# [原著] Radio frequency thermal coagulation therapy for lung tumors : An experimental study

# Radiofrequency thermal coagulation therapy for lung tumors: An experimental study

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## ABSTRACT

We developed a thermal coagulation therapy using radiofrequency dielectric warming as a local therapy for lung tumors and conducted a basic study of the procedure involved. A fine needle was percutaneously inserted into the tumor and radiofrequency waves emanating from the tip of the needle were converted to thermal energy, which serves to denature and necrotize the tumor cells. In order to evaluate the cytocidal effect of this method, cells of tumor tissue subjected to thermal coagulation were cultured and their morphology was evaluated histologically.

Twenty-three male Japanese white rabbits weighing 2 ~ 3 kg were used. A cell suspension of rabbit VX-2 tumor ($10^6$/0.3 ml/rabbit) was injected through the ear veins of the rabbits and 70 tumors with a diameter of 2 ~ 3 mm exposing on the pleural surface of the lung were collected 2 weeks later and used as implanted lung tumors. For thermal coagulation, the current was supplied for 10 seconds (N = 19), 20 seconds (N = 17) and 30 seconds (N = 26); while 0 second (N = 8) served as a control. Among the 70 tumors, 30 were used for cell culture and 40 for tissue specimens. In the culture study, cells from a single tumor were cultured and a growth curve was plotted. The number of viable cells grown following current supply fell significantly in comparison with the group with no current supply (Student's t-test, $p < 0.001$). There was no significant difference in viable cell count, however, among groups receiving current supply of various durations (10 seconds, 20 seconds, and 30 seconds). As for the effect on growth capacity, there was no significant difference between viable cell counts immediately after current supply and those 14 days later in groups receiving current supply for 10 seconds and 20 seconds. In the group receiving current supply for 30 seconds, viable cell count decreased significantly both immediately after current supply and 14 days later ($p < 0.05$). The temperature in the marginal part of the tumors was 64.8℃ on average at the time of dielectric warming. In tissue specimens, destruction of pulmonary alveolar structure accompanied by very slight emphysematous change in the pulmonary tissue surrounding the tumors was observed in 5 of 40 specimens (13%). From these results, it was concluded that this could serve as a new therapeutic procedure for lung cancer tumors. *Ryukyu Med. J., 17(4)203~209, 1997*

Key words: Lung tumor, Radiofrequency, Coagulation therapy

## INTRODUCTION

Presently, surgical resection is the therapeutic method of first choice for malignant tumors of the lung[1]. For quite a few patients, however, chemotherapy, radiotherapy, or immunotherapy, etc., is selected since they cannot be subjected to surgical resection due to a poor general condition, including insufficient pulmonary functions[2].

Since solitary tumors in the lung are surrounded by lung tissue that contains air, which is of low thermal conductivity, the heat reservoir effect following warming is high. It was thus considered that a high-temperature zone could be generated without elevating the temperature of surrounding tissues. Percutaneous insertion of a fine needle into lung tumors is a standard lung biopsy method in the field of interventional radiology[3]. Therefore, it was considered that an efficient local thermal coagulation therapy could be developed for solitary tumors in the lung, by applying radiofrequency dielectric thermal

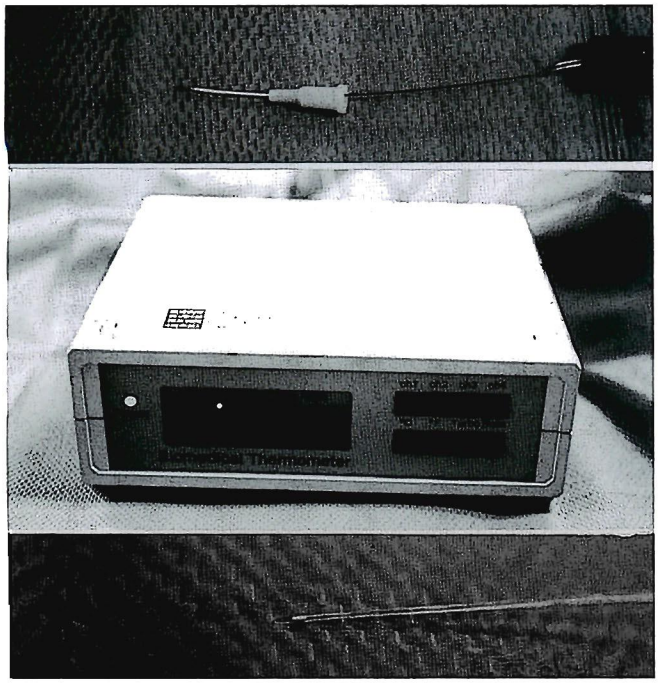

Fig. 1a. Puncture needle.
1b: Optical fiber thermometer.
1c: The tip of optical fiber thermometer.

energy through a fine needle. At present, local thermal coagulation therapy utilizing radiofrequency dielectric warming is applied clinically to hepatic cancer[4], hepatic metastasis of malignant tumors[5], brain tumors[6], and osteoid tumors[7]. The only therapeutic method that has been reported for lung cancer utilizing radiofrequency is hyperthermia therapy using radiofrequency dielectric warming[8].

We therefore developed a local thermal coagulation therapy using radiofrequency dielectric warming as a less-invasive and replicable therapeutic method for malignant tumors of the lung.In this study, we conducted basic experiments on this new therapeutic method to evaluate its usefulness.

## MATERIALS AND METHODS

1) Radiofrequency Generator

A Surgitron FFPF (Ellman, New York, USA) with a frequency of 3.8 MHz was used as a radiofrequency generator and experiments were conducted at an output of 15 W. The radiofrequency inducing probe was a stainless steel wire 0.016 inch (0.41 mm) in diameter. A polyester tube (Telmo, Tokyo) with an outer diameter of 24 G (0.56 mm) was used as an insulating material to coat the wire. A section 2 ~ 3 mm in length was left uninsulated (Fig. 1a). A counter-electrode plate was tightly attached to the back of the rabbit.

2 ) Experimental Animals

Twenty-three male Japanese white rabbits weighing 2~3 kg were used in the experiment.

The feeding and management of experimental animals and experimental methods used in this study comply with the rules established by the "Committee for Experimental Animals of the University of the Ryukyus" and were approved by the Committee (Approval No. 1267).

3) Lung Tumor Model

Experimental lung tumors used were made by injecting 0.3 ml of cell suspension of VX-2 tumor ($3 \times 10^6$ cell /ml) prepared according to Sekiguchi et al.[9] into the ear vein to inoculate the rabbits with tumors. The inoculated animals were fed for 2 weeks.

4) Radiofrequency Thermal Coagulation

Fifty mg/kg of Ketamine (Sankyo, Tokyo) and 0.5 mg/kg of Ceractar (Bayer, Leverkusen, Germany) were administered intramuscularly to the rabbits to induce anesthesia. The lungs were then exposed one by one by thoracotomy. The cervical trachea was incised and a 5 Fr. catheter (Clinical Supply, Gifu) was inserted. After that, the trachea was ligated and air was injected through the catheter to expand the lungs. A radiofrequency probe of 2~3 mm length was then inserted into the implanted VX-2 lung tumor, which was exposed on the pleura, and radiofrequency dielectric warming was begun.

5) Measurement of Tissue Temperature

The temperature distribution in the tumor and surrounding lung tissue was measured by an optical fiber thermometer (Biomedical Thermometer, Yamamoto Vinytar, Osaka) 10 seconds (N = 3), 20 seconds (N = 3) and 30 seconds (N = 3) after the current supply (Fig. 1b, c). Temperature was measured in the marginal area of the tumor, and in the lung tissue 5 mm and 10 mm away from the needle.

6) Histological Findings

After warming by supplying current for various periods of time [10 seconds (N = 13), 20 seconds (N = 9), and 30 seconds (N = 18)] , tissues were fixed in 10 percent formalin solution and embedded in paraffin for histological examination. The paraffin block was cut into 4 $\mu$ m thick sections and stained with hematoxylin-eosin. Histological changes were evaluated for the zone within 0.5 mm from the puncture needle and areas 0.5 mm or farther away from it.

7) Cytocidal Effect

Cytocidal effect was evaluated after supplying current for 10 seconds (N = 6), 20 seconds (N = 8), and 30 seconds (N = 8).

For this, a suspension of single tumor cells was prepared from the tumor tissue immediately after current

Table 1 Measurement of tissue temperature

| Duration of warming (seconds) | Marginal zones (℃) | Surrounding areas (℃) |
|---|---|---|
| 10 | 61.3(51.4~72.6) | 38.6(37.3~39.7) |
| 20 | 68.5(60.5~75.2) | 38.1(36.4~39.3) |
| 30 | 64.6(59.4~69.7) | 38.1(37.0~39.7) |

(Body temperature 32.0℃, Rectal temperature 36.0℃)

supply (Naniwa *et al.*[10]) and the number of viable cells was counted. The tumor and the surrounding lung tissue were immersed in 2 ml of 0.1% collagenase (Sigma, St. Louis, USA) solution in a dish immediately following current supply, and a 0.1% collagenase solution was injected into 10 or more sites on the boundary between the tumor and adjoining lung tissue. Then, the tumor was separated from the lung tissue using a pair of tweezers. The tumor was transferred to another dish, cut into small pieces, resuspended in 1 ml of collagenase solution, and kept warm at 37℃ for 60 minutes. Two ml of culture medium was added to halt the reaction of the collagenase. Single cells were obtained by pipetting. The cells were then centrifuged at 800 rpm for 10 minutes for precipitation. The supernatant was discarded and 1 ml of culture medium was added to prepare a suspension of tumor single cells. Then, 0.1% eosin solution (Eosin Y, Merk, Darumstadt, Germany) was added to count the number of viable cells under a microscope at a magnification of 200. The culture medium used was prepared by adding 10% FCS (Biowhittaker, Maryland, USA), 100 $\mu$g/ml of streptomycin (Meiji, Tokyo), and 100 $\mu$g/ml of penicil lin (Meiji, Tokyo) to HAM-F12 (Sigma, St. Louis, USA).

While counting, when no viable cells were observed in any of the 9 visual fields of a modified Neubauer cytometer (Hishigaki, Tokyo), it was considered that 1 or less viable cells were present in all visual fields. The lower measurement limit was $2.2 \times 10^3$ cell/ml.

8) Effect on Growth Capacity

Cells were cultured according to the technique of Sekiguchi *et al.*[9] to examine the growth capacity of viable cells in the tumors.

Current was supplied for various periods of time [10 seconds (N = 6), 20 seconds (N = 8), and 30 seconds (N = 8)]. Seven ml of culture medium was added to 1 ml of a single cell suspension similar to that described above to make 8 ml. Each 2 ml of the mixture was poured into 4 wells (24-well plate, Falcon 3047, New Jersey, USA) and then cultured in a $CO_2$ incubator (Hirasawa CPD-170, Tokyo). The number of viable cells in a well was counted on days 4, 7, 11, and 14 and expressed by viable cell number/single tumor. One ml of the culture medium was added on day 7. Tumors into which the radiofrequency probe had been inserted without subsequent application of radiofrequency were used as a control group (N = 8).

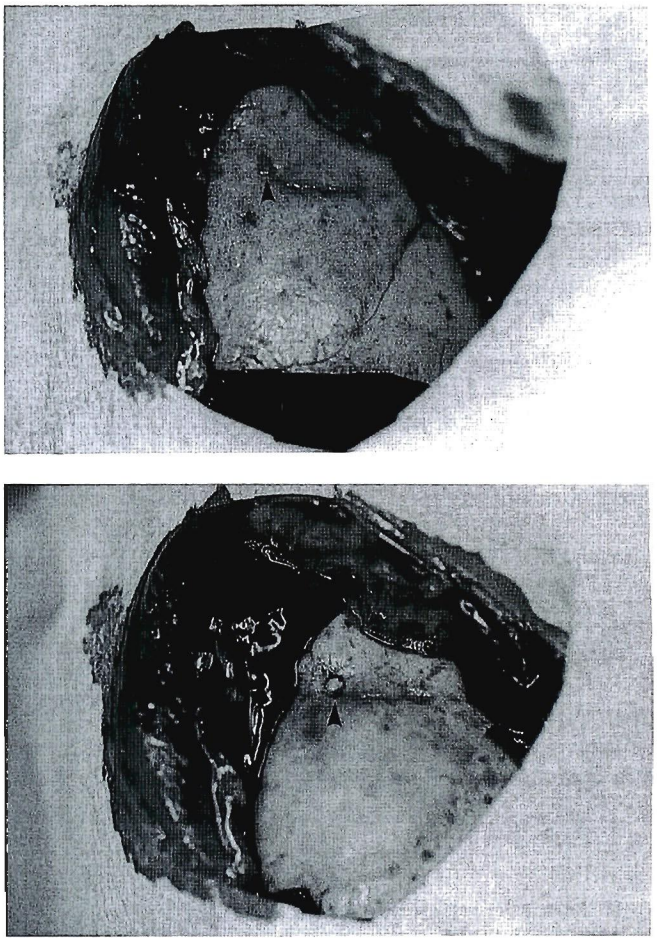

Fig. 2. A gross view of the lung tumor.
a: Before the insertion. The surface of the tumor is semitransparent white.
b: After the radiofrequency (RF) supply. The entire surface of the tumor is whitish as compared with the surrounding lung tissue.

## RESULTS

1) Preparation of Experimental Tumors

In all 23 rabbits, multiple implanted lung tumors with a diameter of 1 ~ 3 mm were observed in bilateral lungs.

2) Measurement of Tissue Temperature (Table 1)

The surface body temperature of the rabbits was 32.0℃ and the rectal temperature was 36℃. Temperatures in marginal zones of the tumors were 51.4~72.6℃ (61.3℃ average) after dielectric warming for 10 seconds, 60.5~75.2℃ (68.5℃ average) after 20 seconds, and 59.4 ~69.7℃ (64.6℃average) after 30 seconds. Temperatures of areas 5 mm away from the puncture needle were 37.3

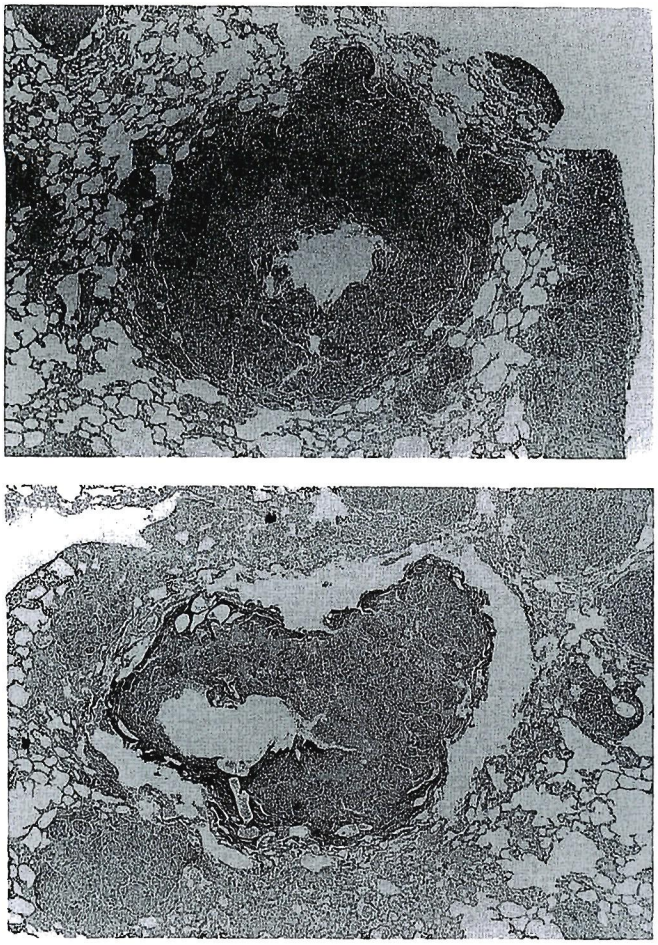

Fig. 3. Histopathologic findings of the tumor.
a: Before RF supply. Although the track of the needle insertion can be seen in the center, no destruction of the tumor tissue was observed (HE staining, ×10).
b: After RF supply. The structure of the tumor is destroyed. The alveoli around the tumor is little changed (HE staining, ×10).

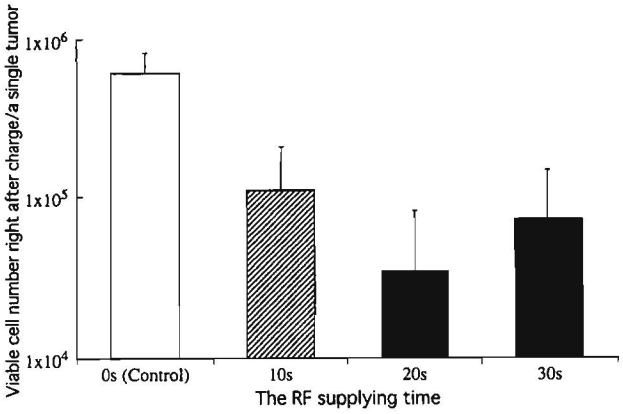


Fig. 4. The viable cell number immediately after charge in a single tumor treated with RF thermal coagulation therapy. RF energy was supplied for 10 seconds (hatched bar: N = 6), 20 seconds (solid bar: N = 8) or 30 seconds (spotted bar: N = 8) with 15 Watt, 3.8MHz. The control (open bar: N = 8) was not supplied with RF energy. One standard deviation (S.D.) is shown as a vertical line.

~39.7℃ (38.6℃ average) after dielectric warming for 10 seconds, 36.4~39.3℃ (38.1℃ average) after 20 seconds, and 37.0~39.7℃ (38.1℃ average) after 30 seconds. Temperatures of parts 10 mm away from the puncture needle were 34.4~36.5℃ (35.1℃ average) after dielectric warming for 10 seconds, 34.7~36.8℃ (35.5℃ average) after 20 seconds, and 34.8~36.9℃ (35.7℃ average) after 30 seconds.

3) Morphologic Changes

The color of all tumors (N = 40) changed from semitransparent white before current supply to opaque white following current supply, regardless of the duration of the current supply. However, no obvious change was observed in surrounding lung tissue (Fig. 2a, b).

4) Histological Changes

In all specimens, tumor cells were destroyed or denatured over several layers in zones surrounding the puncture needle track (Fig. 3a, b). However, in all specimens, there were cells without clear evidence of cellular death in zones within 0.5 mm from the puncture needle track. In the areas 0.5 mm or farther away from the puncture needle, clear destruction or denaturation of the tumor structure was observed in 10 of 13 specimens (76%) in the group receiving current supply for 10 seconds, in 8 of 9 (88%) in the 20-second group, and 15 of 18 (83%) in the 30-second group.

In the lung tissue surrounding the tumor (in the range of 0.1~0.7 mm/0.27 mm average/from the margin of the tumor), the following changes were observed in 1 of 13 specimens (8%) in the group receiving current supply for 10 seconds, 2 of 9 (22%) in the 20-second group, and 2 of 18 (11%) in the 30-second group: pulmonary alveolar structure was destroyed, causing emphysematous changes, and denatured lung cells strongly stained by hematoxylin were present, which appeared to be attached to the tumor tissue on its boundary (Fig. 3b). These changes were not observed, however, in areas 0.7 mm or more away from the puncture needle track.

5) Effect on Growth Capacity

The number of residual viable cells immediately after radiofrequency current supply was $(11 \pm 10) \times 10^4$ cells/tumor (mean ± S.D.) for the group receiving current supply for 10 seconds (N = 6), $(3.4 \pm 4.7) \times 10^4$ cells/tumor for the group receiving current supply for 20 seconds (N = 8), $(7.2 \pm 7.6) \times 10^4$ cells/tumor for the group receiving current supply for 30 seconds (N = 8), and $(62 \pm 20) \times 10^4$ cells/tumor for the control group (N = 8). There was a significant difference in the viable cell count between the groups receiving current supply (N = 22) and the control group (N = 8; Student's t-

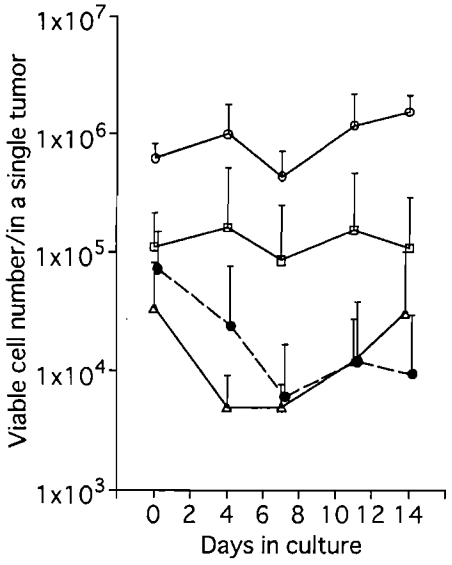


Fig. 5. The effect of the RF thermal coagulation therapy on the tumor cell growth in vitro. VX-2 tumors were treated with the RF for 10 seconds ( □ ; N = 6), 20 seconds (△ ; N = 8) or 30 seconds ( ●; N = 8) and no treated ( ○; N = 8) . One standard deviation (S.D.) is shown as a vertical line.

test, $p < 0.001$). No significant difference in viable cell count was observed, however, among the groups receiving different current supply (Fig. 4).

The results of cell culture are shown in Figure 5. In the group receiving current supply for 10 seconds, no significant change (increase/decrease) in viable cell count was observed between the observations made immediately after current supply and 14 days later, although the viable cell count varied during the culture period. Viable cells increased throughout the culture period in 1 of 6 specimens. During the culture period (on day 4 and day 7), values below the lower measurement limit were obtained in one specimen. The viable cell counts did not fall below the lower measurement limit on day 14 in any of the specimens. In the 20-second current supply group, viable cells tended to decrease on day 4 and day 7, and then to increase from day 7 to day 14. No significant increase or decrease in viable cell count was observed between observations made immediately after current supply. The viable cells were more on day 14 than immediately after the current supply in 1 of 8 specimens. The viable cell count fell below the lower measurement limit during the culture period (days 4, 7, and 11) in 3 specimens. In one specimen, the viable cell count on day 14 was below the lower measurement limit.

In the 30-second current supply group, the tumor cell count decreased up to day 7 ($p < 0.05$) and then exhibited slight increase/decrease on days 10 and 14, with no significant difference from day 7. Viable cell count immediately after current supply was significantly decreased as compared with that of 14 days later ($p < 0.05$). There were fewer viable cells on day 14 than immediately after current supply in all specimens. The viable cell count on day 14 was below the lower measurement limit in 6 of 8 specimens.

In the control group, tumor cells increased from the day of insertion to day 4, decreased in number from day 4 to day 7, and increased again from day 7 to day 14. There was a significant increase in the viable cell count ($p < 0.001$) as compared with that immediately after insertion and day 14. In the control group, the maximum tumor cell count per well was $2.4 \times 10^6$ cell/well. Tumor cells with this experimental system seemed to reach confluence at about $2\sim3 \times 10^6$/well.

## DISCUSSION

We have conducted a basic study of thermal coagulation therapy. For this, we developed a local thermal coagulation therapy using radiofrequency dielectric warming as a less-invasive, replicable therapeutic method for malignant tumors of the lung.

VX-2 tumors are a form of transplantable primary cancer first prepared from rabbit papilloma by Rous *et al.* in 1935. This tumor is characterized by extremely high local malignancy and causes hematogenous lung metastasis[11]. Intravenous injection of VX-2 cell suspension nearly always causes multiple lung metastases. Rabbits developing multiple metastases survive only about 2～3 weeks after intravenous injection. In our experimental system, we used rabbits surviving 2 weeks after intravenous VX-2 injection and having metastatic lung tumors about 2～3 mm in diameter.

A radiofrequency probe was inserted into the lung tumor to induce radiofrequency dielectric warming. After supplying electricity for 30 seconds or more, tissues in the vicinity of the probe were carbonized and the temperature around the tumor margins decreased. Thus, current was supplied for 10～30 seconds in this experimental system.

Microscopic examination of the cytocidal effect immediately following therapy showed that some tumor cells were not killed microscopically in all specimens. Therefore, a dye exclusion method by eosin staining was used to confirm cell viability. As a result, residual viable cells were found in the tumors.

This method utilizes the fact that dead cells are stained by eosin dye and is an excellent means of identifying dead cells. The cellular death was defined as disorder of the cell membrane. The inherent disadvantage of this is that cells not stained by dye are deemed to have growth capacity even if they are suffering from disorders[12]. This is thought to be the reason why the viable cell counts immediately after receiving the current supply were not related to the growth curves obtained later. Consequently, it was considered necessary to study presence/absence of regeneration of residual tumor cells for judging the cytocidal effect over all regions of the tumor. In this experimental system, cell cultures were grown since the

rabbits could not survive for a long time.

During the 14-day cell culture period a significant decrease in viable cell count in the group receiving current supply for 30 seconds was observed. In this group, the viable cell count was below the lower measurement limit on day 14 in 6 of 8 specimens. In the remaining 2 specimens, it was also lower on day 14 in comparison with that immediately after current supply, and no regeneration was observed during the culture period. It was considered that the thermal energy generated by the current supply caused an irrevocable disorder in the tumor cells so as to induce cellular death[12] and apoptosis by heat shock[13]. The thermal energy released also damaged the growth capacity of tumor cells that did not die.

Consequently, the cytocidal effect of thermal energy obtained by supplying radiofrequency for 30 seconds was considered to be appropriate. However, there was a possibility that residual tumor cells could begin to regenaration on day 14 or thereafter. It was thus considered necessary to perform repeated thermal coagulation therapy. In the group receiving current supply for 20 seconds, viable cell counts decreased until day 4, but then increased from day 7. This was because cells that had been damaged, but not killed, were restored after the renewal of tumor cells retaining growth capacity[12]. In the group receiving current for 10 seconds, slight increases or decreases in viable cell counts immediately after current supply and on 14 days later were noted. This was considered to be due to a combination of an increase in cellular death due to damage by heat energy and a decrease in growth rate[12].

In this experimental system, gross changes due to local warming were localized in the tumors. Histological denaturation of surrounding lung tissue by heat was also localized in the vicinity of the tumors. Destruction of pulmonary alveolar structures was most frequently observed when the current was supplied for 20 seconds. According to Brend *et al.*, histological changes following burning of the lungs appeared after a latent period. Findings such as inflammation, interstitial edema and pneumonia did not appear until the animals survived for at least several hours after burning[14, 15]. In this experiment, the rabbits were killed immediately after supplying the current, so the changes in the surrounding lung tissue due to thermal energy may have been incomplete. Further investigation is required before this methodology can be applied in a clinical setting.

This percutaneous thermal coagulation therapy for lung tumors is based on an established technique for percutaneous needle biopsy of lung tumors[3], and is considered to be clinically applicable.

## CONCLUSIONS

1) High-temperature regions obtained through thermal coagulation therapy were localized in tumors and their vicinities, while destruction of pulmonary alveolar structure was very mild. Some papers have reported, however, that thermal denaturation of lung tissue appears at a later stage. Thus, further studies are necessary before clinical application of this method.

2) In the group receiving current supply for 30 seconds, during the 14-day cell culture period, the number of tumor cells decreased significantly. The number of cells did not increase in comparison with that immediately after current supply in any specimen. The therapeutic effect was thus considered to be proved.

3) There is a possibility that regenaration may occur following day 14, so repeated therapy may be required.

4) This therapeutic method is considered to be effective for lung tumors.

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## REFERENCES

1 ) Boring C.C., Squires T.S. and Tong T.: Cancer Statistics, 1992. CA Cancer J. Clin. 42: 19-43,1992.
2 ) Oho K.: Trachea, bronchus and lung.: Standard Surgery (Mutou T., Tanabe T. eds), 315-343, Igakusyoin, Tokyo, 1991 (in Japanese).
3 ) Wescott J.L.: Percutaneous transthoracic needle biopsy. Radiology 169: 593-601, 1988.
4 ) Rossi S., Fornari F. and Buscarini L.: Percutaneous ultrasound-guided radiofrequency electrocautery for the treatment of small hepatocellular carcinoma. J. Intervent Radiol. 8: 97-103, 1993.
5 ) Solbiati L., Lerace T. and Goldberg S.N.: Percutaneous US-guided RF tissue ablation of liver metastasis: long term follow up (abstr.). Radiology 197 (P): 199, 1995.
6 ) Anzai Y., Lufkin R., DeSalles A., Hamilton D.R., Farahani K. and Black K.L.: Preliminary experience with MR-guided thermal ablation of brain tumor. Am. J. Neuroradiol. 16: 39-48, 1995.
7 ) Rosenthal D.I., Springfield D.S., Gebhardt M.C., Rosenberg A.E. and Markin H.J.: Osteoid osteoma: percutaneous radio-frequency ablation. Radiology 197: 451-454, 1995.
8 ) Matsuda T.: Clinical procedure of heating: lung and mediastinum: Hyperthermia manual (Matsuda T.

ed), 69-78, Maguburosu,, Tokyo, 1991 (in Japanese).
9 ) Sekiguchi M.: Cell isolation from human patient: Isolation and cultivation of functional cell (Mitsui Y., Takagi R., Ichihara A., Sekiguchi M. and Matsumura T. eds), 391-416, Maruzen, Tokyo, 1987 (in Japanese).
10) Naniwa M.: Isolation and cultivation of normal cell: Isolation and cultivation of functional cell (Mitsui Y., Takagi R., Ichihara A., Sekiguchi M. and Matsumura T. eds), 380-390, Maruzen, Tokyo, 1987 (in Japanese).
11) Komatsu T.: An experimental study of chemoemobolization for VX2 bone tumors. J. Jpn. Orthop. Assoc. 69: 30-42 1995 (in Japanese with English summary).
12) Takeda T.: Cellular damage and viable test: Animal cell culture: A practical approach (Mitsui Y. ed), pp 225-263, Maruzen, Tokyo, 1990 (in Japanese).
13) Thompson C.B.: Apoptosis in the pathogenesis and treatment of disease. Science 267: 1456-1462, 1995.
14) Brinkmann B. and Puschel K.: Heat injuries to the respiratory system. Virchows Arch. A. Path. Anat. and Histol. 379: 299-311, 1978.
15) Hasleton P.S., McWilliam L. and Haboubi N.Y.: The lung parenchyma in burns. Histopathology 7: 333-347, 1983.