Diluted hydrochloric acid generates larger radiofrequency ablation lesions in excised porcine livers

Rong-Guang Luo, Fei Fao, Jin-Hua Huang, Yang-Kui Gu, Xiong-Ying Jiang, Ying-jie Huang

PURPOSE
This study evaluated the influence of continuous infusion of diluted hydrochloric acid during radiofrequency ablation (RFA) on the size of ablated lesions.

MATERIALS AND METHODS
Experiments were performed in 20 excised porcine livers using three different treatment modalities: (1) normal saline-enhanced RFA (NS-RFA), which was normal saline pumped into ablated tissue during RFA; (2) diluted hydrochloric acid ablation (DHAA), which was 3 mol/L dilute hydrochloric acid (HCl) injected into hepatic tissue without RFA; and (3) HCl-enhanced RFA (HCl-RFA), which was 3 mol/L diluted HCl continuously infused into ablated tissue during RFA. We produced 20 HCl-RFA and NS-RFA lesions, respectively, using a monopolar perfusion electrode connected to a commercially available radiofrequency generator, and 20 DHAA lesions using an 18-gauge Chiba needle. The ablated lesions were evaluated both macroscopically and histologically. Dimensions of lesions were compared among HCl-RFA, NS-RFA, and DHAA.

RESULTS
The ablated lesions had an elliptical-like shape and were well-demarcated with normal liver tissue. The mean volume, longitudinal diameter, and transverse diameter of NS-RFA lesions were 11.2±0.29 cm³, 3.49±0.07 cm, and 2.48±0.03 cm, those of HCl-RFA lesions were 58.14±3.05 cm³, 5.51±0.05 cm, and 4.49±0.11 cm, and those of DHAA lesions were 4.41±0.16 cm³, 2.43±0.08 cm, and 1.8±0.03 cm, respectively. The mean dimensions of HCl-RFA lesions were the largest among the three types of ablation (P<0.001).

CONCLUSION
Under the present experimental conditions, the continuous infusion of diluted HCl during RFA can generate larger ablated lesions than NS-RFA or DHAA in excised porcine livers.

During the past two decades, radiofrequency ablation (RFA) had become a promising image-guided intervention accepted worldwide as a safe and effective treatment for hepatocellular carcinoma (HCC) and focal metastatic liver tumors in patients who are not candidates for hepatic resection or who refuse surgery (1–3). Radiofrequency waves of 460–500 kHz result in frictional heat, which is distributed from the electrodes by conduction into the tissue (4–5). Experimental studies have shown that higher local temperatures induce increased ablation lesion sizes (6). However, a major limitation of this approach has been achieving an acceptable coagulation size due to charring around the electrodes, because charring reduces conductivity and consequently makes it much more difficult to effectively convey energy into the depth of the tissue. As a result, larger tumors had tended to have higher recurrence rates, mostly because the volume of the RFA lesions did not encompass the entire tumor or the required safety margin of 0.5–1.0 cm (7–9), which has compromised the broader applicability of RFA.

Many strategies currently exist for overcoming this limitation, including improvements to devices and methods. Among them, continuous infusion of fluid during RFA has been one of the most used methods for enlarging ablated lesions (10, 11). We previously had compared the size of the ablation lesions created by continuous infusion of normal saline during RFA (NS-RFA) and continuous infusion with diluted hydrochloric acid (HCl) during RFA (HCl-RFA) (12). Diluted HCl has been shown to be a highly effective chemical ablation agent (13). Our results showed that HCl-RFA can produce larger ablation lesions compared to NS-RFA using the same radiofrequency parameters. However, the results of that study did not rule out the possibility that the increase in the size of ablation lesions was simply caused by the diluted HCl. Therefore, in this study we evaluated the influence of continuous infusion of diluted-HCl during RFA on the size of the ablated lesion.

Materials and methods

RFA system
The RFA system consists of a radiofrequency generator (Model 1500X, AngioDynamics, RITA Medical Systems, Queensbury, New York, USA) coupled with a monopolar perfusion electrode (UniBlate, RITA Medical Systems). The UniBlate is a 17-gauge, open monopolar perfused electrode, with a largest active tip exposure of 2.5 cm. The monopolar electrode consists of an insulated primary trocar with a temperature sensor and two infusion holes at the distal end. Liquids are pumped through these two holes into the ablated tissues at a rate of 0.2 mL/min by a peristaltic pump, which cools the electrode tip and reduces charring of the surrounding tissue that might decrease tissue conductivity and block radiofrequency energy. The chemical ablation needle used in the study...
was an 18-gauge Chiba needle. Liquids used in this study were normal saline for NS-RFA and 3 mol/L diluted HCl for HCl-RFA and diluted hydrochloric acid ablation (DHAA).

**RFA protocol**

We obtained 20 fresh, entire porcine livers from normal pigs at a local slaughterhouse that weighed between 1.5 and 2 kg. All excised porcine livers were exposed under room temperature at approximately 25°C. An entire porcine liver has five lobes. We chose three lobes that had a thickness greater than 5 cm to carry out ablation. During the experiments, the excised porcine livers were placed on a large metal plate connected to the grounding pads of the RITA RFA system, and the tips of the radiofrequency electrodes or Chiba needle were inserted into the tissue at a depth of least 5 cm.

We created one NS-RFA lesion, one DHAA lesion, and one HCl-RFA lesion in each complete porcine liver *ex vivo*. According to the manufacturer’s recommended protocol, the duration of ablation was set to 15 min at a temperature of 103°C and power of 30 W for NS-RFA or HCl-RFA. The infusion dosage of normal saline (during NS-RFA) or diluted HCl (during HCl-RFA) was 3 mL (0.2 mL/min×15 min). In addition, 3 mL diluted HCl was injected into liver tissues for DHAA using a Chiba needle with the same infusion speed as the NS-RFA and HCl-RFA.

**Evaluating the size and shape of ablation lesions**

After ablation, we dissected each ablation lesion along the axis of the electrode or needle insertion. The longitudinal diameter (along the electrode or needle) and transverse diameter (90° to the electrode or needle) were measured with a ruler by three investigators who reached consensus on each dimension.

The volume of the ablation lesion was calculated by approximating it to an ellipsoid using the formula (11, 14–16): $V = \frac{1}{6} \pi \times LD \times TD^2$, where $V$ is volume, $\pi$ is the circumference of a circle divided by its diameter, $LD$ is longitudinal diameter, and $TD$ is transverse diameter.

Gross morphological characteristics were assessed visually, and histological features were assessed from 4-µm-thick slides with hematoxylin and eosin (H-E) staining under light microscopy.

**Statistical methods**

Data were reported as the mean±standard deviation. Ablation lesion dimensions were compared among NS-RFA, DHAA, and HCl-RFA with the Kruskal-Wallis rank sum test for several independent samples using a commercially available software (Statistical Package for Social Sciences, version 15.0, SPSS Inc., Chicago, Illinois, USA). The alpha was set at 0.05.

**Results**

**Macroscopic features**

All procedures were successful and 20 lesions were created by NS-RFA, DHAA, and HCl-RFA, respectively. The NS-RFA lesions were approximately ellipse-like in shape and existed in well-demarcated areas within the liver tissue. The central zone, whose diameter was slightly larger than the radiofrequency electrode, was a hollow space created by the vaporization of tissue and withdrawal of the electrode. Beyond the central area was a belt of pale or brown coagulated tissues, and reddish tissue was in the outermost belt of the lesion, which was bordered by normal liver tissue (Fig. 1a). The mean volume, longitudinal diameter, and transverse diameter of NS-RFA lesions were 11.24±0.29 cm³, 2.48±0.03 cm, and 2.43±0.08 cm, and 1.86±0.03 cm, respectively (Table). In contrast, HCl-RFA lesions were also ellipse-like in shape, but the central portion was jelly-like and the tract of the radiofrequency electrode was unclear. Surrounding the jelly-like tissue was off-white or dust color coagulation necrosis, and beyond this area was a ring of reddish coagulated tissues that was bordered by normal liver tissue that was reddish in color (Fig. 1c). The mean volume, longitudinal diameter, and transverse diameter were 58.14±3.05 cm³, 5.51±0.05 cm, and 4.49±0.11 cm, respectively (Table). Ablation lesions created with HCl-RFA were significantly larger than those created with NS-RFA or DHAA ($P < 0.001$). In particular, the mean volume of ablation lesions created by HCl-RFA were more than four times larger than ablation lesions created by NS-RFA and 14 times larger than ablation lesions created by DHAA.

**Histopathological findings**

Microscopically, the NS-RFA lesions showed a central complete destruction of the parenchyma, including a small blank area where tissue has been lost (Fig. 2a). Surrounding the central portion were coagulation necrosis and a peripheral hemorrhagic rim. Coagulation necrosis created by NS-RFA revealed a zone of altered cellular morphology, which was best characterized as a heat effect that consisted of degenerated shrunken hepatocytes with pyknotic nuclei of the liver tissue (Fig. 2b). This region did not meet the classical criteria of coagulation necrosis, but the cells were clearly different from normal hepatocytes. There was no sharp division between ablated lesions and normal liver tissue (Fig. 2c). The coagulation lesions created by DHAA were similar to those of NS-RFA, except that

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DHAA, diluted hydrochloric acid ablation; HCl-RFA, diluted hydrochloric acid-enhanced radiofrequency ablation; NS-RFA, normal saline-enhanced radiofrequency ablation.

*Kruskal-Wallis rank sum test.

Data are presented as mean±standard deviation.
there was no central charred zone. Coagulation necrosis created by DHAA also appeared as irregular and degenerated shrunken hepatic cells with pyknotic nuclei (Fig. 3a). However, the extent of hepatocyte damage in the lesions induced by DHAA was more substantial than the lesions produced by NS-RFA, and there was a sharp division between ablated lesions and normal liver tissue (Fig. 3b). In contrast, the damage of liver cells created by HCl-RFA was more serious than that induced by NS-RFA and DHAA. The central jelly-like tissue appeared as a homogeneous red dye-like substance (Fig. 4a), and coagulation necrosis in the outer layer of HCl-RFA lesions was similar to that in NS-RFA lesions (Fig. 4b). No sharp distinction was observed between the ablated lesions and normal liver tissue (Fig. 4c).

**Discussion**

Open hepatic resection can completely remove tumors and provides a potentially curative therapy for patients with HCC or focal metastatic liver tumors. Unfortunately, 70%–85% of these patients are not candidates for surgery, either because of poor hepatic reserve of extrahepatic comorbidities (17–19). Image-guided percutaneous ablation is currently accepted as the best therapeutic choice for patients with liver malignant tumors when surgery is contraindicated, such as RFA and microwave ablation (MWA) as well as chemical ablation with ethanol or acetic acid (PEA). PEA has been the seminal technique used for local ablation; however, the injected ethanol does not always accomplish complete tumor necrosis because of its unequal distribution within the tumor, especially with intratumoral septa. Tumors treated with this approach have a high local recurrence rate that may reach 33% in lesions smaller than 3 cm and 43% in lesions exceeding 3 cm (20).
Because acetic acid is a noxious chemical, percutaneous acetic acid injection (PAI) has also been proposed as an alternative to ethanol in order to decrease the number of treatment sessions needed (21). RFA destroys biological tissues with electromagnetic waves at frequencies between 460 and 500 kHz. This frequency is high enough to cause molecular frictional heating without stimulating neuromuscular reactions and electrolysis, and is low enough to confine energy transmission to a more controllable tissue mass without generating excessive radiation (4–5). Randomized trials had found that RFA has a greater anticancer effect than PAI and leads to better local control of the disease (22–25). Moreover, RFA offered a distinct survival benefit compared to PAI, which has established RFA as the standard percutaneous technique (26, 27). However, the major limitation of this approach is achieving an acceptable coagulation size (9) due to charring that occurs around the electrodes that increases tissue impedance and, which makes it much more difficult to effectively transfer energy into the depth of the tissue (28). In addition, abutting large blood vessels (3 mm or greater) reduce the effective heat produced by radiofrequency due to perfusion-mediated tissue cooling within the area to be ablated, which consequently lowers RFA efficacy (29). Seki et al. (30) previously reported that MWA was another technique for ablation in HCC. Although some scholars believed that MWA was a promising approach, no randomized clinical trial was conducted to show a statistically significant difference between RFA and MWA. Moreover, RFA has similar limitations as MWA, such as a decrease in the efficiency of energy transfer for tumors adjacent to large feeding blood vessels. In addition, the ablation lesion size is also limited with MWA, which compromises the broader applicability of the technique.

In this study, we pumped diluted HCl into the area of ablation during RFA using monopolar perfusion electrodes in order to enlarge the ablation volume, which achieved simultaneous chemical and thermal ablation. Our result showed that HCl-RFA lesions were significantly larger than NS-RFA or DHAA lesions. Several factors may explain this difference. First, the ionic concentration of 3 mol/L HCl is higher than that of normal saline, and the perfusion of the dielectric solution of HCl during HCl-RFA probably increased the electrical conductivity of the tissue. Therefore, compared to NS-RFA, HCl-RFA may have created more molecular friction, which would generate more energy and enlarge the ablated volume. Therefore, in a future study we will explore the impact of the ablated lesion size with the injection of different concentrations of dilute HCl and saline during RFA to confirm our hypothesis. Second, it is known that HCl is the aqueous solution of hydrogen chloride and transforms back into hydrogen chloride when HCl is heated. Therefore, the rising temperatures during HCl-RFA may have evaporated the HCl solution and transformed it into hydrogen chloride. Hydrogen chloride is gas, and gas would increase tissue impedance. In contrast, however, as the hydrogen chloride moved outward, it would have encountered fluid in the tissues and changed back into HCl, thereby inducing chemical ablation and enlarging the lesion volume. This hypothesis will require further investigation.

Several studies have reported that after RFA, the tissue appears to be almost unchanged microscopically (5, 31). Thermal coagulation necrosis differs from classic coagulation necrosis in that cells retain their original shape and the nuclei do not change, which makes it difficult to visualize by H-E staining. In fact, these results were reported in the literature as a “ghost phenomenon,” and cells showing thermal coagulation necrosis were referred to as “ghost cells” (5, 32–34). Nicotinamide adenine dinucleotide (NADH) vital staining can be used to identify the viability of hepatocytes with normal morphology (5, 16, 34). However, NADH vital staining is more complicated than H-E staining. Therefore, using H-E staining with careful observation, we found that most hepatocytes killed by NS-RFA appeared as coagulation necrosis microscopically. Our results also showed that HCl-RFA produced more obviously coagulation necrosis than NS-RFA. The coagulation necrosis created with HCl-RFA was easily observed by H-E staining, and thus it was not necessary to perform NADH vital staining to identify cell viability.

A theoretical, yet untested, oncologic concern regarding the perfusion electrodes used in RFA is that saline contaminated with viable tumor cells may leak from the electrode track and cause peritoneal or track seeding. Another unexplored concern is that the saline may increase intratumor pressure and force tumor cells into the circulation, thereby causing lymphatic or hematogenous seeding (5, 35). As a chemical ablation agent, HCl might reduce or even eliminate both of these risks, regardless if they occur with normal saline.

Our study had some limitations. We ablated normal tissue in ex vivo porcine livers and did not ablate tissue in vivo.
or use metastatic and HCC tumor tissue. Therefore, the distribution of infused liquid (normal saline or 3 mol/L HCl) differed due to the different tissue architecture. In addition, without the cooling effect of blood flow, the ablated volume in ex vivo tissue might be larger than that produced in tissue in vivo. Hence, our results were preliminary and provide only a reference for further research. Although HCl is safe and effective when it is used in chemical ablation, the safety of HCl-RFA still requires additional studies. We will address these questions in future studies.

In conclusion, our results have shown that HCl-RFA can create ablation lesions with markedly larger volumes of coagulation necrosis compared to those from NS-RFA or DHAA in excised porcine livers.

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Conflict of interest disclosure
Authors declared no conflicts of interest.

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