCER •

Combined transarterial chemoembolization and arterial administration of *Bletilla striata* in treatment of liver tumor in rats

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Abstract

AIM: To evaluate and compare the effect of combined transarterial chemoembolization (TACE) and arterial administration of *Bletilla striata* (a Chinese traditional medicine against liver tumor) *versus* TACE alone for the treatment of hepatocellular carcinoma (HCC) in ACI rats.

METHODS: Subcapsular implantation of a solid Morris hepatoma 3 924A (2 mm^3) in the liver was carried out in 30 male ACI rats. Tumor volume (V1) was measured by magnetic resonance imaging (MRI) on day 13 after implantation. The following different agents of interventional treatment were injected after retrograde catheterization via gastroduodenal artery (on day 14), namely, (A) TACE (0.1 mg mitomycin + 0.1 ml Lipiodol) + *Bletilla striata* (1.0 mg) ($n=10$); (B) TACE + *Bletilla striata* (1.0 mg) + ligation of hepatic artery ($n=10$), (C) TACE alone (control group, $n=10$). Tumor volume (V2) was assessed by MRI (on day 13 after treatment) and the tumor growth ratio (V2/V1) was calculated.

RESULTS: The mean tumor volume before (V1) and after (V2) treatment was 0.0355 cm^3 and 0.2248 cm^3 in group A, 0.0374 cm^3 and 0.0573 cm^3 in group B, 0.0380 cm^3 and 0.3674 cm^3 in group C, respectively. The mean ratio (V2/V1) was 6.2791 in group A, 1.5324 in group B and 9.1382 in group C. Compared with the control group (group C), group B showed significant inhibition of tumor growth ($P<0.01$), while group A did not ($P>0.05$). None of the animals died during implantation or in the postoperative period.

CONCLUSION: Combination of TACE and arterial administration of *Bletilla striata* plus ligation of hepatic artery is more effective than TACE alone in the treatment of HCC in rats.

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INTRODUCTION

HCC is a highly malignant tumor with a very high morbidity and mortality rate worldwide, carrying a poor prognosis due to its rapid infiltrating growth and complicating liver cirrhosis[1,2].

Surgical resection, liver transplantation and cryosurgery are regarded as potentially curative treatment for HCC[3-5], but most patients are not suitable candidates[6-9]. Thus, the local interventional therapy of liver tumor has been rapidly evolving currently, which includes transarterial chemoembolization (TACE), percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), laser-induced thermotherapy (LITT) and microwave coagulation therapy (MCT)[10-19]. TACE is one of the most common forms of interventional therapies and seems most effective against encapsulated small HCCs without extracapsular invasion, whereas in large HCCs, viable residual tumor cells remain and the tumor frequently recurs[20-22]. Moreover, in patients with large lesions, multiple TACE sessions are necessary to control tumor growth but may increase the risk of worsening hepatic function through damage to noncancerous liver parenchyma[8,21,23].

In the past years, locoregional Chinese medicinal therapy for treating unresectable HCC has been reported with encouraging results, especially for inhibiting arterial collaterals of liver tumor and recurrence of HCC[24,25]. Such an adjuvant treatment in conjunction with TACE has the potential to enhance the therapeutic effect of TACE alone in experimental and clinical studies. However, no experimental study to assess the value and efficacy of this combined therapy in an animal model of HCC has been performed.

The current prospective randomized study was designed to compare the effect of combined TACE and arterial administration of *Bletilla striata* (a Chinese traditional medicine against liver tumor) *versus* TACE alone for the treatment of HCC in ACI rats.

MATERIALS AND METHODS

Tumor
The hepatoma cell line (Morris hepatoma 3924A), a rapidly growing, poorly differentiated hepatocellular carcinoma[26], was used in ACI rats in this study. The hepatoma specimens were obtained from the German Cancer Research Center (DKFZ; Heidelberg, Germany).

Animal
Thirty inbred male ACI-rats (Harlan Winkelmann; Borchen, Germany) weighing 220-260 g were used. The animals were kept under conventional conditions with a temperature of 22±2 ℃, a relative humidity of 55±10 %, a dark-light rhythm

of 12 hr, and had free access to laboratory chow and tap water. All of the experiments on animals were approved by the German government.

Agents

The original material of *Bletilla striata* microspheres is the stem tubers of *Bletilla striata*. *Bletilla striata* microspheres were kindly provided by Tongji Medical College (Wuhan, China). A dose of 1.0 mg *Bletilla striata* microspheres (50 µm) was suspended in 0.5 ml 0.9 % NaCl for 10 minutes before administration.

Anesthesia

The animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (Ketanest, Parke-Davis, Germany; 100 mg/kg), Xylazinhydrochloride (Rompun, Bayer, Germany; 15 mg/kg) and atropine sulfate (Atropinsulfat Braun, Braun, Germany; 0.1 mg/kg) in all interventional and imaging procedures.

Tumor implantation (on day 1)

The technique for tumor implantation was basically similar to that described by Yang *et al*[26] with minor modifications[27-29]. 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 and the abdominal wall was then closed.

Interventional therapy (on day 14)

For interventional studies a second laparotomy was performed. By using a binocular operative microscope (M651, Leica; Wetzler, Germany), a PE-10 polyethylene microcatheter (inner diameter 0.28 mm, outer diameter 0.61 mm; Wenzel; Heidelberg, Germany) was retrogradely inserted into the gastroduodenal artery. Different agents were then injected (20 minutes a duration time) through the microcatheter via hepatic artery using sandwich technique. Administration was as follows:

Group A (*n*=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + *Bletilla striata* (1.0 mg)
Group B (*n*=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) + *Bletilla striata* (1.0 mg)+ligation of A. hepatica propria
Group C (*n*=10): Mitomycin (0.1 mg) + Lipiodol (0.1 ml) (*control group*).

MR imaging and analysis (on day 13 and 27)

A 1.5 Tesla Sonata (Siemens; Erlangen, Germany) supplemented by a wrist coil (Small field of view) was used for MRI before and after therapy (on 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 and no contrast medium was administered. The tumor volume was determined and evaluated in T2-weighted image according to the formula[30]:

$$\text{Tumor volume (mm}^3) = \frac{\text{Largest diameter (mm)} \times [\text{smallest diameter (mm)}]^2}{2}$$

The mean tumor growth ratio (V2/V1) was analyzed by using *t* test for comparing the effect of each therapeutic group with control group respectively. A *P*-value less than 0.05 was considered to indicate a significant difference.

RESULTS

The rate of tumor implantation reached 100 % in all the rats receiving tumor implantation with Morris hepatoma 3924A. None of the animals died during implantation or interventional therapy. A total of 30 individual HCC tumors were seen with unenhanced MR imaging in the livers of 30 rats (100 %) before treatment. The tumors showed homogeneously hypointense on the T1-weighted images and hyperintense on the T2-weighted images. T2-weighted sequences provided significantly higher tumor-liver contrast than T1-weighted sequences, and improved the detectability of intrahepatic metastasis. Intrahepatic metastasis occurred in two of 10 rats (20 %) in group C.

The mean tumor volume before (V1) and after (V2) therapy was 0.0355 cm^3 and 0.2248 cm^3 in group A, 0.0374 cm^3 and 0.0573 cm^3 in group B, 0.0380 cm^3 and 0.3674 cm^3 in group C, respectively. The mean ratio of V2/V1 was 6.2791 in group A, 1.5324 in group B and 9.1382 in group C. Compared with the control group (group C, TACE alone), group B (TACE + *Bletilla striata* + ligation of hepatic artery) showed significant inhibition of tumor growth (*P*<0.01), while group A (TACE + *Bletilla striata*) did not (*P*>0.05).

The tumor volume ratio (V2/V1) in different groups (*n*=30) is shown in Table 1.

Table 1 Tumor volume rate (V2/V1) in different groups (*n*=30)

Rat No.	Group A (BS)	Group B (BS+Lig.)	Group C (control)
1	6.4810	0.8556	5.6284
2	5.7038	1.4565	9.5091
3	6.2490	1.6469	10.5063
4	7.8920	1.3920	7.7416
5	7.8023	1.6577	8.6378
6	7.4781	1.6911	8.2029
7	5.5685	0.9025	8.3670
8	6.8346	1.9530	8.5399*
9	5.5800	1.9636	11.5310
10	3.2015	1.8054	12.7182*

BS: *Bletilla striata*. Lig.: Ligation of hepatic artery; *: tumor with intrahepatic metastasis.

In group B (TACE + *Bletilla striata* + ligation), relative small tumors with the size of 0.52×0.37 mm^2 and 0.44×0.38 mm^2 in diameter were shown in two treated rats, respectively, indicating a minimal response but no tumor growth after therapy compared with that before therapy (Figure 1).

In group C (control group), the tumor volume was generally markedly increased in treated rats compared to untreated. Two rats appeared to be accompanied with intrahepatic metastasis (Figure 2).

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[11,31]. TACE with iodized oil has been shown to result in regression of HCC and reduction of systemic toxicity, thus improving the therapeutic effects[21,32,33]. However, prolongation of the overall survival of patients remains questionable[34]. TACE might ablate a significant portion of the tumor but had a high rate of recurrence[35]. In patients with focal HCC, TACE was well tolerated and provided a survival benefit. However, no apparent benefit of it has been found for patients with diffuse HCC[36,37]. TACE using various embolizers has been well documented to include
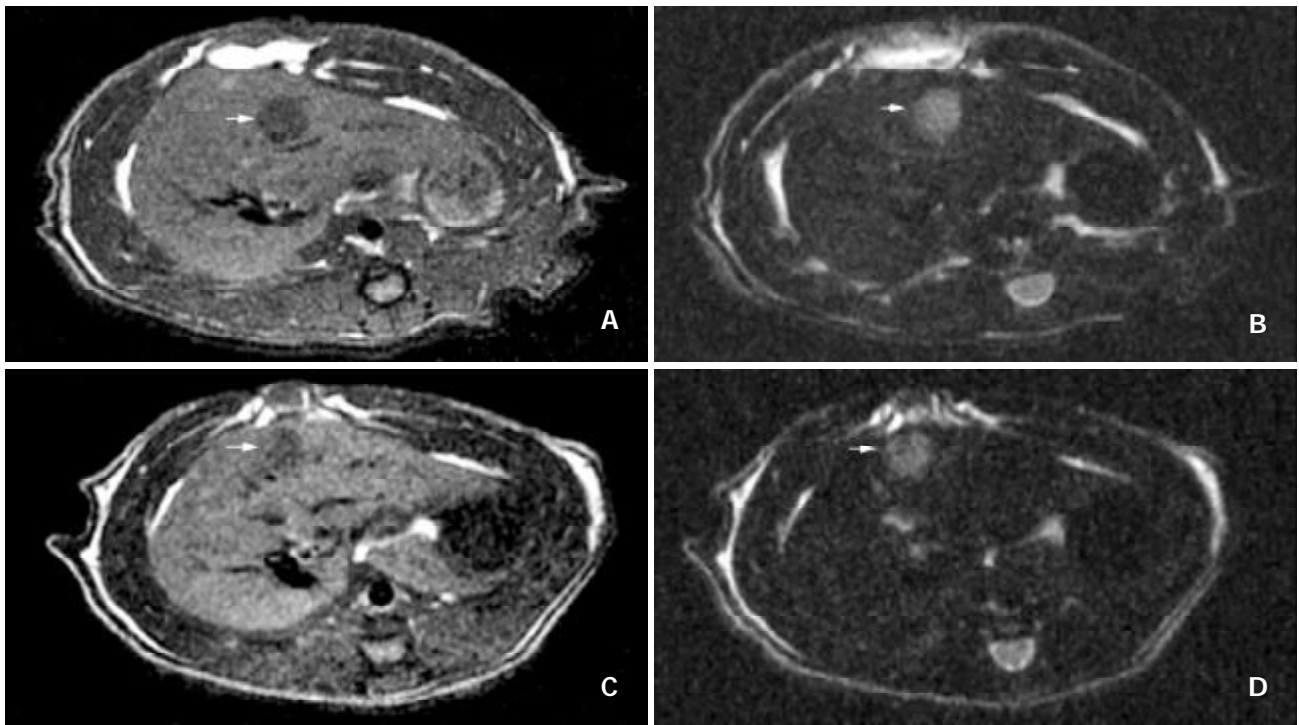
Figure 1 A: Images in a ACI rat with a solid HCC in group B. (a) Pretherapy did not enhance T1-weighted MR imaging with SE sequence (460/15). It shows a small hypointense tumor (arrow) in the left lateral lobe of liver; B: Pretherapeutic unenhanced T2-weighted MR imaging with TSE sequence (3170/99). The hyperintense lesion with a size of 0.44×0.40 mm² (arrow) is well discernible from the surrounding liver tissue. C: Images in a ACI rat with a solid HCC in group B. (a) Posttherapy did not enhance T1-weighted MR imaging with SE sequence (460/15). It shows a small hypointense tumor (arrow) in the left lateral lobe of liver. D: Unenhanced T2-weighted MR imaging with TSE sequence (3170/99) after therapy. It shows the inhomogeneous hyperintense lesion with a size of 0.44×0.38 mm² (arrow) and demonstrates that there is no difference between the tumor volume before and after therapy.
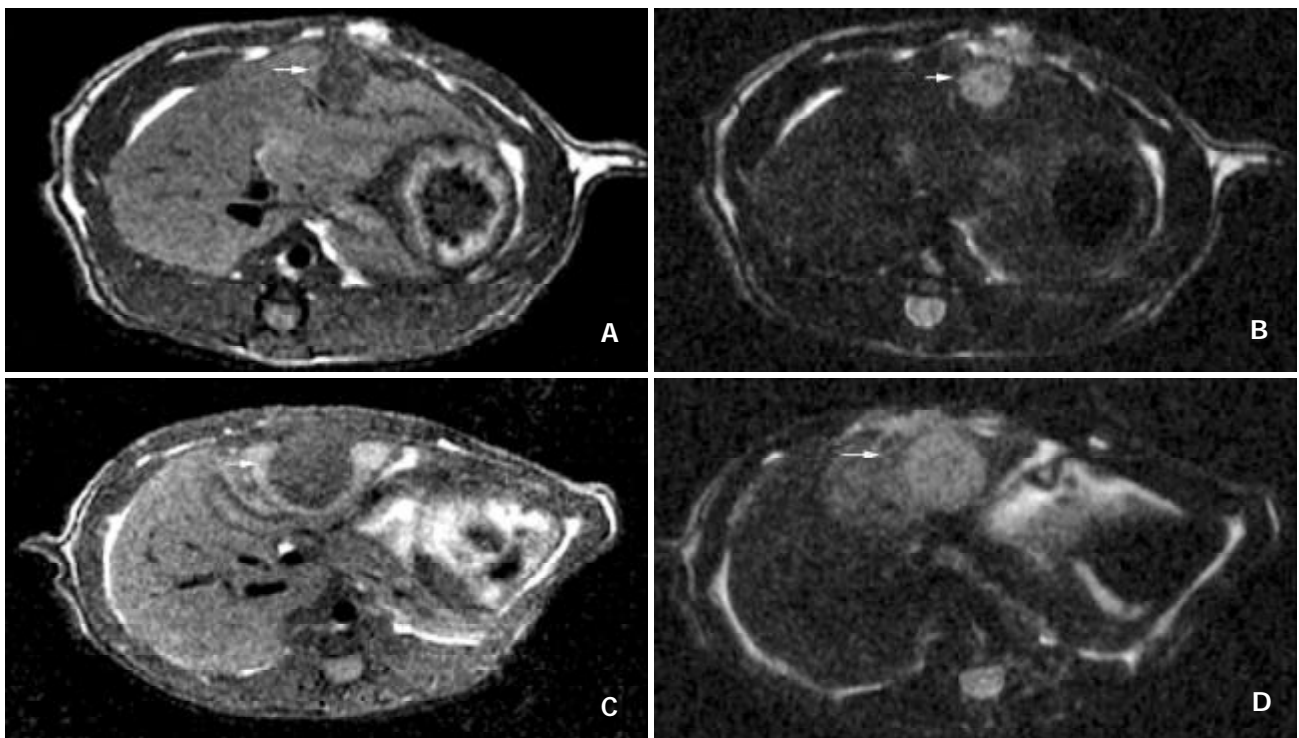

Figure 2 A: Images in a ACI rat with a solid HCC in group C. (a) Pretherapy did not enhance T1-weighted MR imaging with SE sequence (460/15). It shows a small hypointense tumor (arrow) in the left lateral lobe of liver. B: Unenhanced T2-weighted MR imaging with TSE sequence (3170/99) after therapy. The hyperintense lesion with a size of 0.49×0.46 mm² (arrow) is well discernible from the surrounding liver tissues. C: Images in a ACI rat with a solid HCC in group C. (a) Posttherapy did not enhance 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 an iregular hypointense area. D: Unenhanced T2-weighted MR imaging with TSE sequence (3170/99) after therapy. The tumor with a size of 0.96×0.96 mm² had a rapid growth compared with that before therapy. It also shows the unhomogeneous hyperintense area (arrow) corresponding to the intrahepatic metastasis.

controlled studies. However, it is not indicated for patients with thrombosed main portal veins. Its therapeutic effect was also doubtful when the tumor was infiltrative in nature or hypovascular, too large or too small[38,39]. The rapid development of arterial collaterals after these treatments might reduce this therapeutic effect and thus, inhibition of the development of arterial collaterals might be important in enhancing the therapeutic efficacy of these treatments[40]. In addition, in patients with large lesions, multiple TACE sessions were necessary to control tumor growth but might increase the risk of worsening hepatic function through damage to noncancerous liver parenchyma[23]. Moreover, the types and doses of embolic agents have been extremely variable in several reported studies. The major problem with embolic agents were twofold[41]. First, they could often completely obstruct the hepatic artery, leading to difficulties in administration of subsequent courses of hepatic artery chemotherapy. With a relative short half-life of embolic agents, the effectiveness of TACE was not significantly improved. Second, it was easy to aggravate the liver cirrhosis and lead to hepatic failure after repeated TACE. The optimal treatment modality of TACE is still unknown[42].

Today, it is well known that improvement of the overall therapeutic effect of liver malignancies depends on the combined therapies. In the past years, locoregional Chinese medicinal therapy has gained wide acceptance as a safe, palliative and effective treatment even in patients with large HCC and liver cirrhosis in China. *Bletilla striata* (BS) is a common Chinese traditional medicinal herb and is usually used as an embolic material in TACE for HCC. Its compositions are mucilage, starch, and a little volatile oil. In a previous study, Zheng *et al*[24] used BS to embolize the hepatic artery in order to induce ischemic necrosis and shrinkage of tumor. It mainly blocked the trunk of the blood-supplying artery of tumor with a "vascular cast-like appearance". The embolization was extensive and lasted longer, hence a better therapeutic effect[24]. The mechanisms of embolization by BS are attributable to the following factors: non-absorbent property, total mechanical obstruction, no influence on coagulative and anticoagulative systems and secondary obstruction due to injury of the wall of blood vessels[43, 44]. Zheng *et al*[25] have confirmed that BS has an adherent function and can expand slowly in blood flow, leading to mechanical blockade of vessels. It was also hypothesized that BS could slowly diffuse into the liver parenchyma around the tumor as a colloidal form, leading to prolonged anticancer effect and inhibition of collateralisation and metastasis of tumor[25]. Compared with gelfoam embolus, BS had the following characteristics. It could produce extensive and permanent vascular embolization, while it could not be absorbed by body tissue. After embolization, tumor necrosis and shrinkage were significant with less collateral circulation that formed later. The mucilage component of BS is a wide-spectrum anticancer element that might inhibit tumor occurrence and development[45]. 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 transarterial administration of BS might provide a beneficial therapeutic modality for HCC[45]. In our experimental study, the best therapeutic effect was the combined therapy of BS+TACE+ligation of hepatic artery. There was almost no significant difference between the tumor volume before and after therapy (Figure 1). No intrahepatic metastasis was observed in this group. This approach of central- and peripheral chemoembolization is able to increase the inhibition of tumor growth more completely, resulting in local control of tumor growth in rats and has a promising prospect for treating patients with HCC in the future.

In summary, by combining TACE and arterial administration

of *Bletilla striata* plus ligation of hepatic artery for treating HCC in ACI rats, an encouraging result 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 remain unclear and more randomized experimental and clinical studies are required.

REFERENCES

1 **Cha C**, DeMatteo RP, Blumgart LH. Surgery and ablative therapy for hepatocellular carcinoma. *J Clin Gastroenterol* 2002; **35**(5 Suppl 2): S130-S137

2 **Yuen MF**, Cheng CC, Lauder IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. *Hepatology* 2000; **31**: 330-335

3 **Tang ZY**. Treatment of hepatocellular carcinoma. *Digestion* 1998; **59**: 556-562

4 **Franco D**, Usatoff V. Resection of hepatocellular carcinoma. *Hepatogastroenterology* 2001; **48**: 33-36

5 **Durand F**, Belghiti J. Liver transplantation for hepatocellular carcinoma. *Hepatogastroenterology* 2002; **49**: 47-52

6 **Alsowmely AM**, Hodgson HJ. Non-surgical treatment of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2002; **16**: 1-15

7 **Durand F**, Belghiti J. Liver transplantation for hepatocellular carcinoma. *Hepatogastroenterology* 2002; **49**: 47-52

8 **Poon RT**, Fan ST, Tsang FH, Wong J. Locoregional therapies for hepatocellular carcinoma: a critical review from the surgeon's perspective. *Ann Surg* 2002; **235**: 466-486

9 **Sturm JW**, Keese MA, Bonninghoff RG, Wustner M, Post S. Locally ablative therapies of hepatocellular carcinoma. *Onkologie* 2001; **24**(Suppl 5): 35-45

10 **Chen MS**, Li JQ, Zhang YQ, Lu LX, Zhang WZ, Yuan YF, Guo YP, Lin XJ, Li GH. High-dose iodized oil transcatheter arterial chemoembolization for patients with large hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 74-78

11 **Li L**, Wu PH, Li JQ, Zhang WZ, Lin HG, Zhang YQ. Segmental transcatheter arterial embolization for primary hepatocellular carcinoma. *World J Gastroenterol* 1998; **4**: 511-512

12 **Huo TI**, Huang YH, Wu JC, Lee PC, Chang FY, Lee SD. Survival benefit of cirrhotic patients with hepatocellular carcinoma treated by percutaneous ethanol injection as a salvage therapy. *Scand J Gastroenterol* 2002; **37**: 350-355

13 **Teratani T**, Ishikawa T, Shiratori Y, Shiina S, Yoshida H, Imamura M, Obi S, Sato S, Hamamura K, Omata M. Hepatocellular carcinoma in elderly patients: beneficial therapeutic efficacy using percutaneous ethanol injection therapy. *Cancer* 2002; **95**: 816-823

14 **Jiang HC**, Liu LX, Piao DX, Xu J, Zheng M, Zhu AL, Qi SY, Zhang WH, Wu LF. Clinical short-term results of radiofrequency ablation in liver cancers. *World J Gastroenterol* 2002; **8**: 624-630

15 **Allgaier HP**, Galandi D, Zuber I, Blum HE. Radiofrequency thermal ablation of hepatocellular carcinoma. *Dig Dis* 2001; **19**: 301-310

16 **Vogl TJ**, Mack MG, Roggan A, Straub R, Eichler KC, Muller PK, Knappe V, Felix R. Internally cooled power laser for MR-guided interstitial laser-induced thermotherapy of liver lesions: initial clinical results. *Radiology* 1998; **209**: 381-385

17 **Pacella CM**, Bizzarri G, Cecconi P, Caspani B, Magnolfi F, Bianchini A, Anelli V, Pacella S, Rossi Z. Hepatocellular carcinoma: long-term results of combined treatment with laser thermal ablation and transcatheter arterial chemoembolization. *Radiology* 2001; **219**: 669-678

18 **Itamoto T**, Katayama K, Fukuda S, Fukuda T, Yano M, Nakahara H, Okamoto Y, Sugino K, Marubayashi S, Asahara T. Percutaneous microwave coagulation therapy for primary or recurrent hepatocellular carcinoma: long-term results. *Hepatogastroenterology* 2001; **48**: 1401-1405

19 **Seki T**, Tamai T, Nakagawa T, Imamura M, Nishimura A, Yamashiki N, Ikeda K, Inoue K. Combination therapy with transcatheter arterial chemoembolization and percutaneous microwave coagulation therapy for hepatocellular carcinoma. *Cancer* 2000; **89**: 1245-1251

20 **Liad inverted question marko L,** Virgili J, Figueras J, Valls C, Dominguez J, Rafecas A, Torras J, Fabregat J, Guardiola J, Jaurrieta

E. A prognostic index of the survival of patients with unresectable hepatocellular carcinoma after transcatheter arterial chemoembolization. *Cancer* 2000; **88**: 50-57

21   **Llovet JM**, Real MI, Montana X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Sola R, Rodes J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002;**359**: 1734-1739

22   **Vogl TJ**, Trapp M, Schroeder H, Mack M, Schuster A, Schmitt J, Neuhaus P, Felix R. Transarterial chemoembolization for hepatocellular carcinoma: volumetric and morphologic CT criteria for assessment of prognosis and therapeutic success-results from a liver transplantation center. *Radiology* 2000; **214**: 349-357

23   **Bartolozzi C**, Lencioni R, Caramella D, Vignali C, Cioni R, Mazzeo S, Carrai M, Maltinti G, Capria A, Conte PF. Treatment of large HCC: transcatheter arterial chemoembolization combined with percutaneous ethanol injection versus repeated transcatheter arterial chemoembolization. *Radiology* 1995; **197**: 812-818

24   **Zheng C**, Feng G, Liang H. *Bletilla striata* as a vascular embolizing agent in interventional treatment of primary hepatic carcinoma. *Chin Med J* 1998; **111**: 1060-1063

25   **Zheng C**, Feng G, Zhou R. New use of *Bletilla striata* as embolizing agent in the intervention treatment of hepatic carcinoma. *Zhonghua Zhongliu Zazhi* 1996; **18**: 305-307

26   **Yang R**, Rescorla FJ, Reilly CR, Faught PR, Sanghvi NT, Lumeng L, Franklin TD Jr, Grosfeld JL. A reproducible rat liver cancer model for experimental therapy: introducing a technique of intrahepatic tumor implantation. *J Surg Res* 1992; **52**: 193-198

27   **Qian J**, Truebenbach J, Graepler F, Pereira P, Huppert P, Eul T, Wiemann G, Claussen C. Application of poly-lactide-co-glycolide-microspheres in the transarterial chemoembolization in an animal model of hepatocellular carcinoma. *World J Gastroenterol* 2003; **9**: 94-98

28   **Trubenbach J**, Pereira PL, Graepler F, Huppert PE, Eul T, Konig CW, Duda SH, Claussen CD. Animal experiment studies on the effectiveness of permanent occlusion of the hepatic artery in transarterial chemoembolization. *Rofo Fortschr Geb Rontgenstr Neuen Bildgeb Verfahr* 2000; **172**: 274-277

29   **Trubenbach J**, Graepler F, Pereira PL, Ruck P, Lauer U, Gregor M, Claussen CD, Huppert PE. Growth characteristics and imaging properties of the morris hepatoma 3924A in ACI rats: a suitable model for transarterial chemoembolization. *Cardiovasc Intervent Radiol* 2000; **23**: 211-217

30   **Carlsson G**, Gullberg B, Hafstrom L. Estimation of liver tumor volume using different formulas-an experimental study in rats. *J Cancer Res Clin Oncol* 1983; **105**: 20-23

31   **Achenbach T**, Seifert JK, Pitton MB, Schunk K, Junginger T. Chemoembolization for primary liver cancer. *Eur J Surg Oncol* 2002; **28**: 37-41

32   **Yan FH**, Zhou KR, Cheng JM, Wang JH, Yan ZP, Da RR, Fan J, Ji Y. Role and limitation of FMPSPGR dynamic contrast scanning in the follow-up of patients with hepatocellular carcinoma treated by TACE. *World J Gastroenterol* 2002; **8**: 658-662

33   **Fan J**, Ten GJ, He SC, Guo JH, Yang DP, Weng GY. Arterial chemoembolization for hepatocellular carcinoma. *World J Gastroenterol* 1998; **4**: 33-37

34   **Choi BI**, Kim HC, Han JK, Park JH, Kim YI, Kim ST, Lee HS, Kim CY, Han MC. Therapeutic effect of transcatheter oily chemoembolization therapy for encapsulated nodular hepatocellular carcinoma: CT and pathologic findings. *Radiology* 1992; **182**: 709-713

35   **Clavien PA**, Kang KJ, Selzner N, Morse MA, Suhocki PV. Cryosurgery after chemoembolization for hepatocellular carcinoma in patients with cirrhosis. *J Gastrointest Surg* 2002; **6**: 95-101

36   **Lee HS**, Kim JS, Choi IJ, Chung JW, Park JH, Kim CY. The safety and efficacy of transcatheter arterial chemoembolization in the treatment of patients with hepatocellular carcinoma and main portal vein obstruction. A prospective controlled study. *Cancer* 1997; **79**: 2087-2094

37   **Lopez RR Jr,** Pan SH, Hoffman AL, Ramirez C, Rojter SE, Ramos H, McMonigle M, Lois J. Comparison of transarterial chemoembolization in patients with unresectable, diffuse vs focal hepatocellular carcinoma. *Arch Surg* 2002; **137**: 653-657

38   **Lin DY**, Lin SM, Liaw YF. Non-surgical treatment of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1997; **12**: S319-S328

39   **Raoul JL**. Is chemoembolisation of value in inoperable primary hepatocellular carcinoma. *HPB Surg* 1998; **10**: 406-408

40   **Qian J**, Feng GS, Vogl TJ. Combined interventional therapies of hepatocellular carcinoma. *World J Gastroenterol* 2003; **9**:

41   **Iwai K**, Maeda H, Konno T. Use of oily contrast medium for selective drug targeting to tumor: Enhanced therapeutic effect and X-ray image. *Cancer Res* 1984; **44**: 2115-2121

42   **Camma C**, Schepis F, Orlando A, Albanese M, Shahied L, Trevisani F, Andreone P, Craxi A, Cottone M. Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. *Radiology* 2002; **224**: 47-54

43   **Feng XS**, Qiu FZ, Xu Z. Experimental studies of embolization of different hepatotropic blood vessels using *Bletilla striata* in dogs. *J Tongji Med Univ* 1995; **15**: 454-459

44   **Qian J**, Feng G, Liang H. Action of DDPH in the interventional treatment of portal hypertension induced by liver cirrhosis in rabbits. *J Tongji Med Univ* 1998; **18**: 108-112

45   **Feng G**, Kramann B, Zheng C, Zhou R. Comparative study on the long-term effect of permanent embolization of hepatic artery with *Bletilla striata* in patients with primary liver cancer. *J Tongji Med Univ* 1996; **16**: 111-116

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