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High-powered microwave ablation of larger hepatocellular carcinoma: evaluation of recurrence rate and factors related to recurrence

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AIM: To evaluate the safety and efficacy of high-powered (80–100 W) percutaneous microwave ablation (MWA) at a frequency of 2450±10 MHz for treating larger hepatocellular carcinoma (HCC) and to predict the risk factors of local recurrence after high-powered MWA.

MATERIALS AND METHODS: The study was approved by the Institutional Review Board, and informed consent was waived because of the retrospective study design. Forty-five patients with a total of 60 lesions received high-power (80–100 W) MWA at a frequency of 2450±10 MHz through a percutaneous approach that was guided by ultrasound. Of the 60 lesions with a maximum tumour measuring 3–8 cm, 46 lesions were 3–5 cm and 14 were 5–8 cm. The complete ablation rates, local recurrence rates, complications, and short-term survival were analysed. Ten possible risk factors for local recurrence were analysed.

RESULTS: The complete ablation rates were 82.61% for the first ablation and 100% for the second ablation for 3–5 cm lesions. The complete ablation rates were 64.29% (82.61% versus 64.29%, p=0.037) for the first ablation and 85.71% (100% versus 85.71%, p=0.055) for the second ablation for 5–8 cm lesions. Local recurrence was observed in 11 out of the 45 (24.44%) successfully treated patients. The 1-year and 2-year survival rates were 95.56% (43/45) and 86.67% (39/45), respectively. No procedure-related mortality was observed and no major bleeding, liver rupture, or liver abscesses occurred. Univariate analysis showed that a positive correlation existed between the number of lesions (p=0.022), proximity to the risk area (p=0.001), pre-ablation alpha-fetoprotein (AFP) levels (p=0.025), hepatitis B virus (HBV)-DNA replication (p=0.027) and local recurrence. Multivariate analysis identified HBV-DNA (p=0.031) and proximity to the risk area (p=0.039) as the independent prognosis factors causing postoperative HCC local recurrence.

CONCLUSION: High-powered MWA of larger hepatocellular carcinomas appears to be a safe and effective treatment. HBV-DNA and proximity to the risk area appear to be independent predictors of local tumour recurrence.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer worldwide and is the second...
most common cause of cancer-related death.\(^1\) Surgical resection is recognised as a potentially curative treatment for patients with HCC. Unfortunately, the majority of primary HCCs are frequently considered to be unresectable because of the number of tumours, location of tumours, or poor hepatic functional reserve that is a result of underlying liver disease, thus making the resection of a large volume of the liver parenchyma unfeasible.\(^2\) Thermal ablation, including radiofrequency ablation (RFA) and microwave ablation (MWA), are image-guided techniques that are used to treat tumours up to 3 cm in diameter.\(^3\)

With improvements in devices and techniques, thermal ablation has displayed potential for treating HCC measuring 3 cm.\(^4\) Meanwhile, RFA is the therapy of choice in very early and early HCC according to the Barcelona Clinic Liver Cancer (BCLC) classification when patients are not candidates for either liver resection or transplantation;\(^5\) however, RFA has had limited success in treating larger tumours with high local recurrence rates.\(^6\) MWA has some advantages over RFA with regard to energy delivery, such as larger ablation zones, higher treatment temperatures, and less susceptibility to heat-sink effects.\(^7\) Recently, the advanced development of a cooled-shaft antenna has allowed MWA to be performed at much higher power outputs with longer ablation durations. This allows the lesion to be treated with a single application without severe skin burns or severe pain.\(^8\) The present study was undertaken to evaluate the results of higher power output (80–100 W) MWA at a frequency of 2450±10 MHz in patients with larger HCC tumours measuring over 3 cm and to clarify the risk factors of recurrence after MWA.

Materials and methods

Patient enrolment

From January 2012 to June 2013, 45 HCC patients (38 men and seven women with a mean age of 58.41±7.67 years) with 60 lesions underwent percutaneous high-power (80–100 W) MWA at a frequency of 2450±10 MHz. All patients had chronic hepatitis B virus (HBV) infections. Of the 45 patients, 17 had tumours that were within 5 mm of risk areas (diaphragm, gall bladder, blood vessels, and gastrointestinal tract). Not all patients were amenable to surgical resection, and some declined surgical resection; treatment selections were made after a full multidisciplinary discussion. The MWA treatment procedure was explained to both the patient and his/her relatives. Final decisions were made by the patients and relatives, and consent for the ablation was signed by the patient or his/her relatives. The study was approved by the institutional review board. Because of the retrospective nature of this study, informed consent of the patients was waived.

HCC diagnosis was established based on histological evidence or typical findings with contrast-enhanced ultrasonography, contrast-enhanced computed tomography (CT), or magnetic resonance imaging (MRI). The inclusion criteria for the study were no more than three lesions, no treatment undertaken for the lesions before MWA, tumour measuring 3 cm in diameter, and liver function Child–Pugh status score of A or B. Patients with extra-hepatic metastases or vascular invasion were excluded. The demographic characteristic data of these patients are listed in Table 1.

Ablation procedures

An ECO-100C microwave generator (ECO Microwave Electronic Institute, Nanjing, China) at a frequency of 2450±10 MHz and a power output of 0–100 W was used for MWA. The microwave antenna was a 15 cm 14 G water-cooled electrode needle. Ablation therapy was performed at 80–100 W output with one antenna. An overlapping ablative technique with antenna reinsertion was applied to treat tumours to ensure adequate coagulation necrosis. The ablation time was determined according to the ultrasound findings; the size and shape of the hyperechoic zone caused by gas microbubbles appearing in the ablation zone during the MWA procedure was monitored by ultrasound to assess the completion of therapy. Treatment was stopped when the entire target was completely hyperechoic and when the hyperechoic area overlapped the area of the tumour with a 1 cm safety margin.

Anaesthesia

Local anaesthesia with 2% lidocaine (Huibei Tianyao Pharmaceuticals, Huibei, China) and intravenous

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic characteristics of hepatocellular carcinoma (HCC) patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>No. of patients</td>
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<tr>
<td>Gender (male/female)</td>
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<td>HBV e antigen</td>
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<td>Positive</td>
<td>39</td>
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<tr>
<td>Negative</td>
<td>26</td>
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<tr>
<td>HBV-DNA replication</td>
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<tr>
<td>Positive</td>
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<td>Negative</td>
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<td>Child–Pugh classification</td>
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<td>Class B</td>
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<td>Type of tumour</td>
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<tr>
<td>Initial HCC</td>
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<td>Recurrent HCC</td>
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<td>3–5</td>
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<td>5–8</td>
<td>14</td>
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<tr>
<td>No. of tumours</td>
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<td>Multiple</td>
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<td>Diaphragm</td>
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<tr>
<td>Gall bladder</td>
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<td>Blood vessels</td>
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<td>Gastrointestinal tract</td>
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<td>Serum alpha-fetoprotein level (ng/ml)</td>
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<tr>
<td>≥400</td>
<td>11</td>
</tr>
<tr>
<td>&lt;400</td>
<td>34</td>
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</table>

HBV, hepatitis B virus.
Anaesthesia with fentanyl (Yichang Renfu Pharmaceuticals, Huibei, China) was used for percutaneous MWA.

**Ultrasound guidance system**

The whole procedure was guided and constantly monitored by real-time ultrasound (EUB-2000, HITACHI Medical Systems, Tokyo, Japan) at a frequency of 1–5 MHz using a convex array probe. At the end of the procedure, the needle track was coagulated to prevent bleeding from the liver surface.

**Assessment of technical success and follow-up**

Technical success was evaluated by comparing contrast-enhanced ultrasonography or contrast-enhanced CT images performed 3 days after treatment. Complete ablation and technical success were considered to have been achieved if the ablation zone completely covered the tumour and if there was no irregular enhancement at the treatment margin. Incomplete ablation was defined as any irregular contrast enhancement that was found inside or next to the ablation zone. Additional MWA was performed for tumours with incomplete ablation within 1 week.

Contrast-enhanced ultrasound or contrast-enhanced CT of the abdomen was performed 1, 2, and 3 months after the procedure (Figs 1–2). All patients were monitored with contrast-enhanced ultrasound or contrast-enhanced CT of the abdomen every 3–6 months. A new lesion that appeared in or adjacent to the successfully treated lesion or an enlargement of the treated lesion was considered to be local recurrence. The presence of new intrahepatic or extrahepatic tumour nodules was defined as a distant recurrence. Patients with new lesions or a distant recurrence were considered for further treatment.

A major complication was defined as an event that led to substantial morbidity, disability, increase in level of care, hospital admission, or substantially lengthened hospital stay. Major complications, such as skin burns, pneumothorax, haemorrhage, subcapsular haematoma, gall bladder perforation, gastrointestinal perforation, liver abscess, biliary leakage or stricture, and tumour dissemination, were documented.

**Statistical analysis**

Continuous variables were expressed as the mean ± standard deviation (SD). For qualitative variables, the chi-square test or Fisher’s exact probability test was performed. For continuous variables, Student’s t-test was applied. Cumulative recurrence-free survival was determined by using the Kaplan–Meier method with univariate comparisons between groups through the log-rank test. A two-tailed p-value of <0.05 was considered to be statistically significant. Statistical analyses were performed with the statistical package SPSS (version 16.0.01 for Windows, SPSS, 6 Chicago, IL, USA).

**Results**

**Local tumour control**

All patients were successfully treated with MWA. Thirty-eight of 46 lesions (82.61%) in the 3–5 cm group and nine of 14 lesions (64.29%) in the 5–8 cm group showed complete ablation after one treatment with MWA ($p=0.037$). Forty-six of 46 lesions (100%) in the 3–5 cm group and 12 of 14 lesions (85.71%) in the 5–8 cm group showed complete ablation after a second treatment with MWA ($p=0.055$; Table 2). Two of the 14 lesions in the 5–8 cm group were incompletely treated; these patients received additional MWA treatments.

![Figure 1 CT images of a 54-year-old man with a 4.1 cm diameter HCC lesion that is adjacent to the gall bladder and right portal vein. (a) Pretreatment CT image shows hyperattenuation of the lesion during the arterial phase (arrows). (b) One month after MWA treatment, contrast-enhanced CT shows uniform hypo-attenuation in the ablated area (arrows), which indicates complete ablation.](image)
Three days after the procedure, the ablation zone was measured with ultrasound. The average ablation zone of a tumour with a diameter of 3–5 cm was $(4.71 \pm 0.6) \text{ cm} \times (5.48 \pm 0.7) \text{ cm}$. The average ablation zone of a tumour with a diameter of 5–8 cm was $(5.57 \pm 0.6) \text{ cm} \times (8.46 \pm 0.5) \text{ cm}$.

**Recurrence rate and analysis of postoperative recurrence factors**

The mean follow-up period was 15 months (range 3–24 months). Local recurrence was observed in 11 of the 45 (24.44%) successfully treated patients. Seven of 45 patients (15.56%) developed a distant recurrence after the ablation. Of the 18 patients with 25 tumours in the recurrence group who underwent subsequent treatments, 10 tumours in the 3–5 cm group and three tumours in the 5–8 cm group underwent MWA and six tumours in the 3–5 cm group and six tumours in the 5–8 cm group underwent transcatheter arterial chemoembolisation (TACE) plus MWA. Univariate analysis (Table 3) showed that a positive correlation existed between the number of lesions ($p=0.022$), proximity to the risk area ($p=0.001$), pre-ablation alpha-fetoprotein (AFP) levels ($p=0.025$), HBV-DNA replication ($p=0.027$), and early recurrence. There was no significant correlation between recurrence and patient age, sex, diameter of tumour, tumour pathological grade, serum AFP after MWA, or Child–Pugh classification (Table 3).

Multivariate analysis (Table 4) identified that HBV-DNA ($p=0.031$) and proximity to the risk area ($p=0.039$) were significant factors associated with postoperative recurrence.

Three days after the procedure, the ablation zone was measured with ultrasound. The average ablation zone of a tumour with a diameter of 3–5 cm was $(4.71 \pm 0.6) \text{ cm} \times (5.48 \pm 0.7) \text{ cm}$. The average ablation zone of a tumour with a diameter of 5–8 cm was $(5.57 \pm 0.6) \text{ cm} \times (8.46 \pm 0.5) \text{ cm}$.

**Table 2**

<table>
<thead>
<tr>
<th>Complete ablation rate (n,%)</th>
<th>3–5 cm (n=46)</th>
<th>5–8 cm (n=14)</th>
<th>Total (n=60)</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First ablation</td>
<td>38 (82.61)</td>
<td>9 (64.29)</td>
<td>47 (78.33)</td>
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<td>Second ablation</td>
<td>46 (100)</td>
<td>12 (85.71)</td>
<td>58 (96.67)</td>
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**Table 3**

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<tr>
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<td>6</td>
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<tr>
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<td>Serum AFP before MWA (ng/ml)</td>
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<td>$\leq$400</td>
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AFP, alpha-fetoprotein; MWA, microwave ablation; HBV, hepatitis B virus.

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![Figure 2](image-url) CT images of a 65-year-old man with a 3.5 cm diameter HCC lesion that is adjacent to the diaphragm. (a) Pretreatment CT image shows hyperattenuation of the lesion during the arterial phase (arrows). (b) One month after MWA treatment, CT shows uniform hypoattenuation in the ablated area (arrows), which indicates complete ablation.
independent prognosis factors causing postoperative HCC recurrence. The number of lesions and pre-ablation AFP levels \((p > 0.05)\) did not show a significant association. The rate of recurrence of patients with a tumour adjacent to the risk area was significantly higher than the rate of recurrence of patients with a tumour in the general area. The time until recurrence in patients with a tumour adjacent to the risk area was significantly shorter than the time until recurrence in patients with a tumour in the general area \((\chi^2 = 7.107, p = 0.008; \text{Fig } 3)\). The rate of recurrence of patients with positive HBV-DNA replication was significantly higher than the rate of recurrence of patients with negative HBV-DNA replication. The time until recurrence in patients with positive HBV-DNA replication was significantly shorter than the time until recurrence in patients with negative HBV-DNA replication \((\chi^2 = 5.263, p = 0.022; \text{Fig } 3)\).

Complications and survival

No procedure-related mortality was observed. No major haemorrhage, liver rupture, or liver abscess occurred. There were no significant changes in the full blood count, blood glucose levels or electrolytes. Twelve out of the 45 patients \((26.67\%)\) suffered from significant liver transaminase level increases, which usually reverted to normal levels within 1 week. Pain, fever, and asymptomatic pleural effusion were the most common minor complications after the treatment. Grade 1 pain \((\text{World Health Organisation criteria})\) in the upper abdomen was observed after the procedure in 15 patients \((33.3\%, 15/45)\). A low-grade fever was observed after treatment in 26 of the 45 \((57.78\%)\) patients. An asymptomatic pleural effusion was observed in four of the 45 \((8.9\%)\) patients. There were no episodes of skin burning or tumour seeding in the study. The frequency of procedure-related complications in the 3–5 cm group versus the 5–8 cm group is listed in Table 5. The 1-year and 2-year overall survival rates after the initial ablation were 95.56\% \((43/45)\) and 86.67\% \((39/45)\), respectively.

Discussion

Percutaneous MWA delivered using lower-powered devices \((40–60 \text{ W})\) can successfully treat small tumours in patients with HCC\(^{10}\); however, the use of high-powered MWA to ablate larger tumours has been limited because this technique requires longer irradiation times that may induce skin burns. Microwave devices and antennas have been greatly improved; thus, ablating larger areas for shorter times may now be possible. Microwaves generate heat by oscillating dipole water molecules within tissues. Frequencies of 915 and 2450 MHz delivered with single, dual, or triple antennas are currently used for tissue

<table>
<thead>
<tr>
<th>B</th>
<th>SE</th>
<th>Wals</th>
<th>Sig</th>
<th>OR</th>
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<td>1.984</td>
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<td>Serum AFP before MWA</td>
<td>1.250</td>
<td>0.893</td>
<td>1.960</td>
<td>0.162</td>
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</table>

B, regression coefficient; SE, the standard error of regression coefficient; Wals, Wald chi-square value; Sig, \(p\)-value; OR, odds ratio.

Figure 3 Cumulative recurrence-free survival in patients with/without a tumour that is adjacent to a risk area, and cumulative recurrence-free survival in patients with HBV-DNA replication. (a) Cumulative recurrence-free survival in patients with a tumour that is adjacent to a risk area and patients with a tumour in a general area after MCT (Kaplan–Meier method, \(p < 0.05\), log-rank test). (b) Cumulative recurrence-free survival in patients with positive HBV-DNA replication and patients with negative HBV-DNA replication after MCT (Kaplan–Meier method, \(p < 0.05\), log-rank test).
A study found that percutaneous MWA using these two frequencies achieves similar efficacy, but fewer antenna insertions were required at 915 MHz. In the present study, a 2450 MHz internally cooled shaft antenna with a high power output (80–100 W) was used. The antenna was equipped with two channels inside the shaft lumen that were filled with distilled water that was circulated using a peristaltic pump to provide continuous cooling. The low temperature of the antenna shaft allows for the delivery of more energy to the tissue without causing skin burns. Further, a low-temperature antenna shaft can reduce the temperature in the centre to decrease tissue charring and improve energy transfer, leading to a longer and higher-output treatment. These conditions made the remarkable expansion of the ablation zone possible. Specifically, the rate achieved for complete ablation of 3–5 cm lesions was significantly higher compared with that for 5–8 cm lesions (82.61% versus 64.29%, respectively, \( p < 0.05 \)). Similar results were obtained by others who found that the rate for complete ablation in patients (49 of 52) with 3–5 cm tumours is significantly higher compared with that in patients (21 of 28) with 5–8 cm tumours (94.2% versus 75%, respectively, \( p = 0.033 \)).

Using MWA to achieve complete tumour ablation in patients with HCC may depend on factors such as the size, location, and boundary of tumours, as well as the adjacent structure. In the present study, the rates of local and distant recurrences were 24.44 and 15.56%, respectively. It is difficult to determine whether the development of a new HCC lesion is caused by intrahepatic metastasis associated with local recurrence or a multicentric origin. Therefore, it is important to determine the independent risk factors associated with the intrahepatic recurrence of HCC.

In the present study, multivariate analysis identified the area adjacent to the risk area as an independent prognostic factor of postoperative early recurrence. This is attributed to (1) difficulty to achieve a sufficient ablation margin to avoid injury for tumours adjacent to areas at risk, such as a major bile duct or blood vessel. The recurrence rate of tumours adjacent to risk areas increases because of insufficient ablation margins; (2) an important inherent effect of the heat sink of tumours that are adjacent to blood vessels during thermal ablation may influence the effective temperature and cause incomplete ablation. Therefore, the adjunctive use of dextrose solution, carbon dioxide or balloon interposition is suggested, which can separate and protect vital organs. The percutaneous technique allows for the safe and effective performance of the vast majority of ablations.

The combination of MWA with other non-invasive therapies, such as TACE and percutaneous ethyl injection (PEI), may prove to be effective for ablating lesions that are adjacent to risk areas. PEI has the advantage of allowing tumour treatment near organs and tissues at risk and avoids the problem of the heat-sink effect of adjacent vessels. One study performed PEI simultaneously with MWA therapy to treat tumours that were adjacent to both large vessels and bile ducts. TACE followed by RF ablation was used to minimise heat loss because of perfusion-mediated tissue cooling and to increase the therapeutic effect of RFA. Further research is required to determine the efficacy of combining TACE with MWA for lesions that are adjacent to risk areas.

Important unresolved clinical issues include predicting and preventing the extremely high rate of recurrence of HBV-related HCC even after curative treatment. Several factors are associated with an increased risk of HCC recurrence after local ablation therapies, such as tumour multiplicity, size, and portal invasion as well as the levels of AFP and albumin and a patient’s Child–Pugh class. Furthermore, the present study identifies HBV-DNA replication as another independent prognostic factor for early postoperative recurrence of HCC. Similar results in other studies suggest that a high viral load is another risk factor for recurrence and that antiviral therapy is associated with a lower risk of recurrence. Given the strong association between the level of HBV-DNA and cancer recurrence, antiviral therapy may reduce the risk of HCC recurrence after MWA.

After undergoing MWA, four (8.9%) patients developed asymptomatic pleural effusion, which was gradually relieved after treatment within 1 week. These patients had lesions adjacent to the diaphragm; asymptomatic pleural effusion may be caused by the inflammatory effusion of the diaphragm after MWA. Therefore, adjunctively using a dextrose solution may reduce the possibility of damaging the diaphragm. In this study, high-powered MWA, which causes massive coagulation necrosis, may have led to the increase in liver transaminase levels that were detected in 10.99% of the tumours in the 3–5 cm group and 50% of the tumours in the 5–8 cm group. Therefore, it is important to protect liver function after administering high-powered MWA.

The limitations of the present study include its single-centre, retrospective design, and the relatively small number of patients. Therefore, multicentre, prospective randomised controlled studies with more patients are required to document the potential benefits of high-powered MWA therapy for HCC patients with large tumours.

In conclusion, high-powered MWA appears to be a safe and effective treatment for treating larger tumours in HCC patients. High levels of HBV DNA and the proximity of tumours to risk areas appear to be independent prognostic factors of postoperative recurrence. Thus, antiviral therapy and other non-invasive therapies combined with high-powered MWA may reduce the postoperative recurrence of HCC and improve long-term outcomes.

| Table 5 Procedure-related complications. |
|------------------------------------------|------------------|
| 3–5 cm group | 5–8 cm group |
| Pleural effusion | 2 (4.3%) | 2 (14.3%) |
| Liver transaminase levels increase | 5 (10.9%) | 7 (50%) |
| Fever | 15 (32.6%) | 12 (85.7%) |
| Pain | 7 (15.2%) | 10 (71.4%) |

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References

Prognostic factors for survival after transarterial chemoembolization combined with microwave ablation for hepatocellular carcinoma

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Abstract

AIM: To analyze prognostic factors for survival after transarterial chemoembolization (TACE) combined with microwave ablation (MWA) for hepatocellular carcinoma (HCC).

METHODS: Clinical data of 86 patients who underwent TACE combined with MWA between January 2006 and December 2013 were retrospectively analyzed in this study. Survival curves were detected using log-rank test. Univariate analysis was performed using log-rank test with respect to 13 prognostic factors affecting survival. All statistically significant prognostic factors identified by univariate analysis were entered into a Cox proportion hazards regression model to identify independent predictors of survival. P values were two-sided and P < 0.05 was considered statistically significant.

RESULTS: Median follow-up time was 47.6 mo, and median survival time of enrolled patients was 21.5 mo. The 1-, 2-, 3- and 5-year overall survival rates were 72.1%, 44.1%, 31.4% and 13.9%, respectively. Tumor size ($\chi^2 = 14.999, P = 0.000$), Barcelona Clinic Liver Cancer (BCLC) stage ($\chi^2 = 29.765, P = 0.000$), Child-Pugh class ($\chi^2 = 51.820, P = 0.000$), portal vein tumor thrombus (PVTT) ($\chi^2 = 43.086, P = 0.000$), arteriovenous fistula ($\chi^2 = 29.791, P = 0.000$), MWA therapy times ($\chi^2 = 12.920, P = 0.002$), Eastern Cooperative Oncology Group (ECOG) score ($\chi^2 = 28.660, P = 0.000$) and targeted drug usage ($\chi^2 = 10.901, P = 0.001$) were found to be significantly associated with overall survival by univariate analysis. Multivariate analysis identified that tumor size (95%CI: 1.608-4.962, P = 0.000), BCLC stage (95%CI: 1.016-2.208, P = 0.020), PVTT (95%CI: 2.062-9.068, P = 0.000), MWA therapy times (95%CI: 0.402-0.745, P = 0.000), ECOG score (95%CI: 1.012-3.053, P = 0.045) and targeted drug usage (95%CI: 1.335-3.143, P = 0.001) were independent prognostic factors associated with overall survival.

CONCLUSION: Superior performance status, MWA treatment and targeted drug were favorable factors, and large HCC, PVTT and advanced BCLC stage were risk factors for survival after TACE-MWA for HCC.

Key words: Hepatocellular carcinoma; Transarterial chemoembolization; Microwave ablation; Survival; Prognosis

Core tip: Transarterial chemoembolization (TACE) combined with microwave ablation (MWA) has been used more and more widely for treatment of patients with hepatocellular carcinoma (HCC). However, there has been no study designed to analyze prognostic factors for survival after TACE combined with MWA for HCC. In this study, we retrospectively collected clinicopathologic data of 86 patients who were treated by TACE sequen-
tially combined with MWA, and to analyze prognostic factors for survival after the combinational therapy. We hope that our finding could serve as significant information for clinicians and patients in the decision for selecting treatment strategies.


INTRODUCTION

Hepatocellular carcinoma (HCC) is a kind of highly aggressive malignant tumor, and it is one of leading causes of cancer death. HCC ranks fifth in incidence for men and eighth for women and accounts for more than 660000 new cases worldwide annually[3-5]. Surgical resection is still considered the preferred treatment choice for patients with early-stage HCC. However, the majority of HCC patients were diagnosed at intermediate or advanced stage of tumor growth and accompanied with poor hepatic function which was caused by hepatitis, alcoholic liver disease or cirrhosis, and made surgical resection impossible[6-9]. Liver transplantation also offers a palliative treatment option for HCC patients, but the shortage of organ donors limits its application. In recent years, transarterial chemoembolization (TACE) as a palliative treatment has been accepted as the firstly considerable treatment for patients with early-stage HCC[10,11]. However, the long-term outcomes of TACE were not satisfying. The complete necrosis rate of tumor tissue after TACE was just about 10%-20%[8-10]. Hence, in order to improve clinical effectiveness of TACE and provide better prognosis for patients with HCC, alternative treatment strategies are being explored. One such strategy is TACE sequentially combined with microwave ablation (MWA). MWA as a thermal in situ destruction treatment has been proved to be a safe and effective treatment[11,12].

TACE sequentially combined with MWA provides a new treatment choice for HCC. Previous studies had reported that clinical efficacy of combination of TACE and MWA was much better than that of TACE or MWA monotherapy in the treatment of HCC[11,12]. However, to the best of our knowledge, there has been no study designed to analyze prognostic factors for survival after TACE combined with MWA for HCC. In this study, we collected clinicopathologic data of patients who underwent TACE sequentially combined with MWA and to analyze prognostic factors for survival after the combinational therapy. We hope that our finding could serve as significant information for clinicians and patients in the decision for selecting treatment strategies.

MATERIALS AND METHODS

Patients

Over a 5-year follow-up period between January 2006 and December 2013, we studied 86 patients with a formal diagnosis of HCC (according to criteria of the American Association for the Study of Liver Diseases). All included patients were treated by TACE sequentially combined with MWA in the Department of Interventional Radiology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, China. There were 76 males and 10 females, and their age ranged from 15 to 78 years (mean age, 54.9 years). Exclusion criteria were listed as following aspects: (1) patients without a definite diagnosis of HCC; (2) patients with diffuse-type HCC; (3) HCC after surgical treatment or liver transplantation; (4) patients with poor baseline hepatic function or general condition, who were not able to tolerate the treatments; (5) TACE combined with any other interventional procedure besides MWA, such as 125I seed implantation, radiofrequency ablation, and percutaneous ethanol injection; and (6) patients without regularly clinical data for evaluating overall survival and prognostic factors.

Equipment

Main equipment used in this study was listed as follows: digital subtraction angiography (DSA) system (Philips Allura Xper FD20, Amsterdam, Netherlands), Siemens 64-slice spiral computed tomography (CT) system (Somatom 64 Sensation, Muenchen, Germany), ECO-100 water-cooled microwave apparatus and monopole microwave antenna (16G) (Nanjing Eco Medical Equipment Co., Ltd, Nanjing, China).

TACE therapy

All 86 patients were initially treated by TACE. TACE was performed using DSA. Hepatic artery angiography was then performed using the Seldinger technique. Femoral arterial catheterization was conducted through the common hepatic artery or proper hepatic artery. The location, number, size and blood supply of tumors were evaluated. If necessary, microcatheter was super-selectively inserted into hepatic lobe or hepatic segmental artery. Chemoembolization therapy was then performed via the targeted artery using iodized oil emulsion (lipiodol mixed with chemotherapy drugs), and gelatin sponge embolization was used in the treatment of tumors with rich blood supply. Chemotherapeutics were performed using 5-FU/FUDR (0.5-1.0 g), TIP-ADM (10-40 mg), hydroxyamaptocetin (5-10 mg) or mitomycin (2-8 mg), regularly. The amount of iodized oil emulsion, gelatin sponge and medical chemotherapy drugs used in TACE treatment was based on tumor size, lesion extension and tolerance of patients.

MWA therapy

After TACE treatment, a sequential CT-guided MWA procedure was performed based on the response of tumors.
If residual tumors belong to stable situation or partial response such as focal lipiodol-defect region, progression of focal lesion and/or residual lesion with insufficient blood supply or occlusion of the feeding artery, the MWA procedure was applied sequentially with TACE.

During the MWA procedure, a non-enhanced CT scan was first performed to determine puncture pathway. The puncture site was then anesthetized with 2% lidocaine, and a 16G guided needle was inserted into focus of tumor via the puncture pathway. CT scan was performed again to ensure the location of the guided needle. MWA electrode probe was then inserted along the path of the puncture needle to reach the opposite edge of tumor through its center. The microwave power was set at 60-70 Watt and the procedure lasted for 10-20 min. Vital signs such as heart rate, blood pressure and oxygen saturation were monitored during the procedure. After ablation, the electrode probe and puncture needle were pulled out and CT scan was performed to reexamine coagulation area of MWA treatment.

**Follow-up**

In this study, follow-up was done by telephone or clinical visits at monthly interval. Physical examination, hepatic function test, alpha fetal protein (AFP) level and CT/magnetic resonance imaging (MRI) scan were reviewed. According to the result of post-ablation CT scan, incomplete ablation of tumor was reassessed by our multidisciplinary team of radiologists and oncologists in terms of tumor response and hepatic function. Complete response of tumor after further ablation treatment was defined as complete disappearance based on CT or MRI imaging.

**Statistical analysis**

Student’s t test and Fischer’s exact test were used to compare quantitative variables. The results are expressed as mean ± SD. Cumulative survival curve was calculated using the Kaplan-Meier method. The χ² test was used to compare qualitative variables. The differences in the survival curves of different groups were detected using log-rank test. Univariate analysis was performed using log-rank test with respect to 13 prognostic factors affecting survival, including age, gender, BCLC stage, tumor size, portal vein tumor thrombus (PVTT), Child-Pugh class, Eastern Cooperative Oncology Group score, AFP, serum hepatitis B surface antigen, arterio-venous fistula (AVF), MWA therapy times, TACE therapy times and targeted drug usage. All statistically significant prognostic factors identified by univariate analysis were entered into a Cox proportion hazards regression model to identify independent predictors of survival. For all analyses, P-values were two-sided and P < 0.05 was considered statistically significant. All statistical analyses and graphics were performed using SPSS software package (version 19.0, SPSS Inc., Chicago, IL).

**RESULTS**

**Clinicopathological characteristics of patients**

A total of 86 patients with surgically unresectable HCC were included in this study. Mean age of the included patients was 54.9 years (range: 15-78 years). There were 13 (15.1%), 32 (37.2%), 11 (12.8%) and 30 (34.9%) patients with small, nodular-type, massive-type and huge-type HCC, respectively. Branch and trunk portal vein tumor thrombi were detected in 10 (11.6%) and 9 (10.5%) patients, respectively. Serum hepatitis B surface antigen and AFP were positive in 41 (47.7%) and 55 (64.0%) patients, and no case had anti-HCV antibody detected. There were 18 (21.0%) patients with arterio-venous fistula, which was detected by digital subtraction angiography during TACE treatment. Sixty-four (74.4%) patients underwent treatment with targeted drugs such as sorafenib to control progression of tumor. Liver function was evaluated using Child-Pugh standard, and 62 (72.0%) cases were considered class A and 24 (28.0%) considered class B. More details of clinicopathological characteristics of the included patients are shown in Table 1.

**Overall survival**

Median follow-up time in this study was 47.6 mo. Of 86 patients with unresectable HCC, 5 survived and 81 died. Median survival time was 21.5 mo (range: 2-96 mo), and the 1-, 2-, 3- and 5-year overall survival rates were 72.1% (62/86), 44.1% (38/86), 31.4% (27/86) and 13.9% (12/86), respectively. Kaplan-Meier survival curve of 86 patients who were treated by combined TACE-MWA is shown in Figure 1.

**Univariate analysis**

Univariate analysis revealed that tumor size (χ² = 14.999, P = 0.000), BCLC stage (χ² = 29.765, P = 0.000), Child-Pugh class (χ² = 51.820, P = 0.000), PVTT (χ² = 43.086, P = 0.000), AVF (χ² = 29.791, P = 0.000), MWA therapy times (χ² = 12.920, P = 0.002), ECOG score (χ² = 28.660, P = 0.000) and targeted drug usage (χ² = 10.901, P = 0.001) were significantly associated with overall
analysis identified that tumor size (95%CI: 1.608-4.962, \( P = 0.000 \)), BCLC stage (95%CI: 1.016-2.208, \( P = 0.020 \)), PVTT (95%CI: 2.062-9.068, \( P = 0.000 \)), MWA therapy times (95%CI: 0.402-0.745, \( P = 0.000 \)), ECOG score (95%CI: 1.012-3.053, \( P = 0.045 \)) and targeted drug usage (95%CI: 1.335-3.143, \( P = 0.001 \)) were independent prognostic factors associated with overall survival (Table 3).

Survival curves of 86 patients with different characteristics of tumor size, BCLC stage, PVTT, MWA therapy times, ECOG score and targeted drug usage were shown in Figure 2A-F.

### Complications

Both TACE and MWA are minimally invasive treatments. There were no procedure-related mortalities or serious complications. The most common adverse effect was post-embolization syndrome, such as right upper quadrant abdominal pain, vomiting, nausea and fever. Abdominal pain and fever were found in patients who underwent MWA as well. Hepatic dysfunction was the second most common complication, which could be found by biochemical tests and clinical manifestations, including ascites, jaundice or bleeding during the period of follow-up. Symptomatic treatments were necessary for survival of patients who underwent TACE sequentially combined with MWA (Table 2).

### Multivariate analysis

All statistically significant prognostic factors evaluated by univariate analysis were entered into a Cox proportion hazards regression model. The results of multivariate

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### Table 1 Characteristics of the patients included in this study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) &lt; 40</td>
<td>17 (19.8)</td>
</tr>
<tr>
<td>41-50</td>
<td>16 (18.6)</td>
</tr>
<tr>
<td>51-60</td>
<td>29 (33.7)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>24 (27.9)</td>
</tr>
<tr>
<td>Gender Male</td>
<td>76 (88.3)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (11.7)</td>
</tr>
<tr>
<td>BCLC stage A</td>
<td>4 (4.7)</td>
</tr>
<tr>
<td>B</td>
<td>60 (69.7)</td>
</tr>
<tr>
<td>C</td>
<td>22 (25.6)</td>
</tr>
<tr>
<td>Tumor size (TS) Small HCC (TS ≤ 3 cm)</td>
<td>13 (15.1)</td>
</tr>
<tr>
<td>Nodular type (3 cm &lt; TS ≤ 5 cm)</td>
<td>32 (37.2)</td>
</tr>
<tr>
<td>Massive type (5 cm &lt; TS ≤ 10 cm)</td>
<td>11 (12.6)</td>
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<tr>
<td>Huge type (10 cm &lt; TS)</td>
<td>30 (34.9)</td>
</tr>
<tr>
<td>PVTT None</td>
<td>67 (77.9)</td>
</tr>
<tr>
<td>Branch type</td>
<td>10 (11.6)</td>
</tr>
<tr>
<td>Trunk type</td>
<td>9 (10.5)</td>
</tr>
<tr>
<td>Child-Pugh class A</td>
<td>62 (72.0)</td>
</tr>
<tr>
<td>B</td>
<td>24 (28.0)</td>
</tr>
<tr>
<td>ECOG score 0</td>
<td>11 (12.8)</td>
</tr>
<tr>
<td>1</td>
<td>45 (52.3)</td>
</tr>
<tr>
<td>≥ 2</td>
<td>30 (34.9)</td>
</tr>
<tr>
<td>AFP Negative (≤ 20 μg/L)</td>
<td>31 (36.0)</td>
</tr>
<tr>
<td>Positive (&gt; 20 μg/L)</td>
<td>55 (64.0)</td>
</tr>
<tr>
<td>HBsAg Without</td>
<td>45 (52.3)</td>
</tr>
<tr>
<td>With</td>
<td>41 (47.7)</td>
</tr>
<tr>
<td>AVF Without</td>
<td>68 (79.0)</td>
</tr>
<tr>
<td>With</td>
<td>18 (21.0)</td>
</tr>
<tr>
<td>MWA times Once</td>
<td>35 (40.1)</td>
</tr>
<tr>
<td>Twice</td>
<td>24 (27.9)</td>
</tr>
<tr>
<td>≥ Triple</td>
<td>27 (32.0)</td>
</tr>
<tr>
<td>TACE times Once</td>
<td>19 (22.1)</td>
</tr>
<tr>
<td>Twice</td>
<td>16 (18.6)</td>
</tr>
<tr>
<td>Triple</td>
<td>21 (24.4)</td>
</tr>
<tr>
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<td>30 (34.9)</td>
</tr>
<tr>
<td>Targeted drug Without</td>
<td>22 (25.6)</td>
</tr>
<tr>
<td>With</td>
<td>64 (74.4)</td>
</tr>
</tbody>
</table>

BCLC: Barcelona Clinic Liver Cancer; HCC: Hepatocellular carcinoma; PVTT: Portal vein tumor thrombus; ECOG: Eastern Cooperative Oncology Group; AVF: Arterio-venous fistula; MWA: Microwave ablation.

### Table 2 Univariate analysis results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n)</th>
<th>Median survival time (mo)</th>
<th>( \chi^2 )</th>
<th>( P ) value</th>
</tr>
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<tbody>
<tr>
<td>BCLC stage</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>A</td>
<td>4</td>
<td>36 (range: 25-60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>60</td>
<td>25 (range: 3-96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>22</td>
<td>5 (range: 2-60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small HCC (TS ≤ 3 cm)</td>
<td>13 (15.1)</td>
<td>50 (range: 26-63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular type (3 cm &lt; TS ≤ 5 cm)</td>
<td>32 (37.2)</td>
<td>29 (range: 13-96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Massive type (5 cm &lt; TS ≤ 10 cm)</td>
<td>11 (12.6)</td>
<td>12 (range: 4-60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huge type (10 cm &lt; TS)</td>
<td>30 (34.9)</td>
<td>8 (range: 2-43)</td>
<td></td>
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<tr>
<td>PVTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>67</td>
<td>25 (range: 3-96)</td>
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<tr>
<td>Branch type</td>
<td>10</td>
<td>5 (range: 2-40)</td>
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<tr>
<td>Trunk type</td>
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<td>Child-Pugh class</td>
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<tr>
<td>A</td>
<td>62</td>
<td>30 (range: 4-96)</td>
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<tr>
<td>B</td>
<td>24</td>
<td>7 (range: 2-19)</td>
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<td>ECOG score</td>
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<tr>
<td>0</td>
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<td>50 (range: 30-96)</td>
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</tr>
<tr>
<td>1</td>
<td>45</td>
<td>29 (range: 5-84)</td>
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<tr>
<td>≥ 2</td>
<td>30</td>
<td>8 (range: 2-32)</td>
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<tr>
<td>AVF</td>
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<tr>
<td>Without</td>
<td>68</td>
<td>25 (range: 3-96)</td>
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<tr>
<td>With</td>
<td>18</td>
<td>5 (range: 2-40)</td>
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<tr>
<td>MWA times</td>
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<tr>
<td>Once</td>
<td>35</td>
<td>14 (range: 2-60)</td>
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</tr>
<tr>
<td>Twice</td>
<td>24</td>
<td>20 (range: 3-60)</td>
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<td>≥ Triple</td>
<td>27</td>
<td>32 (range: 8-96)</td>
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<td>Targeted drug</td>
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<tr>
<td>Without</td>
<td>22</td>
<td>6 (range: 2-60)</td>
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<tr>
<td>With</td>
<td>64</td>
<td>24 (range: 5-96)</td>
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</tbody>
</table>

BCLC: Barcelona Clinic Liver Cancer; HCC: Hepatocellular carcinoma; PVTT: Portal vein tumor thrombus; ECOG: Eastern Cooperative Oncology Group; AVF: Arterio-venous fistula; MWA: Microwave ablation.

TACE: Transarterial chemoembolization; MWA: Microwave ablation; BCLC: Barcelona Clinic Liver Cancer; ECOG: Eastern Cooperative Oncology Group; AVF: Arterio-venous fistula; TS: Tumor size; PVTT: Portal vein tumor thrombus; HCC: Hepatocellular carcinoma; AFP: Alpha fetal protein; HBsAg: Serum hepatitis B surface antigen.
patients with hepatic dysfunction. No other complications emerged after TACE or MWA treatment.

**DISCUSSION**

Nowadays, TACE sequentially combined with MWA has been widely accepted as an important treatment option for surgically unresectable HCC. To the best of our knowledge, there has been no study designed to analyze prognostic factors for survival after combined TACE-MWA for HCC. In this study, we collected the clinical data of 86 patients who underwent TACE combined with MWA. Overall survival and prognostic factors were analyzed. We found that patients who were treated by TACE combined with MWA had relatively satisfying overall survival. In addition, our study revealed that tumor size, BCLC stage, PVTT, MWA therapy times, ECOG score and targeted drug usage were significantly independent factors affecting overall survival.

Although there was no comparative data in this study, our previous study had reported that combined TACE-MWA treatment was associated with better effectiveness in comparison with TACE monotherapy in HCC. During previous clinical practice and experimental research, some synergistic effects between TACE and MWA could be observed. The advantages of combined TACE-MWA may be explained as following aspects: (1) embolization of tumor vessel by TACE was expected to enlarge thermal coagulation area of MWA by reducing the “cooling effect” of hepatic blood flow; (2) edematous change in tumor tissues after TACE was expected to increase thermal effects of MWA; (3) some hypovascular HCCs, regenerated tumor feeding vessel or mimicking vessel after TACE made it difficult to perform TACE procedure, while imaging-guided MWA can destroy targeted tumors precisely and directly; and (4) TACE sequentially combined with MWA can effectively decrease the liver function impairment caused by TACE monotherapy, and improve the prognosis of patients. All the above aspects indicated that combination of TACE and MWA had advantages in improving overall survival of patients with HCC.

Referring to prognostic factors for survival after combined TACE-MWA for unresectable HCC, we found that tumor size, BCLC stage, PVTT, MWA therapy times, ECOG score and targeted drug usage were independent prognostic factors associated with overall survival of patients.

Complete necrosis rate of tumor tissue after TACE was about 10%, while for large HCC it was much lower in clinical practice. In the treatment of large HCC, it was difficult to reach complete necrosis for MWA. A previous study had reported that 1- and 3-year overall survival rates of patients who underwent MWA treatment were 92% and 72%, respectively. In this study, we found that the effectiveness of combined TACE-MWA in small- and nodular-type HCC was superior to that in massive-type and huge-type HCC. Peng et al. had reported that the overall survival of patients with small HCC, who were treated by combination of TACE and radiofrequency ablation, was much better than that of patients with large-size HCC. Hence, our study revealed that large tumor lesion was associated with an increased risk for poor prognosis of patients with HCC.

In clinical practice, the BCLC staging system has been widely accepted as an important staging system that comprehensively considers tumor size, hepatic function and performance status of patients with HCC. Our analysis showed that overall survival of patients with BCLC stage A HCC was much longer in comparison with patients with BCLC stages B and C. Our study suggested that BCLC stage was an independent prognostic factor for survival after combined TACE-MWA for HCC.

In this study, 10 and 9 of all enrolled patients had branch- and trunk-type PVTT, respectively, which was detected by DSA during TACE treatment. It was obvious that PVTT led to a high risk of intrahepatic and/or extrahepatic metastasis of cancer cells. Additionally, advanced tumor thrombus usually occluded the portal vein, and resulted in portal hypertension and further damage of hepatic function. PVTT was also associated with hepatic arterio-portal fistula. Ngan et al. had reported that hepatic arterio-portal fistula played a critic role in distant metastasis of tumor cells. In analysis of the effect of PVTT on overall survival, we found that PVTT significantly affected median survival time of patients with HCC. The survival time of patients with branch- and trunk-type PVTT was much shorter than that of patients without PVTT. Our study suggested that PVTT was an independent prognostic factor associated with survival.

---

**Table 3** Cox proportion hazards regression multivariate analysis results

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Sig</th>
<th>Exp(B)</th>
<th>Wald</th>
<th>95%CI for Exp(B)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>Tumor size</td>
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<td>0.402</td>
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<tr>
<td>TD</td>
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<td>0.213</td>
<td>0.001</td>
<td>2.107</td>
<td>10.203</td>
<td>1.333</td>
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</table>

PVTT: Portal vein tumor thrombus; BCLC: Barcelona Clinic Liver Cancer; ECOG: Eastern Cooperative Oncology Group; MWA: Microwave ablation; TD: Targeted drugs.
Progression and metastasis of residual tumor cells after TACE or MWA seriously affected long-term survival of patients with HCC. TACE sequentially combined with MWA can significantly enhance the necrosis rate of tumor tissue. Our analysis suggested that MWA treatment times was an independent prognostic factor associated with overall survival.

Good performance status is very important for patients to tolerate interventional therapy and chemotherapeutics. Sorafenib tosylate as a targeted drug has been used more and more widely to control progression of tumor. We found that the prognosis of patients who were treated with targeted drugs was much better in comparison with patients without usage of targeted drugs.

In conclusion, the data of our study indicated that superior performance status, MWA treatment and targeted drug treatment times were independent prognostic factors associated with overall survival.

Figure 2 Survival curves of 86 patients with different prognostic factors. A: Patients with different sizes of hepatocellular carcinoma (HCC); B: Patients with different stages of Barcelona Clinic Liver Cancer; C: Patients with different types of portal vein tumor thrombus; D: Patients with different times of microwave ablation treatment; E: Patients with different Eastern Cooperative Oncology Group scores; F: Patients with and without therapy with targeted drugs.
drug usage were favorable factors, and large HCC, PVTT and advanced BCLC stage were risk factors for survival after TACE-MWA for HCC.

**COMMENTS**

**Background**

Transarterial chemoembolization (TACE) combined with microwave ablation (MWA) has been used more and more widely in the treatment for hepatocellular carcinoma (HCC). Previous studies had reported that combined TACE-MWA was associated with better effectiveness in comparison with TACE or MWA monotherapy. However, there has been no study designed to analyze prognostic factors for survival after combined TACE and MWA for HCC.

**Research frontiers**

In the current report, authors designed an analysis to study prognostic factors for survival after combined TACE-MWA, and the study is the first one to analyze prognostic factors for survival after TACE combined with MWA for HCC.

**Innovations and breakthroughs**

In this study, 13 prognostic factors were enrolled. In univariate analysis, authors found that tumor size, Barcelona Clinic Liver Cancer (BCLC) stage, Child-Pugh class, portal vein tumor thrombus (PVTT), arterial-venous fistula, MWA therapy times, Eastern Cooperative Oncology Group (ECOG) score and targeted drug usage were significantly associated with survival. Multivariate analysis showed that tumor size, BCLC stage, PVTT, MWA therapy times, ECOG score and targeted drug usage were independent prognostic factors associated with overall survival of patients who underwent TACE sequentially combined with MWA.

**Applications**

In this study, authors found that tumor size, BCLC stage, PVTT, MWA therapy times, ECOG score and targeted drug usage independently affected the survival of patients who were treated by combined TACE-MWA. They believe that their finding could serve as significant information for clinicians and patients in the decision for selecting treatment strategies.

**Terminology**

TACE is one of the most widely performed treatments for unresectable HCC, which is a type of interventional radiological treatment. MWA is a thermal in situ destruction technique, and it has been proved to be a safe and effective treatment. MWA has been accepted as one of the best treatment options for early-stage HCC.

**Peer review**

This is an interesting manuscript describing the various factors that impact survival in HCC patients undergoing TACE and MWA. Being a retrospective analysis, it has its inherent drawbacks. However, the results support the outcomes of others where radiofrequency ablation/MWA has been combined with TACE. As expected the outcomes correlate with liver function, tumor size and PVTT. The main limit of the paper is the use of inferential analyses in a small cohort study.

**REFERENCES**


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Original article

Microwave ablation: results in ex vivo and in vivo porcine livers with 2450-MHz cooled-shaft antenna

Zhou Qi, Jin Xing, Jiao De-chao, Zhang Fu-jun, Zhang Liang, Han Xin-wei, Duang Guang-feng, Han Jian-jun and Li Chuan-xing

Keywords: animals; experimental study; liver; microwave ablation

Background Imaging-guided thermal ablation using different energy sources continues to gain favor as a minimally invasive technique for the treatment of primary and metastatic hepatic malignant tumors. This study aimed to evaluate the performance of microwave ablation with 2450-MHz internally cooled-shaft antenna in ex vivo and in vivo porcine livers.

Methods All studies were animal care and ethics committee approved. Microwave ablation was performed using a noncooled or cooled-shaft antenna in 23 ex vivo (92 ablations) and eight in vivo (36 ablations) porcine livers. Diameters of the coagulation zone were observed on gross specimens. The coagulation diameters achieved in different microwave ablation parameter groups were compared. Curve estimation analysis was performed to characterize the relationship between applied power and treatment duration and coagulation diameter (including short-axis and long-axis diameter).

Results Coagulation zones were elliptical and an arrowed-shaped carbonization zone around the shaft was observed in all groups. But the antenna track was also coagulated in the noncooled-shaft antenna groups. In ex vivo livers, the short-axis diameter correlated with the power output in a quadratic curve fashion ($R^2=0.95$) by fixing ablation duration to 10 minutes, and correlated with the ablation duration in a logarithmic curve fashion ($R^2=0.98$) by fixing power output to 80 W. The short-axis reached a relative plateau within 25 minutes. In in vivo livers, short-axis diameter correlated with the coagulation duration in a sigmoidal curve fashion (60 W group $R^2=0.76$, 80 W group $R^2=0.87$), with a relative plateau achieved within 10 minutes for power settings of 60 W and 80 W.

Conclusions The internally cooled microwave antenna may be advantageous to minimize collateral damage. The short-axis diameter enlargement has a plateau by fixing power output.

Imaging-guided thermal ablation using different energy sources (such as radio frequency (RF), microwave, or laser) continues to gain favor as a minimally invasive technique for the treatment of primary and metastatic hepatic malignant tumors.1-5 Regardless of the primary energy source, all of these modalities induce cellular destruction by means of the direct effects of heat, with irreversible cellular damage occurring at temperatures above 50°C when applied for 4–6 minutes and almost instantaneously at temperatures above 60°C.6 Thus, the main difference between modalities lies in the ability to translate energy efficiently into heat throughout the entire tumor ablation target volume. Historically, the greatest attention has been given to the potential of RF ablation, fueled in part by the substantial morbidity and mortality associated with hepatic resection.7,8 Results of studies of RF ablation suggest that the characteristics of the tissue, such as electrical conductivity, can substantially affect and occasionally retard energy deposition and heating throughout a tumor.9 Additionally, exponential rises in electrical impedances of tumor tissue may result from the application of high RF current, thus limiting the total amount of energy that can be delivered into tissue.6 This limits the amount of coagulation that can be achieved. On the other hand, microwave energy deposition is dominated by the absorption caused by the rapid rotation of the polar water molecule and is far less dependent on the electrical conductivities of tissue.10 However, compared with RF, little data are available on the extent of tumor destruction possible with microwave ablation, especially at frequencies near 2450-MHz.11,12 Traditional antennas have been plagued by excessive amounts of reflected power from the antenna. This increases the amount of undesired heating along the needle shaft and
feed cable which may reduce the amount of energy deposited into the tissue and cause collateral damage, such as skin burn.\textsuperscript{13} Internal cooling of the antenna may be a solution to minimize heating of the shaft and feed cable which may decrease tissue charring, facilitate microwave energy deposition, and avoid collateral damage at the same time. There have been previous studies about the tissue damage with cooled-shaft antenna microwave ablation, but they concentrated mostly on the scales and the times of the ablation with the same kind of antenna.\textsuperscript{14-16} With our experience in clinical work, we found that to make the result more meaningful, it is important to compare the difference between different antennas. Thus, the purpose of our study was to characterize the ablative effects on the extent of tissue coagulation achieved with an internally cooled 2450-MHz microwave applicator in both an ex vivo liver model and a perfused in vivo liver models.

METHODS

Microwave coagulation system
A microwave delivery system (ECO-100A; Nanjing Yigao Microwave Electric Institute, Nanjing, China) was used in the experiment. This system consisted of a microwave generator with a frequency of 2450-MHz, a power output of 10–120 W, a flexible low-loss cable and a 14 gauge cooled-shaft antenna. The cooled-shaft antenna, which consisted of a 10 cm long cable connection portion, a 16.5 cm-long shaft coated with Teflon, and a 1.5 cm-long active tip coated with polytetrafluoroethylene, was used to deliver energy to the liver tissue. The antenna shaft contained two lumina that enabled the delivery of 4°C saline solution to the tip of the shaft and the return of the warmed solution to a 500 ml plastic bag outside the body. A steady-flow pump was used to push the chilled saline solution circulating within the lumina of the antenna shaft at 50–60 ml/min. The amount of circulating chilled solution could be adjusted to maintain a mean shaft temperature of (10±2) °C (±standard deviation (SD)).

Ex vivo studies
Single-application microwave ablation was performed at room temperature (25°C) in 22 fresh porcine livers purchased from local markets.

Phase 1: comparing the coagulation achieved along the antenna track by using microwave ablation with noncooled and cooled-shaft antennas. A non-cooled or cooled-shaft antenna was inserted 6–9 cm into the liver tissue. Microwave ablation was performed at 60, 80 and 90 W for 10 minutes. A total of three ablation parameter settings were tested in six livers. Immediately after each ablation procedure, the liver specimen was sectioned along the antenna track and the morphologic features of the track were compared. All procedures were performed by two authors (ZHANG Fu-jun, JIAO De-chao) who had eight and three years of experience with ablation procedures, respectively.

Phase 2: observe the sizes of the coagulation zones induced by cooled antenna microwave ablation at different power output levels. Microwave ablation was performed by using a cooled-shaft antenna at 20, 30, 40, 50, 60, 70, 80, 95 and 100 W for 10 minutes. A total of 10 ablation parameter settings were tested in eight livers. Each ablation parameter group was tested four times, for a total of 40 experiments. After the ablation, the liver specimens were immediately sectioned along the antenna track. The visualized coagulated area was measured with calipers. The coagulation diameter along or perpendicular to the antenna track was measured by consensus between two observers (LI Chuan-xing, JIAO De-chao) who had eight and three years of experience with ablation procedures, respectively. The size of the coagulation zone was described in terms of the short-axis × long-axis coagulation diameter. The coagulation diameters achieved in the different ablation parameter groups were compared. Curve estimation analysis was performed to characterize the relationship between applied power and coagulation diameter (including short axis and long axis diameter).

Phase 3: observe the size of the coagulation zones induced by cooled antenna microwave ablation at different ablation durations. Microwave ablation was performed by using a cooled-shaft antenna at 1, 3, 5, 7.5, 10, 15, 20, 25, 30 and 35 minutes by fixing the power output to 80 W. A total of 10 ablation parameter settings were tested in nine livers. Each ablation parameter group was tested four times, for a total of 40 experiments. The methods used to section the specimens and measure coagulation diameter were identical to those used in the ex vivo experiments. Curve estimation analysis was performed to characterize the relationship between the duration and coagulation diameter.

Phase 4: Temperature measurements were performed in parameter group 60 W/10 min, 70 W/10 min, 80 W/10 min, and 80 W/15 min. Four 20-gauge thermistor probes (response time 1 second, accuracy 0.3°C) were placed 0.5, 1.0, 1.5 and 2.0 cm from the antenna during single application microwave ablation. Temperatures were recorded at 30-second intervals for all the experimental groups.

In vivo studies
Xizang pigs weighing as much as 60.0–70.0 kg were used to study the size of the coagulated areas produced with cooled-antenna microwave ablation in normal livers. Approval from the University Subcommittee on Animal Research Experiments was obtained before the initiation of these studies. General anesthesia was induced in the animals by means of intramuscular injection of ketamine hydrochloride (10–15 mg per kilogram of body weight) and maintained by means of intramuscular injection of pentobarbital sodium (3 g/100 ml, 0.25 ml/kg) and intravenous injection of diazepam (10 mg). Observation
of cardiac and respiratory conditions was made throughout the procedures. A bilateral subcostal incision was used to expose the liver. With normal hepatic blood flow, cooled-shaft antennas were inserted approximately 5.0–5.5 cm into the liver parenchyma. Ultrasonographic guidance (15L8W-s linear transducer, Sequoia 512, Acuson, Mountain View, CA, USA) helped us to avoid inserting the antennas into large intrahepatic vessels. Single applications of microwave energy were performed at 60 W for 3, 5, 10, 15, 20, and 25 minutes, and at 80 W for 3, 5, 10, 15, 20, and 25 minutes. Three experiments were performed for each parameter. Immediately after performing the ablations, we excised the livers for gross pathologic analysis and then sacrificed the animals by incising the inferior vena cava. The methods used to section the specimens and measure coagulation diameter were identical to those used in the ex vivo experiments. All ablation procedures were performed by four authors (JIAO De-chao, DUAN Guang-feng and HAN Jian-jun, ZHANG Fu-jun) with three, two, two and eight years of experience with microwave ablation procedures, respectively. Curve estimation analysis was performed to characterize the relationship between the ablation duration and coagulation diameter.

**Pathologic study**
After measuring the coagulation diameters, the in vivo specimens were fixed in 10% formalin for 24 hours, embedded in paraffin, cut and stained with hematoxylin-eosin, and examined under light microscopy.

**Statistical analysis**
Continuous data were expressed as mean ± standard deviations. In the ex vivo and in vivo studies, an independent Student’s t test was used to compare coagulation diameters between different groups. All statistical analyses were performed using SPSS 10.0 statistical software (SPSS Company, Chicago, IL, USA). A P value <0.05 indicated a statistically significant difference. The sphericity index can be simplified to the short axis/long axis. A perfect sphere has an index of 1.0, and ellipsoids have indexes of less than 1.0. The volume of ablation in this study was defined as the calculated volume of an ellipsoid obtained by using the short axis \(r_1\) and long axis \(r_2\) \((V=4/3\pi r_1^2 r_2/3)\). Multivariate regression analysis employed linear and nonlinear (exponential, logarithmic, sigmoidal and quadratic) regression models were performed with SPSS 10.0 statistical software. The strength of the best-fit regression curves were reported as \(R^2\) computations.

**RESULTS**

**Ex vivo study**
Phase 1: The coagulation zones were elliptical and an arrowed-shaped carbonization zone around the shaft was observed in all groups. But the antenna track was also coagulated in the noncooled-shaft antenna groups (Figure 1). The short-axis diameter correlated with the ablation duration in a sigmoidal curve fashion in the both 60 and 80 W groups. Totally 89.00% and 85.76% of maximum short axis diameters were achieved within 10 minutes in 60 and 80 W groups, respectively.

![Figure 1](image1.png)

**Figure 1.** *Ex vivo* porcine liver in which one application of microwave energy at 80 W for 10 minutes was performed using cooled-shaft antennas (1A) and noncooled-shaft antennas (1B). Microwave energy applied through a noncooled-shaft antenna produced an elliptical coagulated area (1B). The antenna track was also coagulated (arrows). Coagulated area produced by microwave ablation with a cooled-shaft antenna was almost spherical, the antenna track was not coagulated (1A).

**Figure 2.** Graphs show short axis coagulation diameters for microwave ablation of in vivo liver. The short axis diameter correlated with the ablation duration in a sigmoidal curve fashion in the both 60 and 80 W groups. Totally 89.00% and 85.76% of maximum short axis diameters were achieved within 10 minutes in 60 and 80 W groups, respectively.
Table 1. Diameters of *ex vivo* coagulation zone created by noncooled- and cooled-shaft antennas

<table>
<thead>
<tr>
<th>Power/duration (W/min)</th>
<th>Group</th>
<th>Short axis diameter (cm)</th>
<th>Long axis diameter (cm)</th>
<th>Sphericity index</th>
<th>Carbonization length (cm)</th>
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<tr>
<td>60/10</td>
<td>EG</td>
<td>2.51±0.09</td>
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<tr>
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Table 2. Diameters of *ex vivo* coagulation zone created by fixing duration and power output

<table>
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<tr>
<th>Power/ duration (W/min)</th>
<th>Short axis diameter (cm)</th>
<th>Long axis diameter (cm)</th>
<th>Sphericity index</th>
<th>Ablation volume (cm³)</th>
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<td>6.16±0.13</td>
<td>0.72±0.02</td>
<td>63.28±5.25</td>
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</table>

not significant between the 20 and 30 W groups (*P*=0.64), the 20 and 40 W groups (*P*=0.12), the 30 and 40 W groups (*P*=0.27), the 40 and 50 W groups (*P*=0.06), the 50 and 60 W groups (*P*=0.15), the 70 and 80 W groups (*P*=0.06), and the 95 and 100 W groups (*P*=0.84). The difference in long axis diameter was not significant between the 30 and 40 W groups (*P*=0.12), the 40 and 50 W groups (*P*=0.15), and the 50 and 60 W groups (*P*=0.50). Both short axis and long axis diameter correlated with the applied power output in a quadratic curve fashion (multivariate, short axis $R^2=0.95$ and long axis $R^2=0.96$).

Phase 3: We observed the sizes of the coagulation zones induced by cooled antenna microwave ablation at different ablation duration. The mean sizes of coagulation in the excised porcine livers ranged from (1.00±0.15) cm × (2.54±0.11) cm to (4.43±0.15) cm × (6.16±0.13) cm (Table 2). In each ablation duration group, microwave ablation for 35 minutes produced the largest area of coagulation. The difference in short axis diameter was not significant between the 25 and 30 minutes groups (*P*=0.35), and the 25 and 35 minutes groups (*P*=0.08), and the 30 and 35 minutes groups (*P*=0.32). This means the short axis reached a relative plateau within 25 minutes. The difference in long axis diameter was not significant between the 30 and 35 minutes groups (*P*=0.17). The short axis diameter correlated with the coagulation duration in a logarithmic curve fashion (multivariate, $R^2=0.98$). The long axis diameter correlated with the ablation duration in a power curve fashion (multivariate, $R^2=0.98$).

Phase 4: We observed the dynamic thermal distribution in the ablation zones in the 60 W/10 min, 70 W/10 min, 80 W/10 min, and 80 W/15 min parameter setting groups. The dynamic temperature curve was divided into two parts: a rapid rising period and a plateau period, which was conspicuous at 5 mm and 10 mm distance from the antenna. Higher power output and/or shorter distance to the microwave antenna led to a greater slope of dynamic temperature curve, and reaching the relative plateau sooner. The maximum temperature was 125°C at 5 mm from the antenna in the 80 W/15 min group.

In *vivo* study

With fixed power output of 60 and 80 W, we observed the size of the coagulation zones induced by cooled antenna microwave ablation at different coagulation durations. The mean size of coagulation in *in vivo* porcine livers ranged from (1.70±0.20) cm × (2.47±0.15) cm to (3.00±0.40) cm × (4.67±0.21) cm in the 60 W group, and ranged from (1.77±0.21) cm × (3.07±0.25) cm to (3.30±0.26) cm × (5.20±0.30) cm in the 80 W group.
Table 3. Diameters of in vivo coagulation zone created by fixing power output to 60 and 80 W

<table>
<thead>
<tr>
<th>Power/ duration (W/min)</th>
<th>Short axis diameter (cm)</th>
<th>Long axis diameter (cm)</th>
<th>Sphericity index</th>
<th>Ablation volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60/3</td>
<td>1.70±0.20</td>
<td>2.47±0.15</td>
<td>0.69±0.04</td>
<td>3.80±1.10</td>
</tr>
<tr>
<td>60/5</td>
<td>2.03±0.40</td>
<td>3.16±0.32</td>
<td>0.65±0.04</td>
<td>6.03±2.35</td>
</tr>
<tr>
<td>60/10</td>
<td>2.67±0.31</td>
<td>3.73±0.21</td>
<td>0.71±0.02</td>
<td>11.68±2.49</td>
</tr>
<tr>
<td>60/15</td>
<td>2.73±0.23</td>
<td>3.97±0.25</td>
<td>0.67±0.04</td>
<td>12.81±3.16</td>
</tr>
<tr>
<td>60/20</td>
<td>2.87±0.38</td>
<td>4.40±0.27</td>
<td>0.63±0.05</td>
<td>15.41±4.66</td>
</tr>
<tr>
<td>60/25</td>
<td>3.00±0.40</td>
<td>4.67±0.21</td>
<td>0.64±0.08</td>
<td>18.39±5.78</td>
</tr>
<tr>
<td>80/3</td>
<td>1.77±0.21</td>
<td>3.07±0.25</td>
<td>0.58±0.03</td>
<td>5.12±1.62</td>
</tr>
<tr>
<td>80/5</td>
<td>2.27±0.31</td>
<td>3.37±0.21</td>
<td>0.67±0.05</td>
<td>9.27±3.12</td>
</tr>
<tr>
<td>80/10</td>
<td>2.83±0.25</td>
<td>4.37±0.25</td>
<td>0.65±0.02</td>
<td>18.57±4.34</td>
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<tr>
<td>80/15</td>
<td>3.06±0.31</td>
<td>4.63±0.25</td>
<td>0.66±0.03</td>
<td>23.13±5.93</td>
</tr>
<tr>
<td>80/20</td>
<td>3.13±0.31</td>
<td>4.93±0.21</td>
<td>0.63±0.04</td>
<td>25.66±5.87</td>
</tr>
<tr>
<td>80/25</td>
<td>3.30±0.26</td>
<td>5.20±0.30</td>
<td>0.63±0.02</td>
<td>29.95±6.58</td>
</tr>
</tbody>
</table>

Figure 3. Gross specimen of porcine liver tissue treated with 2450 MHz cooled-shaft antenna. An elliptical coagulation zone of 2.83 cm × 4.37 cm was produced with one application of 80 W for 10 minutes and vessels as large as 3.8 mm were completely coagulated (black arrows).

Figure 4. Pathologic changes in three zones. 4A: The central zone showed a thin strip of a central cavity, with the surrounding cellular structure eliminated (HE, original magnification ×200); 4B: The coagulated zone showed cellular degeneration and incomplete necrosis were noted immediately after microwave irradiation (HE, original magnification ×400); 4C: The congestive reaction zone showed tissue congestion, cellular degeneration, and edema were noted immediately after microwave application (HE, original magnification ×400).

DISCUSSION

Of all the thermal ablation modalities, RF is the most commonly adopted. Besides treating liver cancers, clinical application has been expanded to the treatment of neoplasms in other sites. However, the local recurrence rate following treatment remains high, particularly for hepatic colorectal metastases greater than 3.0 cm in diameter. The failure of RF may be caused by its inherent limitations. First, RF ablation has a small zone of active heating, tissue heating is fundamentally restricted by the electrical conductivity of the tissue. Second, due to its primarily passive heating nature, RF is easily affected by the perfusion mediated “heat-sink” effect, and overt deflection of the coagulation zone can occur in the vicinity of vessels. Third, if multiple

Pathologic study

Three zones were present (Figure 3). First, the central zone, measuring 2–5 mm in diameter, appeared grossly as a dark brown charred band. Microscopically, the central zone showed a thin strip of a central cavity, with the surrounding cellular structure eliminated. Second, the coagulated zone was a pale brown, hard, dense area around the central zone. Microscopically, cellular degeneration and incomplete necrosis were noted immediately after microwave irradiation. Third, the congestive reaction zone was thick and light red, and located adjacent to the coagulated zone. Microscopically, tissue congestion, cellular degeneration, and edema were noted immediately after microwave application (Figure 4).
electrodes are employed simultaneously, electrical interference will be countered which may make the coagulation zone including the temperature, the size and the shape unpredictable. Microwave, though less often adopted at present, is a promising new modality for tumor ablation. In the past, microwave ablation was only performed in the Far East. Encouraging therapeutic results have been obtained both for primary and metastatic liver cancers. As the potential benefits of microwave became more apparent in recent years, Western investigators also began to do experimental and clinical researches.

A major limitation of the current microwave ablation system is that its efficiency is subjected to power feedback due to impedance mismatches between the antenna and the surrounding tissue. This may cause elongation of the coagulation zone along the shaft due to thermal conduction, resulting in collateral damage to normal liver parenchyma and skin burn. The new cooled-shaft antenna is specially designed to limit impedance mismatches and subsequent power feedback. Furthermore, the antenna adopts a design similar to that of internally cooled RF electrodes which can prevent over heating of the shaft during ablation. Thus, it is our hypothesis that the undesired elongation of coagulation along the needle shaft can be prevented, avoiding possible collateral damage. Moreover, as charring along the needle shaft may be decreased or postponed by the circulating water, more energy may be deposited in a direction perpendicular to the shaft, expanding the short-axis diameter of coagulation. However, In our ex vivo phase 1 study, the differences in short-axis for 60 W/10 min, 80 W/10 min and 90 W/10 min parameter setting groups, were not significant between the noncooled-shaft antennas (CG) and cooled-shaft antennas group (EG) (P >0.05). Lack of significant differences may have been due to small sample size and requires our further study. But results told us that, compared with noncooled shaft antenna, the application of an internally cooled- shaft antenna did not reduce the short axis diameter. However, the differences in long axis and sphericity index were significant between control groups and experimental groups (P <0.05). As expected, the undesired extension of coagulation along the needle shaft was not encountered in this study. This may prevent collateral damage to the normal liver tissue and avoid complications, such as skin burn when used percutaneously. Kuang et al compared the coagulation achieved along the antenna track with non-cooled and cooled-shaft antennas (60 W/5 min), and demonstrated that the thermal effects on the antenna track outside the coagulation zone were observed only in non-cooled-shaft antennas. We had the same results. Of course, internal cooling may not always be beneficial. The risk of tumor cell seeding along the needle tract may be increased, thus, for clinical trials, coagulating the needle track when withdrawing the antenna may be necessary. Questions of whether microwave ablation using internally cooled antenna has a higher rate of local recurrence need to be answered by future clinical studies.

We correlated the power output and ablation duration with lesion size in the ex vivo porcine liver experiments. The results showed that the short axis diameter correlated with the applied power output in a quadratic curve fashion (R^2 = 0.95) by fixing the duration at 10 minutes. Short axis and long axis diameters increased with the increasing power output, when the power output increased from 20 W to 100 W, short axis and long axis diameter increased by 0.84 times and 1.53 times respectively and 1.53 times respectively. It means that the increase of power may have a greater effect on long axis elongation. The short axis diameter correlated with the ablation duration in a logarithmic curve fashion (R^2=0.98) by fixing power output to 80 W, and the short axis diameter reached its relative plateau within 25 minutes. Actually, it had already reached a near best effect around 10 minutes. This means that the short axis diameter would not increase even if we prolonged the ablation duration after 25 minutes, although long-axis diameter also tended to increase in the ex vivo porcine experiments. That might give us some clues to the perfect timing to balance the curative effect and the damage.

Perhaps the most important finding in this study is that the short axis diameter correlated with the coagulation duration in a sigmoidal curve fashion (60 W group R^2=0.76, 80 W group R^2=0.87) by fixing power output to 60 or 80 W in in vivo porcine livers, and short axis diameter reached its relative plateau within 10 minutes. Hines-Peralta et al first correlated the duration of the ablation (2–20 minutes) and the amount of power delivered to tissues (50–150 W) with the lesion size. Eight time increments (2, 4, 6, 8, 10, 12, 16 and 20 minutes) and five power increments (50, 75, 100, 125 and 150 W) were used, constituting 40 time-power combinations, and for each combination trials were performed in triplicate, resulting in a total of 120 ablations. The results showed that both the applied power and the treatment duration correlated well with the coagulation diameters (short and long axis) in a sigmoid curve fashion. For power settings of 75 W or higher, 90% of the maximum short coagulation diameter was achieved within 8 minutes. In our in vivo study, we get the same sigmoid curve fashion as Hines-Peralta’s experimental results. But the two studies also had differences. For example: (1) Different microwave antenna: we used a 1.5 cm-long active tip coated with polytetrafluoroethylene, but a 5.7 mm diameter ceramic microwave antenna was used in Hines Peralta’s experiment. (2) Different aims in ex vivo study were the fundamental difference: we compared the coagulation diameters achieved along the antenna track by using microwave ablation with noncooled and cooled-shaft antennas in an ex vivo study to prove that non cooled-shaft antennas caused elongation of the coagulation zone along the shaft due to thermal conduction, resulting in collateral damage to normal liver parenchyma and skin burn. On the other hand, the results...
from Hines Peralta’s work were mainly comparative with the in vivo study. (3) Selection of parameters: In our work, we chose the 60 W and 80 W for the in vivo study, which are the most common parameters in our clinical practice. In the Hines Peralta paper, the applied power varied from 50 W to 150 W using 50W increments in vivo. In our in vivo porcine liver study, 89.00% and 85.76% of maximum short axis diameters were achieved within 10 minutes in the 60 and 80 W groups, respectively. Microwave is highly efficient for tissue heating; the tissue surrounding the antenna will be coagulated rapidly. The loss of water content can affect tissue permittivity, hampering microwave energy deposition. Thus, a balance between energy deposition and perfusion-mediated cooling only takes several minutes to complete. Prolonged application could not result in significant enlargement of the coagulation diameter for in vivo tissue. Wang et al. reported that, to ensure adequate treatment and ease of use, 10 minutes is suitable for observing the effectiveness of microwave ablation. In clinical terms, this may likely translate to greater ease and faster and more efficient positioning, which, coupled with shorter treatment duration, may decrease total treatment time. Given the findings from prior microwave research, it is likely that even greater volumes can be ablated with this system, if necessary, by simultaneously applying energy to an array of applicators. However, this possibility will require further study.

The short-axis diameter for in vivo ablation is significantly smaller than that of the corresponding ex vivo ablation for the same parameter settings. This could be easily explained by the “heat-sink” effect. The in vivo study showed the plateau time was 10 minutes, but it did not mean that the results from the ex vivo study were not significant. As we know, combined therapy with transcatheter arterial chemoembolization and percutaneous microwave coagulation has been a regular treatment for hepatocellular carcinoma. The tumor blood supply after combined therapy should be between that of normal tissue and the ex vivo liver. The maximum ablation duration was 25 minutes in one ablation session. Given that increased short axis coagulation diameter is more important for ablating a liver tumor with the intent to create a spherical coagulation zone, 80 W/10–25 min is a suitable parameter combination, and we could adopt an “overlap” method or a microwave antenna array to treat tumors ≥3 cm. Further clinical studies are warranted to observe the therapeutic efficacy of the antenna.

This study has some limitations. First, similar to other experimental studies, this study used normal porcine liver as the proxy for human liver tumors. The size and morphologic characteristic might not extrapolate to clinical practice, as liver tumors, as well as cirrhotic liver have different tissue compositions and vascularity. Second, our study has a small sample sizes for making many of the comparisons. Many of the results need to be interpreted carefully, pending further corroboration from larger studies. Third, due to engineering differences, the geometry of ablation zones may be specific to this antenna and cannot be applied to other antennas.

In conclusion, our internally cooled microwave antenna can produce clinically usable coagulation diameters without undesired extension of coagulation along the shaft. The design of internal cooling may prevent collateral damage, like skin burns, during ablation. Microwave ablation at 2450-MHz can achieve large zones of coagulation in a relatively short time, which may ultimately prove advantageous for imaging-guided tumor ablation in the clinical setting.

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Targeted percutaneous microwave ablation at the pulmonary lesion combined with mediastinal radiotherapy with or without concurrent chemotherapy in locally advanced non-small cell lung cancer evaluation in a randomized comparison study

Xinglu Xu1 · Xin Ye2 · Gang Liu1 · Tingping Zhang1

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Abstract Concurrent chemoradiotherapy is the standard treatment for patients with locally advanced lung cancer. The most common dose-limiting adverse effect of thoracic radiotherapy (RT) is radiation pneumonia (RP). A randomized comparison study was designed to investigate targeted percutaneous microwave ablation at pulmonary lesion combined with mediastinal RT with or without chemotherapy (ablation group) in comparison with RT (target volume includes pulmonary tumor and mediastinal node) with or without chemotherapy (RT group) for the treatment of locally advanced non-small cell lung cancers (NSCLCs). From 2009 to 2012, patients with stage IIIA or IIIB NSCLCs who refused to undergo surgery or were not suitable for surgery were enrolled. Patients were randomly assigned to the RT group \( (n = 47) \) or ablation group \( (n = 51) \). Primary outcomes were the incidence of RP and curative effectiveness (complete response, partial response, and stable disease); secondary outcome was the 2-year overall survival (OS). Fifteen patients (31.9 %) in the RT and two (3.9 %) in the ablation group experienced RP \( (P < 0.001) \). The ratio of effective cases was 85.1 versus 80.4 % for mediastinal lymph node \( (P = 0.843) \) and 83.0 versus 100 % for pulmonary tumors \( (P = 0.503) \), respectively, for the RT and ablation groups. Kaplan–Meier analysis demonstrated 2-year OS rate of NSCLC patients in ablation group was higher than RT group, but no statistical difference \( (\log\text{-}rank\ \text{test}, \ P = 0.297) \). Percutaneous microwave ablation followed by RT for inoperable stage III NSCLCs may result in a lower rate of RP and better local control than radical RT treatments.

Keywords Non-small cell lung cancer · Radiotherapy · Radiation pneumonitis · Microwave ablation

Introduction

Lung cancer is the main cause of death due to cancer in both men and women worldwide accounting for 1.82 million cases and 1.6 million deaths in 2012 [1]. In 70 to 80 % of patients, the diagnosis is made at an advanced stage [2, 3]. A high proportion, 80 %, of lung cancer cases are classified as non-small cell lung cancer (NSCLC) [4]. Although the management of NSCLC has continued to improve over the past 5 years, due to late diagnosis the prognosis remains poor with a 5-year survival rate of about 16 % [5]. NSCLC can be classed according to its degree of invasiveness into four stages. Early stages I and II of NSCLC are generally treatable with surgical resection, but stage IIIA when the disease has spread to the lymph nodes in the middle of the chest is the point at which multimodal therapy needs to be considered [6]. Locally advanced NSCLC (LA-NSCLC) is a heterogeneous disease, encompassing stage IIIA. Many patients with LA-NSCLC are not suitable for surgery [6]. But therapy for stages IIIA and IIIB is associated with high rates of distant metastasis, local recurrence and toxicity [7]. The use of modern radiotherapy (RT) techniques has improved outcomes in some patients with limited metastatic disease [8].

Concurrent chemoradiotherapy is the recommended standard treatment modality for patients with locally advanced lung cancer [9]. However, RT risks adverse effects due to the sensitive nature of local tissues,
commonly resulting in radiation pneumonia (RP) [10], and RP is the most common dose-limiting adverse effect of thoracic RT. Carefully targeted RT is intended to prevent RP by methods such as three-dimensional conformal radiotherapy (3DCRT), intensity-modulated radiotherapy (IMRT), and radiofrequency ablation minimizing the damage to normal tissue when a high dose is delivered to the tumor [11, 12], although RP is often inevitable especially in multiple mediastinal lymph nodes and/or larger pulmonary tumors, and sometimes, it is serious [13].

Another thermal ablative technique, involving microwaves, has also been considered recently in the treatment of lung tumors [14–16]. Although less studied than radiofrequency ablation, microwave ablation may have some advantages in that the microwave energy is deployed over a larger zone with higher temperatures produced faster [17]. This would allow larger tumors to be ablated with a higher degree of accuracy than with radiofrequency ablation, as shown in an animal model [18]. Thus, some studies have successfully used microwave ablation to treat unresectable NSCLC and found it to be a safe and reliable method [19–21]. However, it remains unclear whether microwave ablation provides significant benefits when compared to RT, in particular in terms of the incidence of RP resulting from treatment. Therefore, the aim of this study was to investigate targeted percutaneous microwave ablation at pulmonary lesions combined with mediastinal radiotherapy with or without chemotherapy in LA-NSCLC, in contrast to concurrent chemoradiotherapy or radiotherapy alone. The treatment methods were compared in terms of the ratio of RP and treatment effectiveness [complete response (CR), partial response (PR), and stable disease (SD) described as effectiveness] 3 months after RT. The secondary outcome was the 2-year overall survival (OS).

Materials and methods

Patients

The study population was consecutively recruited from 132 patients with stage IIIA or IIIB NSCLCs who refused to undergo surgery or were not suitable for surgery at the Qilu Hospital from January 2009 to September 2012. The inclusion criteria were: (1) Karnofsky performance scale (KPS) score >60 points; (2) pulmonary reserve greater than severe obstructive or severe restrictive ventilatory dysfunction, and (3) cell pathology or histopathology confirmed the stage IIIA or IIIB NSCLC. The exclusion criteria were: (1) KPS score ≤60 points, (2) pulmonary reserve was severe obstructive or severe restrictive ventilatory dysfunction, (3) severe liver and kidney dysfunction, and (4) severe coagulopathy. According to the exclusion criteria, a total of 34 patients were excluded, so finally 98 patients with IIIA or IIIB NSCLC were finally enrolled (Fig. 1).

The diagnoses of all the patients were confirmed using the pathological results of bronchofibroscopy or percutaneous lung biopsy; in addition, the diagnoses in 54 patients were further confirmed using the pathological examinations of mediastinal lymph nodes obtained by mediastinoscopy or percutaneous biopsy [22].

The patients were randomly (simple randomization) assigned to the targeted percutaneous microwave ablation at pulmonary lesion combined with mediastinal radiotherapy with or without chemotherapy (ablation group n = 51) or radiotherapy (target volume includes pulmonary tumor and mediastinal node) with or without chemotherapy (radiotherapy group n = 47). This prospective cohort study was approved by the local ethics committee and institutional review board (Pingyi branch of Qilu Hospital affiliated to Shandong University, China). (Approval ID: 200901116), and all participants provided written informed consent.

Computed tomography guidance

A 16-slice spiral computed tomography (CT) machine (SOMATOM Emotion, Siemens, Germany) was used for imaging guidance. An outline of the positions of adjacent organs was made during conventional preoperative CT scanning. Based on the location of the lesion and its relationship with adjacent and vital structures, the optimal position of the patient and insertion sites of the required instrument were identified (Fig. 2a).

Interventions for the ablation group

The patients in the ablation group all received targeted percutaneous microwave ablation at the pulmonary lesions combined with mediastinal radiotherapy with or without chemotherapy. The microwave ablation was performed either before or at 1 week after mediastinal RT. The microwave ablation system was a CO-100C Microwave System (Nanjing Eco Microwave System Co., Ltd., China) with a disposable microwave ablation probe: ECO-100AL8 (Nanjing Eco Microwave System Co., Ltd.). The puncture site for the ablation antenna was determined according to the size, shape, and adjacent relationships of the tumors; the pattern of ablation (single-point or multipoint ablation) was also determined accordingly. Reconstruction of the CT image was done to reassure the ablation site after the puncture (Fig. 2b), and then, the microwave device was turned on. The parameters were as follows: power of 60–75 W and time for a single-point ablation of 4–8 min. Bubble formation could be observed inside the tumor.
during the ablation (Fig. 2c). Enhanced CT scanning of the chest was performed in the patients 1 month later to exclude the existence of insufficient ablation regions; secondary ablation was performed if there were enhanced regions in the soft tissues (Fig. 2d).

Ablations were followed or preceded by 3DCRT standard fraction external beam RT, once per day, five times per week using a linear accelerator (BJ6B; Beijing Medical Apparatus and Instrument Institute, China) and treatment planning system (Topslane Venus, Shanghai, China). The RT covered the enlarged mediastinal lymph nodes but not the tumor in the lungs. Single RT was provided if the patient was older than 70 years, had a history of cardiopulmonary disease, a poor cardiopulmonary reserve, or was in a generally good condition but refused chemotherapy. Otherwise the patients underwent concurrent chemoradiotherapy. The chemotherapy administered included a schema of paclitaxel (Yangtze River Pharmaceutical Group, Jiangsu, China) plus cisplatin (Haosen Pharmaceutical Group, Jiangsu, China) for two cycles.

**Interventions for the radiotherapy group**

Patients in the radiotherapy group also received either single RT or concurrent chemoradiotherapy as for the ablation group. 3DCRT (conventional fractionated radiotherapy, once per day, five times per week) was provided for the enlarged mediastinal lymph nodes as well as the tumor in the lungs. Chemotherapy drugs included paclitaxel plus cisplatin (two cycles).

**Outcomes**

Primary outcomes were the incidence of RP and curative effectiveness. CT scanning of the chest was performed at 3 months after RT, and the Response Evaluation Criteria In Solid Tumors criteria were used for evaluating the
treatment effects [23] [complete response (CR), partial response (PR), and stable disease (SD) were classified as effective, while progressive disease (PD) was classified as progression]. Remission of the tumor in the lungs and of mediastinal lymph nodes and the rate of incidence of RP (according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer evaluation criteria, namely Subjective, Objective, Management, Analytic Scales, acute RP ≥ 2 was included for the calculation) were evaluated [24].

Secondary outcome was the 2-year overall survival (OS). The patients were followed up by telephone once a month and every 3 months by imaging (chest abdomen enhanced CT, brain MRI) within 6 months after treatment. After 6 months, the patients were followed up once every 3 months. The rate of 2-year OS was calculated; the OS was measured from the time of diagnosis to death.

Statistical analysis

Statistical software SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Data were expressed as median (min, max) and compared using Mann–Whitney U for independent samples. Categorical variables were compared using the Fisher’s exact test or Pearson Chi-square test, as appropriate. The Kaplan–Meier and log-rank tests were used for survival analysis. The threshold of statistical significant was \( P < 0.05 \).

Results

Baseline characteristics

Patients’ baseline characteristics are given in Table 1. There were 65 men and 33 women with an age range of 42–76 years recruited between January 2009 and September 2012. Among the 98 patients, 35, 42, 9, and 12 patients were diagnosed with squamous carcinoma, adenocarcinoma, adenosquamous carcinoma, and large cell carcinoma, respectively. Thirty-nine patients were diagnosed with stage IIIA carcinoma (including 12 with T1N2M0, 8 with T2N2M0, 7 with T3N1M0, and 12 with T3N2M0 carcinoma) and 59 patients were diagnosed with stage IIIB carcinoma (including 5 with T4N2M0, 11 with T4N3M0, 17 with T1N3M0, 14 with T2N3M0, and 12 with T3N3M0 carcinoma).

Fifty-one patients were assigned to the ablation group, 18 of those patients received single radiotherapy (DT5400-6000cGy/27-30f), while 33 patients received concurrent chemoradiotherapy (DT5600-6000cGy/28-30f). Forty-seven patients were included in the radiotherapy group. Fifteen patients received single radiotherapy (DT5600-6000cGy/28-30f over 6 weeks) due to their age and cardiopulmonary functions, and 32 patients received concurrent chemoradiotherapy (DT5600-6000cGy/28-30f over 6 weeks). There were no significant differences between the groups in terms of gender, TNM stage, histology or the number of patients receiving either radiotherapy alone or concurrent chemoradiotherapy (Table 1).
Curative effectiveness and incidence of RP

Enhanced CT scanning of the chest was performed at 3 months after chemotherapy to evaluate the rate of remission of the pulmonary tumor and mediastinal lymph nodes as an indication of staging. In the mediastinal lymph node, curative effectiveness was found in 40 (85.1 %) patients in the radiotherapy group including 16, 19, and 5 patients who had CR, PR, and SD, respectively, while 41 (80.4 %) suggested curative effectiveness in the ablation group with 14, 22, and 5 patients who had CR, PR, and SD, respectively. PD was found in 7 (14.9 %) patients in the radiotherapy group and 10 (19.6 %) in the ablation group. Chi-square test was used to compare the differences between the two groups, and the results did not show any significant difference ($P = 0.843$; Table 2).

In the pulmonary lesion, curative effectiveness was suggested in 39 (83.0 %) patients in the radiotherapy group with 6, 22, and 11 patients who had CR, PR, and SD, respectively. In the ablation group, curative effectiveness was suggested in 51 (100 %) patients and 12, 23, and 16 who had CR, PR, and SD, respectively. The differences between the two groups were not significant ($P = 0.503$). PD was found in 8 (17.0 %) patients in the radiotherapy group and 0 (0 %) in the ablation group (Table 2). In many patients, the tumor in the lungs disappeared completely, and the patients lived for a long time (Fig. 2e, f).

In the pulmonary lesion, CT scanning examination of mediastinal lymph node or pulmonary tumor at 3 months after the treatment procedure

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Radiotherapy ($n = 47$)</th>
<th>Ablation ($n = 51$)</th>
<th>$P$ value*</th>
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<tbody>
<tr>
<td>Mediastinal lymph node, n (%)</td>
<td></td>
<td></td>
<td>0.843</td>
</tr>
<tr>
<td>CR</td>
<td>16 (34.0)</td>
<td>14 (27.5)</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>19 (40.4)</td>
<td>22 (43.1)</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>5 (10.6)</td>
<td>5 (9.8)</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>7 (14.9)</td>
<td>10 (19.6)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary tumor, n (%)</td>
<td></td>
<td></td>
<td>0.503</td>
</tr>
<tr>
<td>CR</td>
<td>6 (12.8)</td>
<td>12 (23.5)</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>22 (46.8)</td>
<td>23 (45.1)</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>11 (23.4)</td>
<td>16 (31.4)</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>8 (17.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

CR complete response, CT computed tomography, PD progressive disease, PR partial response, SD stable disease

* CR versus PR versus SD
Two-year OS

The patients were followed up for 2 years. No patients were lost to follow-up. Kaplan–Meier survival curves demonstrated 2-year OS rate of NSCLC patients in ablation group was higher than radiotherapy group, but no statistical difference (log-rank test, \( P = 0.297 \)) (Fig. 3).

Adverse events in relation to microwave ablation

It was also found that the rate of incidence of pneumothorax was 17.6 % in the ablation group, and drainage was required for three cases. Nine patients had a small amount of hemoptysis during surgery or postoperatively in the ablation group, and symptomatic remission occurred after treatment. There were no severe or fatal cases.

Discussion

Concurrent chemoradiation is the method recommended by the National Comprehensive Cancer Network guideline for the treatment of stage III NSCLS [25, 26]. However, RP is often unavoidable using this method. Microwave ablation has shown some promise in treating lung cancer [19–21] and may result in lower rates of RP. The aim of this study was to directly compare, in terms of the incidence of RP and curative effectiveness, microwave ablation in combination with mediastinal RT and chemotherapy with the recommended method of radiotherapy and chemotherapy. The results show that the incidence of RP was lower in the ablation group compared with the radiotherapy group, and the results were statistically significant. However, curative effectiveness was similar in both groups, but no patients experienced PD in the ablation group and this was significantly different to the radiotherapy group. Two-year OS was also similar between groups.

It has previously been suggested that the major factor influencing the development of RP is the ratio of irradiated pulmonary volume to the overall pulmonary volume, namely the value of V20 [13]. Adjusting the target region, and the angle and direction of the x-ray could reduce the irradiated volume; however, in some cases where the mass is large, mass is located at the lung periphery, and multiple mediastinal lymph nodes are involved that need to be irradiated, the V20 could not be reduced and thus the risk of developing RP is very high.

Minimally invasive treatments have been introduced in recent years, and of these, percutaneous ablation seems to be an attractive option in lung cancer [27, 28]. Image-guided ablation has been recently introduced as a safe, alternative treatment of localized disease in selected patients. The three main ablative treatments currently used in the lung are radiofrequency ablation, microwave ablation, and percutaneous cryotherapy [29]. Yang et al. [20] found that percutaneous microwave ablation is safe and effective for the treatment of medically inoperable stage I peripheral NSCLC. Microwave ablation may be safely and effectively used as a therapeutic tool for the treatment of pulmonary metastases [30]. Several studies have used combined treatment of percutaneous image-guided microwave ablation and RT for patients with inoperable stage I/II NSCLC. With low rates of complication, combined treatment may result in an improved survival compared with either modality alone [31].

In the present study, in the ablation group microwave ablation was used to treat the pulmonary tumors and 3DCRT plus concurrent chemotherapy was used to treat the mediastinal lymph nodes. The incidence of RP in the ablation group was lower than in the radiotherapy group. The reason for this might be the reduced radiation volume of the lungs. In contrast, the effectiveness of the treatment,

<table>
<thead>
<tr>
<th>Advance event</th>
<th>Radiotherapy (( n = 47 ))</th>
<th>Ablation (( n = 51 ))</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation pneumonia, ( n ) (%)</td>
<td>15 (31.9)</td>
<td>2 (3.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig. 3 Kaplan–Meier curves showing the cumulative survival rate of patients with locally advanced non-small cell lung cancer (NSCLC) treated with radiotherapy concurrent chemotherapy (radiotherapy group) (blue curve) or targeted percutaneous microwave ablation at pulmonary lesion combined with mediastinal radiotherapy concurrent chemotherapy (ablation group) (green curve) (log-rank test, \( P = 0.297 \))
which was evaluated at 3 months after RT, showed no significant difference in the mediastinal tumor between the two groups, while the remission rate of the pulmonary tumor was higher in the ablation group than in the radiotherapy group. The 2-year OS rate was also not significantly different between the two groups. These results were in accordance with previous findings [32].

Although the 2-year OS rate was not significantly different between the two groups, the lower rate of incidence of RP in the combination group suggested that the patients in the combination group had better quality of life. However to date, it is not clear whether the higher remission rate of the pulmonary tumor and the lower rate of incidence of RP could improve the survival rate, and further studies with longer-term follow-ups are needed to evaluate this hypothesis.

This study has some limitations. The sample size was quite small and was based in a single center so the results need to be confirmed in a larger study. Quality of life and other measures of patient experience of the treatment were not directly studied so should be considered in future to truly evaluate the value of microwave ablation for treatment of NSCLC. The follow-up period of 2 years was quite short. A longer follow-up period may provide some difference between the groups in terms of survival rates.

Percutaneous microwave ablation in combination with mediastinal radiotherapy, with or without chemotherapy, compared to radiotherapy of the pulmonary lesion and mediastinal region, with or without chemotherapy, suggests that both methods achieve similar outcomes in terms of curative effectiveness and two-year OS. However, there was a significantly lower incidence of RP in the patients treated with microwave ablation. These results suggest that microwave ablation should be considered for the treatment of inoperable stage III NSCLCs.

Compliance with ethical standards  

Conflict of interest  

None.

References


Local microwave ablation with continued EGFR tyrosine kinase inhibitor as a treatment strategy in advanced non-small cell lung cancers that developed extra-central nervous system oligoprogressive disease during EGFR tyrosine kinase inhibitor treatment

A pilot study

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Abstract
The non-small cell lung cancer (NSCLC) patients that experienced good clinical response to epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKIs) will ultimately develop acquired resistance. This retrospective study was performed to explore the potential survival benefit of microwave ablation (MWA) therapy in epidermal growth factor receptor (EGFR)-mutant NSCLC that developed extra-central nervous system (CNS) oligoprogressive disease during TKI treatment.

We retrospectively analyzed 54 NSCLC patients with EGFR mutations who showed a clinical benefit from initial EGFR-TKI therapy and developed extra-CNS oligoprogressive disease at our institutions. Twenty eight patients received MWA as a local therapy for the metastatic sites and continued on the same TKIs (MWA group). The following 26 patients received systemic chemotherapy after progression (chemotherapy group). The progression-free survival (PFS1) was calculated from initiation of targeted therapy to first progression. Progression-free survival (PFS2) was defined from first progression to second progression after MWA or chemotherapy. Overall survival (OS) was calculated from the time of diagnosis to the date of last follow-up or death.

The median PFS1 for both groups was similar (median 12.6 vs. 12.9 months, HR 0.63). However, the MWA group patients had a significantly longer PFS2 (median 8.8 vs. 5.8 months, hazards ratio [HR] 0.357) and better OS (median 27.7 vs. 20.0, HR 0.238) in comparison with chemotherapy group. Multivariate analysis and the internal validation identified MWA as the main favorable prognostic factor for PFS2 and OS. In the MWA group, the median PFS2 for complete ablation was significantly longer than that for incomplete ablation (11 vs. 4.2 months, HR 0.29, \( P < 0.05 \)).

MWA with continued EGFR inhibition might be associated with favorable progression-free survival (PFS) and OS in patients with extra-CNS oligometastatic disease. MWA as a local therapy for extra-CNS oligometastatic disease should be considered for NSCLC with acquired resistance to EGFR-TKIs.

Abbreviations:
BS = bone scintigraphy, CT = computed tomography, EGFR = epidermal growth factor receptor, MRI = magnetic resonance imaging, MWA = microwave ablation, NSCLC = non-small cell lung cancer, OS = overall survival, PET/CT = positron emission tomography/CT, PFS = progression-free survival, RECIST = response evaluation criteria in solid tumors, TKI = tyrosine kinase inhibitor.
1. Introduction

Activating epidermal growth factor receptor (EGFR) mutations occur in 10 to 20% of patients with non-small cell lung cancer (NSCLC) in North American and European populations and in up to 60% among Asians populations.\[1\] Treatment of EGFR-mutant NSCLC with specific tyrosine kinase inhibitor (TKI) that target EGFR, such as gefitinib, erlotinib or afatinib, has led to remarkable tumor shrinkage and improvement in progression-free survival (PFS) and quality of life compared with standard chemotherapy.\[2-4\] Based on the results of numerous studies, EGFR sensitizing mutant advanced NSCLC patients should receive first-line epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKIs) treatment.\[3,5-7\]

Despite the initial advancement of these agents, most patients ultimately develop acquired resistance to such TKIs after 1 to 2 years.\[3,4,8,9\] Several resistant mechanisms have been identified, such as T790M missense mutation, amplification of MET, activation of alternative pathways (IGF-1, HGF, PI3CA, AXL), and, in rare instances, transformation to small-cell histology.\[10-16\] Although studies of second-generation, irreversible EGFR-TKIs and other novel agents are ongoing, there are no currently approved targeted therapies specific for treatment of such patients upon progression. Cytotoxic chemotherapy alone used to be the standard therapeutic option at the time of progression while continuation of TKI therapy by itself or in combination with chemotherapy seems to provide continued clinical benefit.\[17-19\] It may be in accordance with the tumor heterogeneity in the development of resistance to targeted therapies and minor tumor cell populations will still be sensitive to the previous EGFR-TKIs.

Different patterns of progressive disease may represent different biological mechanisms. So, it is important to distinguish among these patterns as different therapeutic strategies may apply. Considering the growth rate and number of growing tumour lesions, progressive disease during TKI treatment can be generally distinguished in 3 patterns: intracranial disease progression, development of 1 or few distant metastatic sites while the patient remains asymptomatic, and systematic and/or symptomatic disease progression.\[20,21\] The first two fall into the general term of oligoprogressive disease. Typically, the definition of oligoprogressive disease refers to the presence of fewer than 5 discrete metastatic sites. Local therapy with continued EGFR inhibition has been shown to be effective for treating patients with oligoprogressive disease and associated with long PFS and OS.\[22,23\] As a new thermal ablation method, microwave ablation (MWA) for NSCLC is likely to be increasingly used, given its theoretical advantages over radiofrequency ablation (RFA) including a less severe heat sink effect and faster, greater heating.\[24\] We previously reported that advanced and medically inoperable stage I NSCLC could benefit from MWA therapy. Major complications as a result of MWA are rare and tolerable.\[25-28\] Therefore, we performed the retrospective multicenter study to explore the potential benefit of MWA in treating EGFR acquired resistance NSCLC with extra-CNS oligoprogressive disease.

2. Materials and methods

2.1. Patients and eligibility

We conducted a retrospective multicenter study at Shandong Provincial Hospital Affiliated to Shandong University, Sun Yat-sen University Cancer Center, Jinan Military General Hospital of Chinese People’s Liberation Army and Weifang People’s Hospital Affiliated to Weifang Medical University between March 2010 and January 2016. Patients eligible for inclusion in this retrospective analysis included histologically or cytologically proven EGFR-mutant NSCLC treated with erlotinib or gefitinib, objective clinical benefit from erlotinib or gefitinib monotherapy, experienced progression of disease despite the maintenance of erlotinib or gefitinib. EGFR mutations (exon 19 deletions or exon 21 L858R mutations) were examined either through direct sequencing or allele-specific polymerase chain reaction assays. Objective clinical benefit of EGFR-TKIs was defined by either: complete or partial response (CR or PR), or durable stable disease (≥6 months) according to the criteria for acquired resistance proposed by Jackman et al.\[9\] We collected initial baseline clinical characteristics including sex, age at diagnosis, smoking status, tumor histology, prior therapy, date of diagnosis of any known extra-CNS involvement and sites of metastatic disease of all enrolled patients by retrospective collection from electronic records.

This study was performed in accordance with the declaration of Helsinki and the ethical guidelines and regulations of China. The protocol was approved by the ethics committees of each center before the initiation of enrollment. Written informed consent was obtained from all patients before enrollment in the study.

2.2. Treatment and follow-up

All EGFR-mutant patients received erlotinib or gefitinib starting at 150 mg or 250 mg once a day. Patients received routine chest computed tomography (CT) every 1 to 2 months to assess the local response according to the response evaluation criteria in solid tumors.\[25\] Additional procedures including CT, magnetic resonance imaging, bone scintigraphy and positron emission tomography/CT were applied to evaluate extrapulmonary symptoms and metastatic sites. All patients randomly received MWA or chemotherapy after progression on EGFR-TKIs. In the MWA group, once the acquired resistance was identified patients received MWA followed by the same targeted therapy. After 1 month of MWA, a new baseline for surveillance was established. At 1, 3, 6, 9, and 12 months after MWA, patients received CT scan to assess their response to treatment and to identify adverse events. The patients then underwent imaging every 3 months surveillance thereafter. Sites of first progression were documented. Patients in the chemotherapy group received 2 to 6 cycles of platinum-based systemic chemotherapy followed by the same EGFR-TKI after acquired resistance, and detailed chemotherapy regimens are listed in the Table S1, http://links.lww.com/MD/B67. Chemotherapy was paused until disease progression or unacceptable toxicity.
2.3. MWA procedure

Instrumentation: (1) MTC-3C microwave ablation system (Nanjing Qi Ya Research Institute of Microwave Electric, China. Registration standard: YZB/country 1408–2003. No: SFDA (III) 20073251059) or (2) ECO-2450B microwave ablation system (Nanjing, ECO Microwave Institute, China. Registration standard: YZB/country1475–2013. No: SFDA (III) 20112251456) was used for MWA treatment. Generally, we set the microwave emission frequency at 2450±50MHz and ablation power between 60 and 80 W. The effective length of the antenna is 100 to 180 mm and 14G to 20G outside diameter. In addition, a water circulation cooling system was applied to reduce the antenna surface temperature. For tumors ≤3.3 cm and 3.6 to 5.0 cm, we used single or double antennae, respectively [25,26,30]. The whole procedures of MWA were performed under CT guidance and the detailed procedures were described in our previous publications [25,26]. All patients received a CT scan immediately after MWA to monitor the shape and size of the lesions, as well as to determine any potential complications including pneumothorax, bleeding or others. The proposed ablative margin was 0.5 cm.

2.4. Statistical analysis

Pearson’s Chi-square (or Fisher’s exact) and t-test were used to determined differences between groups. Median progression-free survival (PFS1) was calculated from time of initiation of targeted therapy to first progression of disease or clinical progression (as assessed by clinician). PFS2 was defined from the time of first progression to second progression after MWA or chemotherapy. OS was calculated from the time of diagnosis to the date of last follow-up or death from any case. Survival curves were estimated using Kaplan–Meier method. Patients who did not experience progression or death during the study time were censored at the time of the last available follow-up. Univariable and multivariable Cox proportional hazards regression models were used to assess the association between each of the variables and PFS2 or OS. For the multivariable analysis model, we include treatment strategy variable, which was statistically significant on univariable analysis.

We used bootstrapping for bias correction (n = 1000 bootstrap samples). Random samples (sample size was equivalent to original cohort) were drawn from the original data set with replacement. Then, we applied the same Cox model, which was derived from the original cohort to the bootstrap samples. One thousand bootstraping-based model performance indices were independently generated by repeating this process 1000 times. The mean value of the 1000 performance indices is the bias-corrected estimate, which is considered to estimate the nomogram performance that could be expected in a separate but similar patient population. The average and standard deviation of these 1000 bootstrap samples of the regression parameter were calculated as the bootstrap estimate and standard error (SE) of the parameter. Statistical analysis was performed using SPSS for Windows Version 17.0 (IBM, Chicago, IL) and R software version 3.1.1 (The R Foundation for Statistical Computing). All analyses were two sided with a \(P\)-value < 0.05 considered statistically significant.

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>MWA group (n = 28)</th>
<th>Chemotherapy group (n = 26)</th>
<th>( \chi^2/t )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>11 (39.3)</td>
<td>7 (26.9)</td>
<td>0.927</td>
<td>0.336</td>
</tr>
<tr>
<td>Age</td>
<td>61.5±11.3</td>
<td>59.8±10.5</td>
<td>0.568</td>
<td>0.572</td>
</tr>
<tr>
<td>Smoking history</td>
<td>4 (14.3)</td>
<td>4 (15.4)</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Stage at initial diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I–II</td>
<td>3 (10.7)</td>
<td>1 (3.8)</td>
<td>3.073</td>
<td>0.381</td>
</tr>
<tr>
<td>III</td>
<td>3 (10.7)</td>
<td>2 (7.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>22 (78.6)</td>
<td>23 (88.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR mutation type</td>
<td></td>
<td></td>
<td>0.271</td>
<td>0.673</td>
</tr>
<tr>
<td>Exon 19 deletion</td>
<td>14 (50.0)</td>
<td>12 (46.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 21 L858R</td>
<td>11 (39.3)</td>
<td>10 (38.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (10.7)</td>
<td>4 (15.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best response to TKI</td>
<td></td>
<td></td>
<td>1.305</td>
<td>0.521</td>
</tr>
<tr>
<td>Complete response</td>
<td>1 (3.6)</td>
<td>2 (7.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial response</td>
<td>17 (60.7)</td>
<td>18 (69.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>10 (35.7)</td>
<td>6 (23.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site of metastatic disease after targeted therapy</td>
<td></td>
<td></td>
<td>7.317</td>
<td>0.198</td>
</tr>
<tr>
<td>Lung</td>
<td>15 (53.6)</td>
<td>14 (53.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>2 (7.1)</td>
<td>1 (3.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>4 (14.3)</td>
<td>3 (11.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>5 (17.9)</td>
<td>2 (7.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pleura</td>
<td>2 (7.1)</td>
<td>2 (7.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>0 (0)</td>
<td>4 (15.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EGFR = epidermal growth factor receptor, MWA = microwave ablation, TKI = tyrosine kinase inhibitor.
(<5 sites of disease) at the time of PFS1 and the pattern of progression for these patients is also summarized in Table 1. There was no significant difference in age, sex, smoking history, stage at diagnosis, best response to TKI, and sites of metastatic disease between the MWA group and chemotherapy group.

### 3.2. Treatment procedures and complications after MWA

All patients with extra-CNS oligoprogressive disease received MWA followed by continuation of targeted therapy in the present study. The median time from the first progression to MWA was 3 weeks. Thirty-one metastatic sites in 28 cases were ablated. The mean size of the metastatic sites was 3.1 cm (range, 1–6.4 cm). Most MWAs were well tolerated. No patient died during the procedure or within 30 days after MWA. Pain was the common side effect and 10 patients (35.7%) suffered moderate pain after MWA, but no severe post-ablation pain occurred. Post-ablation syndrome including fever (under 38.5°C), fatigue, general malaise, nausea, and vomiting, etc. occurred in 9 patients. Postoperative pneumothorax was observed in 10 patients (35.7%), in which 2 of them received chest tube drainage. Five patients (17.8%) had pleural effusion (of which 1 case underwent chest tube insertion) after the MWA. Hemoptysis occurred in 2 cases (7.1%) and the conventional application of hemostatic agents including snake venom thrombin, glucocorticoids, could effectively stop bleeding. The average length of hospital stay was 4.3 days (3–17 days).

### 3.3. Outcomes

The median follow-up for this study was 23.5 months (range, 7–70 months). Among the 28 patients in the MWA group, 11 of them progressed after MWA during the study period, and 7 patients died. While 21 of 26 patients in the chemotherapy group died. The difference of PFS1 in both groups was not significant (12.6 months vs. 12.9 months, HR 0.63, CI 0.350–1.142, P = 0.13) (Fig. 1A). However, the patients in MWA group had a significantly longer PFS2 than those in chemotherapy group (8.8 months vs. 5.8 months, HR 0.357, CI 0.169–0.751, P = 0.007) (Figs. 1B and 2). In addition, the OS in MWA group was also longer than that in chemotherapy group (27.7 months vs. 20.0 months, HR 0.238, CI 0.112–0.505, P = 0.0002) (Fig. 1C).

The variables listed in Table 1 were examined (Cox Regression) for their association with PFS2 and OS. Results of the univariate analyses are shown in Table 2. Treatment strategy with univariate significance (P < 0.05) was selected for the multivariate analysis, and the detailed results and the hazard ratios of the multivariate analysis estimated by Cox regression are listed in Table 2. We confirmed the results of the multivariate analysis by using the bootstrap resampling technique mentioned above. We observed the closeness of the bootstrap estimates to those obtained in the final Cox model, which validated the final results (Table 3).

In the MWA group, the overall complete ablation rate achieved 78.6% (22 of 26). The median PFS2 for complete ablation was significantly longer than that for incomplete ablation (11 vs. 4.2 months, hazard ratio: 0.2874, 95% CI of the ratio: 0.062–1.339, P < 0.05). The difference of OS between the two groups was not significant (39 vs. 26.4, P > 0.05) (Fig. 3).
Treatment Variables

Multivariable Cox regression analysis for PFS2 and OS

4. Discussion

The past decade witnessed the dramatic alteration in the treatment of NSCLC with the identification of somatic gene mutations that underlie tumor initiation and maintenance. EGFR mutations were first identified in lung cancer after clinical benefit to EGFR tyrosine kinase inhibitors was observed. First-line TKI therapy is recommended in NSCLC harboring an EGFR mutation. Unfortunately, most patients who initially benefit from erlotinib or gefitinib ultimately developed acquired resistance to these drugs, and there are limited options for these patients. Patients treated with erlotinib or gefitinib monotherapy in this study had a median PFS1 of 12.6 months, which is similar with that of chemotherapy group (12.9 months) and consistent with the data of East Asian patients, indicating that we do not seem to have with literature precedent, especially with the data of East Asian patients.

Additional disease control was observed. However, there is little published data about the use of MWA for oligoprogressive therapies is suitable for local ablative therapy and continuation of the targeted agents, and is associated with more than 6 months of additional disease control.[22] Moreover, Yu et al.[23] reported EGFR-mutant lung cancers with acquired resistance to EGFR-TKI therapy are amenable to local therapy including surgery or radiation followed by continued targeted therapy, and 10 months additional disease control was observed. However, there is little published data about the use of MWA for oligoprogressive disease progression (oligoprogressive disease), local therapy to these sites with surgery or radiation, in combination with ongoing use of the same TKI, might also be clinically beneficial. It was recently reported that Oncogene-addicted NSCLC with CNS and/or extra-CNS oligoprogressive disease on relevant targeted

Table 2

Results of Cox regression analyses.

Univariate Cox regression analysis for PFS2 and OS

Table 3

Summary of variables for 1000 bootstrap samples.

PFS2 OS

Treatment

Figure 3. Kaplan-Meier survival curves of PFS2 and OS between complete ablation and incomplete ablation. The median PFS2 was 11 months for complete ablation and 4.2 months for incomplete ablation (HR 0.287, CI 0.536–0.657, P < 0.05). CI = confidence interval, HR = hazard ratio, OS = overall survival.
disease on therapy. This is the first study to evaluate the efficacy and safety of MWA as a local therapy for patients with TKI acquired resistance. In this study, the MWA group had a similar PFS1 with that of chemotherapy group (control group). However, the PFS2 and OS were significantly longer in patients of MWA group compared with chemotherapy group. Our data suggest that MWA in combination with continuation of the TKIs may be associated with longer PFS and OS in patients with EGFR-mutation on targeted therapy who developed less than two systemic progressive lesions. These results expand on the role of local therapy in EGFR-mutant NSCLC that developed extra-CNS oligoprogressive disease during TKI treatment and suggest that these patients may benefit from the MWA.

The mechanism of acquired resistance to EGFR-TKIs in NSCLC is still not well understood. Several molecular mechanisms have been elucidated and generally can be classified into 3 main categories: alterations in EGFR itself, activation of bypass signaling pathways, and phenotypic transformation.\(^{[34]}\)

The most frequent mechanism of acquired resistance to EGFR-TKIs is the secondary T790M mutation in EGFR which is identified in approximately 50 to 60% of patients. However, in metastatic cancers, biological heterogeneity among tumour cells pre-exists at the time of clinical presentation and tumours resistant to EGFR-TKIs may be composed of a heterogeneous mix of TKI-sensitive and TKI-resistant cells. Continuation of targeted therapy can lead to ongoing benefit in nonprogressing clones, which have not developed acquired resistance. Our data indicated that the efficacy of MWA together with continuation of TKIs may be superior to TKIs treatment alone, suggesting local microwave ablative therapy of the resistant clone before widespread dissemination is associated with prolonged disease control.

In the present study, the most common complication for MWA therapy was pneumothorax, followed by pleural effusion and hemothorax. Complications can be controlled by proper treatments and there is no patient died during or within 30 days after the procedure. Therefore, MWA is a safe and well tolerable strategy for NSCLC patients.

Certainly, our study does have some limitations. The main limitation of this study lies in its retrospective nature and relatively small number of treated patients. Further limitations include the absence of pathological proof in metastatic diseases and identification of the acquired resistance molecular characteristics. Prospective multicenter evaluation of MWA with large sample size is needed to define more clearly the effectiveness and safety of this treatment strategy.

Despite of these limitations, our results show that MWA with continued EGFR inhibition may extend disease control by over 8 months in patients with oligoprogressive disease. MWA as a local therapy for oligoprogressive disease should be considered for NSCLC with acquired resistance to EGFR-TKIs.

References


Effectiveness of Ultrasound-guided Percutaneous Microwave Ablation for Symptomatic Uterine Fibroids: A Multicenter Study in China

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Effectiveness of Ultrasound-guided Percutaneous Microwave Ablation for Symptomatic Uterine Fibroids: A Multicenter Study in China

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Effectiveness of Ultrasound-guided Percutaneous Microwave Ablation for Symptomatic Uterine Fibroids: A Multicenter Study in China

Abstract

Purpose: To evaluate the clinical efficacy of ultrasound-guided percutaneous microwave ablation therapy (PMWA) for symptomatic uterine fibroids in a multicenter study.

Materials and Methods: Patients with symptomatic uterine fibroids who underwent PMWA at multiple treatment centers in China between January 2013 and August 2015 were prospectively studied to compare the reduction rate of uterine fibroids, hemoglobin level and uterine fibroid symptom and health-related quality of life questionnaire (UFS-QOL) scores before and at 3, 6, and 12 months after ablation.

Results: A total of 311 patients (405 leiomyomas) from 8 treatment centers underwent the treatment (age, 29–55 years; mean ± SD, 41 ± 5.11 years). The mean diameter of the myomas ranged from 2.03 to 12.50 cm (mean, 5.10 ± 1.28 cm) and the volume ranged from 4.40 to 1022.14 cm³ (mean, 95.01 ± 70.29 cm³). Forty-eight myomas were identified as FIGO type 1/2 fibroids, 256 as type 3/4 fibroids, and 101 as type 5/6 fibroids. The mean ablation rate was 86.6% (54.0%–100%). The mean reduction rate was 63.5%, 78.5% and 86.7% at 3, 6, and 12 months posttreatment, respectively. The hemoglobin level increased significantly from 88.84 ± 9.31 g/L before treatment to 107.14 ± 13.32 g/L, 116.05 ± 7.66 g/L, and 117.79 ± 6.51 g/L at 3, 6, and 12 months posttreatment, respectively (P = 0.000). The SSS and HRQL scores were also significantly improved posttreatment compared with before treatment (P = 0.000).

Conclusion: PMWA is an effective, minimally invasive treatment for symptomatic leiomyomas that can significantly improve the quality of life of patients.

Keywords: leiomyoma; microwaves; ablation; ultrasonography; interventional; multicenter study
Introduction

Uterine leiomyoma is the most common gynecologic benign tumor. Uterine fibroids are the most common myomas in women of childbearing age, with a reported incidence of 20–77% [1, 2]. Most myomas are asymptomatic, but they can cause certain significant symptoms such as menorrhagia, secondary anemia and pelvic pressure. At present, the primary treatment methods are administration of gonadotropin-releasing hormone analogs, myomectomy, hysterectomy and uterine artery embolization. In addition, as an ever-expanding alternative, thermal ablation therapy includes microwave ablation, high-intensity focused ultrasonography, and radiofrequency ablation [1, 3]. Patients who wish to preserve the uterus opt for minimally invasive therapies such as uterine artery embolization and in situ ultrasound (US)-guided thermal ablation.

US-guided percutaneous microwave ablation (PMWA) therapy utilizes electromagnetic microwave heat to induce cellular death via coagulation necrosis. Single-center research studies have confirmed the safety and efficacy of this treatment; it has been shown that this treatment is effective, minimally invasive and uterus-conserving with effective relief of clinical symptoms after therapy [4-11]. However, no multicenter study with a large sample has been conducted to confirm the conclusions of these single-center investigations. To fill this gap, we have conducted a prospective study across multiple treatment centers in China to evaluate the clinical efficacy of PMWA for the treatment of uterine fibroids.

Materials and Methods

This study has been approved by the Ethics Committee of the Chinese PLA General Hospital (ratification No. 2012036, registration No. ChiCTR-ONC-12002968).

Enrollment criteria for the treatment centers

One of the criteria was that the staff at the hospital should be trained in standard operating procedures and data collection at the Interventional Ultrasound Department of the Chinese PLA General Hospital (the standard procedures are set by multiple centers together). The second criterion was that the annual number of uterine fibroid
cases treated at each center should be at least 20.

**Patient enrollment criteria**

Patients diagnosed with uterine fibroids through MRI and US at each treatment center were recruited between January 2013 and August 2015. The inclusion criteria were (1) leiomyoma-related symptoms such as menorrhagia, secondary anemia, and urinary frequency; (2) a strong desire to preserve the uterus; (3) no response to medication or other conservative treatment; (4) no use of hormonal drugs within the first 3 months of ablation; (5) absence of peri-menopausal signs; and (6) availability of a safe abdominal puncture path. The exclusion criteria were (1) menstruation, pregnancy, or lactation; (2) plans for future pregnancy; (3) fibroids that grew rapidly in the short term; (4) cervical intraepithelial neoplasia (CIN) III; (5) malignant neoplasm of any organ; (6) uncontrolled acute pelvic inflammation; (7) dysfunction of vital organs (e.g., heart, brain, liver or kidney); and (8) severe coagulation disorder.

**Instruments**

The instruments used were a KV2000 MW tumor coagulator (Nanjing Kangyou Medical Instruments Co., China) and an ECO-100C Microwave System (Nanjing ECO Microwave System Co. Ltd., China).

**Sonography system**

The Acuson Sequoia 512 (Siemens AG, Germany), Acuson S2000 (Siemens AG, Germany), LOGIQ E9 (General Electric Company, America), MyLab_Twice (Esaote S.p.A., Italy), and HI_VISION_Preirus (Hitachi, Ltd., Japan) were used.

**Contrast agent**

SonoVue (Bracco, Italy) was used.

**Preparation for the procedure**

(1) Before ablation, all the patients were required to understand the principles, plan, expected effects and complications of the treatment and to provide their consent by signing an informed consent form. (2) Patients underwent routine blood, urine and stool tests; a test to measure bleeding and clotting time; electrocardiography; chest radiography; contrast-enhanced MRI; and the thin-cytologic test. (3) Conventional ultrasonography and contrast-enhanced ultrasonography (CEUS) were performed to
assess the position, size and blood supply of the myomas.

**Methods used to induce anesthesia**

Anesthesia was induced via intravenous conscious sedation (induction: midazolam 1.0 mg, sufentanil 10 µg, propofol 1.0 mg/kg; maintenance: propofol 4-10 mg/kg·h) and local infiltration (2% lidocaine 3.0-5.0 ml).

**Treatment procedures**

Patients adopted a supine position for the treatment. Under US guidance, one or two microwave antennas were inserted into the fibroid, depending on its size. The output energy of the microwave was set to induce ablation. The entire ablation process was monitored via real-time ultrasonography. The ablation procedure was discontinued when the hyper-echo (caused by microbubbles during microwave emission and roughly representing the ablation field [12]) covered the entire nodule. CEUS was performed immediately to preliminarily evaluate the ablation effect. If the contrast enhancement was found to be within the lesion, supplemental ablation was immediately performed.

**Effectiveness assessment**

1. **Assessment of the ablation effect:** The myoma length, width and height were measured via CEUS before ablation and immediately after ablation; then, the volume of the fibroid and the ablation zone were calculated. The formulae were as follows:
   
   \[ \text{Mean diameter} = \frac{(\text{length} + \text{width} + \text{height})}{3}; \]
   
   \[ \text{Volume} = \frac{4}{3} \times \pi \times r^3, \text{ where } r \text{ is the mean radius (mean diameter/2)}; \]
   
   \[ \text{Ablation rate} = \frac{\text{volume of the non-enhanced field in the lesion immediately after ablation}}{\text{volume of the lesion before ablation}} \times 100\%. \]

2. **Assessment of the clinical effect:**
   
   (1) **Assessment times:** 3, 6, and 12 months after ablation
   
   (2) **Assessment indicators:**

   1) Fibroid volume: Calculated via conventional ultrasonography according to the above formulae for Mean diameter and Volume.

   \[ \text{Fibroid reduction rate} = (\text{Volume before therapy} - \text{Volume after} \]

   3, 6, and 12 months after ablation.
therapy)/\textit{Volume} before therapy \times 100\%.

2) Hemoglobin levels before and after treatment.

3) The uterine fibroid symptom and health-related quality of life questionnaire (UFS-QOL) as reported by Spies et al. in 2002 [13]. This instrument includes a symptom severity score (SSS) and a health-related quality of life (HRQL) score. The patients completed the questionnaire by themselves before and after treatment.

\textbf{Statistical analysis}

Each treatment center uploaded monthly data of the eligible via e-mail. All statistical analyses were performed using SPSS version 17.0. The quantitative data were described as means ± SD and were tested using the \textit{t}-test if the data followed a normal distribution; if not, the Wilcoxon signed rank sum test was used. \(P < 0.05\) was considered to indicate statistical significance.

\textbf{Results}

\textbf{General characteristics}

Eight Chinese treatment centers treating a total of 344 patients were included. Six patients were excluded due to the lack of a safe window. Twenty-three of the enrolled patients were lost to follow-up. Therefore, 311 patients were ultimately included in the statistical analysis. The age of the patients ranged from 29 to 55 years (mean, 41 ± 5.11 years). Two hundred thirty-two patients had one fibroid, 48 patients had two fibroids, and 31 patients had three or more fibroids. A total of 405 leiomyomas were treated. Thirty-seven leiomyomas were not treated because their mean diameter was less than 2 cm. The mean diameter ranged from 2.03 to 12.50 cm (mean ± SD, 5.10 ± 1.28 cm), and the volume ranged from 4.40 to 1022.14 cm\(^3\) (mean ± SD, 95.01 ± 70.29 cm\(^3\)). Among all leiomyomas, 48 were identified as FIGO type [14] 1/2 fibroids, 256 as type 3/4 fibroids, and 101 as type 5/6 fibroids. Two patients with large fibroids (mean diameters of 12.5 cm and 10.8 cm) and two patients with more than 10 fibroids were treated twice to ensure that the ablation was successful. The other patients received only one round of treatment, during which the lesion was completely ablated.
The CEUS examination conducted after ablation showed no enhancement in the ablation area, but a circular enhancement was observed at the periphery of the fibroid (Figure 1). The time to discharge ranged from 4 to 7 days (mean ± SD, 5.43 ± 0.89 days). For 46 patients, discharge of necrotic masses from the vagina occurred 7–270 days after ablation. Among these patients, 21 had type 1/2 fibroids and 25 had type 3/4 fibroids. The mean volume of the mass was 6.91 ± 8.73 cm$^3$ and the volume of the largest mass was 32.97 cm$^3$. The masses were discharged entirely in ten patients with type 1/2 fibroids and four patients with type 3/4 fibroids, while masses were discharged partially in the other patients.

**Effectiveness of the ablation**

The mean ablation rate of the myomas was 86.6% (54.0–100%).

**Clinical efficacy**

The fibroid volumes, hemoglobin levels, and UFS-QOL scores are shown in Table 1. The differences in these parameters between baseline and posttreatment were statistically significant (P < 0.05). The mean myoma reduction rate was 63.5%, 78.5% and 86.7% at 3, 6, and 12 months after treatment, respectively.

**Complications and side effects**

No major complications occurred during ablation and follow-up. Twenty-seven patients (8.68%) experienced grade 1 (Common Terminology Criteria for Adverse Events (CTCAE) v4.0 of the National Cancer Institute) lower abdominal pain, which can be tolerated and which disappeared within 12 hours. Nineteen patients (6.11%) had a small amount of vaginal secretion. These secretions resolved within 20 days after ablation.

**Discussion**

PMWA is a minimally invasive treatment that involves in situ inactivation of tumors. In response to the applied electrical field of microwaves, the rotation of dipole molecules and ionic polarization produce friction and heat, thus inducing cellular death via coagulation necrosis [15]. This technology has been widely used to treat solid tumors of the liver, spleen, thyroid, and other organs [16-18] [19-24]. In 2011,
Zhang et al. [5] studied PMWA therapy for uterine fibroids and confirmed its safety and efficacy.

To avoid the subjective bias of a single-center study, this study summarizes data from multiple treatment centers and objectively evaluates the clinical efficacy of PMWA. The effectiveness of ablation was evaluated via CEUS. At present, contrast-enhanced MRI is considered the gold standard for evaluating the effect of thermal ablation. A pathological study showed that the coagulated necrotic area corresponded to the zone of non-contrast enhancement [15, 25]. CEUS can objectively reflect perfusion of the microcirculation in tissue. Studies have shown high consistency between CEUS and contrast-enhanced MRI with regard to evaluation of the ablation effect, and CEUS could therefore be used as an alternative to contrast-enhanced MRI for evaluation of the ablation zone [26, 27]. Moreover, CEUS is a convenient, real-time, dynamic procedure, so residual lesions can be found immediately after the treatment, after which further ablation treatment can be performed.

Uterine leiomyoma is a benign lesion, so the main purpose of treatment is to relieve clinical symptoms and improve patient quality of life. In this study, the mean ablation rate was 86.6%. In some fibroids, the ablation rate was less than 60%. This is because only partial ablation was performed in some cases depending on the location and size of the fibroid; in particular, complete ablation was not performed in the case of fibroids that were in a relatively unsafe position such as close to the bowel or bladder. Partial ablation was primarily undertaken to ease clinical symptoms and avoid serious complications.

The volume of the myomas decreased significantly after treatment. The reduction rate at 6 months after ablation was 78.5%, which is consistent with that of a previous single-center study [5]. The reduction rate at 12 months was 86.7%, which is slightly lower than the rate of 93.1% reported previously but higher than that of 74.5% reported over the same period for intramural leiomyomas [11]. This is because the percentage of patients with complete sarcoid discharges in the present study was 4.50%, which is lower than the percentage of 10.0% reported in a previous study [5].

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Type 1/2 myomas have a higher reduction rate because necrotic tissue can be discharged through the natural passage of the uterine cavity (the vagina), which is not possible for type 5/6 and most type 3/4 myomas, as there is no route of passage outside the body.

Improvement in clinical symptoms and quality of life is an important indication of treatment efficacy. Abnormal uterine bleeding is reported to be primarily relevant to the position of the myomas, especially type 1/2 myomas that are in or partially intruding into the endometrial cavity. Pelvic pressure increases with an increase in the size of a myoma, and decreases in urinary frequency and urgency are observed following a decrease in myoma volume after therapy [1, 2, 28-30]. After PMWA treatment, the fibroid volume decreased significantly and the area of the endometrium of the uterus also decreased, resulting in the relief of clinical symptoms such as menorrhagia. Thus, the hemoglobin level was significantly increased, pelvic pressure was reduced, and the SSS scores were significantly decreased. Therefore, patient quality of life was improved and the HRQL scores were significantly increased. At 12 months after treatment, the SSS and HRQL scores of the patients were similar to those of healthy women (22.5 ± 21.1 and 86.4 ± 17.7, respectively) [13]. This finding confirms the findings of single-center studies, as the clinical efficacy of PMWA has been shown to be reliable.

During ablation and 12 months of follow-up, no major complications occurred. The discharge of necrotic tissue was considered to be a cure for the disease [7]. Vaginal secretion could be caused by liquefaction of the necrotic tissue and by irritation and inflammation of the endometrium.

In summary, PMWA is a convenient, effective, and minimally invasive therapy for FIGO type 1–6 uterine fibroids. PMWA has the added advantage of rapid recovery, and it significantly improves patient quality of life. These characteristics make PMWA therapy worthy of popularization and application as a minimally invasive treatment method for uterine fibroids.
Declaration of interest

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References


Table 1. Fibroid volumes, hemoglobin levels, and UFS-QOL scores before and after ablation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>Pre-ablation</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
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<tr>
<td>V (cm³)</td>
<td>95.01 ± 70.29</td>
<td>36.85 ± 31.21*</td>
<td>26.12 ± 23.50*</td>
<td>17.30 ± 16.98*</td>
<td></td>
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<tr>
<td>Hb (g/L)</td>
<td>88.84 ± 9.31</td>
<td>107.14 ± 13.32*</td>
<td>116.05 ± 7.66*</td>
<td>117.79 ± 6.51*</td>
<td></td>
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<tr>
<td>SSS</td>
<td>49.22 ± 14.35</td>
<td>20.96 ± 9.95</td>
<td>16.70 ± 8.51</td>
<td>13.14 ± 6.10</td>
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<tr>
<td>HRQL</td>
<td>58.37 ± 14.56</td>
<td>78.66 ± 13.16*</td>
<td>84.62 ± 8.71*</td>
<td>87.71 ± 7.00*</td>
<td></td>
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*P < 0.001 (compared with the pre-ablation value)

V = tumor volume, Hb = hemoglobin level, SSS = symptom severity score, HRQL = health-related quality of life score
Figure 1. Examinations of a patient with fibroids before and after ablation.

(A) MRI (T2WI) before ablation showed the fibroid. (B) Before ablation, the CEUS image showed non-homogeneous enhancement within the fibroid. (C) During ablation, microwave energy diffused from the antenna tip. (D) After ablation, no contrast enhancement was observed in the ablation area. (E) Contrast-enhanced MRI three days after ablation showed the ablation zone.
RESEARCH ARTICLE

Evaluation of the Therapeutic Efficacy of Sequential Therapy Involving Percutaneous Microwave Ablation in Combination with $^{131}$I-Hypericin Using the VX2 Rabbit Breast Solid Tumor Model

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Abstract

Purpose

Combination of percutaneous microwave ablation (PMWA) and intravenous injection of $^{131}$I-hypericin (IIIH) may bear potential as a mini-invasive treatment for tumor. The objective of this study was to assess the effect of PMWA and IIIH in breast tumor growth.

Methods

Ten New Zealand White rabbits bearing VX2 breast carcinomas were randomly divided into two groups (each 5 examples) and processed using PMWA followed by IIIH and IIIH alone. The IIIH activity was evaluated using planar scintigraphy, autoradiography and biodistribution analysis. The maximum effective safe dose of IIIH was found through 48 rabbits with VX2 breast tumor, which were randomized into six groups (n=8 per group). Subsequently, a further 75 rabbits bearing VX2 breast solid tumors were randomly divided into five groups (each 15 examples) and treated as follows: A, no treatment group; B, PMWA alone; C, IIIH alone; D, PMWA+IIIH×1 (at 8 h post-PMWA); and E, PMWA+IIIH×2 (at 8 h and at 8 days post-PMWA). The therapeutic effect was assessed by measurement of tumor size and performation of positron emission tomography/computed tomograph (PET/CT) scans, liver and renal function tests and Kaplan-Meier survival analysis.

Results

The planar scintigraphy findings suggested a significant uptake of $^{131}$I in necrotic tumor tissue. The autoradiography gray scales indicated higher selective uptake of IIIH by necrotic tissue, with significant differences between the groups with and those without necrotic tumor tissue ($P<0.05$). The maximum effective safe dose of IIIH was 1mCi/kg. The PET/CT scans and tumor size measurement suggested improvements in treatment groups at all time points ($P<0.01$). Significant differences were detected among Groups A, B, D and E.
Lower levels of lung metastasis were detected in Groups D and E ($P < 0.05$). There were no abnormalities in liver and renal functions tests or other reported side effects.

**Conclusion**

IIIH exhibited selective uptake by necrotic tumor tissue. Sequential therapy involving PMWA+IIIH was successfully inhibiting tumor growth and prolonging survival.

**Introduction**

Overall survival was not significantly different between breast conserving treatment and radical mastectomy in stage II breast, which was verified by EORTC 10801 trial [1]. Breast conserving surgery is currently accepted as the therapy of choice by numerous surgeons and patients. It has been confirmed that breast conserving surgery followed by postoperative breast adjuvant radiotherapy is the normal treatment for proper patients with early breast tumor. However, the cosmetic results and complications that occur after the use of the standard breast-conserving therapy protocol, which consists of lumpectomy and a 6-week adjuvant radiotherapy course [2–4], pose a major concern [5]. Therefore, there is a need for a more minimally invasive treatment protocol.

Compared with traditional surgical therapy, the image-guided ablative therapies, which contain microwave ablation, radiofrequency ablation, laser ablation, high-intensity focused ultrasound and cryoablation, have distinct advantages that cover superior cosmetic results, less complications and mortality, cooperativity with other cancer treatments, the capacity to complete ablative treatment in an outpatient basis, convenience for simultaneous imaging monitoring, smaller expense of treatment and reproducible [6]. Compared to the other thermal ablative modalities, percutaneous microwave ablation (PMWA) has particular advantages, such as preferable heating of cystic masses, bigger tumor ablation range, uniformly higher temperatures inside the tumor, shorter ablation duration, advanced convection profile, capacity to use multiple applicators and minor pain [7–10]. At present, PMWA has been extensively used for the treatment of tumors located in the lung, liver, bone, adrenal gland and kidney. Moreover, due to the special anatomical location of breast where is between ectopectoralis and skin, microwave ablation is more suitable for breast tumor than others solid tumors. We have proved that US-guided PMWA is a promising treatment for solitary breast cancers [11].

Similar to the standard process that breast conservation therapy has to be followed by intraoperative or postoperative radiotherapy [4], the microwave ablation also has to be followed by a postoperative radiotherapy or others ionization therapy. Compared to conventional postoperative adjuvant radiotherapy, intraoperative radiotherapy has the probability to be a hopeful modality for early breast cancer treated using breast conserving therapy, attributable to decrease of the normal tissues exposure to radiation, reduction of the radiotherapy duration and results in a lower local recurrence rate [12,13]. Tumor necrosis therapy (TNT) which was first reported by Epstein et al. was that $^{131}$I-labeled monoclonal antibodies to intracellular antigens, which are integral structural components and are retained by degenerating cells, can kill the viable tumor cell around the necrosis tumor tissue [14]. Therefore, the use of tumor necrosis therapy (TNT) after the completion of the microwave ablation may has the same effect as intraoperative radiotherapy.

Hypericin that is the main component of *Hypericum perforatum* has been widely used in folk medicine. Hypericin can be extracted from the common St. JohnsWort (*Hypericum perforatum*).
species), as well as be synthetised from the anthraquinone derivative emodin. Hypericin was recently shown to be an agent exhibiting a specific affinity for necrotic tissue that is potentially useful for tumor necrosis therapy (TNT) \[15\]; Therefore, radiolabeled hypericin can be used with targeted radiotherapy to target the surviving tumor tissue adjacent to necrotic tissue \[15\], exerting an effect similar to that of intraoperative radiotherapy following surgical resection.

Collectively, therapeutic efficacy of sequential therapy involving percutaneous microwave ablation in combination with \(^{131}\)I-hypericin was evaluated by a rabbit VX2 breast solid tumor model.

**Materials and Methods**

**Experimental design**

Initially, 10 rabbits bearing the VX2 breast solid tumor were randomly divided into two groups (each 5 examples) by picking numbers out of a hat; PMWA group was treated using PMWA followed by injection of \(^{131}\)I-hypericin (IIIH), and the control group was treated using IIIH alone. Subsequently, planar scintigraphy, biodistribution analysis and autoradiograph were performed to verify the affinity of IIIH for necrotic tumor tissue (Fig. 1A).

To find the maximum effective safe dose of IIIH, 48 rabbits bearing VX2 solid tumors were randomized into six groups (n = 8 per group). Then the rabbits pretreated by PMWA were treated by IIIH of various density (0.125mCi/kg, 0.25mCi/kg, 0.5mCi/kg/kg, 1mCi/kg, 2mCi/kg and isometric PBS) at 8 hours after PMWA. Positron emission tomography/computed tomography (PET/CT) was fulfilled to three rabbits, which were randomly chosen from every group by picking numbers out of a hat, to assess the therapeutic effect at days 4, 8 16 after IIIH. Additionally, white blood cell count of all rabbit from every group was detected to assess the reaction in each group at 24 hours, 48 hours, 72 hours and seven days after IIIH.

To evaluate the effects of sequential therapy involving PMWA and IIIH on tumor growth, a further 75 animals were randomly divided into five groups (each 15 examples) by picking numbers out of a hat as follows: A, no treatment group; B, the PMWA alone group, with an ablation zone that covered the tumor and was monitored using two-dimensional ultrasound; C, the IIIH alone group; D, PMWA followed by a single IIIH treatment via the ear vein at the dose of 1mCi/kg at 8h after PWMA; and E, PWMA followed by two IIIH treatments via the ear vein at a dose of 1mCi/kg at 8h and 8 days after completion of PMWA. PET/CT was subsequently fulfilled to assess the therapeutic effect in each group prior to therapy, and at days 4, 8 and 16 after therapy. The Kaplan-Meier method was used for survival analysis. (Fig. 1B)

In the study, n refers to the number of animals, with one acquisition from each rabbit, with mean and standard deviation obtained from each group used for each protocol (five or fifteen animals each group in respective experiment part).

According to the literature \[16,17\], experimental animals grouping and sample size were determined.

**Animal model**

All experimental procedures were approved by the Institutional Animal Care and Use commit-ttee of Nanjing Medical University and conformed with Guide for the Care and Use of Laboratory Animals: Eighth Edition. In our study, Adult female New Zealand White rabbits weighing 2.0–3.0 kg were employed. The VX2 cell line used in this study was gained from the Surgery Department of our hospital. At first, the VX2 tumors that was cryo conserved and gained from the Surgery Department of our hospital were thawed. Second, the thawed VX2 tumors were cut into pieces and innoculated to the upper leg musculature of a New Zealand White rabbit to produce the tumor carrier rabbit from which the tumor material was taken after the
Fig 1. Flow chart of this study. (A) The verification of $^{131}$I-hypericin affinity to necrotic tumor tissue. (B) Evaluation of the therapeutic efficacy of sequential therapy involving percutaneous microwave ablation (PMWA) in combination with intravenous injection of $^{131}$I-hypericin (IIIH) using the VX2 rabbit breast solid tumor model.

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innoculated tumor was formed. Third, the tumor tissue was cut into small strips (1.5 × 1.5 × 6 mm) and was then injected in subcutaneously underneath the right second nipple using a 16-gauge needle. All of the above process were performed after the rabbit was anesthetized through an intravenous injection of pentobarbital sodium (0.8 to 1.2 ml/kg at a concentration of 30 mg/ml; Sigma, St. Louis, MO, USA) [18]. The rabbits were treated when the tumor size reached 2.0–2.5cm in diameter, as monitored using calipers at 30 days post-inoculation. If tumors had not reached or had exceeded the required size range by this time, they were excluded from further study prior to treatment. The rabbits were monitored every 2–3 days and were allowed to live to the humane endpoint when they had lost >10% of their initial body weight. Rabbits were sacrificed using intravenous injection of pentobarbital sodium (3.5 to 4ml/kg at a concentration of 30 mg/ml; Sigma, St. Louis, MO, USA) when the endpoint came.

Every rabbit was raised in a standard cage with 45 cm height and 0.28 m² floor size in a temperature of 22°C and humidity of 55±5%. And the day and night alternation per 12 hours was provided with lights on at 8:30pm. And 15 fresh air changes per hour was performed.

**Drug preparation**

131I-hypericin was produced using a standard iodogen method with a labeling yield of >92% [19], which was determined by ascending paper chromatography. The specific activity of 131I-hypericin was 900MBq/mg; For the sake of intravenous injection at various dose, the 131I-hypericin was diluted in water and polyethylene glycol 400 (volume ratio, 80:20);

**PMWA protocol**

The microwave system (Nanjing Yigao Microwave Institute, Nanjing, China) consisted of a microwave generator and a hollow water-cooled-shaft antenna, a flexible coaxial cable and a microwave generator. For this study, an output power of 40W and an electromagnetic field of 2.5MHz were selected. The duration of microwave irradiation was 2.5 min, over which almost all of the tumor was ablated. After the same anesthesia as preparation of animal model was completed, the rabbits were positioned supine and sterilized. Subsequently, the water-cooled-shaft antenna (2mm in diameter) was penetrated into the tumor along its long axis, guided by two-dimensional ultrasonography.

**In Vivo Planar Scintigraphy**

After radioiodination with a labeling efficiency of approximately 92%, the uptake of IIH by necrotic tumor tissue was visualized in vivo by means of single-photon emission computed tomography (SPECT) scanning (SKYLight ECT, Philips, The Netherlands). Images were gained at 2 h post-injection of IIH in two standard projections (anterior and posterior) after the rabbit was fixed on the camera bed in the supine position. A window of 20% centered on the energy peak of 131I (364 keV) and a matrix of 128 ×128 pixels were selected. Imaging data were obtained for 15 min.

**Autoradiography**

In the case of each tumor collected at 24 h post injection, all specimens were snap-frozen with optimum-cutting-temperature compound (Sakura Finetek, Torrance, CA, USA) and sectioned into 6–8 consecutive 5-mm slices. Subsequently, the sections were stained for autoradiography and dried at 40°C in open air. Subsequently, the sections were exposed and photographed on BAS-SR 2025 Fuji phosphorous film (Fuji Medical Systems, Hanover Park, IL, USA), which
was then scanned through the FLA5100 Multifunctional Imaging System (Fuji Medical Systems, Hanover Park, IL, USA).

**Biodistribution**

The rabbits were sacrificed using intravenous injection of pentobarbital sodium (3.5 to 4ml/kg at a concentration of 30 mg/ml; Sigma, St. Louis, MO, USA) immediately after the SPECT imaging session. Lung, liver, kidney, heart, spleen, brain, stomach, intestine, blood, bone, muscle and tumor tissues were removed and weighed; radioactivity was measured using a Cobra γ-counter (Packard Cobra, MN, USA). The uptake of IIIH in the various organs was assessed as the T/NT [T and NT represent the uptake of IIIH by the target organ and the non-target organ (e.g., muscle), respectively].

**Measurement of tumor size**

The rabbit weight and tumor sizes (measured by means of calipers) were recorded prior to treatment and on days 4, 8 and 16 after treatment. Tumor volume was then calculated in mm$^3$ according to the approximation $V = \frac{1}{2}ab^2$, where $a$ is the long axis and $b$ is the short axis of the tumor in mm [20]. The tumor size change ratio was assessed by means of the value of the tumor size post-treatment divided by the tumor size prior to treatment in respective groups.

**In Vivo PET/CT experiment**

After anesthetized, the rabbits were immobilized in the supine position with their axis coincidence with the PET scanner in the center of the field (Biograph 64 TruePoint PET-CT: Siemens, Munich, Germany). At 1 h after an ear vein injection of fluorodeoxyglucose 18F-FDG at a dose of 14.8 MBq/kg, the rabbits received a prone scan for 20 min. The specific parameters were as follows: a slice thickness of 5 mm; 120 kV; 80 mA; and 7 min per bed position. Before 18F-FDG PET/CT scanning, all the selected tumor-bearing rabbits must be fasting for at least 6 h. The standardized uptake values (SUV) were determined.

**Measurement of hepatorenal function and white blood cell count**

Auto-biochemical analyzer (Roche, Basel, Switzerland) was performed to measure the hepatorenal including glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), blood urea nitrogen (BUN) and creatinine. White blood cell count was performed by blood cells analyzer. (Beckman Coulter, California, USA) Blood samples were collected from the marginal ear vein before and after treatment (i.e. on days 8, 16 and 32).

**Statistical analysis**

SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA) was applied to statistical analysis. Quantitative data were expressed as the mean ± standard deviation. The independent samples t-test was applied to statistical analysis of the autoradiography gray scale and biodistribution between the PMWA+IIIH and the IIIH alone groups.

Analysis of variance (ANOVA) was performed for comparison of repeated measurement data among the various groups, namely GPT,GOT, BUN, creatinine, SUV, white blood cell count and the tumor change ratio. If the Mauchly test of sphericity was found to be significant, we used the Green house-Geisser correction. In cases where significant differences were revealed by means of ANOVA, a least significant difference (LSD) was performed for pairwise comparison.
The Kaplan-Meier method was used for survival analysis. \( P \) values < 0.05 were considered as existence of statistics difference.

**Results**

The animals’ health status was monitored throughout the experiments by a health surveillance programme according to Institutional Animal Care and Use Committee (IACUC) guidelines [21]. The rabbits were free of all viral, bacterial, and parasitic pathogens listed in the IACUC recommendations.

The confirmation of the maximum effective safe dose of IIIH

As shown in Fig. 2A, the treatment groups all have the apparent therapeutic effect (\( F = 472.117, P < 0.01 \)), which was revealed by repeated ANOVA measurements of the SUV for all the indicated time points. The three groups of which dosage are more than 0.5mCi/kg have

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**Fig. 2.** Changes of SUV (standardized uptake value) and white blood cell count in each treatment group with various density IIIH. (A) Compared with the other groups, the larger dose groups (0.5mCi/kg, 1mCi/kg, 2mCi/kg). (B) The white blood cell count in 2mCi/kg group is apparently less than any of the other groups (** represents the presence of statistic, \( P < 0.01 \)).

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the better therapeutic effect than the other groups, which were demonstrated through LSD test. And there is no statistics differences among the three groups of which dosage are more than 0.5mCi/kg ($P > 0.05$).

As shown in Fig. 2B, the apparent decrease of white blood cell count appeared when dosage of IIIH arrived 2mCi/kg, which was demonstrated through LSD test. Therefore 1mCi/kg of IIIH was confirmed as the maximum effective safe dose.

**Assessment of IIIH uptake using Planar Scintigraphy and Autoradiography**

Planar scintigraphy was performed to achieve optimal imaging; the uptake of IIIH was detected at 2 h after injection. The group treated with PMWA exhibited high uptake of IIIH at necrotic tumor sites; however, the group that was not treated using PMWA exhibited no uptake of IIIH at the tumor sites. Representative images are shown in Fig. 3A. Autoradiography was used to obtain evidence of IIIH selectivity for tumor necrosis (Fig. 3B). The gray scale was significantly higher ($7.55 \times 10^6 \pm 8.59 \times 10^4$) in the PMWA treatment group compared to that in the group that did not undergo PMWA treatment ($1.91 \times 10^6 \pm 1.12 \times 10^4$) ($t = 117.278; P < 0.05$; Fig. 3C).

**Biodistribution of IIIH**

At 135 min after injection of IIIH, the T/NT ratio was found to be significantly higher ($7.41 \pm 0.15$) in the groups that underwent PMWA treatment compared to that in the groups that did not ($1.34 \pm 0.05$) ($t = 40.99; P < 0.05$). There were no differences in the T/NT for liver, spleen, heart, lung, kidney and pancreas between the groups that underwent PMWA treatment and those that did not (Fig. 4).

**Tumor size change ratio**

Tumors had a pretreatment volume of $6373.7 \pm 1656.5$ m³, with no significant difference in size among the groups. As shown in Fig. 5, there were apparent statistical differences for all treatment groups revealed by repeated ANOVA measurements regarding the tumor size change ratio ($F = 124.48; P < 0.01$) at all indicated time points. Furthermore, there were significant time-group interactions regarding the tumor size change ratio ($F = 52.70; P < 0.01$). The main effect of time exhibited statistically significant differences ($F = 5.96; P < 0.01$).

Using the LSD test, significant differences were observed between any two groups ($P < 0.01$), with the exception of Groups A and C ($P = 0.51$ and $P > 0.05$, respectively). In Groups A and C, the tumors exhibited an apparent trend to increase in size with time. Tumor size in Groups D and E decreased between days 0 and 16 after treatment using PMWA and IIIH, whereas tumor size in Group B gradually increased, after an initial decrease on day 4 post-treatment. Furthermore, the tumor size in Group E decreased more rapidly compared to that in Group D after day 8.

**PET/CT for the evaluation of the efficacy of sequential therapy**

Representative images are shown in Fig. 6A. There were apparent therapeutic improvements in Groups B, D and E revealed by ANOVA regarding repeated measurements of the SUV for all the indicated time points ($F = 314.59; P < 0.01$). As shown in Fig. 6B, there was a significant time-group interaction ($F = 55.70; P < 0.01$). The main effect of time exhibited statistically differences ($F = 286.48; P < 0.01$). A significant difference ($P < 0.01$) was scanned through the LSD test.
Fig 3. Differences in uptake of $^{131}$I-hypericin in the control group and PMWA group. (A) In vivo planar scintigraphy. The arrows indicate the VX2 tumors, which had been inoculated underneath the right second nipple. The radioactivity overtly accumulated in the necrotic tumors in the PMWA group; By contrast, no radioactivity accumulated in the tumors in the control group. (B and C) The gray scale regarding IIH was significantly higher. There was a significant difference in the gray scale between the control group and the PMWA groups.

doi:10.1371/journal.pone.0120303.g003
There was no significant difference in SUV among groups prior to treatment. The SUV in Groups B, D and E had evidently decreased at day 4 after treatment using PMWA. The SUV in Group B had no significant difference between days 4 and 16 after PMWA treatment. The SUV in Group D had no significant difference between days 8 and 16 after administration of PMWA. In Group E the SUV evidently decreased between days 0 and 16 after treatment using PMWA, whereas the SUV in Groups B and D gradually reached a plateau at days 4 and 16, respectively.

Survival times of rabbits with VX2 breast solid tumor implants

No significant difference was observed in the Kaplan-Meier survival curves for Groups A and C. The mean survival times of animals in Groups B, D and E were longer compared to that in Group A. The Kaplan-Meier curves revealed that survival times in Groups B, D and E were significantly higher than no treatment Group A \((P<0.05)\) (Fig. 7). The animals in Groups B, D and E survived for 12, 18 and 26 days, respectively, which was longer than no treatment group.

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Fig 4. The biodistribution of $^{131}$I-hypericin in PMWA group and control group. The uptake of $^{131}$I-hypericin by the tumor was significantly higher in the PMWA group \((7.41\pm0.15)\) compared to that in the control group treated with IIIH alone \((1.34\pm0.05)\) \((t = 40.989; P<0.05)\). No significant difference was observed in normal organs uptake between the two groups. T, target; NT, non-target

doi:10.1371/journal.pone.0120303.g004

Fig 5. Change in tumor size with time after treatment. A statistically significant difference was observed between any two of the groups \((P<0.05)\), with the exception of groups A and C. The tumor size decreased in Groups D and E, whereas it increased in groups A, C and B, after an initial decrease. The tumor shrinkage was more prominent in group E compared to that in group D.

doi:10.1371/journal.pone.0120303.g005
Fig 6. Representative results from positron emission tomography/computed tomography scans. (A) The arrows indicate the VX2 tumors, which were inoculated underneath the right second nipple. In groups B and D, fluordeoxyglucose (18F-FDG) uptake was clearly decreased and reached a plateau on day 4 and 8, respectively. In groups B, D and E, ring-shaped accumulation of 18F-FDG is observed at the site of percutaneous microwave ablation treatment. (B) In Group E, 18F-FDG accumulation was decreased overtly between days 0 and 16 (P<0.01). SUV, standardized uptake value.

doi:10.1371/journal.pone.0120303.g006
There had no VX2 allograft-recipient rabbit survived for >60 days in Group A. The survival rate in Group E was 50% at day 66, which was the longest among the five groups (Fig. 7).

Metastasis, complications and the results of the hepatorenal function test

The vast majority of tumor metastasis was mainly detected in the lung and lymph nodes, and occurred in 90% of the rabbits in Groups A and C, whereas the incidence of lung metastasis in lung and lymph node in group D and group E is less than in group A and group C ($P<0.05$). And tumor ulcerating in group D and group E is less than in group A and group C ($P<0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
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<tbody>
<tr>
<td>Lung metastasis</td>
<td>14</td>
<td>11</td>
<td>13</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>13</td>
<td>12</td>
<td>14</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Tumor ulcerating</td>
<td>12</td>
<td>7</td>
<td>12</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Puncture path infection</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The tumor metastasis in lung and lymph node in group D and group E is less than in group A and group C ($P<0.05$). And tumor ulcerating in group D and group E is less than in group A and group C ($P<0.05$).
and lymph metastasis was significantly decreased in Groups D and E (table 1) \(P<0.05\). Most rabbits died of tumor lung metastasis.

The tumor metastasis in lung and lymph node in group D and group E is less than in group A and group C \(P<0.05\). And tumor ulcerating in group D and group E is less than in group A and group C \(P<0.05\).

None of the rabbits exhibited overt clinical signs of toxicity, for example, gastrointestinal bleeding, diarrhoea, and changes in hair or skin in response to treatment with PMWA or IIIH. No significant difference was observed regarding the liver and renal function tests among the five groups \(P>0.05\) (Fig. 8).

**Discussion**

Currently, for ablative experiment, as most of the equipment is not designed specifically for animal experiment but for human study, experimental animal size is a considerable problem. Thus, an animal model larger than the mouse or rat was required. The VX2 tumor is a highly malignant squamous cell solid tumor which has already been used widely for the study of local treatment for solid tumor, such as breast [18,22], lung [16], liver [23] and kidney [24]. The VX2 tumor is the only tumor model which is easy to be innoculated in larger animals like rabbit. Therefore, this tumor was selected as the model for use in our study. In addition, the result of this study has the general significance for others solid tumors.
In the present study, we demonstrated that treatment using PMWA followed by one or two administrations of IIIH at an effective dose resulted in a promising therapeutic effect in rabbits bearing VX2 breast tumors.

Our results are consistent with the previously reported research in a rodent bearing VX2 liver tumor, in which the residual tumor tissue was eliminated by the IIIH that targeted the necrosis tissue produced by vascular disrupting agent combretastatin A-4 phosphate (CA4P)[23].

Concerning TNT, relevant clinical trials that a $^{131}$I-radiolabeled monoclonal antibody was used to treat and diagnose solid tumors, such as lung and brain cancer, is currently under way in United States and China[14,25]. When compared to macromolecular agents, such as monoclonal antibodies, peptides or small organic molecules, small molecules such as hypericin exhibit superior permeability and a lower uptake rate by the reticuloendothelial system[23,26].

In the present study, hypericin was labeled with $^{131}$I to form $^{131}$I-hypericin, which emits both $\beta$ and $\gamma$ radiation. $^{131}$I-hypericin has been used for the treatment of liver rhabdomyosarcomas[23]. $^{131}$I is a radioactive isotope with a relevant short half-life of 8 days and a 2mm $\beta$-particle penetration depth, which means higher safety and feasibility. Therefore, the use of IIIH followed by PMWA may exert an effect similar to that of intraoperative radiotherapy following dissection in breast cancer patients. And so on, each effective dose can cause a centrifugal inactivated range to residual viable tumor tissue.

It has been reported that FDG-PET was more sensitive than morphologic imaging in detecting breast residual tumor and recurrence [27]. In addition, $^{18}$F-FDG-PET is more applicable than the other evaluation methods on early assessment of treatment in sensitivity and accuracy. In our study, the quick and significant decrease of SUV at VX2 tumor sites was observed soon after the effective treatment was implemented, which was shown in PET-CT scanning result.

The maximum effective safe dose of IIIH was found to be 1 mCi/kg body weight in our study. Routine tests for blood and hepatorenal function, measurement of weight and hematoxylin-eosin (HE) staining of liver tissue were performed to investigate the toxicity of IIIH (data not shown). When the IIIH dose was 1 mCi/kg, the body weight profile and routine tests for blood and hepatorenal function gave results that were in the normal range; abnormal changes in HE staining of liver tissue were not seen (data not shown).

There were certain limitations to our study. First, the mechanism underlying the affinity of hypericin for necrotic tumor tissue has not been fully elucidated. Second, the power at which PMWA is administered and the duration of treatment requires further optimization to achieve the optimal ablation area covering the entire tumor margin without normal tissue damage. Third, large animal breast tumor models are limited. Whether there is a similar therapeutic effect exists in other animal models remains to be determined.

In summary, in the rabbit VX2 breast solid tumor model, sequential therapy involving PMWA and IIIH decreased tumor growth and improved survival. As such, PMWA followed by IIIH bears great potential to replace traditional surgery, chemotherapy and radiotherapy as a primary curative therapy or palliative care therapy. Although further investigations are required to optimize the delivery parameters regarding PMWA and the dose of IIIH, this sequential therapy will likely continue to receive considerable attention as a minimally invasive treatment for malignancy.

**Acknowledgments**

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Author Contributions
Conceived and designed the experiments: MZ XAL XMZ WBZ TSX SW. Performed the experiments: MZ XAL XMZ WBZ TSX SW. Analyzed the data: MZ TSX. Contributed reagents/materials/analysis tools: MZ XAL XMZ WBZ TSX SW. Wrote the paper: MZ XAL XMZ WBZ TSX SW.

References


A retrospective study comparing endovenous laser ablation and microwave ablation for great saphenous varicose veins

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Abstract. – BACKGROUND, Endo-venous laser or microwave ablation is a minimally invasive surgery for treating varicose veins of lower limbs.

AIM, The aim of our study was to determine whether endovenous microwave ablation of the greater saphenous vein was associated with better effectiveness and less complications than the endovenous laser ablation.

MATERIALS AND METHODS, From July 2008 to June 2011, 259 cases (306 limbs) of varicose veins were assigned to endovenous laser ablation (n=138, 163 limbs) or endovenous microwave ablation (n=121, 143 limbs).

RESULTS, Through analysis there was no significant difference of the operating time, length of hospital stay and Aberdeen score in the two groups. The recanalization rate was statistically higher in the laser group than that in the microwave group. The ecchymosis complication was significantly lower in microwave ablation than that of laser ablation group. However, the skin burn and paralysis complications were significantly lower in the laser ablation than that of microwave ablation group.

CONCLUSIONS, Endo-venous microwave ablation is an effective alternative to laser ablation for treatment of varicose veins, associated with higher occlusion rate and without serious complications.

Key Words:
Saphenous vein, Varicose vein, Laser ablation, Microwave ablation.

Introduction

Varicose veins are a common disorder causing significant impairment in health quality of life. Symptoms include aching, discomfort, pruritus and muscle cramps. A considerable proportion of patients even develop the complication of refractory ulceration. Conventional treatment of greater saphenous vein (GSV) incompetence involves surgical ligation at the saphenofemoral junction (SFJ) and stripping. However, the risks of bleeding, paresthesia, infection, scarring, increased hospital costs and prolonged recovery have been reported. As a result, more attentions are paid on the development of minimally invasive alternatives to replace of surgical treatment. Among these, the endovenous laser ablation (EVLA) is now the most widely used procedure and approximately occlusion rate of 95% could be achieved in several randomized studies. EVLA allows delivery of laser energy directly into the blood vessel lumen to produce endothelial and vein wall damage with subsequent fibrosis. The EVLA treatment has been proved to be less invasive than conventional surgery due to lower complication rate and more tolerance for patients. Interestingly, we have recently noticed a small amount of studies concerning the microwave to be the energy source for endovenous ablation. The endovenous microwave ablation (EVMWA) for treatment of varicose veins was considered safe and related with good clinical outcomes. The aim of our study was to determine whether EVMWA of the GSV was associated with better effectiveness and less complications than EVLA.

Patients and Methods

Patient Selection

From July 2008 to June 2011, 259 cases (306 limbs) of varicose veins had been admitted in the Department of Vascular Surgery at Shanghai First People’s Hospital and underwent the endovenous therapies. Among them, 138 cases (163 limbs) were treated by EVLA and 121 cases (143 limbs) were assigned to the EVMWA procedure.
Preoperatively, patients were asked to complete the Aberdeen Varicose Vein Questionnaire (AVVQ), which has been shown to be a valid measure of quality of life for patients with varicose veins\textsuperscript{14}. There was no significant difference at baseline between the two groups (Table I).

All cases were diagnosed as varicose vein by the clinical pathway containing detail inquiry of history, Trendelenburg’s test, Pether’s test, Doppler’s inspection and high speed spiral CT venography. The clinical severity of the varicose disease was graded according to the clinical, etiological, anatomical and pathophysiological (CEAP) scoring system\textsuperscript{15}. CEAP classes C0-C6 varicose veins were included in this study (Table II). Patients who had a history of deep vein thrombosis, peripheral artery diseases, serious systemic diseases, or pregnancy were excluded. In addition, the patients who refused to participate in this study were also excluded.

Table I. Baseline characteristics of the treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>EVMWA group</th>
<th>EVLA Group</th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td>121 cases (143 limbs)</td>
<td>138 cases (163 limbs)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.3 (22-79)</td>
<td>56.8 (25-88)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Sex ratio (M:F)</td>
<td>59/62</td>
<td>65/73</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Left/Right limbs</td>
<td>75/68</td>
<td>86/77</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Aberdeen score</td>
<td>13.76 ± 1.32</td>
<td>13.44 ± 1.29</td>
<td>&gt; 0.05</td>
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</table>
formed using the Student’s t tests. If the data were not normally distributed, median (interquartile range) values were presented, with analysis using the Mann-Whitney U test for unrelated samples and Wilcoxon signed rank test for paired data. Friedman test was used to analyze multiple related samples across the study interval. Categorical data were analyzed by means of X² test. p < 0.05 was considered statistically significant.

Results

Totally two hundred and fifty nine cases (306 limbs) were included in our study, of them, 138 cases (163 limbs) underwent EVLA and 121 cases (143 limbs) underwent EVMWA. All operations were performed successfully. The operating time and length of hospital stay were similar in both groups and no statistical difference was found in the improvement of Aberdeen score (Table III). Our study revealed a little higher occlusion rate in EVMWA group than that of EVLA group (Table IV). The main postoperative complications were ecchymosis, skin burns, paresthesia and scleroma. No deep vein thrombosis or pulmonary embolism (PE) was observed in our study (Table V). All patients were submissive for the first follow-up examination. However, in the second follow up, the lost rate was up to 18.8% (24 cases lost) and 19.6% (37 cases lost) in EVMWA group and EVLA group, respectively.

Discussion

Since more than one hundred years, the standard treatment of varicose veins has historically been high ligation and stripping of the GSV. However, it is associated with significant pain, prolonged postoperative recovery in some patients, risks of infection, hematoma, and nerve injury. In recent years, minimally invasive endovenous ablation techniques have been developed as.

<table>
<thead>
<tr>
<th>EVMWA group</th>
<th>EVLA group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating time (min)</td>
<td>27.5 ± 6.3</td>
<td>26.7 ± 5.6</td>
</tr>
<tr>
<td>Length of hospital days</td>
<td>2.3 ± 0.3</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Postoperative Aberdeen score</td>
<td>10.8 ± 1.3</td>
<td>11.1 ± 1.2</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>EVMWA group</th>
<th>EVLA group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week postoperatively</td>
<td>1 (0.76%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>6 months postoperatively</td>
<td>5 (5.2%)</td>
<td>11 (9.9%)</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>EVMWA group</th>
<th>EVLA group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecchymosis</td>
<td>23 (17.4%)</td>
<td>35 (21.5%)</td>
</tr>
<tr>
<td>Skin burns</td>
<td>12 (9.9%)</td>
<td>9 (6.5%)</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>13 (10.74%)</td>
<td>8 (5.8%)</td>
</tr>
<tr>
<td>Scleroma</td>
<td>23 (17.4%)</td>
<td>29 (17.6%)</td>
</tr>
<tr>
<td>DVT</td>
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alternatives to conventional surgery. Currently literatures have mainly concerned the endovenous thermal treatment, EVLA. Min et al. reported 97% of GSVs were closed 1 week after initial treatment with endovenous laser ablation and 93.4% of GSVs have remained closed followed for 2 years. In fact, the efficacy of EVLA in our center is similar to that of being reported, with the occlusion rate of 98% one week postoperatively and 90.1% six months after the treatment. However, the mechanisms of this technique remain obscure. It is suggested to involve protein denaturation and destruction of cell structure, which was confirmed by histological studies. In addition, it is also deduced that EVLA causes permanent vein closure through a high-temperature photothermolytic process at the point of contact between the vein and the laser. In 2009, Subwongcharoen et al. found that EVMWA appeared to be another extremely safe and effective technique for treatment of varicose veins and the best ablation effect could be obtained while microwave generator with 50-W power setting. EVMWA uses dielectric hysteresis to produce direct volume heating of tissue. Our EVMWA treatment also indicated a high occlusion rate could be achieved one week (99.24%) and six months (94.8%) after the procedure.

Furthermore, a significant higher occlusion rate could be observed in EVMWA group than EVLA group regardless of one week or six months follow up (p < 0.01). This may result from the following reasons. The laser fiber is very brittle and easily broken so that we have to use the Seldinger technique to change in the fiber. The microwave probe is more flexible than laser fiber, and it can be smoothly inserted into vessel and reach the SFJ without the help of a catheter and guide wire. In addition, the microwave generator can produce much higher energy than that of laser generator. According to the reference concerning the EVMWA, the microwave power was set to 50W when the saphenous trunk was being ablated, which was much higher than that of EVLA. The occlusion rate of target veins depends on the thoroughness of endothelial damage, which correlates positively to the thermal energy received by the vein wall. Due to more energy provided by the microwave, the occlusion rate after operation was significantly higher in the EVMWA group than that in the EVLA group.

The major complications secondary to the endovenous thermal ablation were ecchymosis, skin burns, paresthesia and scleroma. The extreme temperature of laser fiber tip can reach more than 800°C and the direct contact of fiber tip with the vessel wall can easily cause the perforation of vein wall. The temperature at the tip of microwave probe is usually around 80°C, which seldom creates an ulceration, and even perforation. The perforation of vessel wall leads to intraoperative bleeding subcutaneously, with the manifestation of ecchymosis. Thus, the ecchymosis was shown more severe in EVLA than that of EVMWA group. The energy of laser concentrates on the tip of fiber while a considerable part of energy was distributed in the full fiber of microwave. We could not accurately control the energy at the tip of the microwave fiber, and the released heat from the remaining part of fiber may cause thermal damage to the tissues around the vessel. The main adverse effects of thermal injury contain skin burn and paresthesia. The saphenous nerve is just close to the vein, resulting in more thermal damage to the saphenous nerve in the EVMWA group.

Conclusions

EVMWA is an effective alternative to EVLA for treatment of varicose veins, related with higher occlusion rate and without serious complications.

References


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