RESEARCH ARTICLE

^{131}I-Labeled-Metuximab Plus Transarterial Chemoembolization in Combination Therapy for Unresectable Hepatocellular Carcinoma: Results from a Multicenter Phase IV Clinical Study

Jun Ma[1,2], Jian-Hua Wang[1]*

Abstract

Objective: This study evaluated the safety and objective response of combining ^{131}I-labeled-metuximab (Licartin) with transarterial chemoembolization (TACE) in the treatment of unresectable hepatocellular carcinoma (HCC). Materials and Methods: In a multicenter open-label clinical trial, 341 enrolled patients with stage III/IV HCC according to TNM criteria were nonrandomly assigned to a trial group (n=167) and a control group (n=174), undergoing TACE following hepatic intra-arterial injection of licartin or TACE alone from July 2007 to July 2009. Radiopharmaceutical distribution was evaluated. The primary endpoint was overall survival; secondary endpoints included time-to-progression (TTP), toxicity and adverse events (AEs). Results: The radiobiological distribution demonstrated better localization of licartin in liver tumors than other tissues (P<0.01). The organ absorbed doses to liver and red marrow were 3.19±1.01 Gy and 0.55±0.22 Gy, respectively. The 1-year survival rate was significantly higher [79.47% *vs.* 65.59%, hazard ratio (HR), 0.598, P=0.041] and TTP significantly improved (6.82±1.28 *vs.* 4.7±1.14 months, P=0.037) compared with the control group. Patients at stage III achieved more benefit of one year survival than stage IV in the trial group (86.9% *vs.* 53.8%, P<0.001). There were significant different toxicities in leukocytopenia, thrombocytopenia and increased total bilirubin level [P<0.001, P=0.013, P<0.01, relative risk (RR) 1.63, 1.33, 1.43], but no differences in severe AEs of upper GI hemorrhage and severe liver dysfunction between the groups (5.39% *vs.* 2.3%, P=0.136). Conclusions: Owing to excellent tumor-targeting, promised efficacy and favourable toxicity profile, the novel combination therapy of licartin and TACE could be applied in patients with unresectable HCC.

Keywords: Hepatocellular carcinoma - iodine radioisotopes - antibody monoclonal - radioimmunotherapy - clinical study

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Introduction

Hepatocellular carcinoma (HCC) represent the major histological subtype among primary liver cancers is one of the most common malignancies and the second leading cause of cancer-related death in males, and more than 80% HCC rate evolved from liver cirrhosis that is closely related with the chronic hepatitis B virus infection in Asia (Jemal et al., 2011). Due to the HCC characterized by a rapid clinical progression and multiple foci occurring against a background of cirrhosis, more than half of the patients have advanced disease at diagnosis, only a small portion have the opportunity to undergo curative therapy, including surgical resection, liver transplantation and radiofrequency ablation. And transarterial chemoembolization (TACE) has remained a mainstay non-surgical therapy for HCC. However, the patients with vascular invasion, extra-hepatic metastasis and hepatic dysfunction tended to gain fewer benefits and their long-term efficacies were unsatisfactory (Forner et al., 2012; Lencioni and Crocetti, 2012).

In recent years, internal radiotherapy by hepatic intra-arterial injection of radiopharmaceuticals has been developed (Lambert and Van de Wiele, 2005; Lau et al., 2008; Salem et al., 2013), which allow selective retention of radionuclide within the liver tumor tissue, for examlpe radioembolization with radionuclide iodine 131 (^{131}I)-labelled lipiodol or yttrium 90 (^{90}Y) microshperes. In particular, radioimmunotherapy (RIT) using radiolabeled monoclonal antibodies (mAb) has emerged as a therapeutic option for HCC, which enhance the effectiveness of the radionuclide concentrated at the tumor site by mAb binding to the cell surface antigens expressed on tumor cells, and damage tumor cell's DNA by the low linear energy transfer (LET) radiation emitted from radionuclide and eventually lead to tumor cell growth inhibition and death while produce fewer toxic side-effects in normal organs (Kassis and Adelstein, 2005; Sgouros, 2005; Goldenberg and Sharkey, 2006; Zanzonico and Divgi, 2008). Follow this principle, ^{131}I-labeled metuximab (Licartin) as a national class I new drug has achieved efficacies of RIT in the treatment of HCC in

[1]*Department of Interventional Radiology, Zhongshan Hospital, Fudan University,* [2]*Department of Radiology, Tongji Hospital, Tongji University School of Medicine, Shanghai, China *For correspondence: jhdoccn@163.com*

pahse II clinical trial (Chen et al., 2006). Metuximab is one kind of mouse-derived anti-human HCC monoclonal antibody fragment HAb18F(ab´)$_2$, as shown by tissue biodistribution studies performed in both experimental animals and humans (Xu et al., 2007a). As a specific antigen of HCC, targeted antigen HAb18G/CD147 is correlated with invasion and metastasis of HCC. And it has the biological characteristic of expressing at a high level in HCC tissue.

However, RIT has achieved success primarily in the treatment of lymphoma, for solid tumors which tend to be more radioresistant in nature, and RIT has not yielded significant results largely due to insufficient dosing to the tumor (Sgouros, 2005; Lambert and Van de Wiele, 2005; Goldenberg and Sharkey, 2006; Zanzonico and Divgi, 2008). In addition, there is more risk of radiation-induced liver and adjacent organ injury if whole-liver delivered radiation doses greater than 30-35 gray (Gy) over 3 weeks (Salem et al., 2013; Aitken and Hawkins, 2014). Furthermore, in several clinical studies assessed the therapeutic efficacy of radiolabelled mAbs for treating HCC (Zeng et al., 1998; Lambert and Van de Wiele, 2005), such as ^{131}I-hepama-1 and polyclonal ^{131}I-antiferritin, has been recognized the more clinical advantages resulted from the combined therapy approach, including external radiotherapy, chemotherapy and hepatic ligation. Therefore, combination treatment with TACE could be considered as an alternative to improve the therapeutic efficacy of RIT for patient with HCC (Lambert and Van de Wiele, 2005; Goldenberg and Sharkey, 2006; Aitken and Hawkins, 2014).

For this purpose, the multicenter phase IV clinical study was designed to evaluate the safety and clinical efficacies of Licartin in combination with TACE as a new approach for the treatment of unresectable HCC. Whether should allow maximun and sustained concentration of licartin in the tumor, with minimal systemic exposure, with calibrated tumor vessel obstruction, provides promising effectiveness.

Materials and Methods

Subjects

It was designed as a multi-center, open-label and synchronically controlled clinical study. Taking into consideration a dropout rate of less than 10%, a total of 355 subjects were recruited and divided into the trial (n=173) and control (n=182) groups. All patients were diagnosed definitely through clinicopathological examinations as intermediate and advanced HCC (TNM staging, Union for International Cancer Control 5.0, 1997), aged 18-85 years. The inclusion criteria were Karnofsky performance score (KPS) ≥70 points, hepatic function Child-Pugh class A/B, non-total occlusion of portal vein trunk and tumor occupation rate <70%, no history of severe cardiac/renal/hematological disease or hypersensitivity to biological preparations. And pregnant and lactating women and those with positive Licartin skin test were excluded. The exclusion criteria were those cases not conforming to the inclusion criteria, lacking major observation parameters, violating this protocol and impossible to judge efficacies due to severe adverse event (SAE). The present study was approved by both State Food & Drug Administration (SFDA) and Ethics Committee of Affiliated Zhongshan Hospital, Fudan University. And all subjects agreed and signed the form of informed consent.

Drugs and major instruments

Licartin was supplied by Huasun Biotechnology Limited (Chengdu, China) (GYZZ: S20060064, packaging specification: 1 aliquot per person, containing metuximab 5 mg), radioactive chemical purity ≥95%, immunological activity ≥50%, one-time injection dose of pyrogenic endotoxin ≤5EU/ml (EU: endotoxin measurement, 1 ng=2.5 EU), PH 7.0-7.4. DSA (German Simens AXIOM Artis), CRC-15R type dose calibrator (Capintec Inc, NJ, USA), SN-695 type radioimmunity, γ-scintigrapher (Shanghai Hesuo Rihuan Photoelectric Instrument Co., Ltd, China), SPECT apparatus Marconi IRIX type III (USA) and SPECT/CT Dutch Philips Precedence.

Dosing methods

The trial group received the combined therapies of Licartin and TACE. The Seldinger method was employed to insert a 5F transfemoral catheter into common hepatic artery. And vascular contrasting was used to ascertain the tumor site and its status of blood status. The tumor-supplying branches of hepatic artery were identified. The injection dose of Licartin was adjusted according to patient body weight (27.75 MBq/kg). Within 5-10 min after injection, the mixed chemotherapeutic agents of lipiodol emulsion and gelatin sponge were added. All patients used medication only after confirming a negative skin test before dosing and Day 3 pre-dosing. And compound iodine solution (0.5 ml, tid) was taken orally from Day 3 pre-dosing to Day 7 post-dosing. After therapy, they were placed under a radiological quarantine and observed for 2 days inside a solitary room. A second therapy was administered 4 weeks later. The control group received TACE alone. Except for an injection of Licartin, other steps were similar as above.

Pharmacokinetics and biological distribution

For the trial group, the residuals method was employed for pharmacokinetic analysis. After an injection of Licartin, blood samples were collected from 9 patients at 5 min, 0.5, 2, 4, 24, 48, 72, 120 and 168 h and urinary specimens daily within 7 days. γ counter was used to measure the serum and urinary radioactivity concentrations. The radioactivity-time curves of blood and urine were constructed by SPSS statistical software. The major evaluation parameters included half-life ($T_{1/2}$), area under curve ($AUC_{0-\infty}$), maximal plasma concentration (C_{max}) and maximal plasma concentration time (t_{max}). Based upon the urinary radioactivity data, the percentages (%ID) of total radioactivity over injection dose at all timepoints were calculated to analyze the renal drug clearance rates. And radionuclide imaging and ROI method were employed to calculate the hepatic tissue radioactivity ratio of tumor/non-tumor (T/NT). After an injection of Licartin, all subjects underwent radionuclide imaging and a reconstruction of integrated hepatic tomographic scans

for analyzing the biological distribution. Medical internal radionuclide dose (MIRD) (Lau et al., 2008; Salem et al., 2013) was used to calculate the absorption doses for all major organs. The blood radioactivity-time curve was plotted to calculate the blood radioactive intensity. Supposing the red marrow/blood radioactivity ratio at 0.3, the blood radioactivity intensity was converted into the red marrow radioactivity intensity to calculate the absorption doses.

Evaluations of clinical efficacies

The primary evaluation parameter was the patients' overall survival (OS), i.e. duration from Day 1 accepting study medication until death. And the secondary evaluation parameter was time to progression (TTP), i.e. from the start of the first study medication until objective tumor progression. Karnofsky performance score (KPS) was used to measure the quality-of-life. That is to say, comparing the changes of patient KPS score at pre-therapy, 1 month and 2 months post-therapy to evaluate the effects of medication on patient qualify-of-life. Also the changes of alpha fetoprotein (AFP) were assessed. Overall efficacy evaluations were conducted for the subjects taking study medication at least once and having complete post-therapy follow-up data. The follow-up period was counted from a patient on continuous therapy to disease progression and death.

Evaluations of safety

The major evaluation parameters included the changes of adverse event (AE) and hepatic functions. All patients underwent physical examinations and received the examinations of blood routine, hepatic functions and AFP before therapy and Day 7, Month 1 and Month 2 after Day 1 of medication. And imaging follow-ups were conducted at Month 6, Month 9 and Month 12. National Cancer Institute-Common Toxicity Criteria, Version 2.0 (NCI-CTC 2.0) was used to judge the extent. And follow-ups were continued until symptomatic disappearance or stable disease. And any instance of severe adverse event (SAE) should be timely reported to the competent authority. In the present study, the time frame was from the subjects' accepting therapy until Day 28.

Statistical analysis

The SPSS statistical software was used. And the results were expressed as mean ± standard deviation ($\bar{x}\pm s$). ANOVA of repeated measurement data was used to analyze the distribution of Licartin in all tissues and organs at different timepoints in the trial group. And T/NT values were statistically tested. The evaluations of overall response rate were made for the patients taking medication at least once, having post-therapy data and receiving follow-ups at least once. And t test, X^2 test, exact probability, rank-sum test, life table method and Cox proportional hazard regression model were employed for survival analyses. The inter-group comparisons were made for AFP, KPS, incidence of toxic responses and severity. Relative risk (RR), survival rate and hazard ratio (HR) were used for statistical analyses. The test level was set at α=0.05.

Results

General profiles

The present study started from July 2007 until July 2009. Eight large-scaled domestic hospitals with the relevant departments treating malignant tumors participated. A total of 14 patients were recruited (trial group, n=6; control group, n=8). Those not completing the first follow-up were eliminated so that there were a total of 341 evaluable subjects. They were divided into trial (n=167) and control (n=174) groups. Except for gender composition, the baseline characteristics of both groups were consistent (age, Child-Pugh stage, AFP, KPS, TNM stage, refer to ` 1). And there were stage III (n=91) and stage IV (n=76) in the trial group.

Pharmacokinetics and biological distribution

The trial group's pharmacokinetics conformed to the 2-chamber open model. Major parameters: mean absorption phase $T_{1/2a}$ was 1.96 h, distribution phase $T_{1/2\alpha}$ 19.07 h, elimination phase $T_{1/2\beta}$ 57.09 h, C_{max} 2.113×10^{9} $min^{-1}\cdot L^{-1}$, reached C_{max} at 4.1 h, $AUC_{0-\infty}$ 1.302×10^{11} $h\cdot min^{-1}\cdot L^{-1}$. The renal clearance rate of radioactive substances decreased over time. At Day 3 post-therapy, the accumulated urinary radioactivity intensity accounted for 35.6% of injection dose; at Day 5, 46.3%; at Day 7, 52.2%. In the trial group, 15 patients underwent post-therapy SPECT to perform systemic radionuclide imaging at different timepoints and another 14 underwent SPECT/CT to reconstruct integrated images of hepatic scanning at Day 5 (Figure 1). Radioactivity was consistently concentrated within hepatic tumor zones. It had a sparse distribution within other body tissues, such as heart and spleen. Simultaneously it was found that lipiodol had excellent depositions within lesions. In conjunctions with the *in vivo* distribution of Licartin in all tissues and organs at different timepoints, marked statistical differences of T/NT value

Table 1. Baseline Patient Profiles (n=341)

Profiles	Trial group (n=167)	Control group (n=174)	P value*
Age, yrs			0.52
$\bar{x}\pm s$	52.19±11.828	51.32±12.887	
Range	22-81	23-85	
Gender, percentage (%)			<0.001
Male	141(84.43)	172(98.85)	
Female	26(15.57)	2(1.15)	
Child grading, (%)			0.985
Class A	146(87.4)	152(87.4)	
Class B	21(12.6)	22(12.6)	
AFP (ng/ml)			0.092
$\bar{x}\pm s$	581.55±342.18	539.32±301.90	
Range	1.05-1210	3.66-1210	
KPS score, (%)			0.317
Grade I (90 points)	108(64.7)	103(59.2)	
Grade II (80 points)	59(35.3)	71(40.8)	
TNM staging, (%)			0.664
Stage III	91(54.5)	99(56.9)	
Stage IV	76(45.5)	75(43.1)	

*t test, exact probability method and X^2 test were used for comparing the inter-group statistical difference. P<0.05 was considered as having significant difference

existed in other organs (P<0.01), maximal hepatic T/NT was 2.88±1.02 at 3 h, 2.15±0.53 at 65 h, 1.81±0.39 at 120 h and 1.64±0.4 at 168 h. And the values of T/NT for other organs were 4.02-24.06 at other timepoints. It indicated that Licartin was predominantly targeted to concentrate within hepatic tumor tissue. During an early post-therapeutic phase, there was a higher concentration and tended to decline slowly over time (Table 2). According to the calculations, the internal absorption doses of major organs for 12 patients were as follows: hepatic (3.19±1.01) Gy, spleen (3.65±2.41) Gy, pulmonary (0.97±0.23) Gy, renal (0.96±0.35) Gy and thyroid (3.61±2.40) Gy. Among which, the red marrow absorption dose was (0.55±0.09) Gy for 7 patients.

Clinical efficacies

Up to July 2009, the follow-up period was 6-15 months. There were a total of 91 fatal events for two groups. Since it failed to reach 50%, statistical analysis was performed only on the 6-month and 1-year survival rates for two groups. Life table method, long-rank test and Cox proportional hazard regression model were used. When two groups were compared, 6-month survival rate had insignificant difference (87.97% *vs*. 83.23%, *P*=0.225). However, 1-year survival rate increased markedly [79.47% *vs*. 65.59%, HR=0.598, 95% confidence interval (CI) 0.391-0.914, P=0.041] (Figure 2). In the trial group, stage III versus stage IV patients had more significant difference in 1-year survival rate (86.94% *vs*. 53.79%, P<0.001), TTP elongated markedly in the trial group (6.82±1.28 *vs*. 4.7±1.14 months, *P*=0.037). X^2 test was used to compare the changing percentages of KPS scores before and after therapy for two groups. As compared with the control group, the trial group improved (7.2% *vs*. 3.4%), stabilized (71.9% *vs*. 76.4%) and decreased (21% *vs*. 20.1%) at Month 1. At Month 2, it improved (18.6% *vs*. 27%), stabilized (64.1% *vs*. 54%) and decreased (17.4% *vs*. 19%). All percentages had insignificant difference (*P*=0.283, *P*=0.12). At Month 1 post-therapy, X^2 test revealed insignificant difference when AFP decreased (29.3% *vs*. 25.3%), non-altered (48.5% *vs*. 45.4%) and increased (22.2% *vs*. 29.3%) (*P*=0.304).

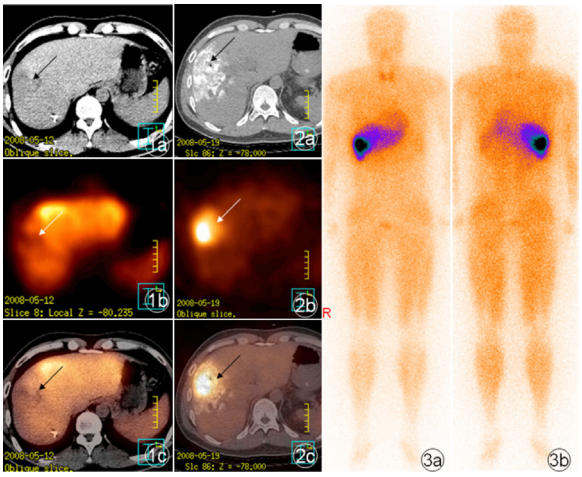

Figure 1. A male HCC Patient Aged 40 years. 1 ^{99}Tcm-PHY hepatic colloid SPECT/CT tomographic image showed right hepatic lobe carcinoma (1c integrated hepatic tomographic image as indicated by arrow head); 2 SPECT/CT hepatic tomographic image at Day 5 post-therapy showed there was an excellent deposit of lipiodol in right hepatic lobe tumor regions and radioactivity concentration predominantly in lesions (2c integrated image as indicated by arrow head); 3 Systemic SPECT imaging (a: anterior and b: posterior) at Day 5 post-therapy showed that radioactivity was predominantly concentrated in hepatic and right hepatic lobe tumor regions. And there was a small amount of radioactive concentration in thyroid

Adverse events

For two groups, the severity of AE was predominantly grade 1/2. And the most common AEs were marrow suppression, hepatic impairment, fever, pain, abdominal distension, nausea and vomiting. The major grade 3 AEs included thrombocytopenia (4.8% *vs*. 0.6%) and elevated total bilirubin (3% *vs*. 1.1%) (Table 3). The results of X^2 test showed that as compared with the control group, the incidence of AEs, such as leucopenia, thrombocytopenia and elevated total bilirubin, increased markedly in the

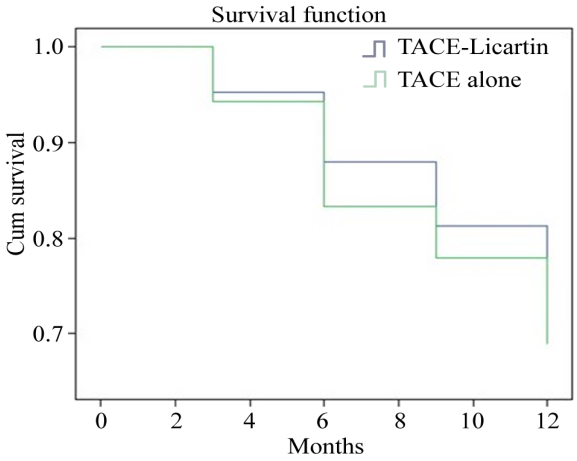

Figure 2. Survival Curve of Life Table Method for Licartin plus TACE versus TACE alone *(*P*=0.041). *Log-rank test for comparing the statistical difference of survival curve. *P*<0.05 was considered having significant difference

Table 2. The *in vivo* Distribution Status of Licartin at Different Timepoints after a Combined Therapy of TACE * (%)

Organs	3 h	70 h	120 h	168 h
Whole body	100.00±0.00	51.14±14.73	31.14±9.05	20.92±7.80
Liver	28.52±4.54	10.62±3.85	4.50±1.89	2.16±1.01
Spleen	3.97±2.90	1.34±0.98	0.57±0.44	0.25±0.17
Lungs	5.01±1.23	1.51±0.48	0.64±0.30	0.28±0.17
Kidney	0.80±0.57	0.36±0.17	0.18±0.12	0.06±0.06
Thyroid	0.29±0.16	0.13±0.07	0.08±0.07	0.06±0.05

* ANOVA analysis of repeated measurement data, F=6.583, *P*<0.01

Table 3. Grading of AE Occurring in Patients (n=341)

AE	Trial group (n=167)		Control group(n=174)		*P* value*
	All (%)	Grade 3 (%)	All (%)	Grade 3 (%)	
Hematological system					
Leucopenia	97(58.1)	3(1.8)	62(35.6)	3(1.7)	<0.001
Neutropenia	6(3.6)	0(0)	4(2.3)	0(0)	0.479
Anemia (erythrocyte)	14(8.4)	0(0)	12(6.9)	0(0)	0.605
thrombocytopenia	93(55.7)	8(4.8)	73(42)	1(0.6)	0.013
Clinical biochemistry					
Total bilirubin	81(48.5)	5(3)	59(33.9)	2(1.1)	0.044
AST	50(29.9)	2(1.2)	49(28.2)	1(0.6)	0.58
ALT	66(39.5)	2(1.2)	58(34.7)	2(1.1)	0.697
Albumin	50(29.9)	3(1.8)	50(28.7)	2(1.1)	0.807
Clinical symptoms					
Nausea/ vomiting	98(57.5)	0(0)	89(51.1)	0(0)	0.162
Fever	130(77.8)	0(0)	139(79.9)	0(0)	0.644
Pain/abdominal distension	114(68.3)	0(0)	123(70.7)	0(0)	0.627
Upper GI hemorrhage	4(2.4)	3(1.8)	2(1.1)	2(1.1)	0.382

*AE grading criteria were NCI-CTC 2.0. *X^2 test for comparing inter-group statistical difference. $P<0.05$ was considered having significant difference

Table 4. Relationship of Baseline Levels and Incidence of AE for Two Patient Groups * (n=341)

Baseline level	Trial group (n=167)		Control group (n=174)		P value*
	Grade 0%	Grade 1-3%	Grade 0%	Grade 1-3%	
Leucocyte					0.792
Normal	35(38.9)	55(61.1)	73(66.4)	37(33.6)	
Abnormal	35(45.5)	42(54.5)	40(62.5)	24(37.5)	
Platelet					0.298
Normal	44(44.4)	55(55.6)	69(63.3)	40(36.7)	
Abnormal	32(47.1)	36(52.9)	32(49.2)	33(50.8)	
Total bilirubin					0.146
Normal	53(47.7)	58(52.3)	76(64.4)	42(35.6)	
Abnormal	33(58.9)	23(41.1)	39(69.6)	17(30.4)	

*Stratified X^2 test for comparing inter-group statistical difference. $P<0.05$ was considered having significant difference

trial group ($P<0.001$, $P=0.013$, $P<0.01$). The values of RR for occurring AE were 1.63 (95%CI 1.294-2.053), 1.33 (95%CI 1.066-1.653) and 1.43 (95%CI 1.107-1.849) respectively. According to the stratified X^2 test, the baseline levels of leucocyte, platelet and total bilirubin were not correlated with the incidence of AE for both groups ($P=0.792$, $P=0.298$, $P=0.146$) (Table 4). In two groups, leucopenia occurred mainly at Day 20 post-therapy. And thrombocytopenia appeared at Days 3-7 post-therapy and returned gradually to normal at Month 2. There was an onset of transient hepatic impairment at Day 7 post-therapy. Within 1-2 months, it returned basically to the pre-therapy baseline level. The result of X^2 test revealed insignificant difference in the incidence of non-fatal SAE for both groups (5.39% *vs*. 2.3%, $P=0.136$). In the trial group, there were upper GI hemorrhage (n=3), severe jaundice (n=5) and severe ascites (n=1). And the control group had upper GI hemorrhage (n=2) and severe jaundice (n=2). Since the patients had a hemorrhagic history of hepatic cirrhosis esophagastric varices, these events were not correlated with study medication.

Discussion

It is currently agreed that the major anti-tumor mechanism of RIT lies in the radionuclide activity of labeled antibody (Kassis and Adelstein, 2005; Sgouros, 2005; Goldenberg and Sharkey, 2006; Zanzonico and Divgi, 2008). The antibody may infiltrate into tumor tissue and conjugate specifically with tumor antigen so as to enable a targeted concentration of radionuclide in tumor tissue. Since antibody fragment has a small molecule and the predominant blood supply of HCC is hepatic artery, the injection route of hepatic artery might boost the tumor targeting of internal irradiation (Lambert and Van de Wiele, 2005). The radionuclide ^{131}I-labeled metuximab is a F(ab´)^2 fragment of mouse-derived HAb18 antibody. Due to its small molecular weight, it may infiltrate easily into tumor tissue. Capable of conjugating with tumor antigen, it has a low antigenicity and becomes rapidly cleared by normal tissues. Hence, a higher value of T/NT was achieved. During the phase I clinical study, a single dosing via hepatic artery had no marked difference in preliminary radioactive concentration between hepatic and tumor tissues. However, the value of hepatic T/NT was >1 and had a peak of 1.09 at 192 h (Zhang et al., 2006). Different from previous researches, the present study applied dosing via hepatic artery and lipiodol emulsion mixed with chemotherapeutic agents was used for embolizing the tumor-supplying branch of hepatic artery. On radionuclide imaging, there was a marked radioactive concentration in hepatic tumor tissue during an early phase. And the mean hepatic T/NT peaked up to 2.88 at 3 h and declined to 1.64 at 168 h. And it was sparsely distributed in other body tissues. Thus there is a pharmacokinetic feature of a high concentration in tumor and a low concentration in plasma. It may reduce the non-specific reticulo-endothelial uptake of normal tissue, block the tumor blood supply, extend the retention time of radionuclide ^{131}I in tumor tissue to facilitate the focused targeting of radionuclide into hepatic tumor tissue.

During radiotherapy, dosage rate and irradiation dose

are important pro-apoptotic factors of tumor cell. Since radionuclide ^{131}I emits β-ray via low LET (maximal energy of 610 KeV, a maximal range of 2 mm). The emission is continuous and decreases exponentially. For non-uniformly distributed low dosage rate (LDR) , the biological effects (Juweid et al., 1997; Kassis and Adelstein, 2005; Alloni et al., 2014) of radiation are mainly manifested as non-fatal injury of cellular DNA and an induction of apoptosis and proliferative mortality. And the magnitude of this effect is closely correlated with the targeting of radionuclide. Therefore enhancing the tumor-targeting of RIT not only improves accumulated tumor irradiation doses but also potentates the biological effects of irradiation. In the present study, the mean absorption dose of intra-hepatic irradiation for combined therapy was markedly higher than that of a single dosing via hepatic artery for phase II clinical study (3.19 *vs*. 2.08 Gy) (Chen et al., 2006). And as compared with TACE alone and phase II clinical study, the patient survival rate markedly increased. Especially 1-year overall survival rate increased by 14% versus the former. And mortality risk decreased by 40%. It was correlated with both TACE-induced tumor ischemia & necrosis and biological effects of concurrent RIT irradiation. Though referring to external irradiation dosage, the therapy of internal irradiation has yet to obtain a more accurate dose-effect relationship (Sgouros, 2005; Chen et al., 2006). However, a postoperative injection of ^{131}I-lipiodol via hepatic artery for adjunct therapy. The 3-year patient survival rate was 86% and median tumor-free survival period 57 months (Lau et al., 2008). And, as further confirmed by a randomized controlled study after hepatic transplantation, Licartin was effective in preventing the recurrence and metastasis of HCC (Xu et al., 2007b). It indicated that the characteristic LDR irradiation of internal radiotherapy demonstrated a definite dose-effect relationship for small lesions and micro-metastatic foci of HCC (Kassis and Adelstein, 2005; Lambert and Van de Wiele, 2005; Goldenberg and Sharkey, 2006). As demonstrated by the results of *in vitro* experiments, during an irradiation dose range of 2-10 Gy, LDR irradiation could induce marked cell apoptosis and suppress tumor growth (Sgouros, 2005; Alloni et al., 2014). An injection of ^{131}I-hepama-1 mAb via hepatic artery plus ligation of hepatic artery could lower AFP by 52% and shrink tumor by 78% (Zeng et al., 1998). As compared with TACE alone, the patients on combined therapy showed insignificant difference in the changes of AFP. However, TTP elongated by 45%. Especially the stage III patients with <3 cm multi-nodule accounting for 75.82% (69/91) had significant survival benefits. This was not only related with direct blocking actions of low LET radionuclide on tumor cell cycle G_2/M and indirect biological effects of DNA-damaging ray. And it was also related with oxygenation and heightened radiation sensitivity of hypoxic tumor cell after TACE (Jiang and Zeng, 2013; Aitken and Hawkins, 2014). And Licartin might block the signal transduction pathways of target antigen so as to blunt the invasion and metastasis of HCC (Xu et al., 2007a).

In the present study, the patients on combined therapy tended to have more instances of bone marrow suppression and hepatic impairment. As compared with TACE alone, the occurring AE rates of leucocyte, platelet and total bilirubin increased by 22.5%, 13.7% and 14.6% respectively. And the value of RR was greater for leucopenia. However, the serious toxicity of platelet was dominating. It was close to grade 3 platelet AE (4.49%) of phase II clinical study. Blood toxicities were correlated with the doses of Licartin. And the intermediate dose of 27.75 MBq/kg offered a higher safety (Chen et al., 2006). It was due to the fact that bone marrow is a major dose-limiting organ for RIT irradiation (Sgouros, 2005; Goldenberg and Sharkey, 2006). Although the red marrow's mean absorption dose of 0.55 Gy for combined therapy here was much lower than the limiting dose of 1.5-2.0 Gy. And multi-factor analysis of hematological toxicities of RIT indicated this (Juweid et al., 1997). Besides irradiation dose of red marrow, other influencing factors, such as baseline platelet/leucocyte count, bone metastasis and preliminary chemotherapy, may be not neglected. However, our study revealed that the baseline patient levels were not correlated with the occurring rate of AE. And, during the course of irradiation therapy, chemotherapeutic agents not only had the toxicities of killing tumor cells, but also acted as an irradiation sensitizer (Jiang and Zeng, 2013; Aitken and Hawkins, 2014) to cause the unpleasant effects of bone marrow. Their injuries were probably derived from radionuclide ^{131}I due to its noticeable effects of emitting high-energy γ ray (364 KeV, 82%) upon normal organs. Furthermore, as compared with phase II clinical study, the patients on combined therapy had more instances of total bilirubin AE and severe hepatic impairment (8.74%, n=2). It was probably due to the fact that adding TACE might cause ischemic injury of normal hepatic tissue and extend the retention time of ^{131}I in tumor tissue so as to promote the occurrence of radioactive injuries of hepatic tissue. However, the non-uniform organ distribution of radioactivity intensity enabled a better tolerance of low LET irradiation (Lambert and Van de Wiele, 2005; Chiesa et al., 2012). In the present study, the absorption doses of internal irradiation for all major organs were much lower than the limiting radioactive doses. And a comparison of incidence of SAE and KPS scores before and after therapy revealed insignificant difference for two groups. Therefore we think that treating HCC patients with the above doses of Licartin plus TACE offered higher tolerance and safety.

At present, the importance of TACE in the treatment of intermediate and advanced HCC has been stressed with the Chinese Diagnostic & Therapeutic Guidelines on Primary HCC (2011 ed.) and many international guidelines (Bruix et al., 2011; Ministry of Health of the People's Republic of China, 2011; European Association For The Study Of The Liver and European Organisation For Research And Treatment Of Cancer, 2012). Especially in accordance with the Barcelona Clinic Liver Cancer (BCLC), TACE has become a standard therapy for intermediate HCC (Llovet et al., 2008; Forner et al., 2012; Aitken and Hawkins, 2014). And tumor status and hepatic function staging are key influencing factors for selecting different therapeutic modalities and achieving long-term efficacies in progressive HCC patients. In 2012, Pinter et al. (2012) published a retrospective study of combining TACE and

sorafenib in the treatment of progressive HCC patients. Their median OS showed benefits despite the presence of vascular invasion or extra-hepatic metastasis (9.2 *vs*. 7.4 months, *P*=0.377). Especially those with Child Pug class A hepatic function gained more benefits (14 *vs*. 9.7 months, *P*=0.449). And multi-factor analysis revealed no correlation between vascular invasion and OS. In the present study, TNM stage IV patients received combined therapy to achieve a >50% chance of 1-year survival. And there was no greater instance of SAE. Therefore combined therapy may become one of therapeutic options for progressive HCC. Furthermore, the combination therapy had also overcome the limitations of TACE alone. In 2012, Wu et al. (2012) conducted a single-center study and found that, as compared with TACE alone, intermediate HCC patients had markedly elongated median OS after undergoing the combined therapy of Licartin plus TACE (26.7 *vs*. 20.6 months, *P*=0.038). And it was also one of independent predicating factors for patient prognosis. In conjunctions with the present study, we think that it may help more intermediate and advanced HCC patients to gain clinical benefits.

Firstly it was designed as a multi-center non-randomized control study. In evidence-based medicine, its category of evidence was lower than that of randomized control study (Llovet et al., 2008). The data of patient OS are expected to provide more proof of survival benefits. And the tumor status and hepatic function stage of the patients, especially those with Child Pug B class, are to be evaluated for safety benefits. Furthermore, although both phase II clinical study and a single-center report of Wu et al. suggested that single and double dosing of Licartin had no differences in clinical efficacies (Chen et al., 2006; Wu et al., 2012), further studies are warranted to examine the effects of Licartin dosing frequency on objective efficacies.

In conclusion, the combination therapy of Licartin and TACE has specifically targeted the intermediate and advanced HCC patients. It has promising objective efficacies and tolerability profile. Therefore it may be a safe and effective therapeutic option for HCC.

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