Original Paper

Radiofrequency Ablation of Hepatic Tissue: A New Experimental Animal Model

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ABBREVIATIONS: Radiofrequency Ablation (RFA); Hepatocellular Carcinoma (HCC)

ABSTRACT

Background/Aims: Experimental radiofrequency ablation has been already performed in healthy livers of porcine models, but not in less expensive and easy-to-manage rats, being so far unavailable devices capable of delivering radiofrequency ablation in the 20-30g liver of such small animals.

Methodology: We experimented a modified system of radiofrequency ablation of liver tissue in rat models developing a custom-made needle-micro-electrode of very small dimensions (0.3*2mm) and an electrode-tip cooling technique, based on saline solution infusion.

We adjusted duration (seconds) and power (watts) of radiofrequency ablation letting them range between 5-50 seconds and 5-25 W, respectively, to obtain the greatest lesions with the least side effects. After sacrificing the animals, an accurate histological examination of the liver was made.

Results: It is possible to establish beforehand the diameter of thermal liver lesion on the basis of joules of applied energy. The greatest increase of liver thermal lesion diameter (8mm) is obtained with a 250-joule (10 W for 25 seconds) thermal energy cooling the electrode-tissue interface.

Conclusions: Experimental radiofrequency ablation in rat liver is an effective and cheap way to study its effects on healthy hepatic tissues. It might be the first step to treat experimentally caused liver tumors.

INTRODUCTION

Radiofrequency ablation (RFA) is a relatively new therapeutic modality for the treatment of non-radically resectable hepatic tumors and, in experimental way, also for renal, pulmonary, brain, musculoskeletal system, thyroid and parathyroid glands, pancreas, lung and breast cancers (1-4).

While short-term clinical efficacy of this treatment has been proved, RFA cannot yet be considered a new type of radical treatment for solid tumors because of lack of long-term results and of some data regarding histological and molecular effects of radiofrequency on the focal lesion and the surrounding healthy tissues.

The devices utilized to perform RFA have been significantly ameliorated in the past few years and new possible clinical applications are foreseeable in the near future. A major role for RFA is predictable in the treatment of patients with hepatocellular carcinoma (HCC). In fact, recent studies demonstrated that RFA is quickly turning out to be one of the most important non-surgical options for liver human tumors (5-7).

Thus, whereas short-term clinical efficacy of RFA on HCC has been proved, at present poor information is available about effects on tumor and healthy hepatic tissue. Since histologic and immunohistochemical study of hepatic tumors treated with RFA in a large subset of patients is almost impossible, experimental model is given a central role for the evaluation of long-term effects of radiofrequency on tumoral tissues. In the past, experimental RFA on porcine liver has been reported (2,3,8), but they have proved to be expensive and hard to manage.

Instead, rats are cheaper experimental models and a higher number of animals (and procedures) can be included in the same protocol study; by this way, it is possible to investigate the effects of RFA according to each variable (power, time of delivery, intensity, time passed since the completion of the procedure, etc.). Several murine experimental models of hepatic primary (9-11) and metastatic (12-15) tumors have been reported in the literature and they all may be effectively used to investigate the effects of RFA. Unfortunately, the weight of the rat liver is 20-30g and no device has so far been available to deliver radiofrequency with adequate power and intensity in such a small organ. In the present study, we like to report the results of a new experimental model of radiofrequency experimentally performed in the rat model.

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METHODOLOGY

Approval for this protocol was obtained by the Ethical Committee of the Catholic University of Rome.

Four male healthy rats (Weight, 250-260g, age 14 weeks) were used.

Before starting the RFA procedure, anesthesia was induced by an intramuscular injection of a mixture of 2cc of Ketamine and 1cc of Medetomidine.

Open laparotomy was performed through midline xifo-pubic incision and the needle-electrode was directly inserted into the liver **(Figure 1)**.

RFA Procedure

The RFA lesions were produced by applying RF energy between an interventional mini needle-electrode (diameter 0.3mm, length 2mm) and a large auto-adhesive patch electrode applied to the animal's back. RF energy was delivered with the prototype of a custom made generator (N.Gi.C. srl, RF ATS), with three different RF outputs of 500, 750 and 1000kHz, microprocessor-controlled timing of onset and offset of energy delivery and telemetry of voltage, current and impedance.

The amount of thermal energy (joule) administered after placing the RF needle-electrode in separate locations of the liver of the rat, varied as a function of both the duration of application (ranging between 5 and 50 seconds) and the power of RFA applied (ranging between 5 and 25 Watts). The above parameters were differently coupled to find out the best combination of energy giving the desired lesion dimension without undesirable side effects, such as the "popcorn" effect or the electrode carbonization.

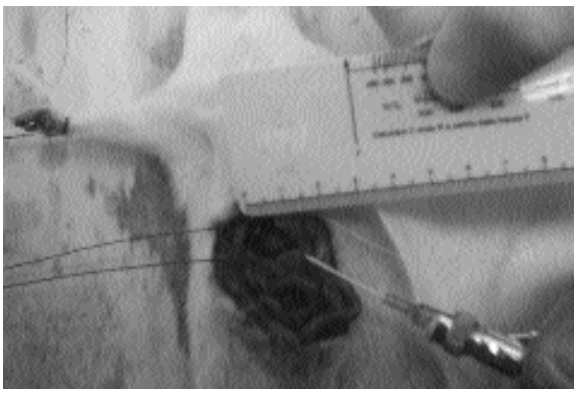

FIGURE 1 Photograph showing open laparotomy of the rat; an ablation needle micro-electrode inserted directly into the liver is seen.

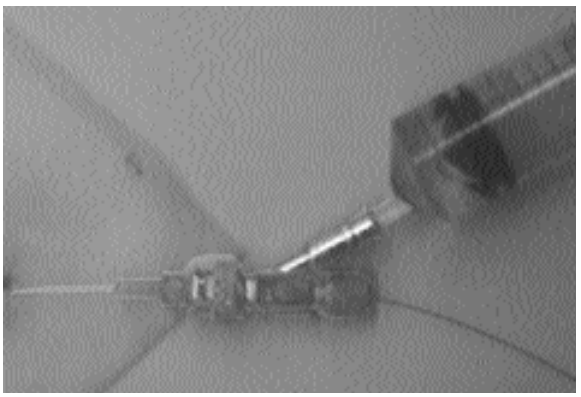

FIGURE 2 Photograph showing the custom-made ablation micro-electrode (diameter 0.3mm, length 2mm), internally cooled with infusion of saline solution through an insulated platinum wire inserted in a plastic catheter. Electrode tip is also seen.

In order to prevent carbonization of the needle-electrode after delivering RF with 100 joules energy, we developed a simple custom-made ablation micro-electrode of very small dimensions (0.3*2 mm). It consisted of an insulated platinum wire inserted in a plastic catheter, with a two-way stopcock allowing electrode's tip cooling with saline and adjustment of the active electrode protrusion with millimeter precision **(Figure 2)**. With this device it was possible to induce areas of coagulation necrosis of proper and reproducible size.

Gross Examination

After treatment, the animals were sacrificed by decapitation; the liver was removed *en bloc* and fixed in 10% buffered formaldehyde; after the gross observation **(Figure 3)**, samples of the RFA liver lesion were embedded in paraffin and then stained with hematoxylin-eosin for microscopic examination.

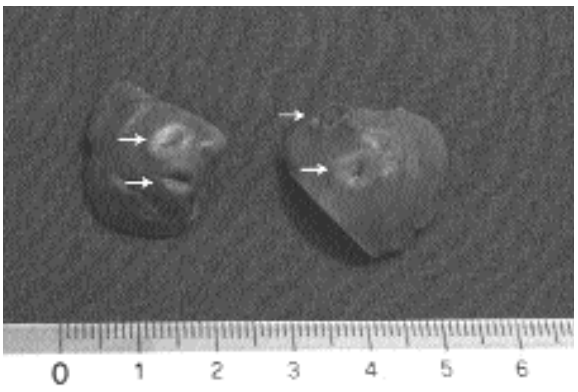

FIGURE 3 Two gross specimens of rat liver after 250 joule of RFA. Four thermal, umbilical lesions (arrows) of 7-8mm in diameter are seen.

TABLE 1 First Session of RFA in Two Rats Hepatic Models

I RFA session in separate liver locations	Watt of power	Time (seconds)	Cooled saline solution (mm)	Joule	Diameter of thermal lesions
I	5	5	no	25	not measurable
II	5	10	no	50	1
III	10	5	no	50	1
IV	10	10	no	100	1.5
V	10	10	yes	100	2.5
VI	20	10	yes	200	5
VII	25	10	yes	250	7
VIII	5	50	yes	250	7
IX	10	25	yes	250	7
X	10	25	yes	250	7

Statistical Analysis

All variables were expressed as mean ±SD. The reproducibility of the lesion size was evaluated by computing the variation coefficient (SD*100/mean).

RESULTS

We performed 25 RFAs in separate locations of the liver of 4 rats. In the first RFA session (first two rats) we applied an amount of thermal energy within a range of 25-250 joule **(Table 1)**. Without cooling with saline solution infusion during the RFA session, the diameter of thermal lesion after RFA was 1mm and 1.5mm with an amount of thermal energy of 50 and 100 joule, respectively. Otherwise, the use of saline infusion to continuously cool the needle-electrode's tip, allowed us to obtain a thermal lesion of 2.5-7mm in diameter, with an amount of thermal energy ranging from 100 to 250 joule **(Table 1)**. Therefore, since a thermal energy of 250 joule is ideal to achieve a maximum of lesions diameter, we decided to test this amount of energy in other sites of the rat's liver after guaranteeing the cooling of the needle tip with cooled saline infusion. We adopted three different modalities of application of energy: in the first one we applied 25 W of power for 10 seconds, in the second one 5 W for 50 seconds and in the third one 10 W for 25 seconds **(Table 1)**. The thermal lesion diameter was the same in every modality of RFA, but the two rats tolerated better the second and third modality (no reflex muscle contraction was observed).

To confirm our observations we performed another RFA session in other two rats only using the third modality of thermal energy application (second session of RFA).

TABLE 2 Second Session of RFA in Two Rats Hepatic Models

II RFA session	Watt	Seconds	Cooled saline solution infused	Joule	(mm) Diam (mm) mean±SD	# Lesions
Rat 1	10	25	yes	250	7.25±0.41	6
Rat 2	10	25	yes	250	7.27±0.36	9

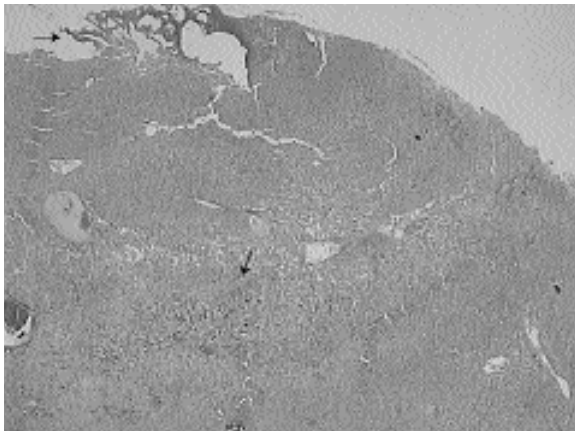

FIGURE 4 Microscopic examination revealing the area of thermal lesion with 6.5x2mm dimensions in which artifacts of thermal electro-coagulation type (horizontal arrow) and extravasated erythrocytes in intercellular spaces (oblique arrow) are observed (original magnification x40).

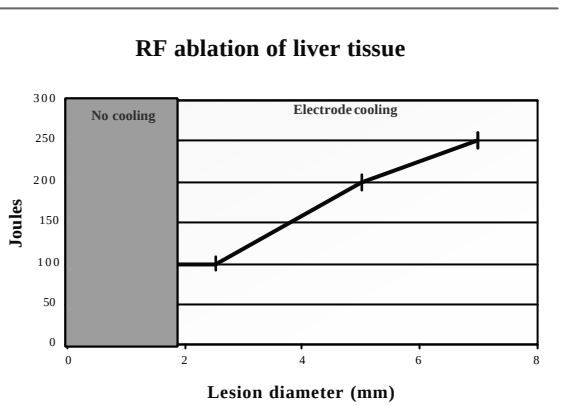

FIGURE 5 Graph showing a linear correlation between RFA energy (joule) and diameter of thermal lesion both with cooling of the needle electrode and with no cooling. The RFA was administrated with an energy that varied from 25 to 100 joule without electrode cooling and from 100 to 250 joule with electrode cooling. The thermal lesion in the rat liver was measured after sacrificing the animal.

The mean size of 15 RFA liver lesions was 7.26mm ±0.37 (mean ±SD) **(Table 2)**.

The reproducibility of the lesion size was 5.11 evaluated by computing the variation coefficient (SD*100/mean).

On histologic examination, we observed an area of variable dimensions in which there are tissue loss and artefacts from thermal electro-coagulation, in its center, and viable cells with blood red cells extravasated in intercellular spaces, peripherally **(Figure 4)**.

DISCUSSION

Radiofrequency is a largely used therapeutic modality for solid non-surgically resectable tumors. Its use as an alternative to surgical excision is now an attractive topic in scientific circles. However, experimental confirmations are needed to consider RFA as a potential radical treatment. The animal model represents an excellent experimental field for the pre-clinical development of medical devices including radiofrequency generators. RFA has only been tested in big and medium animal models (16-20). The rat is a widely utilized experimental animal because of its physical resistance and low costs, but the small size of its liver does not allow it to be considered suitable to test invasive techniques such as RFA.

The availability of an inexpensive and easy-to-manage experimental model could clarify several aspects of RFA. The present study reports for the first time the feasibility and effectiveness of RFA carried out in the rat liver. Our method is inexpensive and easy to perform. Since a large number of animals can be treated, effects of radiofrequency can be evaluated according to all potential variables.

It is well known that for any given electrode size and tissue contact area, RF lesion size is a function of RFA power and duration of application. These parameters are limited when the tissue electrode interface reaches 100°C; due to increasing impedance, proteins denaturation leads to the appearance of a coagulum on the electrode catheter. Cooling the electrode by saline

irrigation helps to prevent coagulum formation and allows the creation of deeper and larger lesions in animal models (21,22).

Of course, expandable needle-electrodes (about 14 gauge) used for human liver ablation cannot be utilized in the rat model due to the small size of the liver (maximum 4cm of transverse diameter). The needle is too big and the power too high to be adjusted according to dimension and weight of the organ.

We, therefore, decided to prepare an electrode which could be safely inserted in the liver of the rat. Adequate power was obtained using a custom made generator (N.Gi.C. srl, RF ATS).

The first attempts failed because of a too rapid desiccation of the surrounding hepatic tissue creating "pop-corn" effect. This consists of carbonization with impedance rising at the electrode-tissue interface and with considerable limitation of heat propagation into the tissue around the electrode and limited extension of coagulative necrosis. The problem was solved by cooling the electrode tip during RF delivery with saline solution chilled at room temperature at approximately 20-30cc/min **(Figure 2)**.

In this way, we performed several successful procedures; we could confirm the evidence of strict correlation between the amount of total energy applied and the diameter of thermal liver lesions in the rat liver model **(Figure 5)**. We found in a reproducible way that the diameter of the thermal liver lesion can be predicted with a good approximation, on the basis of the thermal energy administered during the procedure.

We also observed that thermal lesions of the same size (7-8mm diameter) could be obtained by applying the same amount of thermal energy (250 joule), independently of the different combination between duration and power pulse used to achieve that energy level (25 W of power for 10 seconds, or 5 W for 50 seconds, or 10 W for 25 seconds). The macroscopic findings were also confirmed by the histological comparison of the lesions obtained with the three different modalities, which gave superimposable results. However, the latter two modalities were clearly free from side effects on the animals, whereas the use of the highest power involved a sort of galvanic side effect observed as a strong abdominal contraction of the animal at the start of the RF application. For this reason we preferred to use the third modality (10W for 25 seconds) as the best compromise between avoiding unwanted side effects and pursuing the shortest possible ablation time.

Demonstrating that RFA in the rat is technically feasible, as we did in the present study, can lead to a second step of research consisting of an experimental RFA of animal tumor. Effects of RFA in humans are only evaluated with radiological follow-up. The experimental execution of RFA in neoplastic hepatic tissue of the rat should allow one to define the histological and biological effects determined by radiofrequency in healthy and neoplastic tissues.

In conclusion, in spite of some initial technical difficulties due to the size of the animals, RFA performed in rat's liver offers significant advantages such as lower costs, easier procedures and the possibility of control of the experimentation results within a short time. The present paper points out for the first time that RFA can be safely performed in very small experimental animal models. This result is an introductory step for the RFA's use in these animals affected by experimental hepatic tumors.

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