

## Effect of preoperative transcatheter arterial chemoembolization on tumor cell activity in hepatocellular carcinoma

HUANG Jiefu 黄洁夫, HE Xiaoshun 何晓顺, LIN Xiaojun 林晓军, ZHANG Changqing 张长清 and LI Jingqing 李锦清

**Keywords :** *hepatocellular carcinoma · transcatheter arterial chemoembolization · P53 · proliferate cell antigen*

**Objective** To evaluate the role of preoperative transcatheter arterial chemoembolization ( TACE ) as a palliative approach in hepatocellular carcinoma ( HCC ).

**Methods** From January 1992 to December 1998 , 279 patients with HCC underwent curative liver resection. One to five courses of TACE prior to liver resection were performed in 117 patients ( TACE group ) , while the other 162 patients received only liver resection ( control group ). All 279 specimens of resected tumors were submitted to the following assessments : PCNA and expression of P53 protein. All specimens from the TACE group were examined for downstaging or necrosis of tumors.

**Results** In the TACE group , gross inspection revealed downstaging or necrosis of tumor in all cases. Total necrosis ( 100% ) of tumor was observed in 11.1% of 117 patients , > 90% but incomplete necrosis in 15.4% , 50% – 90% necrosis in 46.2% and < 50% necrosis in 27.3%. Microscopically , extensive and homogenous coagulative necrosis was observed. Viable cancer cells were also present within and outside the tumor capsule in 111 cases. In the remaining 6 cases , the tumor necrosed completely. In control group , necrosis was observed in 8.0% of 162 cases and reduction of tumor size was < 20%. Microscopically , viable HCC cells were noted in all cases. There was no statistical difference in expression of P53 protein between the TACE and control group. High labeling index of PCNA was significantly higher in the TACE group.

**Conclusions** TACE has a marked antitumor effect resulting in various degree of tumor necrosis , but only a small proportion of tumors show complete necrosis. Since the residual tumor cells following preoperative TACE may have more aggressive behavior , we conclude that sequential liver resection is the preferred therapy whenever feasible and preoperative TACE

should be avoided in resectable HCC.

*Chin Med J 2000 ; 113( 5 ):446-448*

Liver resection is still the most effective treatment available for patients with hepatocellular cell ( HCC ) at present. During the last two decades , screening high-risk population for HCC using ultrasonography and serum alpha-fetoprotein ( AFP ) levels has increased the number of patients with HCC detected at early stages. Nevertheless , because of the severity of the underlying liver cirrhosis , the location of the tumor within the liver , or the extent of liver involvement , less than 30% are resectable at the initial diagnosis. The overall long-term results after resections of HCC have been disappointing , since tumor recurrence is frequent. The 1- and 2-year recurrence rates following liver resection even for a small HCC are very high , reaching 33% and 50% , respectively.<sup>1</sup> Transcatheter arterial chemoembolization ( TACE ) was developed as a palliative treatment for patients with unresectable lesions and some studies showed that preoperative TACE reduced tumor size , increased resectability , and prevented dissemination of cancer cells during surgery , correlated with improved survival.<sup>2</sup> TACE has also been used as an adjunctive therapy with liver resection or liver transplantation in an attempt to shrink the tumor. Unfortunately , a less clear advantage was found in more recent studies. Two prospective randomized trials of TACE for unresectable HCC failed to demonstrate any survival benefit.<sup>3</sup> The discrepancies among the older and newer trials may be due to the heterogeneity of the enrolled patients as well as the specific TACE methods used , making the overall results. This study assessed the effect of preoperative TACE on the labeling index of PCNA , expression of P53 protein and downstaging or necrosis of

Liver Cancer Research Center , Sun Yat-Sen University of Medical Sciences , Guangzhou 510089 , China ( Huang JF , He XS , Lin XJ , Zhang CQ and Li JQ )

Correspondence to : Prof. Huang Jiefu , Sun Yat-Sen University of Medical Sciences , Guangzhou 510089 , China ( Tel : 020-87333606. Fax : 020-87331679. Email : Jeffrey@gzsums.edu.cn )

tumor in resected tumor specimens. We evaluated the role of TACE as an adjunctive modality in the treatment of HCC.

## METHODS

### Patients

From January 1992 to December 1998, a total of 279 patients with HCC were referred to our center for surgery. There were 263 men and 16 women with mean age of 50 years (range, 29 to 65). A diagnosis of HCC was obtained for all patients by preoperative US or/and computed tomography (CT) and confirmed by biopsy.

### Surgical procedure

The patients were randomly divided into two groups. In the TACE group, 117 patients underwent 1–5 courses chemoembolization prior to liver resection. In the control group, 162 patients received initial liver resection without preoperative TACE. The extent of liver resection was carried out based on the location of tumor, the severity of concomitant liver cirrhosis and preoperative liver reserve function.

### Histopathologic studies

Surgical specimens were microscopically examined for features of tumor, such as size and number of nodules, distance from the surgical margin and necrosis, which was defined as complete if no viable cells were found in all nodules. Tumor size and number were measured on preoperative US or CT. The size of the nodule on the resected specimens was taken as the final size.

### Immunohistochemical staining

The formalin-fixed, paraffin-embedded specimens were examined immunohistochemically using anti-monoclonal antibody Pc-10 and anti-P53 monoclonal antibody Do-7 (LSAB kit Dako). The deparaffinized tissue sections were treated in a microwave oven before immunohistochemical staining for P53.<sup>4</sup>

The number of positive nuclei were semiquantitatively evaluated by counting the number of positive nuclei in 8–10 random medium power ( $\times 100$ ) fields. Four degrees of positive nuclei of proliferate cell antigen (PCNA) were identified: “+”: < 25%, “++”: 25%–50%, “+++”: 51%–75%, and “++++”: > 75%. Four degrees of positive nuclei of P53 were identified: “-”: < 25% or no positive cell, “+”: 25%–29%, “++”: 30%–70%, and “+++”: > 70%.

### PCR/SSCP method

Single-stranded conformational polymorphism analysis of polymerase chain reaction products (PCR/SSCP) method was used to examine mutations of the P53 gene in exons 5–8.<sup>5</sup>

## Statistical analysis

Data were analyzed by the Chi-square test. A *P* value < 0.05 was considered statistically significant.

## RESULTS

### Histopathologic findings

In the TACE group, gross inspection revealed a downstaging or necrosis of tumor to variable extent in all cases. Total necrosis of tumor was observed in 13 cases (13/117, 11.1%), over 90% but incomplete necrosis was seen in 18 cases (18/117, 15.4%), 50%–90% necrosis in 54 cases (54/117, 46.2%) and 32 cases (32/117, 27.3%) had a less than 50% tumor necrosis. Microscopically, extensive and homogenous coagulative necrosis was found in the tumor. However, viable cancer cells were also present within and outside the tumor capsule in 111 cases. In the remaining 6 cases, the tumor necrosed completely.

In the control group, necrosis was observed in 13 (13/162, 8.0%) cases. The reduction of tumor volume is less than 20%. Microscopically, viable HCC cells were noted in all cases.

### Mutations of P53 gene, expression of P53 protein and labeling index of PCNA

Using the PCR/SSCP technique, mutations of the P53 gene were detected in 40 cases in TACE group. Out of them, 33 cases had mutations in exon 5, 5 cases in exon 6, 18 cases in exon 7 and 15 cases in exon 8. High or positive expression of P53 protein was present in each of these 40 cases.

In the control group, mutations of P53 gene were found in 59 cases (59/162, 36.4%). Out of them, 39 cases had mutations in exon 5, 7 cases in exon 6, 23 cases in exon 7 and 18 cases in exon 8. High or positive expression of P53 protein was detected in 53 cases. There is no statistical difference in the expression of P53 protein between TACE and control groups. High labeling index of PCNA was significantly more frequent in TACE group (Table).

**Table.** Expressions of PCNA and P53 protein in resected HCC

Groups	n	Labeling index of PCNA		P53 expression	
		+ and ++	+++ and ++++	- and +	++ and +++
TACE	111	39 (35.1%)	72 (64.9%)	26 (23.4%)	85 (76.6%)
Control	162	97 (59.9%)	65 (40.1%)*	33 (20.4%)	129 (79.6%)#

+ and ++: high labeling index of PCNA; +++ and ++++: low labeling index of PCNA; - and +: negative or low expression; ++ and +++: high P53 expression. \*  $\chi^2 = 16.1284$ , *P* = 0.0001, compared with TACE group; #  $\chi^2 = 0.3624$ , *P* = 0.5472, compared with TACE group.

## DISCUSSION

HCC is one of the most common malignant neoplasms. For patients with early disease, liver resection or liver transplantation is the most effective form of treatments. Unfortunately, only a minor proportion of the patients currently diagnosed with HCC may benefit from these radical options. The majority of the HCC patients will be treated with palliative approaches to improve the resectability rate and prolong survival.

The rationale for treating HCC by TACE evolved from the observation that, in contrast with liver parenchyma, about 90% of blood supply of this neoplasm are from the hepatic artery. The obstruction of blood supply results in extensive tumor necrosis caused by ischemia. The addition of chemotherapy aimed to enhance the antitumor effect of the ischemia. Despite its theoretical advantages, it is commonly accepted that TACE induced only partial necrosis of the neoplastic tissue and limited size reduction of the tumor mass.<sup>6</sup> Moreover, the efficacy of TACE is related to the following factors: the type of the tumor blood supply, macroscopic and microscopic patterns of tumor growth, the timing and experience with TACE, and the choice of embolization materials and anti-cancer agents. Wang<sup>7</sup> reported the results of 112 patients with undergoing TACE. Medium survival was 14.6 months and 1-, 2-, 3-, and 4-year survival rates were 93.7%, 56.0%, 31.2% and 7.7%, respectively. Arii et al<sup>8</sup> collected 11 379 cases of HCC in Japan, and reported that the 3- and 5-year survival rates following TACE were 19.5% and 8.0%, respectively. These results indicate that long-term survival following TACE remains disappointing. The negative results for TACE are commonly linked to incomplete tumor necrosis and that the blood supply of some tumors derives from the portal vein. The present study confirmed that TACE is able to induce extensive (> 50%) tumor necrosis in 73% of the patients, but only 5% had complete tumor necrosis.

Recent studies revealed that the postoperative recurrence of HCC is closely related to the biological behaviors of the tumor. Tumors with a high labeling index of PCNA are inclined to have invasive growth, progress rapidly and have a poor prognosis.<sup>5</sup> Uchida et al<sup>9</sup> described a retrospective study of highly selective TACE in a series of small HCC (< 2 cm). Tumor necrosis was observed in only 80% of resected tumors. Viable HCC cells were found in resected tumors though serum AFP levels decreased to the normal range.

The current study showed there was no statistical difference in expression of the P53 protein between the TACE

and control groups. Interestingly, high labeling index of PCNA was significantly more frequent in the TACE group, indicating that residual HCC cells become more proliferative following chemoembolization. The mechanism of this phenomenon may be related to the heterogeneity of the cells forming of tumor. Malignant hepatocytes are less sensitive to hypoxia than normal hepatocytes, and this reduced sensitivity is mediated through P53, which is less frequently abnormal in well-differentiated HCC. TACE may affect mainly well-differentiated cells, leaving in place the poorly differentiated ones which exhibit more aggressive behavior and a more malignant phenotype.<sup>10</sup> From this point of view, we believe that preoperative TACE is not justified for resectable HCC since it is unlikely to induce complete tumor necrosis and be more likely to increase the aggressive behavior of HCC, resulting in rapid dissemination. Preoperative TACE may only be suitable for unresectable or marginally resectable HCC. Accordingly, sequential liver resection is strongly recommended whenever feasible.

## REFERENCES

1. Lin DY, Lin SM, Liaw YF. Non-surgical treatment of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1997 ;12 :S319-S328.
2. Uchida M, Kohno H, Kubota H, et al. Role of preoperative transcatheter arterial oily chemoembolization for resectable hepatocellular carcinoma. *World J Surg* 1996 20 :326-331.
3. Pelletier G, Ducreux M, Gay F, et al. Treatment of unresectable hepatocellular carcinoma with lipiodol chemoembolization: a multicenter randomized trial. *J Hepatol* 1998 29 :129-134.
4. Feng KT, Zhang CQ, Zhang JX, et al. A new immunohistochemical staining method for P53 protein. *Chin J Diagn Pathol* 1994 ;1 :50-56.
5. Ng IO, Chung LP, Tsang SW, et al. P53 gene mutation spectrum in hepatocellular carcinomas in Hong Kong Chinese. *Oncogene* 1994 9 :985-990.
6. Liang LJ, Lu MD, Huang JF, et al. The clinical and pathological features of resected hepatocellular carcinoma after hepatic arterial chemoembolization. *Cancer* 1993 ;12 :148-150.
7. Wang F. Interventional treatment for the recurrence primary liver carcinoma (analysis of 112 cases). *Chin J Pract Surg* 1998 ;15 :146-147.
8. Arii S, Okamoto E, Imamura I. Registries in Japan: current status of hepatocellular carcinoma in Japan. *Liver Cancer Study Group of Japan. Semin Surg Oncol* 1996 ;12 :204-211.
9. Uchida H, Iatsuo N, Sakaguchi H, et al. Chemoembolization therapy in small hepatocellular carcinoma. *Gan To Kagaku Ryoho* 1996 23 :840-848.
10. Bruix J, Llovet JM, Castells A, et al. Transarterial embolization versus symptomatic treatment in patients with advanced hepatocellular carcinoma: results of a randomized, controlled trial in a single institution. *Hepatology* 1998 27 :1578-1581.

( Received February 4, 1999 )

本文编辑:潘玲/曹琳冰