Radiofrequency ablation of small hepatocellular carcinoma with intravenous pegylated liposomal doxorubicin

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Abstract

Purpose Synergy between radiofrequency ablation (RFA) and chemotherapy was demonstrated for liver malignancy. We assess the efficacy of intravenous pegylated liposomal doxorubicin (PLD) for RFA in patients with small hepatocellular carcinoma (HCC).

Methods This study was designed as a non-randomized control trial. Patients received either PLD (20 mg) intravenously before RFA, or standard RFA alone. Computed tomography was performed immediately and 4 weeks after RFA to obtain ablative diameter, area and volume for each tumor. The changes in ablation size were analyzed by paired images for each tumor. All patients were followed up regularly.

Results A total of 24 patients with 29 HCCs, including 12 patients with 16 tumors (mean 2.2 cm $\pm$ 0.9) in the PLD and RFA group, and 12 patients with 13 tumors (2.4 cm $\pm$ 0.5) in the RFA alone group, were enrolled. The ablative diameter, area and volume significantly decreased 4 weeks after RFA. The ablative volume decrease was significantly greater for the RFA alone group than for the combination

group (26.1 vs. 12.1%, $p = 0.018$). The 3-year cumulative tumor progression and survival rates did not differ significantly between the two groups.

Conclusion Intravenous PLD before RFA reduced contraction of ablative volume and might have no impact on tumor progression and survival in patients with small HCC after RFA.

Keywords Hepatocellular carcinoma · Radiofrequency ablation · Pegylated liposomal doxorubicin

Introduction

Radiofrequency thermal ablation (RFA) therapy is being successfully used in the treatment of focal liver lesions, including hepatocellular carcinoma (HCC) and metastasis [1, 2]. For HCC, several randomized controlled trials and systemic review have demonstrated the greater therapeutic effectiveness of RFA than percutaneous ethanol injection in terms of both survival and local recurrence [3–6]. However, current RFA therapies have been less effective for treatment of larger HCC, with local recurrence in 65 and 75% of tumors >3.5 and 5 cm in diameter, respectively [7]. Furthermore, an increased incidence in the detection of progressive local tumor growth with further long-term follow-up suggests the presence of residual patches of untreated tumor. Biophysical limitations inherent to the procedure, such as perfusion-mediated tissue cooling, have prevented uniform heating of the entire tumor [8]. As a result, strategies to increase the completeness of tumor destruction, even for small HCC, are needed.

In an attempt to overcome the limitations of current therapy, combined RFA with other adjuvants, including percutaneous ethanol injection, saline infusion and

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embolization, are being investigated to promote greater heating volume and larger zones of contiguous tissue destruction [9–11]. These modalities aim to modulate either hepatic perfusion or electric conductivity during RFA [8, 12]. An alternate approach is a combination of therapies with RFA and chemotherapeutic adjuvants. RFA at a temperature lower than 50°C induces reversible microvascular stasis and increases endothelial permeability and cytotoxicity of some chemotherapeutic agents [13, 14]. On the basis of this synergistic antineoplastic effect, it has been demonstrated that combined RFA and intravenous liposomal doxorubicin increases intratumoral accumulation of doxorubicin, increases coagulation necrosis, reduces tumor growth and increases animal survival in rat breast tumor model studies [15]. A pilot clinical study has also suggested that adjuvant liposomal doxorubicin chemotherapy increases tumor destruction, in contrast to RFA alone, in a variety of focal liver tumors [16]. However, the synergistic antineoplastic effect for HCC may differ from that of liver metastasis. This study investigated the therapeutic effects of intravenous pegylated liposomal doxorubicin (PLD), combined with RFA, in terms of tumor ablation volume, tumor progression and HCC patients' survival rates.

Patients and methods

This study, designed as a non-randomized control trial, was approved by the institutional review board of Chang Gung Memorial Hospital. The inclusion criteria were patients with nodular HCC of 2–4 cm in size, with no more than two tumors each, and liver function reserve in Child-Pugh classification A. Patients were excluded if they had any of the following: uncorrectable bleeding tendency (thrombocyte $<50,000/\text{mm}^3$ or prolonged prothrombin times of more than 4 s of control), medical conditions being contraindications for RFA or PLD (heart failure, defibrillator implant and a history of allergic reaction to doxorubicin), poor renal function unable to tolerate three contrast-enhanced CT studies within a month, previously treated HCC, or indicated and accepted surgical resection. Eligible patients were invited to participate in this study. After a thorough explanation of this study's goals, patients who signed informed consent for PLD before RFA were assigned to the combined treatment group. Patients who signed consent for RFA alone formed the control group.

Radiofrequency ablation and pegylated liposomal doxorubicin infusion

RFA was performed using a 3-cm exposed electrode with an internal cooling system (Radionics, Burlington, MA). All RFA procedures were performed or instructed by one

performer (JH Wang) with assistance of the other authors. The RFA electrode was inserted into the tumor under ultrasonographic guidance to ensure appropriate placement. A standardized regimen of local anesthesia (1% lidocaine) and intramuscular analgesic (pethidine hydrochloride) was used to prevent and relieve pain during RFA. After the electrode was properly positioned, one course of RFA energy was applied with pulsed technique for 12 min. Temperature and tissue impedance were monitored during ablation. At the end of ablation, tissue temperature was obtained from the electrode tip. Each patient received a single session of RFA, during which one or two tumors were treated per protocol. For patients who received PLD before RFA, 20 mg of PLD (Lipo-Dox, TTY Biopharm, Taiwan) was intravenously administered in 250 ml dextrose water within 24 h prior to RFA.

Imaging technique and interpretation

All patients received triple-phase helical computed tomography (CT) within 1 h (immediately) and 4 weeks after RFA. A radiologist (C.T.Y) reviewed all examinations without knowledge of treatment methods. Triple-phase helical CT images of the liver were obtained with a Somatom Volume Zone scanner (Siemens AG, Germany). All images were obtained with a 2.5 mm collimation, 0.75 pitch and 30 s (arterial-dominant phase) and 60 s (portal-dominant phase) delays after beginning of intravenous administration of 100 ml of Angiografin (Schering AG, Germany) at a rate of 3 ml/s by an automated power injector. Serial transverse sections with reformatted 3 mm thickness of the liver were evaluated. Tumor necrosis area measurements were obtained using the standard region of interest tracing measurement by hand, tracing the necrotic borders using the built-in electronic cursor on a workstation. The summation of the measured areas, multiplied by sectional thickness, determined the volume of tumor necrosis. The ablative diameter, area and volume of the thermal lesion, within 1 h and 4 weeks after RFA, were obtained for each tumor. When comparing CT scans obtained immediately after ablation with those 4 weeks later, changes in the ablation diameters and areas were not uniform in different levels and areas, and focal changes were asymmetric. Hence, the change of the ablation was quantified with maximal long and short axis of ablation, and maximal ablation area and volume. The changes in data obtained after 4 weeks, minus that immediately after RFA, were calculated using paired images for each tumor.

Follow-up

All patients were followed up with liver ultrasonography every 2–3 months, and enhanced CT every 6–12 months for tumor recurrence until either the patient's death or loss

of contact with the outpatient department. Local tumor progression was defined as a new lesion at the same segment of the initial tumor; distant tumor progression was defined as a new lesion at a different segment away from the initial tumor. Patients with recurrence received further therapies, including transarterial embolization, RFA, and/or percutaneous ethanol injection.

Statistical analysis

Data were expressed as the mean $\pm$ SD. Categorical data were compared with Fisher's exact test, and continuous ones with Student's *t* test. The diameter, area and volume were obtained and compared on a lesion-by-lesion basis with paired Student's *t* test. Cumulative survival rates and tumor progression rates were obtained by Kaplan–Meier method, and differences in survival curves between the two groups were statistically compared by log-rank test. Statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA) and SigmaStat® 3.1. Survival curves were constructed using SigmaPlot® 9.0. All *p* values were derived from two-tailed tests, and a level of <0.05 was accepted as statistically significant.

Results

A total of 24 patients with 29 HCCs were enrolled into this study. Twelve patients with 16 tumors, 8 men and 4 women, received adjuvant PLD before RFA (the combination group). Twelve patients with 13 tumors, 10 men and 2 women, received RFA alone (the RFA alone group). HCC was diagnosed with pathology and/or cytology for each patient. As much as 24 HCCs were tissue proven and the other 5 were diagnosed with imaging findings. There were four well-differentiated and eight moderately

differentiated HCCs in each group. There was no difference in the pathological differentiation between RFA alone and combination groups. Tumor sizes ranged from 1.0 to 3.5 cm (mean $\pm$ SD 2.2 ± 0.9) in the combination group, and 2.0–3.2 cm (2.4 ± 0.5) in the RFA alone group. There were three tumors abutting large blood vessels in each group. Alpha-fetoprotein (AFP) level ranged from 4 to 86 ng/ml in the combination group and from 3.7 to 3,347 ng/ml in the RFA alone group. The clinical tumor characteristics and electrode tip temperature at the end of ablation are shown in Table 1. No significant differences were noted in age, sex, hepatitis B or C markers, AFP level, tumor diameter and follow-up period between two groups. All RFA procedures were technically successful for each tumor. After the procedures, the temperature measured from the tip of the electrode was $67.4 \pm 7.7^\circ\text{C}$ in the combination group, and $63.9 \pm 4.9^\circ\text{C}$ in the RFA alone group. There were no complications after PLD infusion.

Helical CT findings after RFA

All tumors, except one in the combination group, showed complete tumor ablation after one session of RFA treatment. The only tumor without complete ablation was also the largest in this study (3.8 cm). For all other tumors, the induction of the maximum ablation diameter was greater than the initial tumor diameter. However, compared with tumor diameter, the increase of the ablated diameter was not statistically different between the two groups (mean $\pm$ SD 1.8 ± 0.6 and 1.5 ± 0.5 cm; *p* = 0.161).

Changes of ablation diameter, area and volume 4 weeks after RFA

The tumor diameter, treatment modality, ablative size and change of ablative volume for each tumor are shown in

Table 1 Baseline characteristics of 24 patients with 29 tumors

Variable	RFA + PLD (<i>n</i> = 12)	RFA alone (<i>n</i> = 12)	<i>p</i> value
Age (mean $\pm$ SD, years)	63 $\pm$ 9.8	58 $\pm$ 8.8	0.200
Sex			0.640
M (%)	8 (67.7)	10 (83.3)	
F (%)	4 (33.3)	2 (16.7)	
Etiology			1.000
B (%)	5 (41.7)	6 (50)	
C (%)	5 (41.7)	6 (50)	
B + C (%)	1 (8.3)	0 (0)	
nBnC (%)	1 (8.3)	0 (0)	
Alpha-fetoprotein (median, ng/ml)	10.8	20.6	0.410
Tumor size (mean $\pm$ SD, cm)	2.2 $\pm$ 0.9	2.4 $\pm$ 0.5	0.508
Electrode temp (mean $\pm$ SD, $^\circ\text{C}$)	67.4 $\pm$ 7.7	63.9 $\pm$ 4.9	0.198
Follow-up period (mean $\pm$ SD, months)	32.9 $\pm$ 6.1	28.0 $\pm$ 13.2	0.258

RFA + PLD, radiofrequency ablation and pegylated liposomal doxorubicin; M, male; F, female; temp, temperature; cm, centimeter; B/C/B + C/nBnC, positive hepatitis B virus surface antigen/positive antibody to hepatitis C virus/positive both/neither positive

Table 2 The tumor diameter, treatment modality, coagulation size and change of coagulation volume for 24 patients with 29 lesions

Patient and lesion	Tumor diameter (cm)	Treatment	Immediately (dia/area/vol) (cm/cm ² /cm ³)	4 weeks (dia/area/vol) (cm/cm ² /cm ³)	Volume change (cm ³ /%)
1	3.5	RFA + PLD	4.6/10.2/23.3	3.9/8.4/16.7	−6.6/−28.3
2	2.0	RFA + PLD	4.5/7.4/11.4	3.2/4.5/6.9	−4.5/−39.5
3	2.0	RFA + PLD	3.9/10.2/24.5	3.7/8.7/20.4	−4.1/−16.7
4	2.0	RFA + PLD	4.0/7.0/13.3	3.6/7.1/10.3	−3.0/−22.6
5a	2.0	RFA + PLD	3.4/6.1/9.6	3.9/6.1/9.9	0.3/3.1
5b	1.0	RFA + PLD	3.5/6.5/8.3	4.5/6.5/8	−0.3/−3.6
6	3.5	RFA + PLD	4.6/12.5/29.8	3.6/7.3/25.2	−4.6/−15.4
7	2.4	RFA + PLD	4.1/9.1/14.6	3.9/8.5/13.5	−1.1/−7.5
8a	2.8	RFA + PLD	4.7/10.8/34.3	3.5/9.5/27.6	−6.7/−19.5
8b	1.3	RFA + PLD	3.3/7.1/13.3	2.7/6.7/12.8	−0.5/−3.8
9	2.8	RFA + PLD	3.9/9.2/18.3	3.3/8.3/16.5	−1.8/−9.8
10a	2.1	RFA + PLD	4.7/9.4/15.2	3.8/7.8/15.8	0.6/3.9
10b	1.4	RFA + PLD	3.4/7.4/14.6	3.8/6.9/13.7	−0.9/−6.2
11a	1.0	RFA + PLD	4.2/8.2/13.1	3.5/5.6/10.5	−2.6/−19.8
11b	2.0	RFA + PLD	3.1/7.0/14.4	3.8/6.2/11.6	−2.8/−19.4
12	3.8	RFA + PLD	4.9/13.6/28.7	4.4/13.3/31.9	3.2/11.1
13	3.2	RFA alone	4.3/10.0/24.8	3.8/8.5/18.1	−6.7/−27.0
14	2.0	RFA alone	3.4/6.7/8.3	3.3/6.0/8.7	0.4/4.8
15	3.0	RFA alone	4.5/8.1/14.6	2.1/4.2/7.3	−7.3/−50.0
16	2.9	RFA alone	3.7/12.0/27.9	3.2/8.6/14.7	−13.2/−47.3
17	2.0	RFA alone	3.3/6.3/10.0	3.4/5.6/8.3	−1.7/−17
18	2.9	RFA alone	4.0/9.7/18.2	3.3/6.3/10.0	−8.2/−45.1
19a	2.1	RFA alone	4.2/8.7/20.0	4.0/8.4/17.2	−2.8/−14.0
19b	1.3	RFA alone	2.8/6.4/12.6	2.8/6.3/11.7	−0.9/−7.1
20	2.0	RFA alone	4.4/10/22.9	4.0/9.5/19.4	−3.5/−15.3
21	2.2	RFA alone	4.2/13.6/33.8	3.8/10.2/21.0	−12.8/−37.9
22	2.3	RFA alone	4.0/8.0/13.2	3.3/6.3/9.9	−3.3/−25.0
23	2.7	RFA alone	4.6/8.0/15.4	3.3/6.3/12.4	−3.0/−19.5
24	2.6	RFA alone	3.5/6.5/12	3.2/4.6/7.3	−4.7/−39.2

RFA + PLD, radiofrequency and pegylated liposomal doxorubicin; Immediately, immediately after treatment; 4 weeks, 4 weeks after treatment dia/area/vol, maximum diameter/maximum area/volume

Table 2. In the combination group, ablative volume increased in 3 tumors and decreased in 13 tumors. The increase of ablative volume ranged from 0.3 to 3.6 cm³ and decrease of ablative volume from 0.3 to 6.6 cm³. In the RFA alone group, only one tumor showed an increase of ablative volume. There were significant decreases of ablation diameter, area and volume for each tumor in both groups ($p < 0.05$). The decrease in long axis, short axis and area was 7.2, 8.5 and 13.2%, respectively, in the combination group, and 12.9, 12.8 and 19.7%, respectively, in the RFA alone group. No significant differences were noted. However, the ablative volume had significantly greater decreases in the RFA alone group. The mean decrease of volume in the RFA alone group was significantly greater than that of the combination group (mean $\pm$ SD 5.2 $\pm$ 4.2 cm³ and 26.1 $\pm$ 16.8% vs. 2.2 $\pm$ 2.7 cm³ and 12.1 $\pm$ 13.1%; $p = 0.029$ and 0.018; power = 0.623) (Table 3).

Tumor progression and survival

One patient with the complication of skin implantation was noted in the combination group 4 months after treatment. The 1-, 2- and 3-year cumulative local tumor progression rates were 8.3, 33.2 and 33.2%, respectively, for the combination group, and 0, 12.5 and 12.5%, respectively, for the RFA alone group. The 1-, 2- and 3-year total tumor progression rates were 33.3, 58.3 and 87.5%, respectively, for the combination group, and 20, 41.7 and 41.7%, respectively, for the RFA alone group. There were no statistical differences in local tumor progression and total tumor progression rates between the two groups ($p = 0.342$ and 0.118) (Fig. 1a, b). Seven patients died in the study period including four patients in the combination group and three patients in the RFA alone group. All four patients in the combination group died of tumor progressions. In the RFA alone group, the cause of death was

Table 3 The coagulated diameter, area and volume evaluated by enhanced computed tomography immediately and 4 weeks after radiofrequency ablation (RFA) in the adjuvant pegylated liposomal doxorubicin (RFA + PLD) group and the RFA alone group

	RFA + PLD				RFA			
	Immediately (mean $\pm$ SD)	4 weeks (mean $\pm$ SD)	Δ (mean $\pm$ SD)	Change (%) (mean $\pm$ SD)	Immediately (mean $\pm$ SD)	4 weeks (mean $\pm$ SD)	Δ (mean $\pm$ SD)	Change (%) (mean $\pm$ SD)
Diameter (cm)								
Long axis	4.0 $\pm$ 0.6	3.7 $\pm$ 0.4	0.4 $\pm$ 0.7 [#]	7.2 $\pm$ 17.6	3.9 $\pm$ 0.5	3.4 $\pm$ 0.5	0.5 $\pm$ 0.6 [#]	12.9 $\pm$ 14.4
Short axis	2.8 $\pm$ 0.5	2.5 $\pm$ 0.6	0.3 $\pm$ 0.4 [#]	8.5 $\pm$ 17.1	2.6 $\pm$ 0.5	2.2 $\pm$ 0.4	0.4 $\pm$ 0.3 [#]	12.8 $\pm$ 9.2
Area (cm ²)	8.8 $\pm$ 2.2	7.6 $\pm$ 2.0	1.3 $\pm$ 1.4 [#]	13.2 $\pm$ 13.4	8.8 $\pm$ 2.2	7.0 $\pm$ 1.9	1.8 $\pm$ 1.3 [#]	19.7 $\pm$ 13.6
Volume (cm ³)	17.9 $\pm$ 7.8	15.7 $\pm$ 7.2	2.2 $\pm$ 2.7 ^a	12.1 $\pm$ 13.1 ^b	18.0 $\pm$ 7.6	12.8 $\pm$ 4.8	5.2 $\pm$ 4.2 ^a	26.1 $\pm$ 16.8 ^b

RFA + PLD, radiofrequency and pegylated liposomal doxorubicin; RFA, radiofrequency ablation alone; Immediately, immediately after radiofrequency ablation; 4 weeks, 4 weeks after radiofrequency ablation; Δ , Immediately minus 4 weeks; Change (%), Δ /Immediately

^a $p = 0.029$ based on Student's t test

^b $p = 0.018$ based on Student's t test

[#] $p < 0.05$ based on paired t test

Fig. 1 a, b The cumulative local progression and total tumor progression rates of both groups are analyzed by Kaplan–Meier curve. There were no significant differences in local progression and total progression rates between the combined pegylated liposomal doxorubicin (PLD) with radiofrequency (RF) ablation and the RF ablation alone groups

septic shock in one patient, esophageal varices bleeding in one patient and liver failure due to chronic hepatitis B with flare-up in one patient. The 1-, 2- and 3-year survival rates were 100, 91.7 and 66.7%, respectively, for the combination group, and 100, 70 and 70%, respectively, for the RFA alone group. There were no statistically significant differences ($p = 0.932$; power = 0.089) (Fig. 2).

Discussion

This study aimed to evaluate the efficacy of PLD on RFA. The efficacy was defined as an increase of ablation volume at 4 weeks compared with the ablation volume immediately after RFA. For HCC with RFA alone, the ablation volume contracted with time. However, ablation volume increased with time after combination therapy from studies in animal and human with hepatic metastasis [15, 16]. Only one patient with HCC received combination therapy of liposomal doxorubicin and RFA in the literature [16].

Fig. 2 The cumulative survival rates in both groups were analyzed by Kaplan–Meier curve. Patients with combined pegylated liposomal doxorubicin (PLD) and radiofrequency (RF) ablation had no significantly better survival rates than those with RF ablation alone

Study enrolling more patients before a large randomized trial is necessary to demonstrate the effect of combination treatment. So, we performed this study as a non-randomized trial. This study is, to our best knowledge, the first clinical trial to assess the therapeutic effect of a combination of hyperthermia therapy using RFA and intravenous chemotherapeutic agent for HCC. Compared with RFA alone, we demonstrated that intravenous PLD administration before RFA did not increase ablation volume for small HCC. Although there was significantly reduced contraction of ablative volume in the combination treatment group, PLD administered intravenously before RFA had no impact on local recurrence, distant tumor progression or patient's survival in follow-up.

Combined hyperthermia using ultrasound with PLD has been successful in treating a patient with advanced HCC [17]. Previous clinical study had shown the synergistic effect of RFA and intravenous liposomal doxorubicin in increasing tumor destruction for a variety of liver malignancies, mostly metastatic tumors [16]. However, there was no synergistic effect in increasing the ablative volume for combined PLD with RFA therapy for HCC in our study. These differing results might be explained by differences in ablation protocol, tumor characteristics and tumor size between our study and the previous one. Instead of multiple ablations in a single session with different electrodes, as in the previous study, each tumor was ablated under the instruction of the same performer, with cool-tip electrode in only one course and fixed duration of ablation. Thus, the synergistic effect of PLD was not affected by different performers, the ablative efficiency of different electrodes, and ablation course or duration in our study. The major difference is, while only one tumor in the previous study was HCC, we enrolled only HCC, which might have a different response to hyperthermia and PLD. HCC has a poorer response to PLD than other tumors, which might explain the results in this study [18, 19]. Another difference was that the tumors in this study were small, so that RFA might destroy them completely. Consequently, our study detected no significant increase in ablation volume 4 weeks after combination therapy.

There was no difference in the ablation volume in both groups immediately after RFA as evaluated by CT image. The ablative lesions were progressively smaller due to the replacement of cellular destruction by fibrosis [20]. In this study, there were significant decreases in ablation diameter, area and volume 4 weeks after therapy in patients of both groups. However, a significantly greater decrease of ablative volume was demonstrated for patients with RFA alone. There might have been different responses to PLD for liver tissue and tumor. In this study, the surrounding liver tissue, rather than the tumor, was the target in increasing ablative volume for adjuvant PLD. Hyperthermia increased

microvascular permeability and inhibited phagocytic activity at the periphery of RFA coagulated liver tissue [13]. Addition of PLD further affected the wound healing process of the RFA lesion [21]. Reduced contraction of ablative lesions might be the result of interaction of PLD and hyperthermia at the periphery of the ablative lesion. For patients in the combination treatment group, the ablative area showed progressive regression in the long-term CT images. There was no relation between the degree of ablative area change and occurrence of local tumor progression. PLD might only affect the wound healing process shortly after RFA. There seemed no further impact on the long-term change of ablative area. The clinical significance and mechanism of reduced contraction of ablative volume in the combination group need further clarification.

Instead of the intravenous route in this study, the intra-arterial administration of eluting doxorubicin on a bead has been demonstrated to enhance the effect of RFA for patients with medium to large HCC [22]. Transarterial chemoembolization with RFA has also been shown to be superior to RFA alone in prolongation of patients' survival [10]. The enhancing and beneficial effects are due to obstruction of the nourishing artery by the bead or other embolizing agents and the anti-tumor effect of the accompanying chemotherapeutic agents. However, the extent of the administered chemotherapeutic agents in augmenting RFA effect is obscure. Although intra-arterial administration of chemotherapeutic agents increased regional drug concentrations and might therefore enhance the anti-tumor effect, decreased hepatic perfusion by embolizing agents might be the main cause of the enhanced effect of RFA administered by bead or transarterial chemoembolization. Studies with intra-arterial administration of doxorubicin without embolizing agents were necessary to clarify the role of doxorubicin in effect of RFA on HCC. Instead of local administration in animal study, intravenous doxorubicin had been demonstrated to enhance the effect of RFA on hepatic metastasis in animal and pilot human studies [15, 16]. There were no studies in patients with HCC. So we tried to elucidate the effect of intravenous doxorubicin on RFA effect of HCC. In this study, we demonstrate the limited effect of intravenous PLD in promoting greater ablation volume by RFA. Compared with intra-arterial administration, doxorubicin administered intravenously might obtain a lower drug concentration locally, which might limit its enhancing effect on RFA. Recent introduction of doxorubicin in temperature-sensitive liposome might increase regional drug concentration and enhance its anti-tumor effect when combined with RFA [23].

RFA, subsequent or parallel to chemotherapy, has been demonstrated to delay recurrence and prolong survival for patients with hepatic colorectal metastasis [24, 25]. For small HCC, RFA is comparable to surgical resection with

regard to survival rates [26]; long-term survival, however, has been offset by local recurrence and new lesions. Although intravenous PLD has been shown to increase tumor destruction in two HCC patients undergoing hyperthermia treatment, it is uncertain whether this will lead to delay of recurrence and prolongation of survival [16, 17]. In contrast to hepatic colorectal metastasis, our study has found that administration of PLD before RFA has not proven effective in delaying recurrence and prolonging survival for patients with small HCC. However, this result has limitation due to the relatively small number of patients enrolled and low significance obtained. It might be too early to reach the conclusion that there is no synergistic effect of chemotherapeutic agents and RFA in delaying recurrence and prolonging survival for patients with small HCC. The synergistic effect might be affected by the dosage, timing of administration, circulation time and administrative agents [15, 27]. Studies with more patients, uniform protocol and long-term follow-up are necessary to clarify whether or not PLD before RFA offered survival benefit for patients with HCC.

The mechanism of the synergistic effect for regional hyperthermia by RFA and chemotherapy has been demonstrated in experimental studies in an animal breast cancer model [15]. The interaction between chemotherapeutic agents and the large zone of sub-lethal heating at the periphery of the ablation focus potentially increases tumor destruction diameter. Several mechanisms have been postulated to explain this synergistic effect, including the increase in intratumoral liposomal drug accumulation and improvement in cytotoxicity from doxorubicin and liposome itself [15]. Combined RFA and intravenous liposomal doxorubicin have also demonstrated therapeutic effects in reducing tumor growth rate and prolonging animal survival [28]. Tumor characteristics might affect the synergistic effect of PLD and RFA, so experimental animal studies of HCC to evaluate the effect of combination therapy, and further elucidate its mechanism of anti-neoplastic synergy, are required.

In summary, PLD administered intravenously before RFA reduced contraction of ablative volume at 4 weeks after treatment and might have no impact on tumor progression and survival in patients with small HCC after RFA. Further studies of patients with medium to large HCC are necessary.

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