Development of an Animal Model for Radiofrequency Ablation of Primary, Virally Induced Hepatocellular Carcinoma in the Woodchuck

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ABSTRACT

Purpose: To develop a consistent and reproducible method in an animal model for studies of radiofrequency (RF) ablation of primary hepatocellular carcinoma (HCC).

Materials and Methods: Fifteen woodchucks were inoculated with woodchuck hepatitis virus (WHV) to establish chronic infections. When serum γ-glutamyl transpeptidase levels became elevated, the animals were evaluated with ultrasound, and, in most cases, preoperative magnetic resonance (MR) imaging to confirm tumor development. Ultimately, RF ablation of tumors was performed by using a 1-cm probe with the animal submerged in a water bath for grounding. Ablation effectiveness was evaluated with contrast-enhanced MR imaging and gross and histopathologic analysis.

Results: RF ablation was performed in 15 woodchucks. Modifications were made to the initial study design to adapt methodology for the woodchuck. The last 10 of these animals were treated with a standardized protocol using a 1-cm probe that produced a consistent area of tumor necrosis (mean size of ablation, 10.2 mm $\times$ 13.1 mm) and led to no complications.

Conclusions: A safe, reliable and consistent method was developed to study RF ablation of spontaneous primary HCC using chronically WHV-infected woodchucks, an animal model of hepatitis B virus–induced HCC.

ABBREVIATIONS

HCC = hepatocellular carcinoma, RF = radiofrequency, WHV = woodchuck hepatitis virus

Since the first reports of radiofrequency (RF) ablation in ex vivo studies, RF ablation has been extensively evaluated in animal and clinical investigations (1,2). Animal models have included a variety of species, with swine being one of the most common. The advantages of using swine are the low cost and availability of the animal and the size of the animal, which translates well to clinical work with humans. Although an excellent model in which to evaluate the size of the ablation in normal liver, the swine model does not incorporate the effects of the presence of tumor and background liver disease because there are no spontaneously developing hepatic neoplasms in this model. On the contrary, the woodchuck (or groundhog; *Marmota monax*) is a well recognized animal model of virally induced hepatocellular carcinoma (HCC) (3). Chronic infection with woodchuck hepatitis virus (WHV), a member of the hepadnavirus family, which includes hepatitis B virus, leads to a very high risk of HCC development within 2–4 years

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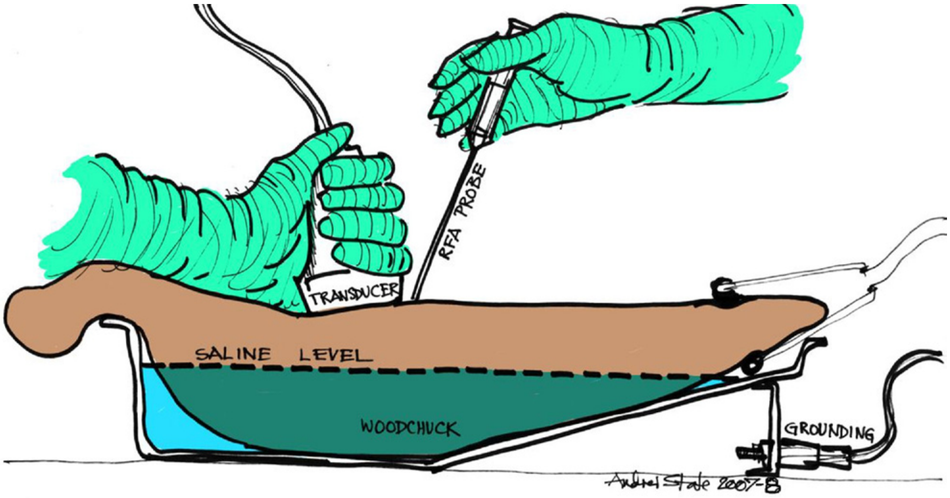


Figure 1. Schematic representation of RF ablation of a woodchuck in a saline solution bath. To prevent skin injury associated with grounding pad burns, the woodchucks were submerged in a saline solution bath within a metal pan. The pan was connected to the generator and served as grounding for the ablation procedure.

after infection (3). The animals are considerably larger than conventional laboratory rodents, weighing 2–5 kg, and develop HCCs that reach nearly 100 cm^3 in size, making them amenable to studies using conventional probes and other instrumentation (4). Therefore, we report our early experience with the use of the woodchuck as an animal model for RF ablation of primary HCC, with magnetic resonance (MR) imaging and pathologic correlation.

MATERIALS AND METHODS

This study was performed as part of a larger study sponsored by the National Institutes of Health comparing traditional ultrasound (US) guidance versus a more sophisticated guidance system with three-dimensional display. This study was overseen and approved by the institutional animal care and use committees of the two participating institutions.

Animals

Twenty woodchucks were purchased from a commercial supplier (Northeastern Wildlife, Harrison, Idaho) as captive-born animals approximately 8 months of age. The animals were injected at birth with a pooled WHV inoculum to establish infection. Animals were tested at approximately 6 months of age for serum WHV polymerase and WHV core antigen to establish that they were chronically infected.

The animals were housed in laboratory animal resources facilities at North Carolina State University. They were kept individually in stainless-steel cages, fed rabbit pellets (Agway, Richmond, Indiana) ad libitum, and given water ad libitum. They were maintained in a 12-hour light/dark cycle and cared for by personnel trained in the care of woodchucks under the conditions specified by the institutional animal care and use committee guidelines.

Blood samples were obtained every 3–4 months to assess levels of WHV DNA levels to verify that the animals remained virus carriers, as well as levels of serum γ-glutamyl transpeptidase, a serum marker for the presence of hepatocellular tumors (5). Animals with γ-glutamyl transpeptidase levels greater than 50 IU/dL were evaluated by US to search for hepatic tumors and evaluate their size and position. When hepatic tumors at least 1 cm in diameter were identified by US, the RF ablation procedure was scheduled within approximately 1 week.

Radiofrequency Ablation Procedure and Postprocedural Care

The RF ablation procedure was performed by an experienced interventional radiologist (C.T.B.). Animals were sedated with 2–5 mg/kg ketamine (100 mg/mL; Fort Dodge Laboratories, Fort Dodge, Iowa) and 0.1–0.2 mg/kg of acepromazine maleate (10 mg/mL; Fort Dodge Laboratories) intramuscularly. When possible, an endotracheal tube was placed, and a nasogastric tube was passed for gastric decompression. General anesthesia was performed by using isoflurane (1%–5%) administered by endotracheal tube (or facemask in cases in which intubation was unsuccessful).

The abdomen was shaved, prepared with iodine and alcohol scrub, and draped with sterile towels. Initially, the animal was placed on a heating pad; pediatric grounding pads (neonatal patient return electrode; Valleylab, Boulder, Colorado) were placed on the caudal back and rear legs. Later, the protocol was modified such that the woodchuck was placed in a metal pan connected to electrodes that were then attached to the grounding wires of the RF generator. The metal pan was filled with warm saline solution (approximately 38°C) and the caudal half of the body was submerged (Fig 1). Heating pads were placed beneath the pan to maintain the temperature of the water bath, and the animal's body temperature was monitored. With the use of US imaging, a 1-cm probe (Soloist/LeVeen; Boston Scientific, Natick, Massachusetts) was placed into the center of the lesion and activated. Because of the size of the woodchuck, a low-energy protocol was used. The generator was started at 10 W and left on for 30 seconds or until roll-off occurred. If roll-off was not achieved, the energy was increased to 20 W and ablation was performed for another 30 seconds or until roll-off occurred. If roll-off was not achieved after the higher energy was used, the ablation was terminated.

After RF ablation, the woodchucks were dried and warmed with heated air (Bair Hugger; Augustine Medical, Eden Prairie, Minnesota) to reestablish body temperature, and recovered in the recovery area. All animals were given 0.05–0.1 mg/kg buprenorphine subcutaneously for postoperative pain control. The woodchucks were monitored during the next 7 days for signs of collateral injury. After approximately 1 week, the animals underwent a follow-up MR examination. After the MR examination, the woodchucks were euthanized with an overdose of pentobarbital (90 mg/kg), and the liver was collected for analysis.

MR Imaging

All MR imaging studies were performed on a 3-T scanner (Allegra; Siemens, Erlangen, Germany) using the standard circularly polarized head coil. The animals were presedated with a mixture of acepromazine and ketamine administered intramuscularly, as described earlier. During imaging, the animals were anesthetized with isoflurane (1%–5%) administered via facemask. The full MR imaging protocol is detailed in the **Appendix** (available online at *www.jvir.org*).

Each scan was evaluated for evidence of ablation, based primarily on lack of enhancement on postcontrast T1-weighted sequences, and size of ablation measured in two dimensions. If there was residual tumor, an estimate of maximum residual tumor thickness was recorded.

Pathologic Assessment

Gross pathologic and histologic examination of the liver was performed by an experienced veterinary pathologist. Tumors were evaluated by using standard rodent classification schemes (6). After euthanasia, the liver was removed and photographed and the treated tumor was measured. Serial sections, approximately 4 mm thick, were taken through the tumors and the adjacent normal parenchyma. Sections were stained with hematoxylin and eosin. The dimensions of the liver tumors and the diameters of RF ablation–induced necrosis were recorded.

RESULTS

Of the initial 20 woodchucks, five were not used in the study. Of these, two recovered from their WHV infection and did not develop tumors before the study concluded, one died from hemorrhage as a result of aortic dissection, and another died from unknown causes. The fifth woodchuck died before treatment, and the tumor was discovered on necropsy.

HCC was treated by RF ablation in 15 woodchucks. Using a conventional human protocol for the first two animals, we discovered that the human grounding pads that had been trimmed to conform to the woodchuck's body became excessively heated and produced significant burns to the abdominal wall and site of attachment. These two animals were euthanized because of the skin injury. The addition of ice packs at the site of the grounding pads was not sufficient to prevent thermal skin injury.

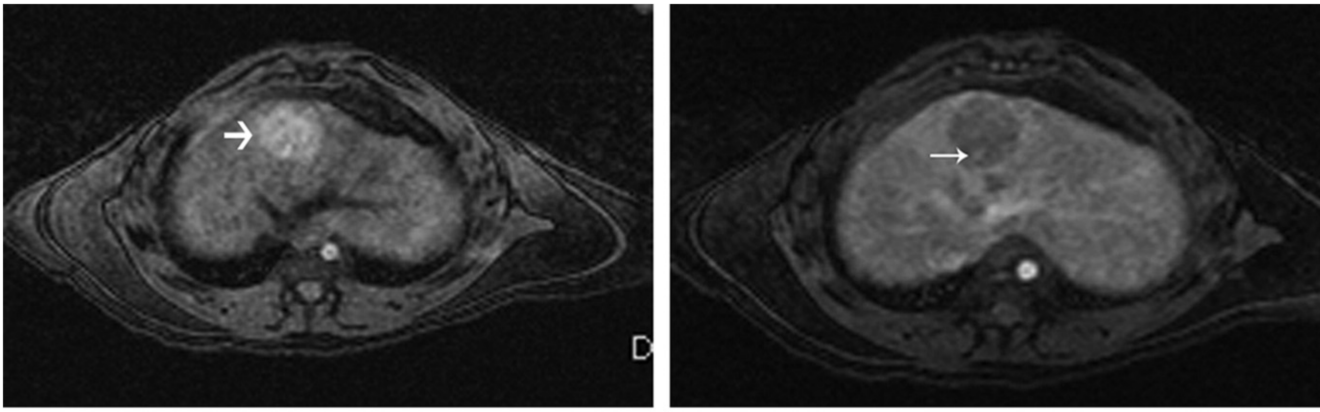
Subsequently, a total of 13 woodchucks were treated. The grounding protocol was altered to increase the surface area involved in grounding the animal. To do this, we immersed the caudal aspect of the woodchuck into warmed saline solution (**Fig 1**) during treatment. This modification eliminated all cutaneous injury associated with grounding. RF ablation was performed by using a 1-cm LeVeen RF ablation probe. Eleven of the 13 animals had postablation MR imaging (**Fig 2**). Two of the woodchucks did not undergo MR imaging: one woodchuck became sluggish, refused to eat in the days after the procedure, and was euthanized before it could undergo MR imaging. Postmortem evaluation revealed that thermal injury from the RF ablation had damaged the gastric wall. The second woodchuck had thermal injury to soft tissue adjacent to the tumor and died during transport to undergo imaging.

In all 11 woodchucks that underwent MR imaging after RF ablation, there was evidence of ablation (**Fig 3**). With the modified protocol, the burn areas were consistent, each approximately 1 cm in diameter (mean, 10.2 mm × 13.1 mm; range, 7–14 mm × 9–23 mm). As all HCCs treated were larger than 1 cm in diameter, there was evidence of residual tumor in all treated lesions with peripheral nodular enhancement (**Fig 4**).

All tumors were confirmed to be HCCs based on typical histologic appearance. Viable tumor tissue was readily discerned from treated tissue. Approximately 1 week after RF ablation, the treated volume was approximately spherical and showed complete loss of viable tissue and replacement with eosinophilic tissue debris and a circumferential rim of edema and early granulation tissue, characterized by an ingrowth of reactive fibroblasts and small-caliber blood vessels. A light infiltration of neutrophils and smaller numbers of lymphocytes were present in the majority of cases. In the two animals that were euthanized or died within 1 day of RF ablation, the treated areas consisted of a clearly defined area of coagulative necrosis rimmed with a mild infiltrate of neutrophils.

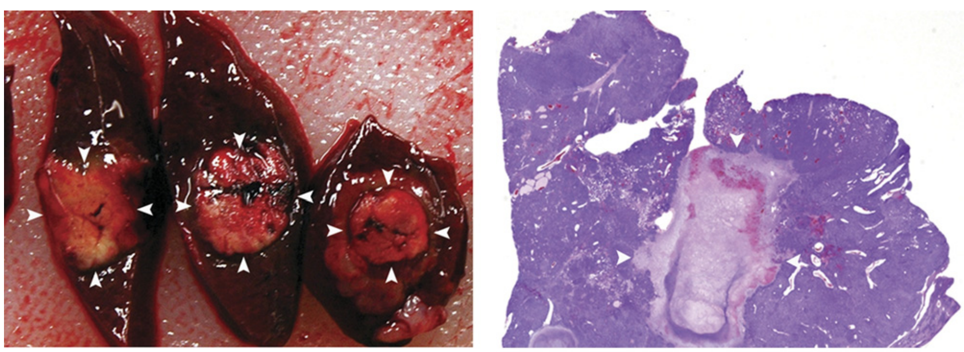
DISCUSSION

Woodchucks are naturally susceptible to infection with WHV, which is closely related to the hepatitis B virus (7).



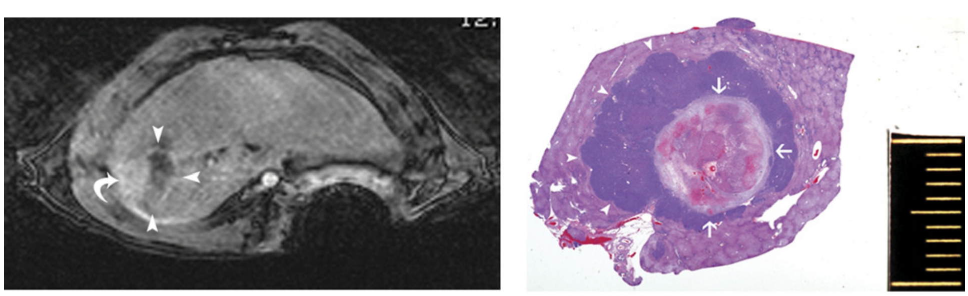
a. b.

Figure 2. MR imaging appearance of treated HCC in a woodchuck model. **(a)** Precontrast T1-weighted image shows a spherical area of hyperintense signal at the site of ablation (arrow). **(b)** After intravenous gadolinium administration, the lesion shows minimal peripheral enhancement with subtle areas of nodularity (arrow).



a. b.

Figure 3. Gross **(a)** and histopathologic **(b)** sections through treated woodchuck liver tumors reveal a central ablation zone within the tumor (arrowheads).



a. b.

Figure 4. **(a)** T1-weighted MR image after gadolinium administration through the level of ablation shows nodular, peripheral enhancement (curved arrow) along the lateral edge of the ablation site (arrowheads) consistent with residual HCC. **(b)** Histologic section through this lesion shows the central treated area (arrows) surrounded by residual basophilic HCC (arrowheads).

Nearly 100% of chronically WHV-infected woodchucks will develop primary HCC within 1–4 years of age, with tumors measuring nearly 100 cm^3 in volume (4,5,7). These tumors are primary liver cancer and possess normal tumor vascularization, differentiating the woodchuck from other animal models that rely on tumor cells that are implanted and grow in the liver.

Thus far, many of the animal models used in studying RF ablation of the liver do not incorporate tumor in the evaluation (8–12). Thus, much of the work evaluating the effectiveness of RF ablation on tumor coagulation relies on explant or pathologic reports. At least one animal study has been reported that did incorporate liver tumors in an animal model with RF ablation: Ahmed et al (12) developed a large animal model by inoculating immunosuppressed dogs with venereal sarcomas (ie, transmissible venereal tumor) to induce tumor development. This model was used to compare the use of RF ablation alone with the use of a combination of RF ablation and acetic acid injection.

The use of the woodchuck in studying a variety of HCC therapies is not new. The association between the WHV and HCC was initially described in 1978, and the woodchuck has served as a laboratory model to study HCC and a variety of treatments since 1980 (13). However, most of the work toward treatment has focused on systemic therapies, such as vaccines, antiviral agents, and immunotherapy. Nevertheless, there is at least one report describing the use of the woodchuck model for percutaneous therapy (7). Gouillat et al (7) treated seven animals with percutaneous alcohol ablation and one animal with laser photocoagulation. Three of the woodchucks treated with alcohol ablation died in the early postoperative period, and the remaining four underwent tumorectomy 1 month later. The single woodchuck treated with photocoagulation was euthanized 1 month after the procedure. The authors concede that this model is limited by the high perioperative mortality rate of the animals (7). They also describe greater fragility of the animals around the hibernation period. However, the woodchuck is now a well established animal model for virally induced HCC (3).

The present work required modification of procedures and protocols to adapt to the smaller size of the woodchucks, which are significantly smaller than even most pediatric patients. Early on, we attempted to perform the RF ablation procedure with algorithms similar to those used in larger animal models and humans. However, this resulted in significant injury to the animals, and the protocol had to be modified. Even neonatal grounding pads proved to pose a challenge because of the small size of the animal's caudal extremities. Also, to use the pads effectively, a large portion of the animal had to be shaved, resulting in considerable heat loss postoperatively. Nevertheless, skin burns at the grounding pad sites remained a problem despite the use of large quantities of tape and ice packs. For this reason, we devised a metal pan that was modified to connect to the electrode return sockets of the RF generator. By submerging the caudal half of the animal in saline solution, sufficient electrical grounding could be achieved without the need to shave the animal and without the risk of grounding pad skin injury.

The second challenge encountered was the development of collateral damage during the ablation. The initial use of higher energy with larger electrodes resulted in a larger volume of ablation than predicted based on the manufacturer's instructions for use. Initially, we opted to reduce the power while continuing to use the larger ablation probe, with the intent of being able to treat larger tumors completely. Unfortunately, this did not fully correct the problem. The feedback system of the LeVeen generator is through impedance, and, when using the lower energy, we did not see the normal increase in impedance; thus, there was no way to determine when sufficient ablation was achieved. Without this feedback, the ablation was carried out for 15 minutes, as this is the typical time required for complete ablation in humans. This resulted in a very large area of thermal injury to the abdominal cavity. From this point onward, we switched our protocol to include the use of a smaller probe with short ablation times of low energy. Using this new target, a consistent 1-cm3 ablation could be achieved with little risk of collateral injury.

The primary limitation of the present study was the restricted size of lesions that could be fully treated. As woodchucks' spontaneous development of tumors in a variety of sizes and locations is seen as the primary advantage to this model, the inability to treat larger lesions is a limitation that should be addressed in the future. We experienced significant injuries to the animals when attempting to fully ablate larger lesions. We postulate that the increased current transmitted between probe and ground when using larger probes and higher energies caused many of the complications encountered when more aggressive ablations were attempted. It remains to be seen whether larger lesions can be fully ablated by using a different technology, such as bipolar RF probes that restrict the current to the site of treatment. Alternatively, another energy delivery method, such as microwave or interstitial laser, may allow for safe ablation of larger tumors. Further investigation is needed to answer this question. Nevertheless, the fact that these animals develop tumors in a variety of sizes and locations potentially makes this an attractive model in which to comparing different treatment strategies. As it has been shown that HCC also has a dominant arterial blood supply in animals as in humans, it is possible that this model may also be used to evaluate transarterial therapies in addition to ablative therapies (14).

In conclusion, the woodchuck does provide an animal model with naturally occurring HCC that can be used to evaluate the efficacy of percutaneous ablation with MR imaging and pathologic evaluation of efficacy of treatment. We have developed a protocol that enables us to consistently create a predictable, controlled RF ablation–induced burn in primary HCC. However, this early work is limited, and further work with evolving technology is needed to develop a model that can be used for the treatment and complete ablation of larger tumors. With this, it may be possible in the future to use the woodchuck as a model in which to compare new minimally invasive therapies or combinations of therapies.

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APPENDIX

Mr Imaging Protocol
MR imaging sequences included T2 HASTE (200 mm × 200 mm FOV, 1 mm × 1 mm × 5 mm voxels, 2,000-ms TR, 65-ms TE, one average, 1:02 TA), T2 TSE with flow compensation (169 mm × 200 mm FOV, 0.9 mm × 0.8 mm × 5 mm voxels, 5,880-ms TR, 84-ms TE, three averages, 5:25 TA), T1 GRE (200 mm × 200 mm FOV, 1.3 mm × 1.0 mm × 4 mm voxels, 2,000-ms TR, 2.83-ms TE, one average, 7:14 TA), DTI with 21 directions (224 mm × 275 mm FOV, 2.1 mm × 2.1 mm × 2.5 mm voxels, 5,100-ms TR, 64-ms TE, two averages, 3:50 TA), T1 TSE with flow compensation (150 mm × 160 mm FOV, 0.6 mm × 0.6 mm × 5 mm voxels, 600 ms TR, 12 ms TE, three averages, 4:47 TA), VIBE (200 mm × 200 mm FOV, 1 mm × 0.8 mm × 2 mm voxels, 5-ms TR, 1.69-ms TE, one average, 0:39 TA) before and after contrast agent injection (intravenous bolus injection, gadodiamide 0.1 mmol/kg), T1 GRE FS 10 minutes after contrast agent injection (250 mm × 219 mm FOV, 1.6 mm × 1.0 mm × 4 mm voxels, 196-ms TR, 2.46-ms TE, one average, 0:28 TA).

Note.—HASTE = half-Fourier acquisition single-shot turbo spin-echo, DTI = diffusion tensor imaging, FOV = field of view, TE = echo time, TR = repetition time, TA = acquisition time, VIBE = volume interpolated breath-hold examination, GRE = gradient recalled echo, FS = fast spin, TSE = turbo spin-echo.