RESEARCH COMMUNICATION

Liver Fibrosis and Five Year Survival of Hepatocellular Cancer Cases Undergoing Transcatheter Arterial Chemo Embolization Using Small Doses

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Abstract

Objective: To investigate liver fibrosis, TGF-β1 levels and curative effects on hepatocellular carcinoma (HCC) with small and conventional dose perfusion chemotherapy by transcatheter arterial chemo embolization (TACE). Methods: Thirty-six hepatocellular carcinoma patients not indicated for surgical resection underwent super-selective transcatheter arterial chemoembolization, divided into small dose (n=15) and conventional dose (n=21) chemotherapy groups. Results: With conventional doses, four indices of liver fibrosis focusing on hyaluronate acide (HA), human procollagen type-III (hPC-III), collagen type-IV (IV-C) and transforming growth factor-βl (TGF-β1) were obviously increased postoperative compared with preoperative (P<0.01); in contrast, with small doses there were no significant differences except for TGF-β1. Five year survival demonstrated no significant differences between the two groups (P>0.05). Conclusion: To hepatocellular carcinoma patients treated by TACE, reducing doses of chemotherapy drugs can reduce progress of liver fibrosis, without impacting on five year survival.

Keywords: Hepatocellular carcinoma - transcatheter arterial chemoembolization - liver fibrosis - survival

Asian Pacific J Cancer Prev, **13**, 1589-1593

Introduction

Hepatocellular carcinoma (HCC) have a higher incidence in malignant tumors. It has replaced carcinoma of stomach to be the second highest incidence in China (Yan, 2002). Trancatheter arterial chemoembolization (TACE) is the chief therapeutic method to middle-late period PHC patients, TACE can promote tumor necrosis by directly kill tumor cells and block tumor's blood supply, so as to achieve the therapeutic purposes.However ,different doses different chemotherapeutics and lipiodol in TACE will damage normal liver parenchyma, induce even aggravate liver fibrosis.But because potential chronic liver injury can't be found by routine examination,which cause clinical can't understand it sufficiently for a long time. Over several years, the phenomenon like progressive chronic liver injury , aggravating liver cirrhosis after more than once TACE graduatelly become a hot problem that some clinical studies pay close attention to (Zhu et al., 2000; Feng et al., 2002; Lu et al., 2004).

Scholars both domestic and overseas found that , one significant reason that cause liver fibrosis was huge doses chemotherapeutics in TACE (Chung et al., 1996; Feng et al., 2002; Lu et al., 2004). Researches demonstrate that normal liver tissue iodized oil embolization can cause or worsen liver fibrosis, so that they advocated super-selective TACE (Xiao et al., 1997). But some research indicated that there was still certain concentration drugs in liver parenchyma (Chung et al., 1996; Bruix 1997) and cell necrosis was visible (Therasse et al., 2000; Kamada et al., 2001) surrounding the iodized oil deposition areas. So only using super-selective intubation treatment to reduce the liver fibrosis that caused by TACE was not enough, because hepatocarcinoma were not that sensitive to chemotherapeutics, and liver fibrosis was caused by huge doses chemotherapeutics drugs in TACE. Then, how small doses chemotherapeutics affected curative effect. However there was little newsreport about the influence of small doses chemotherapeutics to liver fibrosis, especially influence to index of liver fibrosis and curative effect.

Liver fibrosis is liver extrocellular matrix especially the collagenic deposit excessively, is organism'repair response towards hepatic parenchymal damage. Indexes considered to have value are hyaluronate acide (HA), human procollagen type-III (hPC-III), laminin (LN), collagen type-IV (IV-C), these four liver fibrosis indexes combined with Transforming growth factor-βl (TGF-βl) can further reflect liver fibrosis stage,especially HA and hPCIII are most valuable in the diagnosis of early liver fibrosis (Lu and Lu., 2006). HA is the most sensitive index to screen liver fibrosis and liver cirrhosis, hPCIII is collagen type-III precursor, having a positive correlation with the degree of liver fibrosis.In this item we investigate liver fibrosis's development trend and five years survival state's correlation change of HCC patients by means of lessen the doses of chemotherapeutics in TACE.

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Materials and Methods

Study objects and grouping

Collect Hepatocellular Carcinoma (HCC) patients that performed TACE in Three Gorges University Renhe Hospital during Oct 2006 to Oct 2011, all cases were diagnosed by pathologic biopsy. The study selected 36 cases, including male 27, female 9. All patients were grouping into two sets according to Child-Pugh classification standard preoperative, grade A 19 cases, grade B 17 cases. Single tubercle 27 cases, nodosity 9 cases, no diffusion cases. General material of two group patients, the average age, tumor size have no significant differences, and is comparable.

All patients were divided into two groups: low doses group and conventional doses group. Low doses group (group A, n=15) were given low doses chemotherapeutics: patients with tumor diameter less than 5cm were given Mitomycin (MMC) 4mg, Epirubicin (EPI) 10 mg, 5-fluorouracil (5-FU) 250 mg; patients with tumor diameter greater than 5cm were given MMC 6 mg, EPI20mg, 5-FU250 mg. While conventional group were given MMC 10 mg, EPI 40 mg, 5-FU 1000 mg.

Study procedure

Procedure of TACE: Right inguinal region routine disinfection and local anesthesia, intubating from right femoral artery using seldinger technology, all catheters were placed in proper hepatic artery or left and right femoral artery in the course of Infusion chemoembolization; then insert microtubular into blood-supply artery of carcinoma to perform embolization with super-selective intubation technique, so as to maximatily avoid hepatic fibrosis caused by iodized oil deposition in normal liver tissue. All lesions in group A and B were single and multiple nodule, none was diffuse, the doses of Iodized oil adopt maximum dose method (Li et al., 2000). All cases perform CT or MRI examination and one more time TACE one month postoperative, each patient do four times in total.

Serology detection: All patients must draw venous blood off before the first TACE and four weeks after per operation, to detect four index of liver fibrosis (HA,PC-III, IV-C, LN) adopt radio-immunity method, and detect TGF-β1 level with enzyme-linked immunosorbent test. Detecting index of conventional liver function and AFP at the same time.

Efficacy evaluation standard

Evaluate curative effect according to the latest response evaluation criteria in solid tumors(RECIST) that published in "National Cancer Institute of the United States" by Therasse et al. (2000), it include five levels: complete remission (CR), partial remission (PR), stability (SD), progression (PD), effectiveness (CR+PR); then do statistical comparison on the base of five-year survival ratio.

Statistical analysis

Measurement data were demonstrated in the form of mean ± standard deviation, and analyzed with T-test; while enumeration data analyzed with χ^2 test; the analysis of five-year survival ratio adopted Kaplan-Meier estimate and Log-rank test; all statistics completed by statistical software SPSS13.0, $P < 0.05$ have statistical significance.

Results

Four index of liver fibrosis

Serology index value of liver fibrosis of two groups before the first and after the fourth, eighth TACE were in Table 1. The value of each index in two groups has no significant differences preoperative (P>0.05), index of low doses group have no significant differences before and after operation (P>0.05),while conventional doses group obviously elevated compared with preoperative (P<0.05). Four index value of conventional group postoperative is higher than that of low doses group, differences have statistical significance (P<0.05).

TGF-β1 level

TGF-β1 value of two groups preoperative and after the fourth, eighth TACE are in Table 2. The value of two groups has no significant differences preoperative (P>0.05). The value of postoperative had no significant elevation compared to preoperative in low doses group (P>0.05), while in conventional group obviously evaluated($P < 0.01$). Comparison between the two groups after TACE show that TGF-β1 of conventional group is higher than that of low doses group, the difference had statistical significance ($P < 0.05$).

Curative effect evaluation

All cases adopted microtubular super-selective intubation technique in blood-supply artery of carcinoma,

Table 1. Effects of Chemotherapy Drugs of Different Doses (μg/l) on Indexes of Liver Fibrosis in HCC Patients

group	liver fibrosis index	preoperative	after the fourth TACE	after the eighth TACE	t	P
A	HA	283.81±108.33	301.89±115.48	321.16±111.12	1.979	0.068
	PC-III	240.11±82.34	246.16±83.98	254.61±82.22	1.74	0.104
	IV-C	122.20±46.35	128.11±45.20	129.04±48.02	1.937	0.073
	LN	129.86±28.16	130.48±29.48	131.19±30.41	0.34	0.739
B	HA	285.74±118.78	323.79±118.35△	401.28±106.72	6.628	0
	PC-III	242.60±71.80	268.85±87.83△	312.78±77.38*	4.718	0
	IV-C	123.13±40.19	151.63±49.97△	203.33±52.86*	4.268	0
	LN	131.26±33.28	146.58±32.26△	189.32±30.26*	2.715	0.013

Compared with homologous index of group A, △P<0.05 after the fourth TACE and *P<0.01 after the eighth TACE. Index of low doses group compared between before and after treatment, P>0.05, while in conventional doses group P<0.05

Table 2. Influence of Different Doses (μg) of Chemotherapy Drugs to TGF-β1 level in HCC Patients

group	preoperative	after the fourth TACE	after the eighth TACE	t	P
A	88.14 ± 18.07	93.32 ± 17.33	98.56 ± 16.86	1.987	0.071
B	90.42 ± 19.42	101.22 ± 17.90△	120.34 ± 18.23*	3.986	0.001

Compare between the two groups postoperative, △P<0.05, *P<0.01

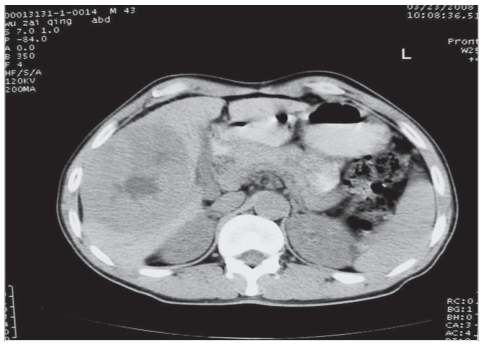

Figure 1. CT Scan Before the First TACE

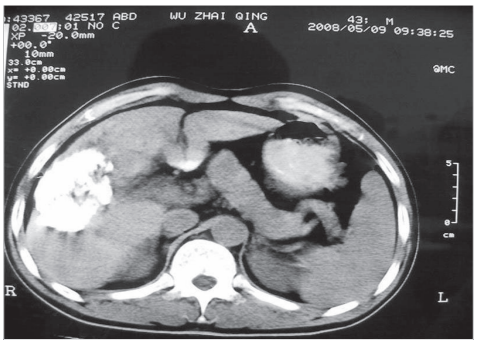

Figure 2. The Size of Tumor Grow Smaller, Non-tumor Areas Without Iodized Oil Deposition

as Figure 1-2 show, iodized oil deposit well in tumor areas, and without iodized oil deposition in non-tumor region, the tumor size obviously grew down after several times of TACE. The situations of five-year curative effect of two groups were showed in Table, there was no CR case in both groups, in low doses group there was 2 PR cases, 10 SD cases and 3 PD cases of 15 cases in total ; while in conventional doses group 4 PR cases, 13 SD cases,4 PD cases of 21 cases in total. The five-year curative effect of two groups were analyzed with Log-rank test, P>0.05, the difference had no statistically significance. It is ranked data analyzed with Rank sum test, the result have no significant differences. Survival curve was showed in Figure 3.

The first and second year survival rate of the two groups were both 100%, the third, forth and fifth year survival rate were 86.67% (13/15), 66.67% (10/15), 40.00% (6/15) in group A and 80.95% (17/21), 61.90% (13/21), 38.09% (8/21) in group B. The two groups five-year survival rate were analyzed with Kaplan-Meier estimate and Log-rank test, P>0.05, the difference had no statistically significance, the survival curve was showed in Figure 3.

Discussion

During TACE, chemotherapeutics and iodinated oil were injected into hepatic artery of HCC carcinoma, which can control the growth of tumor by both killing cancer cells and blocking tumor's blood supply, promote cancer tissue come to necrosis, but they damage normal liver

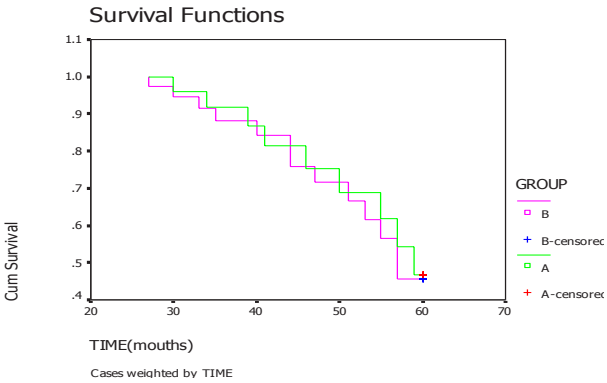

Figure 3. Kaplan-Meier Five-year Survival Curve of Group A and B

parenchyma cell either, almost all chemotherapy drugs could promote cell necrosis through different approaches (Chen et al., 2002), as well in TACE the drugs also aggravate hepatic apoptosis and liver fibrosis in non-tumor areas. Chung's (Chung et al., 2007) research indicated that there was still certain concentration drugs in liver parenchyma surrounding the iodized oil deposition areas. It is thus evident that several drugs released into no cancer tissues were inevitable, which could cause hepatic injury or liver fibrosis. However certain chemotherapeutics is necessary too, which had inhibition and wound function to cancer cells in some extent.

The study detected HCC patients' four index of liver fibrosis treated with different doses of chemotherapeutics in TACE. The results showed that four index of liver fibrosis have no significant differences before and after TACE in HCC patients treated with low doses chemotherapy drugs. While four indexes of liver fibrosis levels in patients treated with the conventional dose chemotherapy were higher than low-dose group after TACE, but five-year curative effect and survival rate have no significant differences. Reducing chemotherapy doses played a positive effect to patients' long-term survival ratio not only have no influence to it's short-term curative effect but also could protect hepatic function. Ikeda et al. (2001) reported 142 hepatocellular liver carcinoma (HCC) patients treated with low doses of chemotherapeutics (MMC 4-20 mg) and gelatin particle ,the result showed that survival rate of low doses group is superior to that of conventional doses group. Camma et al. (2008) summarized several randomized controlled trials in Europe, the study suggested that short-term curative effect improved by decreasing doses of chemotherapeutics. Kamada et al. (2009) reported that PHC patients treated with low doses and conventional doses of chemotherapeutics, patients, 1, 3, 5, 7 years of survival rate were 81%, 41%, 19% and 13%. Thus, TACE with low doses chemotherapy drugs may have important significance to patients' later chronic liver injury and fibrosis and is beneficial to patients' long-term survival. The study showed that four indexes of liver fibrosis of

conventional dose group had an obvious elevation after TACE compared with preoperative, it hint progressive liver injury and liver fibrosis development, it's bad for patients' prognosis.

TGF-β1 is an important regulatory factor in the process of liver fibrosis (Chen et al., 2003). TGFβ-1 can contribute hepatic fibrosis through activating hepatic stellate cells (HSC), inhibiting the degradation of ECM, inhibiting the regeneration of liver cells, and inducing hepatocyte apoptosis. The results show that plasma TGF-β1 levels can reflect the degree of liver fibrosis in some extent.

Currently, there was seldom study on TGF-β1 levels in preoperative and postoperative of TACE. The study observed the change of TGF-β1 levels before and after TACE in HCC patients treated with different doses of chemotherapy drugs. The results showed that TGF-β1 levels were elevated in the HCC patients treated with the low doses of chemotherapy and conventional-dose chemotherapy in TACE, while TGF-β1 levels in patients treated with the conventional dose chemotherapy were higher than low-dose group. After TACE, TGF-β1 levels of the conventional dose group were higher than the low dose group. Therefore, we speculated that reducing the dose of chemotherapy drugs can effectively inhibit the elevation of TGF-β1 levels in HCC patients treated by TACE. TGF-β1 is an important regulatory factor in the process of liver fibrosis, reducing its level can effectively reduce the degree of hepatic fibrosis.

In addition to participating in the process of liver fibrosis, TGF-β1 is a major negative regulatory factors and apoptosis-promoting factor of the epithelial cells, promoting tumor angiogenesis, providing a good local environment for rapid tumor cell growth and metastasis, and leading to Cancer progress. Compared to conventional dose chemotherapy, the low dose TACE in terms can reduce elevating of TGF-β1 levels. So it can play a positive role in inhibiting tumor growth and metastasis in theory.

Using low doses of chemotherapy drugs in TACE could improve survival quality and long-term curative effect for HCC patients: To explore long-term curative effect need large amount of samples and randomised control trial, overall survival (OS) rate for final evaluation index. Some scholars study results presented, conventional doses of chemotherapeutics in TACE could not play more important role in promoting tumor cell necrosis and apoptosis than small doses chemotherapeutics (Lu & Li, 2002). Hu et al. (2002) underwent control trial between small doses and conventional doses group in TACE, the study indicated that the cases in small doses group produce fewer adverse reaction and complications postoperative, liver function recovered well, quality of life and short-term curative effect both improved obviously. Lu wei's study (Lu et al., 2003) indicate that, in super-selective TACE, using small doses chemotherapeutics and using conventional doses chemotherapeutics can gain the same curative effect The study evaluated HCC patients performed TACE with different doses of chemotherapy drugs, the result showed that the difference of five-year curative effect and survival rate had no statistical significance between small doses group and conventional doses group (P>0.05). It indicated that reducing doses of chemotherapy drugs appropriately had no influence to patients' curative effect, it was mainly because of multiple drug-resistant (MDR) of HCC, and the neoplasm effect is mainly caused by embolization of tumor's feeding artery. At the same time small doses of chemotherapy could reduce the risk of liver fibrosis and improve the quality of patients, it was worthy our attention.

Acknowledgements

This study was supported by China Science and Technology Bureau funded projects (NO. A06429).

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