

Safety Limit of Large-Volume Hepatic Radiofrequency Ablation in a Rat Model

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Background: Large-volume hepatic radiofrequency ablation (RFA) has been used to treat large liver tumors, but its safety limit is unknown. This study aimed to investigate the possible systemic responses of large-volume hepatic RFA and to estimate its safety limit in normal and cirrhotic rats.

Hypothesis: Large-volume hepatic RFA causes a significant systemic inflammatory reaction.

Design: Experimental study.

Setting: University teaching hospital.

Intervention: Using the Cool-tip RF System (Radionics, Burlington, Mass), RFA was performed for different percentages of the liver volume by weight in normal and cirrhotic Sprague-Dawley rats.

Main Outcome Measures: Changes in concentrations of serum inflammatory markers (tumor necrosis factor α [TNF- α] and interleukin [IL] 6), functions of various end organs, and survival rates were assessed.

Results: In the normal liver groups, the concentrations of TNF- α and IL-6 were significantly elevated in the early postoperative period when 50% (mean \pm SD TNF- α con-

centration, 130.3 \pm 15.6 pg/mL; mean \pm SD IL-6 concentration, 163.2 \pm 12.2 pg/mL) and 60% (mean \pm SD TNF- α concentration, 145.7 \pm 13.0 pg/mL; mean \pm SD IL-6 concentration, 180.8 \pm 11.0 pg/mL) of the liver volume were ablated compared with the control group (mean \pm SD TNF- α concentration, 30.4 \pm 9.9 pg/mL, $P < .001$; mean \pm SD IL-6 concentration, 28.4 \pm 6.7 pg/mL, $P < .001$). The concentrations of TNF- α and IL-6 in other groups remained similar to those in the control group. Thrombocytopenia, prolonged clotting time, and interstitial pneumonitis occurred when 50% and 60% of the liver volume were ablated. The 4-week survival rates were 100%, 60%, and 0% when 40%, 50%, and 60%, respectively, of the liver volume were ablated. Similar systemic inflammatory responses and poor survival rates were observed among the cirrhotic liver groups when 30% and 40% of the liver volume were ablated.

Conclusions: The normal rats can tolerate RFA of 40% of the liver volume with minimal morbidity and no mortality whereas the cirrhotic rats can only tolerate 20% of the ablated liver volume. Beyond that limit, RFA would cause significant systemic inflammatory responses and poor survival.

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RADIOFREQUENCY ABLATION (RFA) has been used to treat patients with unresectable malignant liver tumors with low morbidity (2.2%-12.7%) and mortality (0.0%-1.4%) rates.¹⁻⁴ It uses high-frequency alternating current (350-500 kHz) to cause thermal destruction of the target lesion by electronic vibration within the lesion. With the advance in modern technology, the volume of ablation by RFA has increased much compared with that of the initial prototype of the radiofrequency electrode.^{5,6} The modern designs of radiofrequency electrodes (saline-enhanced, expand-

able, and cooled-tip electrodes) and the use of the hepatic inflow occlusion technique have rendered RFA feasible in treating large liver tumors (>5 cm). In one study,⁷ RFA was shown to be effective for advanced hepatocellular carcinoma with a maximal size up to 18 cm in diameter.

Clinical studies^{3,8} have shown that large tumor size and the number of RFA sessions were risk factors for major complications after RFA. Therefore, large-volume hepatic RFA carried a significant morbidity that should not be underestimated. A previous animal study⁹ demonstrated that RFA of 30% to 35% of the liver volume caused significant systemic in-

flammatory responses. At present, there is no study to our knowledge on the safety limit of large-volume hepatic RFA. An experimental study¹⁰ showed that hepatic cryotherapy of 35% of the liver volume carried a high mortality rate (43.5%) in a rat model. However, the host survival rate after RFA of such an amount of liver tissue has never been investigated. The present study aimed to evaluate the possible systemic responses and host survival rates after large-volume hepatic RFA using normal and cirrhotic rats to estimate the safety limit of large-volume hepatic RFA. Since serum interleukin (IL) 6 and tumor necrosis factor α (TNF- α) were shown to be the possible cell mediators involved in the systemic inflammatory reactions after cryotherapy,¹¹ these inflammatory markers were chosen in the present study to evaluate the possible systemic responses after large-volume RFA. We hope that our experimental results provide valuable guidance to the clinical use of RFA for large liver tumors.

METHODS

This was an *in vivo* study of 160 Sprague-Dawley rats weighing 350 to 400 g. Approval for the study protocol was obtained from the Institutional Ethics Committee on the Use of Live Animals for Teaching and Research. Radiofrequency ablation was performed on 80 normal and 80 cirrhotic rats using the Cool-tip RF System (Radionics, Burlington, Mass).

NORMAL SPRAGUE-DAWLEY RATS

The rats were kept in the laboratory for a few days with free access to food and water before the experiment. They were divided into 5 groups that underwent different hepatic procedures. Anesthesia was induced by intraperitoneal injection of sodium phenobarbitone (1 μ g of sodium phenobarbitone/1 g of body weight). The rats were placed on a small animal operating table. For the RFA groups (groups 1, 2, and 3), the ground pad of the Cool-tip RF System was attached to the backs of the rats. The abdomen was prepared with povidone-iodine solution, and laparotomy was performed using long midline incision. Avascular attachments to the liver were divided, and all of the lobes of the liver were mobilized. Radiofrequency ablation was carried out using a single 17-gauge cooled-tip electrode with a 2-cm exposed length. Repeated cycles of ablation (4 min/cycle) were performed to ensure complete ablation of the selected liver lobes, and approximately 40%, 50%, and 60% of the liver volume by weight were ablated in groups 1, 2, and 3, respectively (n=17 in each group) (**Figure 1**). Complete ablation of the selected liver lobes was confirmed histologically at the end of the experiment. Sixty percent of the liver volume was resected in group 4 (n=17), and a sham surgical procedure with laparotomy only was performed in group 5 (n=12) as a control.

All of the rats were allowed to recover from anesthesia, and they had free access to food and water after the surgical procedure. The animals were examined once daily for the general health status. In groups 1 through 4, 5 rats in each group were observed for 4 weeks to determine the survival rates. Based on our prior work on systemic responses of hepatic RFA,⁹ serum inflammatory markers were examined at different time points after the surgical procedure (6 hours and 1, 3, and 7 days). At each time point, 3 rats in each group were sacrificed, laparotomy was performed after intraperitoneal injection of phenobarbitone, and serum samples were collected from the infe-

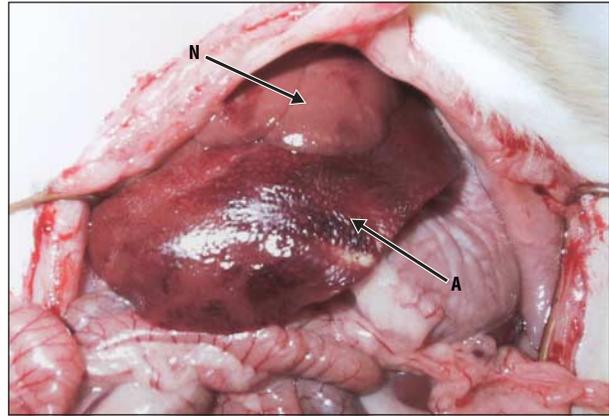


Figure 1. Radiofrequency ablation of normal rat liver (60% of the liver volume by weight). N indicates normal liver; A, ablated liver.

rior vena cava. Each serum sample was taken for systemic inflammatory marker (serum TNF- α and serum IL-6) concentration measurements, intracellular content measurements (lactate dehydrogenase [LDH] and urate concentrations), and assessment of organ functions (liver biochemistry, creatinine concentration, platelet count, and activated coagulation time). In addition, representative tissue samples from the liver remnant, kidney, and lung were taken for paraffin sections, and histological study using hematoxylin-eosin staining was performed.

CIRRHOTIC SPRAGUE-DAWLEY RATS

Iatrogenic liver cirrhosis was induced in 90 Sprague-Dawley rats by phenobarbital induction and weekly intragastric administration of carbon tetrachloride (CCl₄) according to the method described by Proctor and Chatamra.¹² Phenobarbitone was used to increase the sensitivity of the rat liver to CCl₄ by increasing the activity of cytochrome P-450.¹³ It was given in the drinking water at a concentration of 35 mg/dL, and this was accessed freely by the rats. After phenobarbital induction for 14 days, the rats received the first dose of CCl₄. After light anesthesia by inhalation of a halothane and oxygen mixture, a fine (5F) catheter was introduced through the oral cavity and esophagus into the stomach, and the predetermined dosage of CCl₄ was given. It has been shown that a high hepatic concentration of the drug could be achieved to induce liver cirrhosis shortly (about 1.5 hours) after this route of drug administration.¹⁴ One milliliter of CCl₄ was dissolved in 1 mL of corn oil. The initial dosage of the CCl₄ mixture (0.08 mL) was determined according to a previous experiment described by Proctor and Chatamra.¹² Subsequent weekly dosage of the CCl₄ mixture was increased to 0.12 mL (week 2), 0.16 mL (week 3), 0.24 mL (weeks 4-6), and 0.32 mL (week 7). The weight responses of the rats were monitored. The intended weight change was a weight loss of 6% to 9% 2 to 3 days after each CCl₄ administration and a total weight loss of approximately 25% after 7 weeks of CCl₄ administration. Among 90 Sprague-Dawley rats receiving CCl₄ treatment, 10 (11%) died following the fourth through seventh doses of CCl₄. The remaining 80 rats showed finely nodular liver and marked hepatic fibrosis on macroscopical and microscopical examinations, respectively (**Figure 2**).

The rats were divided into 5 groups that underwent different hepatic procedures. The preoperative preparation and operative techniques were similar to those in the normal liver groups. Because of the limited liver reserves, the extent of the RFA procedure in the cirrhotic groups was less than that in the normal groups. Approximately 20%, 30%, and 40% of the liver

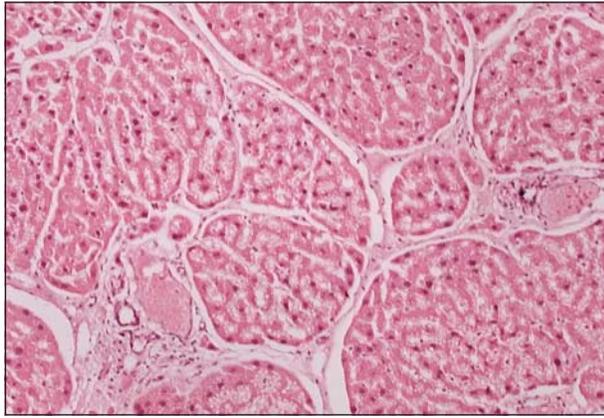


Figure 2. Histological examination of micronodular cirrhosis in Sprague-Dawley rats (original magnification $\times 10$).

volume by weight were ablated by RFA in groups 6, 7, and 8 ($n=17$ in each group), respectively. Forty percent of the liver volume was resected in group 9 ($n=17$). A sham surgical procedure with laparotomy only was performed in group 10 ($n=12$). The same outcome measurements as those in the normal liver groups were performed, including the 4-week survival rates, systemic inflammatory markers (TNF- α and IL-6 concentrations), intracellular contents (LDH and urate concentrations), organ functions (liver biochemistry, creatinine concentration, platelet count, and activated coagulation time), and histological examination of the liver remnant, kidney, and lung.

STATISTICAL ANALYSIS

All of the continuous variables were expressed as mean \pm standard deviation. The statistical software package SPSS version 10 (SPSS, Inc, Chicago, Ill) was used to analyze the results. The unpaired *t* test was used for comparing continuous variables between groups. The overall survival rates were calculated by the Kaplan-Meier method and compared using the log-rank test. A *P* value of less than .05 was considered statistically significant.

RESULTS

All of the hepatic procedures (RFA and hepatectomy) were performed uneventfully in all of the rats. There were no major complications, and all of the rats survived after the surgical procedures.

NORMAL SPRAGUE-DAWLEY RATS

The concentrations of TNF- α and IL-6 were significantly elevated in group 2 (ablation of 50% of the liver volume) (mean \pm SD TNF- α concentration, 130.3 ± 15.6 pg/mL; mean \pm SD IL-6 concentration, 163.2 ± 12.2 pg/mL) and group 3 (ablation of 60% of the liver volume) (mean \pm SD TNF- α concentration, 145.7 ± 13.0 pg/mL; mean \pm SD IL-6 concentration, 180.8 ± 11.0 pg/mL) in the early postoperative period (6 hours) as compared with the control group (mean \pm SD TNF- α concentration, 30.4 ± 9.9 pg/mL, $P < .001$; mean \pm SD IL-6 concentration, 28.4 ± 6.7 pg/mL, $P < .001$). The concentrations of these inflammatory markers in groups 2 and 3 were significantly higher than those in the control group at other time points (1, 3, and 7 days). The corresponding con-

centrations of systemic inflammatory markers at 6 hours in group 1 (ablation of 40% of the liver volume) (mean \pm SD TNF- α concentration, 50.5 ± 14.1 pg/mL; mean \pm SD IL-6 concentration, 60.8 ± 19.2 pg/mL) and group 4 (resection of 60% of the liver volume) (mean \pm SD TNF- α concentration, 41.6 ± 9.8 pg/mL; mean \pm SD IL-6 concentration, 38.9 ± 10.2 pg/mL) remained similar to those in the control group (**Figure 3A and B**). As for the other time points (1, 3, and 7 days), there was no significant difference in the concentration of markers between groups 1 and 4 and the control group. There was no significant change in the concentrations of intracellular contents (LDH and urate) among groups 1 through 4 and the control group. Concerning organ functions, the liver (serum bilirubin concentration) and renal (serum creatinine concentration) functions in groups 1 through 4 did not differ significantly from those in the control group. There were significant decreases in platelet count and significant increases in activated clotting time in group 2 (mean \pm SD platelet count, $80.4 \pm 12.6 \times 10^9/L$; mean \pm SD activated clotting time, 277.4 ± 48.3 seconds) and group 3 (mean \pm SD platelet count, $75.9 \pm 14.5 \times 10^9/L$; mean \pm SD activated clotting time: 280.2 ± 17.7 seconds) at 6 hours after the surgical procedure as compared with the control group (mean \pm SD platelet count, $153.2 \pm 21.0 \times 10^9/L$, $P < .001$; mean \pm SD activated clotting time, 156.7 ± 21.4 seconds, $P < .001$). Similar findings were observed at the other time points (1, 3, and 7 days). The platelet counts and activated clotting times in groups 1 and 4 were similar to those in the control group at all of the time points (**Figure 3C and D**).

The survival rates of different groups are shown in **Figure 4**. All of the rats in group 1 survived for 4 weeks after the surgical procedure (100% 4-week survival rate). However, the overall 4-week survival rates for groups 2 and 3 were significantly reduced (60% and 0%, respectively) ($P < .001$).

Hematoxylin-eosin staining of lung parenchyma in groups 2 and 3 revealed evidence of pneumonitic changes 1 day after the surgical procedure in which there was shrinkage of airspace and a thickened alveolar septum with infiltration of mononuclear cells (**Figure 5A**). This was in contrast to the normal histological appearance of lung parenchyma with preservation of alveolar space in groups 1 and 4 at the same time point (**Figure 5B**). There was no considerable histological change in the liver remnant or kidney in all of the groups of rats.

CIRRHOTIC SPRAGUE-DAWLEY RATS

The pattern of changes in serum parameters of cirrhotic rats was similar to that in the normal rats except that cirrhotic rats could only tolerate RFA of less liver volume. There was a significant increase in the concentrations of TNF- α and IL-6 in group 7 (ablation of 30% of the liver volume) (mean \pm SD TNF- α concentration, 116.5 ± 14.8 pg/mL; mean \pm SD IL-6 concentration, 134.9 ± 15.7 pg/mL) and group 8 (ablation of 40% of the liver volume) (mean \pm SD TNF- α concentration, 124.9 ± 17.2 pg/mL; mean \pm SD IL-6 concentration, 167.2 ± 9.4 pg/mL) at 6 hours after the surgical procedure as compared with the control group (mean \pm SD TNF- α concentration, 26.3 ± 7.3

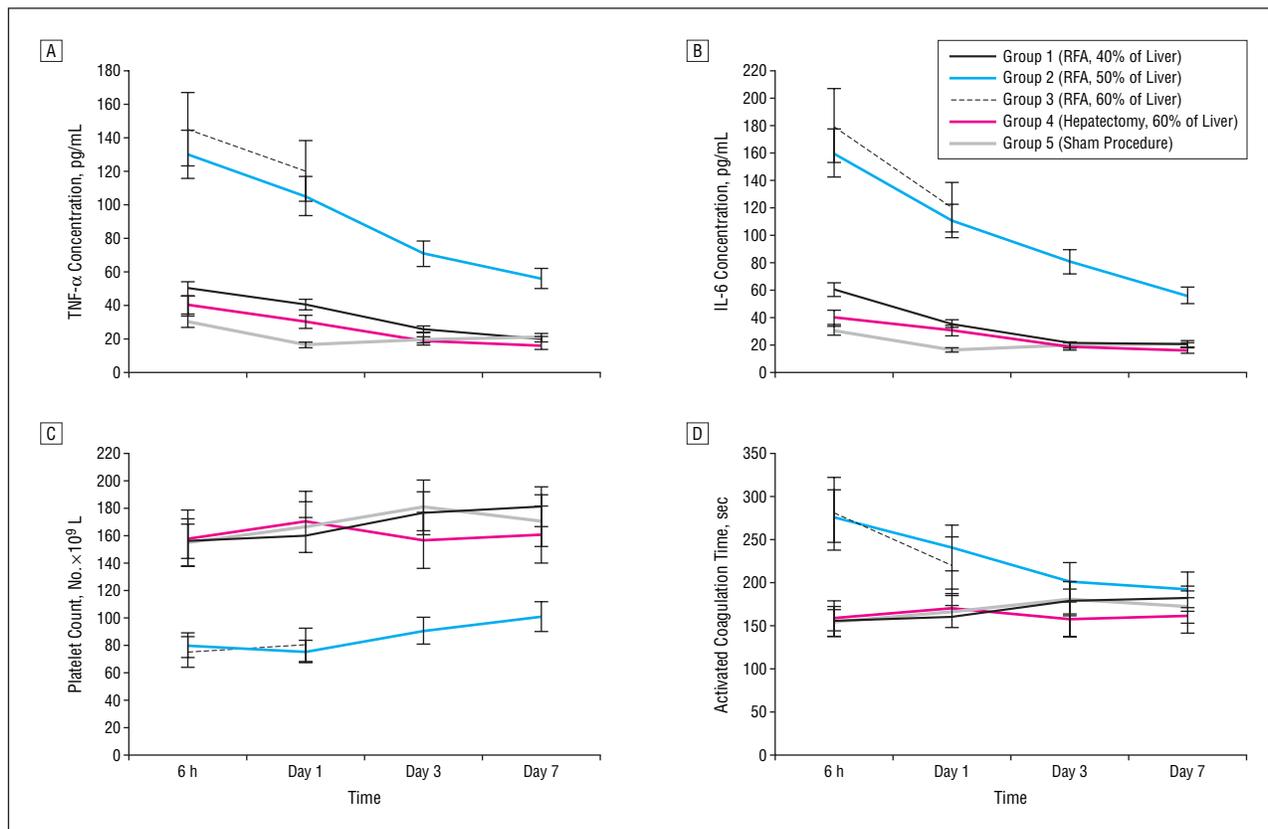


Figure 3. Changes in serum tumor necrosis factor α (TNF- α) concentration (A), serum interleukin 6 (IL-6) concentration (B), platelet count (C), and activated clotting time (D) after different hepatic procedures in rats with normal liver. $P < .001$ for all of the comparisons between groups 2 and 5 at 6 hours and between groups 3 and 5 at 6 hours. RFA indicates radiofrequency ablation.

pg/mL, $P < .001$; mean \pm SD IL-6 concentration, 45.9 ± 9.2 pg/mL, $P < .001$). Similar findings were obtained at the other time points (1, 3, and 7 days). Groups 6 (ablation of 20% of the liver volume) and 9 (resection of 40% of the liver volume) had similar serum TNF- α and IL-6 concentrations compared with the control group at all of the time points (**Figure 6A** and **B**). There were no significant changes among the groups with respect to the concentrations of serum LDH ($P = .49$), urate ($P = .56$), bilirubin ($P = .59$), and creatinine ($P = .61$). Clotting was significantly deranged at 6 hours after the surgical procedure in group 7 (mean \pm SD platelet count, $26.2 \pm 5.4 \times 10^9/L$; mean \pm SD activated clotting time, 340.7 ± 19.2 seconds) and group 8 (mean \pm SD platelet count, $20.3 \pm 6.1 \times 10^9/L$; mean \pm SD activated clotting time, 355.9 ± 21.3 seconds) as compared with the control group (mean \pm SD platelet count, $100.4 \pm 12.9 \times 10^9/L$, $P < .001$; mean \pm SD activated clotting time, 189.6 ± 25.3 seconds, $P < .001$). Similar findings were obtained at the other time points (1, 3, and 7 days) (**Figure 6C** and **D**). The overall 4-week survival rates in groups 6, 7, and 8 were 100%, 40%, and 0%, respectively ($P < .001$) (**Figure 7**).

Cirrhotic rats in groups 7 and 8 had severe interstitial pneumonitis that developed 1 day after the surgical procedure. Such histological changes of the lung were similar to those described in normal rats with ablation of 50% and 60% of the liver volume. Comparatively, groups 6 and 9 had relatively normal lung parenchyma at the same time point. No considerable histological

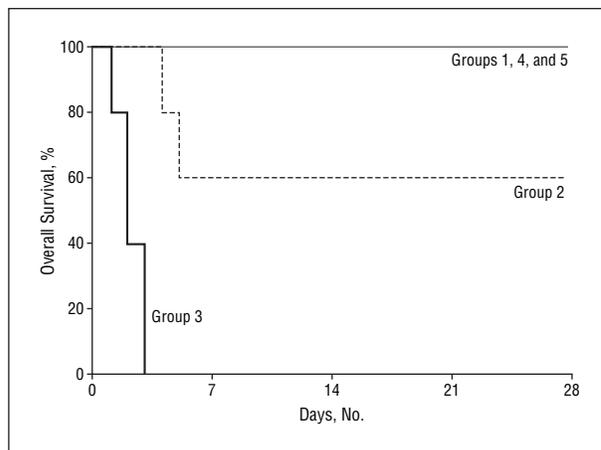


Figure 4. Overall survival rates of normal rats in groups 1 through 5. Group 1 received radiofrequency ablation of 40% of the liver; group 2, radiofrequency ablation of 50% of the liver; group 3, radiofrequency ablation of 60% of the liver; group 4, hepatectomy of 60% of the liver; and group 5, a sham surgical procedure.

change was observed in the liver remnant or kidney in all of the groups.

COMMENT

Radiofrequency ablation was an effective treatment for unresectable malignant liver tumors. Bowles et al⁷ suggested that RFA could be safely and effectively per-

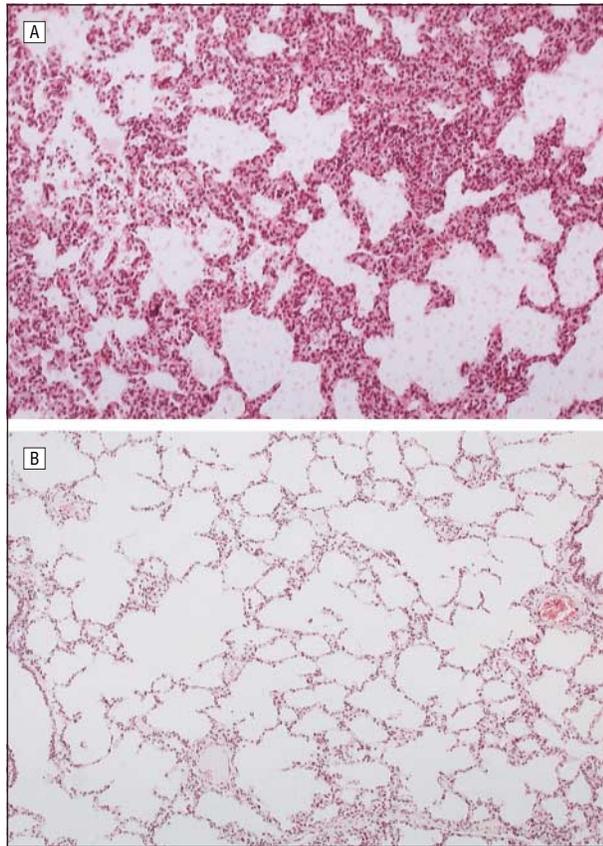


Figure 5. Hematoxylin-eosin staining of lung parenchyma after radiofrequency ablation showed interstitial pneumonitis in group 3 (ablation of 60% of the liver volume) (A) and normal histological appearance in group 1 (ablation of 40% of the liver volume) (B) (original magnification $\times 10$).

formed to control local disease in patients with advanced liver tumors. In that series, patients with large liver tumors (up to 18 cm) were treated by RFA, with the ablated volume more than 3000 mL. With this aggressive local ablation treatment, a favorable outcome for patients could be achieved with low rates of complications (7%) and local recurrence (9%). However, in another clinical study by Bleicher et al,⁸ large tumor size and, hence, the ablated volume were shown to be the only independent poor prognostic factors affecting morbidity after RFA treatment. Hoshida et al¹⁵ have demonstrated that small liver volume ($<600 \text{ mL/m}^2$) and large radiofrequency parenchymal ablation rate were the independent risk factors for adverse events after RFA. Hoshida and colleagues suggested that the limited liver functional reserve after RFA treatment was responsible for the adverse events. In a previous experimental study in a porcine model,⁹ hepatic RFA of 30% to 35% of the liver volume was associated with a significant increase in serum inflammatory marker concentrations and considerable inflammatory changes of the lung. Therefore, hepatic RFA is not absolutely safe, particularly when a significant amount of necrotic tissue is left behind after large-volume ablation. In the literature, the safety limits of large-volume hepatic RFA have not been discussed in detail.

This *in vivo* experiment has evaluated the possible systemic inflammatory reactions after large-volume hepatic RFA using both normal and cirrhotic rat models.

It was found that the rats with normal liver could maximally tolerate RFA of 40% of the liver volume whereas those with cirrhotic liver could only tolerate RFA of 20% of the liver volume with no mortality and no morbidity. Beyond that limit, the mortality rate was high and there was evidence of systemic inflammatory reaction with elevated serum TNF- α and IL-6 concentrations, deranged coagulation profile (thrombocytopenia and prolonged activated clotting time), and interstitial pneumonitis. By comparing the results between RFA and hepatectomy of the same liver volume (60% in normal rats and 40% in cirrhotic rats), the high mortality rate after RFA was probably not related to the liver insufficiency after the surgical procedure but instead to the extent of systemic inflammatory responses to the large-volume ablated liver tissue.

The exact mechanism involved in the systemic inflammatory responses by large-volume RFA is unknown. The fact that intracellular contents were released into systemic circulation after cryotherapy, causing a severe systemic inflammatory reaction, could not be applied to RFA as suggested by the low serum concentrations of LDH and urate after large-volume RFA. Instead, the pathway might be led by the activation of Kupffer cells within the liver remnant by large necrotic radiofrequency-ablated liver tissue. Kupffer cells in the liver represented the largest number of fixed macrophages in the body and were responsible for the mediator release in response to direct liver injury. Within these cells, activation of the transcription factor complex nuclear factor κB would release inflammatory factors, including TNF- α , IL-1, IL-2, IL-6, and IL-8, into the systemic circulation.¹⁶ These factors could result in interstitial pneumonitis and derangement of coagulation through the inflammatory cascade, as shown in this study. Nevertheless, the mechanism through which the Kupffer cells were activated after large-volume RFA remains to be investigated. Thermal injury caused by RFA involved denaturation of cellular protein and lipid bilayers, leading to disintegration of cell membranes and irreversible tissue destruction.¹⁷ The necrotic liver tissue as a result of RFA might extend into the space of Disse, thus stimulating the Kupffer cells in the adjacent nonablated liver parenchyma.

This experiment is the first study to our knowledge documenting the systemic inflammatory responses caused by large-volume hepatic RFA in both normal and cirrhotic rat models. In addition to the previous experimental finding¹⁸ of thermal injury to the portal structures by RFA when hepatic inflow occlusion was applied, large-volume hepatic RFA could be dangerous because of its hazardous effects on the lung and coagulation profile. The estimated safety limit to large-volume RFA in this experiment might provide useful clinical guidance regarding the aggressiveness of this local ablation therapy for unresectable liver tumors. Caution should be used when RFA is performed for large liver tumors in 1 goal. Instead, staged ablation by serial sections of RFA might be another possible approach to minimize the systemic responses by large-volume ablation. Alternatively, the application of anti-inflammatory therapy may be useful to down-regulate the inflammatory cascade caused by RFA.

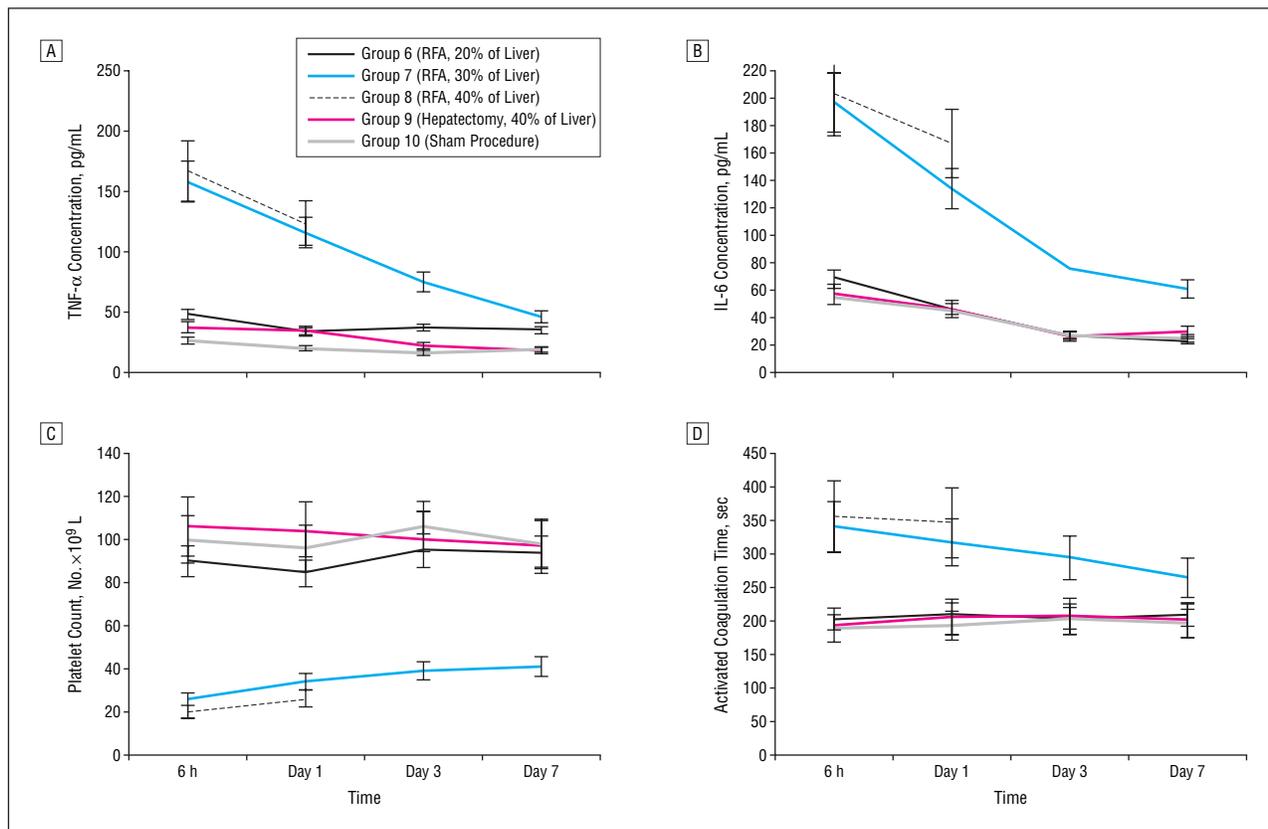


Figure 6. Changes in serum tumor necrosis factor α (TNF- α) concentration (A), serum interleukin-6 (IL-6) concentration (B), platelet count (C), and activated clotting time (D) after different hepatic procedures in rats with cirrhotic liver. $P < .001$ for all of the comparisons between groups 7 and 10 at 6 hours and between groups 8 and 10 at 6 hours. RFA indicates radiofrequency ablation.

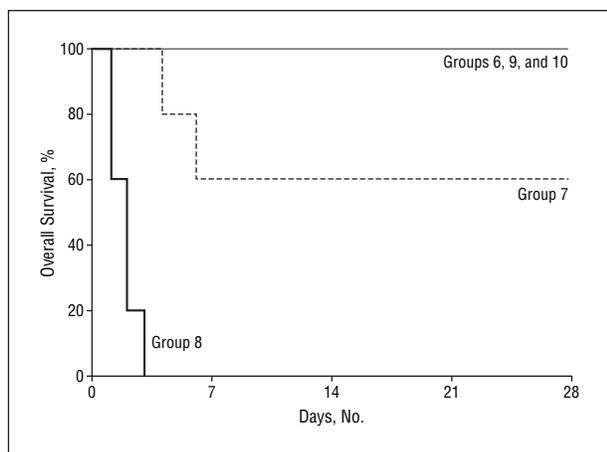


Figure 7. Overall survival rates of cirrhotic rats in groups 6 through 10. Group 6 received radiofrequency ablation of 20% of the liver; group 7, radiofrequency ablation of 30% of the liver; group 8, radiofrequency ablation of 40% of the liver; group 9, hepatectomy of 40% of the liver; and group 10, a sham surgical procedure.

In this experiment, normal as well as cirrhotic liver tissue was used as the substrate for RFA. Further studies on liver tumor models are needed to clarify whether large-volume RFA on malignant liver tumors will produce similar systemic inflammatory responses. In the clinical sphere, comparison of the concentrations of systemic inflammatory markers in patients treated with RFA or liver resection for large tumors would definitely add

value to our understanding of the systemic effects of large-volume hepatic RFA.

In conclusion, normal rats can tolerate RFA of 40% of the liver volume with minimal morbidity and no mortality whereas the cirrhotic rats can only tolerate 20% of the ablated liver volume. Beyond that limit, RFA is associated with significant systemic inflammatory responses affecting the lung and coagulation profile as well as poor survival.

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