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No-touch Radiofrequency Ablation of VX2 Hepatic Tumor In Vivo in Rabbits: A proof of concept study

토끼의 생체 내 VX2 간 종양의 종양-비접촉 고주파 절제술: 개념 증명 연구

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Abstract

Purpose

In a proof of concept study, we attempted to compare no-touch radiofrequency ablation (RFA) technique in a bipolar mode with conventional direct tumor puncture technique regarding local tumor control, peritoneal seeding and tumorigenic factor in rabbit VX2 subcapsular hepatic tumor model.

Materials and Methods

Sixty-two rabbits with VX2 hepatic subcapsular tumor were divided into three groups: Group A (n=25) with direct tumor puncture RFA (dtpRFA); Group B (n=25) with no-touch RFA (ntRFA); Group C (n=12) as a control group. Each three groups were subdivided into two sets either for pathologic analysis (n=24) or CT follow-up until 6-weeks after RFA (n=38). Ultrasonography-guided dtpRFA and ntRFA were performed after nine days of tumor implantation. Local tumor control of RFA was determined by either achievement of complete tumor necrosis on histopathologic exam or absence of local tumor progression (LTP) on follow-up CT and autopsy. The development of peritoneal seeding was also compared among the animal groups. Serum hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6) on day 1, day 2, and day 3 after RFA were measured by ELISA assay for tumorigenic factor evaluation.

Results

Regarding local tumor control, there was a trend with group B (80%, 20/25) for the better ablation than group A (56%, 14/25) (P=0.07). Complete tumor necrosis

was achieved in 54.5% of group A animals (6/11) and 90.9% of group B animals (10/11). Peritoneal seeding after RFA was significantly more common in group A (71.4%, 10/14) than B (21.4%, 3/14) (P=0.021) or group C (0%). The elevation of HGF, VEGF and IL-6 were not detected in all three groups.

Conclusion

NtRFA developed lower rates of peritoneal seeding and showed a tendency to achieve better local tumor control compared to those of dtpRFA.

Keyword : Radiofrequency ablation, RFA; no-touch RFA; direct tumor puncture RFA; peritoneal seeding;

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INTRODUCTION

Radiofrequency ablation (RFA) is currently widely used technique for curative treatment for early-stage hepatocellular carcinoma (HCC) (1). The most frequently utilized RFA technique is a monopolar technique with intra-tumorous electrode placement (2). However, the major limitation of RFA using the conventional monopolar direct tumor puncture technique is relatively high local tumor progression reported between 25-53%, which is related to the limited volume of tumor necrosis (3-5). Therefore, overlapping ablation techniques are frequently used to create sufficient safety margin around the target tumor (6). Also, conventional tumor penetration technique poses potential risks for unwanted tumor seeding with increasing risk of multiple placements of electrodes within the target tumor. Indeed, the achievement of large peritumoral ablation zone while decreasing the risk of intraprocedural tumor cell seeding could be ideal for improving the therapeutic efficacy of RFA for liver malignancies. In that sense, a no-touch technique with multi-bipolar techniques has been suggested to ensure maximum ablative area which creates high-density electrical fields between several pairs of independent electrodes (2, 5, 7, 8). Although there have been sporadic studies which showed promising results of no-touch RFA technique in their retrospective review, only one recent retrospective study (2) compared no-touch RFA with conventional direct tumor puncture technique.

Recently, RFA has been recognized to induce cytokines such as interleukin-6 (IL-6), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) which promote liver regeneration but potentially facilitating unwanted tumor recurrence or distant metastasis (9-13). The cytokines are termed as tumorigenic

factors and may be produced in the residual incompletely treated tumor at the ablative margin and in the healthy liver surrounding the targeted tumor (12, 13). However, there is no study to clarify whether the violation of tumor during the procedure influences the tumorigenic factor release.

One critical application of no-touch RFA using a bipolar mode that has not been explored so far is whether no-touch RFA can provide less development of peritoneal seeding and whether it can show lower tumorigenic factor release compared with conventional monopolar tumor puncture RFA. If these were confirmed, no-touch bipolar RFA could become an attractive alternative to conventional monopolar RFA with direct tumor puncture, especially peripherally located liver malignancies. Thus, in this proof of concept study, we attempted to compare no-touch radiofrequency ablation (RFA) technique in a bipolar mode with conventional direct tumor puncture technique regarding local tumor control (LTC), peritoneal seeding and release of tumorigenic factors in rabbit VX2 subcapsular hepatic tumor model.

MATERIALS AND METHODS

The experimental design is summarized in a flowchart (Figure 1).

Animal care

This study was approved by the Institutional Animal Care and Use Committee at our hospital. Total of 62 adult New Zealand white rabbits weighing 2.5-3 kg were used. During all procedures including sampling, RFA, and computed tomography (CT) follow up, the rabbits were anesthetized with an intravenous injection of $5mgkg^{-1}$ bodyweight tiletamine-zolazepam (Zoletil 50; Virbac, Carros, France).

VX2 Liver Implantation

In addition to 62 rabbits with the hepatic VX2 tumor for the study, 10 rabbits were used as VX2 tumor donor. The VX2 tumor is a virus-induced anaplastic squamous cell carcinoma characterized by hypervascularity with rapid growth (14, 15) and the rabbit VX2 tumor model has been widely employed in numerous RFA-based studies for the treatment of HCC (16-19). The VX2 tumor was transplanted into the hind thigh muscle of donor rabbit and left in situ for 4-6 weeks. When the tumor had grown to more than 5cm, the tumor was harvested and was cut into $4mm^3$ tumor chips. After the tumor chips preparation, midline subxiphoid laparotomy was performed in a total of 62 rabbits for liver tumor implantation. The tumor chip was directly implanted into the subcapsular parenchyma in the left medial lobe of the liver. All rabbits were taken appropriate postoperative care

including analgesics and antibiotics.

Ex vivo test in Bovine Liver for RFA Optimization using Dual Bipolar

We performed an ex vivo test for RFA optimization using the dual bipolar technique for ablation of 1cm size tumor. RFA in a total of 27 bovine liver blocks (size, 5 x 5 x 5 cm^3) which were immersed in a 50 x 20 x 25 cm^3 saline-filled bath. The RFA system utilized in the study was a multichannel radiofrequency (RF) generator system (VIVA multi-RF generator: STARmed, Goyang, Korea) and separable dual electrodes (Dual® electrode; STARmed) with two internally cooled electrodes with a 1-cm active tip each. As each electrode of the dual electrode is connected to a flexible, 50-cm-long cable, those electrodes could be placed in the liver with a diverse inter-electrode distance determined by the size of the tumor According to our previous studies where bipolar RFA induced confluent ablation zone within relatively short time than switching monopolar RFA with less thermal damage to the surrounding structures (20-22), we used bipolar mode for RFA procedure.

In vivo Radiofrequency ablation procedure

Dual RFA electrodes with a 1-cm active tip (STARmed) were placed percutaneously under ultrasonography (US) guidance. For direct tumor puncture (DTP)-RFA group (n=25), one of two electrodes directly penetrated tumor, whereas the other electrode was inserted at the periphery of the tumor. For no-touch RFA group (n=25), both electrodes were inserted at the periphery of the

tumor, not violating the tumor. The only difference between two groups regarding RFA procedure was whether one of the electrodes penetrated the tumor or not. After the RFA procedure, cauterization of the electrode track was performed in both groups. Medical illustration of the in vivo RFA procedure is drawn in Figure 2.

Radiological study

The CT examinations were performed on a multi-detector CT scanner (Discovery CT 750 HD; GE Healthcare, Pewaukee, WI) with 2mL/kg of nonionic contrast medium (Ultravist 370, Bayer, Wayne, NJ, USA) through an auricular vein in the supine position. CT scans included pre, arterial (15 seconds) and portal (30 seconds) phase after contrast injection rate of 1ml/sec with 1mm slice thickness from head to upper thigh. The following CT parameters were used: 150 mA, 140 kVp, 1.0 pitch, and 214 x 214 mm^2 field of view. All 62 rabbits with VX2 tumor implantation underwent CT scans before the RFA (median nine days after implantation (range, 7-12)). In subgroups for pathologic diagnosis (11 rabbits in DTP-RFA and no-touch RFA respectively, two rabbits for control), CT scans were performed after three days post RFA before sacrifice. In subgroups for CT follow-up (14 rabbits in DTP-RFA and no-touch RFA respectively, ten rabbits for control), CT scans were performed every week until 6^{th} week after RFA. Local tumor progression was defined as the appearance of nodular, mass-like, or thick irregular tissue with enhancement adjacent to previous RFA treated site (23). Peritoneal and skin seeding was determined as the appearance of enhancing nodular or thick irregular shaped lesion with interval increment attached to the peritoneum or subcutaneous and skin, respectively(24). Euthanasia and autopsy were also

performed in rabbits for CT follow up to correlate with the CT data after completion of 6 weeks follow up.

Pathologic analysis

Euthanasia was performed on rabbits for pathologic examination three days after RFA. The liver was harvested, and multiple slides of 1cm thickness were cut perpendicular to the RFA needle insertion direction**.** The sliced tissues were embedded in optimal cutting temperature compound (Tissue Tek; Sakura Finetek, Tokyo, Japan), quenched in isopentane and frozen in liquid nitrogen before storage at -80 for nicotinamide adenine dinucleotide (NADH) diaphorase activity evaluation, which reflects viability of the tumor(25). The remnant specimen was fixed in 10% neutral buffered formalin, embedded in paraffin and sliced into 5-um sections for hematoxylin and eosin (H&E) staining for histologic structural evaluation. Pathologic analyses were performed on main tumor nodule and peri-nodular satellite nodule, respectively. Complete local necrosis was defined as entire involvement of both main tumor and all peri-nodular satellite nodules within the ablative zone on H&E staining with no NADH staining(25). To assess the tumorigenic effect, immunohistochemistry was performed to quantify activated Ki-67 positive hepatocytes by using anti-Ki-67 (Ab155580; Abcam, Cambridge, Mass) in the ablated lobe at high-power (x40) microscopy (10).

Local tumor control was determined by either achievement of complete local necrosis on histopathologic examination or absence of local tumor progression (LTP) on follow-up CT and autopsy.

Biochemical analysis for tumorigenic factor

Serum level of IL-6, HGF and VEGF on the pre, 24h, 48h, and 72h after RFA were measured using enzyme-linked immunosorbent assay (ELISA) by using a rabbit kit (Elabscience Biotechnology Co., Wuhan, China). Untreated rabbits served as a control. A total of 14 rabbits (6 rabbits per no-touch RFA and DTP-RFA, respectively, two rabbits for control) were used for the analysis. ELISA assays were performed according to manufacturer's instruction.

Statistical analysis

Data were reported as means ± standard deviation (SD), median (range) or number (percentage, %) as appropriate. Comparison between DTP-RFA and no-touch RFA was performed using Fisher's exact test or Chi-squared test for categorical variables and unpaired Student-t test or Mann-Whitney U test for continuous variables. For continuous variable comparison among three groups (DTP-RFA, no-touch RFA, control), analysis of variance (ANOVA) was performed. All statistical analyses were performed using commercially available statistical software (SPSS software for Windows, version 21.0, SPSS-IBM). P-values of <0.05 were considered statistically significant.

RESULTS

Ex vivo test for RFA optimization

In the ex vivo test, we aimed to find optimal distance and energy where ablation volume reached more than 4000mm^3 with minimum ablation length of 12mm. To satisfy the desired ablative area, when distance was 10mm or 13mm, both 0.5 Kcal and 0.6 Kcal were sufficient. In case of 15mm distance, 0.6 Kcal was required. We applied either 10mm or 13mm distance with 0.5 Kcal for in vivo RFA experiment. Supplementary material 1 summarizes ex vivo test result.

VX2 carcinoma and In vivo RFA procedure

The intrahepatic VX2 tumor was grown for median nine days (range, 7-12), which reached the size of median 8mm in axial longest diameter (range, 5-12) and volume of median 315 mm^3 (range, 75-960) on pre-RFA CT. On pre-RFA CT, there was no significant difference regarding longest mean tumor size between DTP-RFA and no-touch RFA group (DTP RFA,7.9mm; No-touch RFA, 8.3mm; P=0.604). All 62 rabbits were confirmed to have successful VX2 tumor implantation in left medial lobe subscapular area with no iatrogenic tumor seeding on pre-RFA CT.

We applied the result from the ex vivo test for the in vivo RFA procedure and specification as follows; power, 50 watts; the distance between two RFA needle, 10-13mm; energy, 0.51 Kcal (standard deviation, SD, ± 0.07); ablation time, 307.34 seconds (SD, ± 110.34). The number of punctures per procedure between

two groups differed with no-touch group requiring more punctures (DTP-RFA; 2 (median) (range: 2-3), no-touch RFA; 3 (2-5), P<0.001). On post three day post RFA CT, there was no significant difference regarding ablation zone seen as low attenuation in the liver between DTP-RFA and no-touch RFA group (DTP RFA, 7045 mm3; No-touch RFA, 7789 mm3; P=0.517). The tumor characteristics and in vivo RFA specifications are summarized in Table 1.

Local tumor control

Table 2 summarizes the result of the local tumor control in DTP-RFA, and no-touch RFA in both pathologic analysis and 6-week post-RFA CT follow up. There was a tendency of a better result in no-touch RFA than that of DTP-RFA (DTP-RFA 56% (14/25) vs. no-touch RFA 80% (20/25), P=0.069). Regarding complete local necrosis (including main tumor and satellite nodules) on pathologic assessment, DTP-RFA reached 54.5% (6 out of 11) whereas no-touch RFA achieved 90.9% (10 out of 11) (P=0.148). Furthermore, in three of the five rabbits with viable satellite nodules in DTP-RFA group, viable satellite nodules were located more than 5mm distance from the main tumor (Fig.3 (b), supplementary materials 2 and 4) whereas all of satellite nodules in both No-touch RFA (n=1) and control group (n=2) were within 2mm distance from the main tumor. Also, intravascular tumor emboli were noted in one of the rabbits with the satellite nodule in DTP-RFA. (Supplementary material 4). On CT follow up and autopsy, DTP-RFA showed local tumor progression in 42.8% (6 out of 14) while no-touch RFA showed local tumor progression in 28.6% (4 out of 14) LTP, respectively (P=0.694).

CT follow-up for peritoneal seeding

Table 3 summarizes the results of 6-week post-RFA CT follow-up in DTP-RFA, no-touch RFA and control groups. Regarding peritoneal seeding, DTP-RFA had significantly higher incidence than that of no-touch RFA (71.4% vs. 21.4%, P=0.021). Control group showed no evidence of peritoneal seeding or skin seeding. Lymph node metastasis and lung metastasis varied among three groups without statistical significance. Representative cases are presented in figure 4 and figure 5.

Tumorigenic factor

Serum level of HGF, VEGF, and Il-6 was not detectable due to a minimal increase in all three groups (DTP-RFA, no touch RFA and control). Additionally, measurement of the Ki-67 proliferation marker (at three days after ablation) in ablated lobe per high-power microscopy frame did not differ among three groups (DTP-RFA;7.5±3.0, no-touch RFA;8.1±4.1, control; 3.0±1.6, P=0.494) (supplementary material 3).

DISCUSSION

In the current study, we proved that no-touch RFA developed less peritoneal tumor seeding than DTP-RFA in the subcapsular VX2 tumors of the liver in rabbits. No-touch RFA showed the tendency of better local tumor control than DTP-RFA (DTP-RFA 56% (14/25) vs. no-touch RFA 80% (20/25), P=0.069) based on either pathologic assessment or combination of contrast-enhanced CT and autopsy findings. Also, no difference was found regarding the tumorigenic factor elevation between DTP-RFA and no-touch RFA. Considering these results of our study, no-touch RFA may provide better clinical outcome for bridge therapy to liver transplantation for subcapsular HCC when comparing to dtp RFA.

In fact, peritoneal tumor seeding is one of the most unfavorable complications after RFA for liver malignancies such as hepatocellular carcinoma or colorectal liver metastases. Many previous studies reported the various incidence of tumor seeding after RFA for HCC ranging from less than 1.5% (26-29) to 4.0% (30), although a high rate of 12.5% has been reported by one center (31). Most of the previous studies dealt with DTP-RFA technique and suggested various risk factors such as subcapsular location (30, 31), a prior biopsy (27, 29), poorly differentiated tumors (31) and no cauterization of the electrode track (28, 29). Possible mechanisms for peritoneal tumor seeding after DTP-RFA include facilitation of viable cancer cell dissemination by increased intratumoral pressure (24, 26), direct tumor implantation through a needle (28), or direct migration of tumor cells with a little bleeding into the peritoneal cavity (26, 28). As no-touch RFA alternately places probes at the surrounding liver parenchyma to the tumor margin, there must

be reduced risk of direct tumor implantation through the needle or along the bleeding from the tumor. Also, no-touch ablation can induce vessel coagulation around the tumors first, and therefore, we may expect that it causes relatively less intratumoral pressure than DTP-RFA (2) leading to lower peritoneal seeding. Accordingly, recent clinical studies of no-touch RFA reported no peritoneal seeding (2, 5, 32), although most of them are retrospective studies with small sample size.

The incidence of peritoneal seeding in our study was higher (71.4% in DTP-RFA, 21.4% in no-touch RFA) than previous studies on human (1.5% ~ 12.5%). We thought that it might attribute to the subcapsular location of the tumor and the virulence of VX2 carcinoma. Although there is a controversy over the risk of tumor seeding in RFA for subcapsular tumor, there are several studies proving the higher risk of tumor seeding in subcapsular tumors than that of the non-subcapsular tumors (30, 31, 33). Also, VX2 carcinoma is initially developed from a virus-induced anaplastic-squamous cell carcinoma and has been propagated by numerous serial transplantations into different tissues over the years resulting in high virulence and possible sarcomatous change. Given the innate different tumor characteristics of VX2 carcinoma in a rabbit model and those of HCC in human, caution is warranted when interpreting the incidence of peritoneal seeding between studies on rabbits and human.

No-touch RFA is known to improve the rate of complete necrosis resulting from larger ablation volume with ablation forming centripetal direction inducing safety ablative margins. Seror et al. (7) reported enhanced complete necrosis in no-touch RFA (26 out of 29, 89.6%) than in DTP-RFA (14 out of 30, 46.6%) in patients with

HCC. In our experiment, we found a similar trend with no-touch RFA for the better local tumor control rate than DTP-RFA but failed to reach statistical significance(P=0.069). This was probably attributed to the biological features of VX2 tumors developing multiple tiny satellite nodules around the main implanted tumor when they reach more than 2 cm in diameter. On follow-up CT and autopsy, despite complete necrosis of the target tumor, local tumor progression was found from satellite nodules which might be related to the biological aggressiveness of VX2 tumors having an infiltrative growth.

At the same time, of interesting note is that in three rabbits having viable satellite nodules of DTP-RFA group, the satellite nodules were located more than 5mm from the main tumor and one of them developed intravascular tumor emboli. On the contrary, no-touch RFA and control group had satellite nodules within the 2mm distance from the main tumor. This may reflect the facilitated local dissemination and subsequent trend toward poor local control rate DTP-RFA resulting from intratumoral placement of electrodes, intratumoral pressure increase and centrifugal ablation with late vascular perfusion blockage during ablation, recapitulating previous observations that manipulation of the tumor during surgery aggravates postoperative recurrence and distant metastasis (34-36) and that early vascular flow control before the manipulation of tumor improves the survival outcome (37). Further study with larger study volume is needed to clarify whether no-touch RFA can provide a better efficacy of complete local necrosis compared with conventional DTP-RFA.

It is interesting to note that in our study, the number of total punctures per RFA procedure between two techniques differed significantly in our experiment (DTP-

RFA; 2 (median) (range: 2-3), no-touch RFA; 3 (2-5), P<0.001). Also, of interesting observation is that all of three cases with peritoneal seeding in no-touch RFA in our study required more than four punctures due to the repositioning of unsatisfactory initial electrode insertions without intervening RF heating (38). We assume during the repositioning, peri-nodular satellite tumor nodules may have been manipulated resulting in tumor seeding facilitation in the three cases. Albeit with the technical and experimental difficulty, it is noteworthy that statistical difference was still found between no-touch RFA and DTP-RFA. Nonetheless, no-touch RFA is assumed to be a more demanding technique that requires longer learning curve than DTP-RFA (2). Considering that radiologists are accustomed to placing electrode to the central portion of the tumor when an ultrasound-guided biopsy or ablation procedure, it might be expected that there might be a longer learning curve for no-touch RFA placing electrodes in the peritumoral zone with favorable geometry.

Recently, there are increasing experimental evidence that hepatic RFA contribute to the release of the tumorigenic factors which stimulate tumor development, growth, or more aggressive biology at separate sites within the same organ or in the distant tumor. However, in our study, there was no increase of tumorigenic factors including IL-6, HGF and VEGF and no different Ki-67 proliferation index in both RFA groups (no-touch RFA and DTP-RFA) and control group. We contribute the contrary results in our study to the study design on rabbit model, considering previous studies have been conducted on the small animal model (mouse (12, 13) and rat (10, 11, 39)).

There are several limitations to our study to be acknowledged. First, there was technical challenge performing US-guided RFA of VX2 tumor in rabbit liver due to the anatomy of rabbit liver which has five lobes of racemose type, and small size of the tumor. Nevertheless, we strived to mimic real clinical practice and found a statistical difference between DTP-RFA and no-touch RFA regarding the development of peritoneal seeding. Second, another limitation would be a relatively small sample size. Thus, additional study in an expanded number and clinical scenarios is required to acquire high statistical significance. Finally, as noted earlier, using a small animal model with VX2 tumor could be a limitation in extrapolating our data to a clinical setting, as the biological behavior of VX2 tumors may differ from that of human hepatocellular carcinoma. Nonetheless, we believe this study presents a reasonable preclinical proof to show the improved safety with treatment efficiency of no-touch RFA in subcapsular hepatic tumors.

CONCLUSION

In conclusion, no-touch RFA has lower peritoneal seeding rate and tendency to induce better local tumor control compared to those of DTP-RFA which may contribute to better treatment in a subcapsular hepatic tumor and serve as an attractive bridge therapy to liver transplantation

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Table 1. Tumor characteristics and In Vivo RFA specification.

Tumor characteristics	
Initial chip size	$4mm^3$
Location	Left medial lobe sub-capsular area
Days after implantation for RFA (median)	9 (range, 7-12)
Tumor Axial Long on pre-RFA CT (mm) (median)	8 (range, 5-12)
Tumor Volume on pre-RFA CT (mm^3) (median)	315 (range, 75-960)
In Vivo RFA specification	
Type	Dual Bipolar
Power (Watt)	50
Active tip (cm)	1
Distance between two RFA needle (mm)	10-13
Energy (Kcal) †	0.51 (± 0.07)
Time (sec) †	307.34 (± 110.34)

Note.-RFA, radiofrequency ablation; CT, computed tomography.

†Data is presented as mean (±standard deviation).

Table 2. Local tumor control between direct tumor puncture (DTP)-RFA and No-touch RFA.

	DTP-RFA	No-touch RFA	P value
Pathology			
Complete local necrosis*	6/11 (54.5%)	10/11 (90.9%)	0.148
Main tumor mass			
Within ablative zone	8/11 (72.7%)	10/11 (90.9%)	0.586
Abutting ablative margin	3/11 (27.3%)	1/11 (9.1%)	
Peri-tumoral satellite nodule			
Within ablative zone	6/11 (54.5%)	10/11 (90.9%)	0.148
Outside of ablative zone	5/11 (45.5%)	1/11 (9.1%)	
CT follow up			
Local tumor progresion	6/14 (42.8%)	4/14 (28.6%)	0.694
Local tumor control†	14/25 (56.0%)	20/25 (80%)	0.069

Note.-RFA=radiofrequency ablation, CT=computed tomography. H&E=haematoxylin & eosin, NADH=nicotinamide adenine dinucleotide

Data are presented as a number with the percentage in parenthesis.

*** Defined as the entire involvement of both main tumor and all peri-nodular satellite nodules within the ablative zone on H&E staining with no NADH staining.**

† Determined by either achievement of complete local necrosis on histopathologic examination or absence of local tumor progression on follow-up CT.

Table 3. Results of 6-week post-RFA CT follow up in direct tumor puncture (DTP)-RFA, No-touch RFA, and control group.

	DTP-RFA (N=14)	**No-touch RFA (N=14)**	**Control (N=10)**	**P value***
Peritoneal seeding	10 (71.4%)	3 (21.4%)	0 (0.0%)	***0021***
Skin seeding	8 (57.1%)	3 (21.4%)	0 (0.0%)	0.342
Lymph node metastasis	7 (50.0%)	4 (35.7%)	6 (60.0%)	0.187
Lung metastasis	10 (71.4%)	6 (42.8%)	2 (20.0%)	0.382

Note.-RFA= radiofrequency ablation; CT= computed tomography.

Data are presented as a number with the percentage in parenthesis.

*** DTP-RFA vs. No-touch RFA, using Fisher's exact test.**

P values with underline in Italic indicate statistical significance.

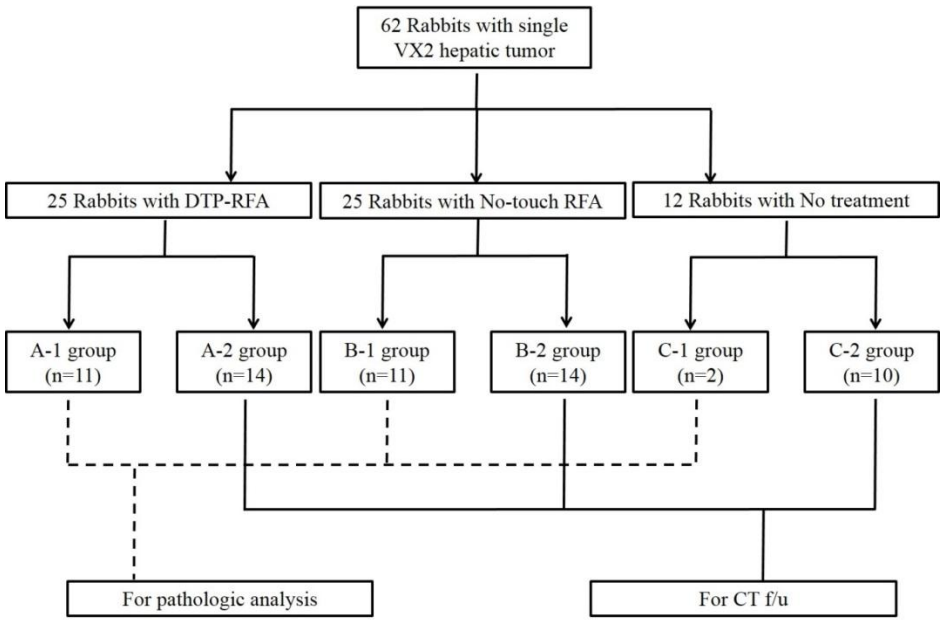


Fig 1. Study protocol

In all, 62 New Zealand white rabbits with confirmed VX2 tumor by computed tomography (CT) on day 9 (range,7-12) were randomized into three groups (DTP-RFA group, No-touch RFA group, and control group) and each group was further divided into two subgroups for pathologic analysis (A-1, B-1, C-1) and CT follow up (A-2, B-2, C-2) for 6 weeks.

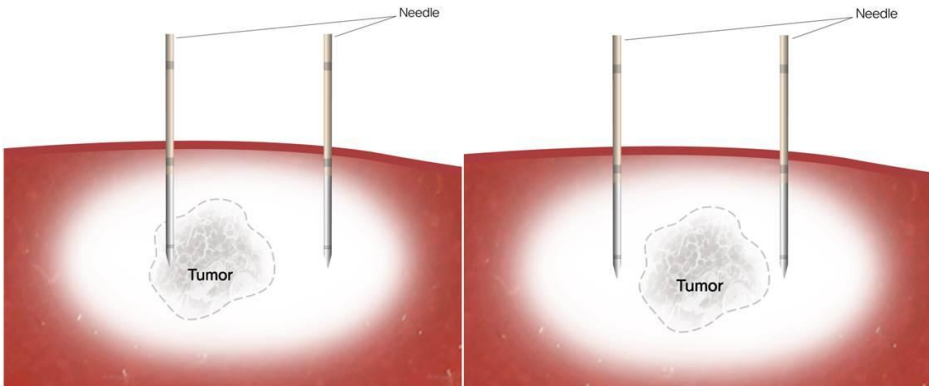


Fig 2. Illustration of DTP-RFA and no-touch RFA

(a) DTP-RFA technique. One of the dual electrodes is directly inserted into the tumor, violating tumor capsule. This technique initiates ablation within the tumor.

(b) No-touch RFA technique. Dual bipolar needles are inserted in the periphery of the tumor, not violating tumor capsule. This technique induces ablation starting from healthy tissue surrounding the tumor, extending centripetally into the tumor.

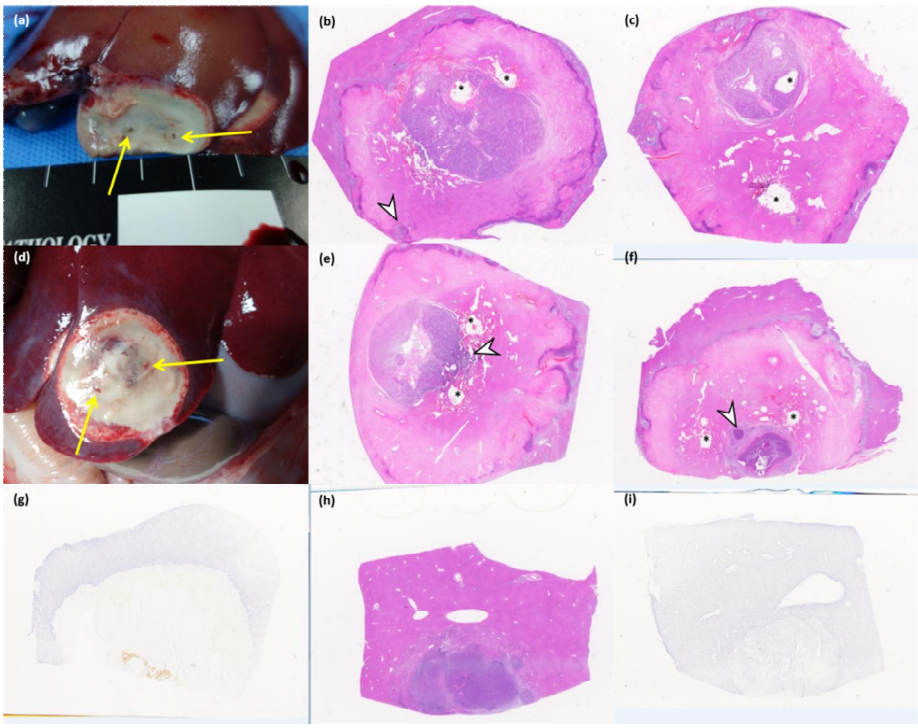


Fig 3. Histopathology in DTP-RFA, no-touch RFA, and control.

(a)-(c), DTP-RFA technique. (a) The gross picture shows one of the bipolar needles (arrow) penetrated the tumor. (b) Hematoxylin and eosin (H&E) staining of the ablation zone reveals needle inserted site (asterisk) with the main mass within the ablation zone. Note that incomplete ablation was performed for satellite tumor nodule (arrowhead). The satellite nodule is located 6mm from the main tumor. (c) Another H&E staining of the ablation zone reveals needle inserted site (asterisk) with satellite nodule (arrowhead) located within the ablation zone. Note that the lateral border of the main tumor abutted ablation margin. (d)-(g), No-touch RFA technique. (d) The gross picture shows dual bipolar needles (dotted arrows) penetrated the periphery of the tumor. (e)(f) H&E staining of ablation zone in two different rabbits reveals needle inserted site (asterisk) with both the main tumor and satellite nodules (arrowhead) completely ablated. (g) Nicotinamide adenine

dinucleotide (NADH) staining of the ablation zone. Contrary to the normal viable cells with NADH staining outside of ablation zone, there is no viability within the ablation zone. (h)-(i), Control group. (h) H&E staining reveals the main tumor with multinodular satellite tumors. Note that all of satellite nodules are located within 2mm from the main tumor (i) NADH staining shows both viable hepatocyte and the main tumor.

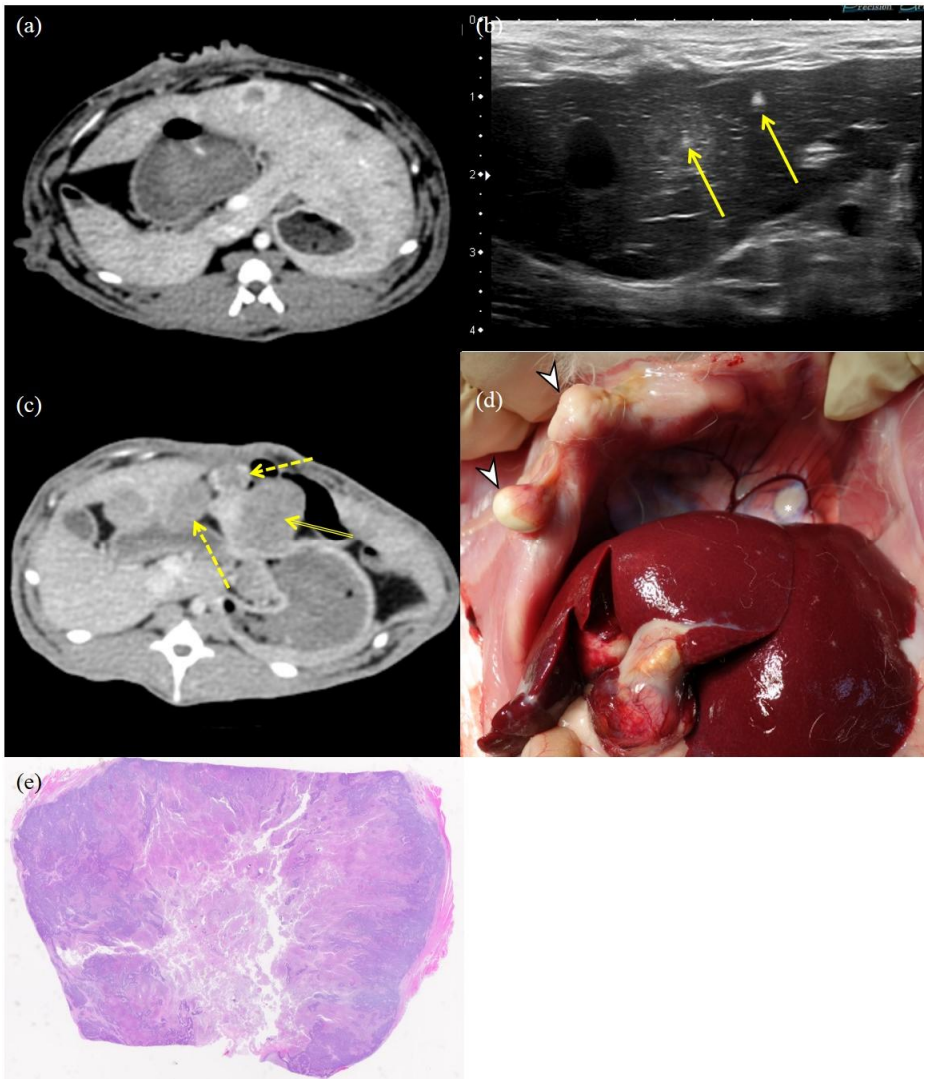


Fig 4. DTP-RFA

(a) Pre-RFA CT shows a 0.8cm peripheral enhancing tumor in left medial lobe subcapsular area. (b) Ultrasonography-guided RFA was performed, and one of two dual bipolar needles (arrows) accurately penetrated the tumor. (c) Post-RFA 6week CT reveals multiple peritoneal seeding nodules (dotted arrows) and local recurrence (double lined arrow) to the inferior aspect of the previous tumor. (d) The gross picture shows peritoneal seeding nodules (arrowheads) and local recurrence. Lung metastasis (asterisk) is also shown. (e) H&E staining of peritoneal seeding

nodules confirmed as VX2 carcinoma.

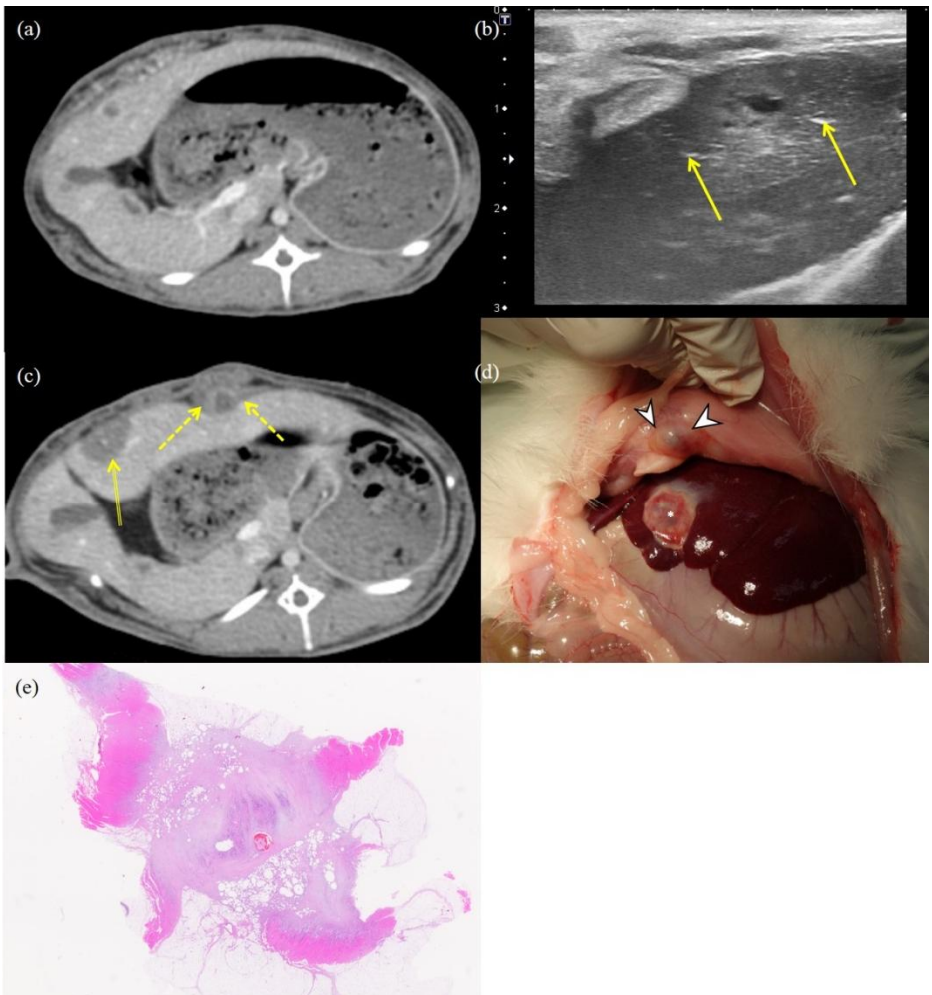


Fig 5. No-touch RFA

(a) Pre-RFA CT shows a 0.7cm peripheral enhancing tumor in left medial lobe subcapsular area. (b) Ultrasonography-guided RFA was performed, and dual bipolar needles (arrows) accurately penetrated the periphery of the tumor. (c) Post-RFA 6week CT reveals complete ablation of the tumor (double line) with a small amount of localized fluid collection with soft tissue anterior to the left lobe of the liver (dotted arrows). (d) The gross picture shows complete necrosis of the tumor (asterisk) and localized peritoneal fluid collection. (arrowh1eads) (e) H&E staining of peritoneal fluid collection confirmed as reactive fibrosis with a few lymphocytes

with no tumor.

Supplementary material 1. Ex Vivo test in Bovine Liver for RFA optimization using Dual Bipolar.

Distance	Energy (Kcal)	Time(sec)	Axial Long(mm)	Axial Short (mm)	Height (mm)	Volume (mm^3)
10mm	0.4	143	18	13.1	14.4	3400
	0.5	133	19.3	14.0	15.4	4160
	0.6	19.4	19.9	14.1	15.2	4260
13mm	0.4	81	19.8	12.1	12.9	3090
	0.5	109	21.7	12.5	14.9	4040
	0.6	130	22.0	13.6	14.6	4370
15mm	0.4	61	24.1	10.0	11.9	2870
	0.5	89	23.1	11.3	12.6	3290
	0.6	123	24.2	12.9	14.1	4400

Note.-RFA, radiofrequency ablation;

Supplementary material 2. The distance between satellite nodule outside the ablative zone and the main tumor.

	Direct tumor puncture-RFA (N=5)	No-touch RFA (N=1)	Control (N=2)
Distance (mm)	5 (2-8)	2	2 (1-2)

Note.-RFA, radiofrequency ablation;

Data is presented as median (range if possible).

Supplementary material 3. The Ki-67 proliferation marker in the ablated lobe.

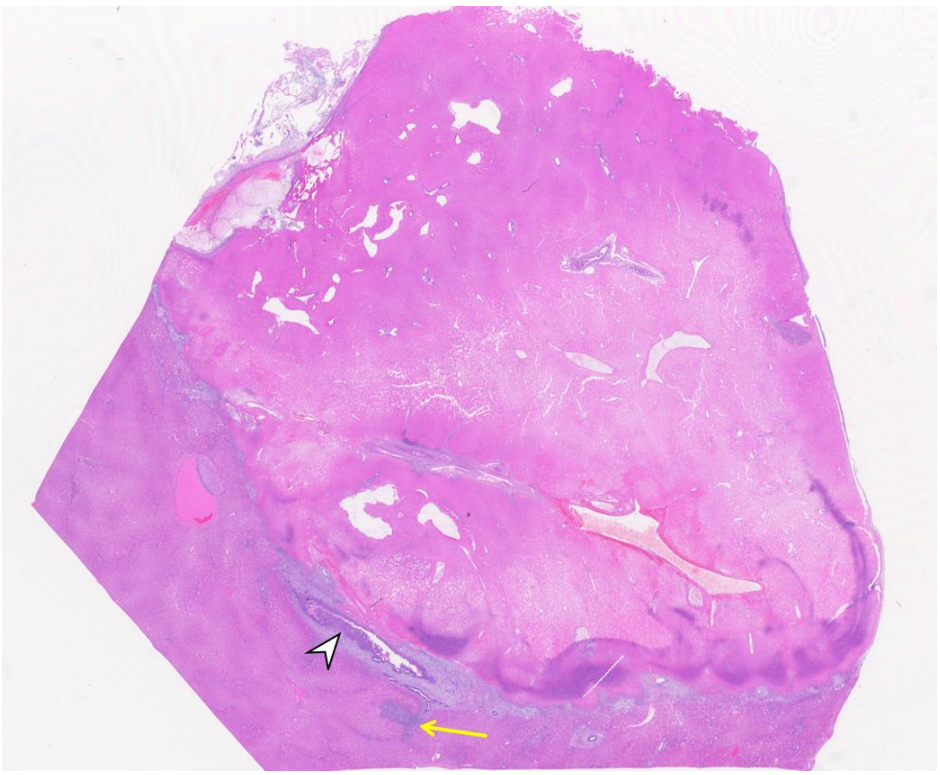
	Direct tumor puncture-RFA RFA (N=11)	No-touch RFA (N=11)	Control (N=2)	P value
Ki-67 index (cells per x40 frame) †	7.5±3.0	8.1±4.1	3.0±1.6	0.494††

Note.-RFA, radiofrequency ablation;

† Data is presented as mean (±standard deviation).

†† Using Analysis of Variance (ANOVA).

Supplementary material 4. Satellite tumor outside the ablative zone with intravascular tumor emboli in direct tumor puncture-RFA.



[Figure legend]

Hematoxylin and eosin (H&E) staining of the lower ablation zone reveals satellite tumor (arrow) and intravascular tumor emboli (arrowhead) outside the ablative zone. The distance between satellite tumor and main tumor (not shown in the slide) was 7mm.

국문 초록

소아 토끼의 생체 내 VX2 간 종양의 종양-비접촉 고주파 절제술: 개념 증명 연구

목적

개념 증명 연구에서 우리는 양극성 모드의 종양-비접촉 고주파 절제술 (RFA, radiofrequency ablation) 기술을 토끼 생체 내 VX2 간 종양모델의 국소 종양 조절, 복막 파종 및 종양 형성 인자에 대한 기존의 직접 종양 천자 RFA와 비교하고자 하였다.

재료 및 방법

VX2 간 종양을 가진 62 마리의 토끼를 3 개의 그룹으로 나누었다 : A 군 (25 명), 직접 종양 천자 RFA (dtpRFA); 그룹 B (n = 25), 종양-비접촉 RFA (ntRFA); 대조군으로 C 군 (n = 12). 세 그룹은 각각 RFA 후 6 주까지 병리학적 분석 (n = 24) 또는 CT 추적 관찰 (n = 38)을 위해 두 세트로 세분되었다. 초음파 삽입 유도 된 dtpRFA와 ntRFA는 종양 삽입 후 9 일째 시행되었다. RFA의 국소 종양 조절은 조직 병리학적 검사에서의 완전한 종양 괴사의 달성 또는 추적 CT 및 부검에서 국소 종양 진행 (LTP)의 부재에 의해 결정되었다. 복막 파종의 발달도 동물 집단간에 비교되었다. RFA 후 1 일, 2 일 및 3 일째에 혈장 내피 성장 인자 (VEGF) 및 인터루킨 -6 (IL-6)을 종양 원성 인자 평가를 위한 ELISA 분석법으로 측정 하였다.

결과

국소 종양 억제와 관련하여 B 군 (80 %, 20/25)은 A 군 (56 %, 14/25)보다 나은 제거율을 보였다 (P = 0.07). 완전 종양 괴사는 A 군 동물의 54.5 % (6/11) 및 B 군 동물의 90.9 % (10/11)에서 달성되었다. RFA 후 복막 파종은 B 군 (21.4 %, 3/14) (P = 0.021) 또는 C 군 (0 %)보다 A 군 (71.4 %, 10/14)에서 유의하게 흔하였다. HGF, VEGF 및 IL-6의 상승은 세 군 모두에서 발견되지 않았다.

결론

NtRFA는 복막 파종 율이 낮았으며 dtpRFA보다 우수한 국소 종양 대조군을 얻는 경향을 보였다.