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Application of trifluoroacetic acid as a theranostic agent for chemical ablation of solid tissue

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Abstract

Purpose—To evaluate trifluoroacetic acid (TFA) as a theranostic chemical ablation agent and determine the efficacy of TFA for both non-invasive imaging and tissue destruction.

Materials and Methods—Fluorine-19 magnetic resonance imaging (¹⁹F-MRI) was optimized at 7 T using a custom-built volume coil. Fluorine images were acquired with both rapid acquisition with relaxation enhancement and balanced steady-state free precession (bSSFP) sequences with varying parameters to determine the optimal sequence for TFA. The theranostic efficacy of chemical ablation was examined by injecting TFA (100 μ L; 0.25, 0.5, 1.0, and 2.0M) into ex vivo porcine liver. ¹⁹F- and proton-MRI were acquired and superimposed to visualize distribution of TFA in tissue and quantify sensitivity. Tissue damage was evaluated with gross examination, histology, and fluorescence microscopy.

Results—The optimal ¹⁹F-MRI sequence was found to be bSSFP with a repetition time of 2.5 ms and flip angle of 70°. The minimum imageable TFA concentration was determined to be 6.7 ± 0.5 mM per minute of scan time ($0.63 \times 0.63 \times 5.00$ mm voxel) and real-time imaging (temporal resolution of at least 1 s^{-1}) was achieved with 2M TFA both in vitro and in ex vivo tissue. TFA successfully coagulated tissue and damage was locally confined. In addition to hepatic cord disruption, cytoskeletal collapse and chromatin clumping were observed in severely damaged areas in tissues treated with 0.5M or higher TFA concentrations.

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Conclusion—TFA was determined to be a theranostic agent for chemical ablation of solid tissue. Ablation was both efficacious and imageable in ex vivo healthy tissue, even at low concentrations or with high temporal resolution.

Keywords

Hepatocellular carcinoma; tissue ablation; chemical ablation; fluorine-19 MRI; interventional MRI

Introduction

Prognosis for hepatocellular carcinoma is extremely poor without intervention (1,2) and for patients treated by ablation methods, local recurrence is frequently a challenge. Residual tumor is found upon pathological examination in up to 50% of resected livers in patients who are treated and subsequently receive a liver transplant (3,4). Tumors treated with thermal methods such as radiofrequency ablation and located near high flow blood vessels are prone to heat sink effects that reduce peak temperatures. This can lead to incomplete treatment and, in certain instances, incomplete tumor eradication provokes an aggressive, detrimental tumor response (5).

Chemical ablation (CA), although largely supplanted by thermal methods, is not subject to this limitation. An important issue in CA, however, is the potential of an ablative agent to flow along paths of least resistance. This can result in irregular lesion shapes, non-target injury, and systemic toxicity (6). Monitoring and accurately mapping the distribution of the instilled material is essential. Knowing the delivered dose is fundamentally important in order to understand the biological effects of a procedure and its optimization. Further, to translate a procedure to the clinic, control and safe administration of a therapy should be well understood. Imaging should help answer both these questions.

Acetic acid has been investigated as an alternative to the much more common ethanol (4,7,8). It diffuses through tissue more effectively and for a given concentration is more effective than ethanol, but tracking the distribution of acetic acid in vivo is challenging (9,10). Trifluoroacetic acid (TFA) imaged using fluorine-19 MRI (^{19}F -MRI) in combination with proton anatomic MRI may be a useful strategy for understanding and optimizing CA. Substituting some or all of the acetic acid with TFA in CA would allow for monitoring the distribution of the injected material with essentially no background signal. Historically, the use of TFA in ^{19}F -MRI has been limited primarily to serving as a reference standard (11) or optimization agent (12). Most studies use ^{19}F -MRI to image fluorinated drugs or cells labeled with other fluorinated compounds. These studies inevitably involve very low ^{19}F concentrations and, hence, very low MR signal intensities (13–15). Therapeutic applications of TFA in CA could involve much higher ^{19}F concentrations, resulting in significantly increased MR signal with the potential for real-time imaging. This manuscript reports the initial results of using TFA as an imageable therapeutic agent in phantoms and ex vivo tissue to determine the optimal ^{19}F -MRI sequence and respective sensitivity limit as well as the mechanism and extent of tissue damage.

Materials and Methods

Evaluation of imaging potential

A. TFA phantom imaging—A phantom was constructed containing five nuclear magnetic resonance tubes (5 mm diameter) of varying concentrations of TFA (31.25, 62.50, 125.0, 250.0, and 500.0mM). MRI was performed on a 7 T Biospec 70/30 (Bruker Corp.; Billerica, MA, USA) using a custom-built ^{19}F volume coil (RAPID MR International; Columbus, OH, USA) tuned to 282.56 MHz. Axial ^{19}F images of the phantom were acquired with rapid acquisition with relaxation enhancement (RARE) and balanced steady-state free precession (bSSFP) sequences using a 20×20 -mm field of view. For the RARE sequence, various repetition times (TR; 0.1, 0.5, 1.0, 2.0, 3.0, 3.8, 5.0, 6.0, 7.5, 10, 15, and 30 s) and echo train lengths (ETL; 16 and 32) were applied to determine optimal sequence parameters. For the bSSFP sequence, various repetition times (2.50, 3.75, 5.00, 7.50, and 10 ms) and flip angles (50° to 90° in 5° increments) were applied. The optimal parameters were defined as those that yielded maximum signal-to-noise ratio (SNR) efficiency (SNR divided by square root of time). Voxel size was fixed at $0.63 \times 0.63 \times 5.00$ mm. All sequences were acquired for 30 s and then repeated twice for a total of three measurements. Proton (^1H) images were acquired with a RARE sequence (TR = 2.5 s, ETL = 8, matrix size = 128×128 , number of signal averages = 8), and the ^1H and ^{19}F images were then superimposed using ImageJ (v1.50i; National Institutes of Health, Bethesda, MD, USA). The SNR of the varying TFA concentrations (from the optimal sequence) was fit to a straight line to determine the sensitivity limit of the system, assuming a conservative minimum necessary SNR of 5 (16). The possibility of real-time imaging of TFA injection was evaluated by injecting 2M TFA into a flexible tube (3.2 mm inner diameter) while imaging at ten frames per second with cine ^{19}F -MRI using the optimal bSSFP sequence. All measurements were acquired at bore temperature (approximately 12°C).

B. Liver procurement and treatment—All procedures using laboratory animals were approved by the Institutional Animal Care and Use Committee. Porcine livers without tumors were procured by en bloc viscerectomy from heparinized donors after euthanasia. Following hepatectomy, the liver lobes were divided and sectioned into smaller pieces (approximately $2 \times 2 \times 0.5$ cm). A single dose of 2.0M TFA (700 μL) was injected into a single tissue section during cine ^{19}F -MRI. The treated liver section was then imaged with MRI post-injection, photographed, and prepared for histology.

C. Ex vivo MRI—Ex vivo ^{19}F -MRI was performed as above for phantom imaging using the optimal bSSFP sequence with $2 \times 2 \times 10$ mm voxel size (slab imaging). During injection, images were acquired at 1.4 images per second. Post-injection images were acquired with signal averaging yielding scan times of 0.73, 21.8, and 43.7 s. Proton images were also acquired with a RARE sequence (same as for phantom imaging but with a 32-mm field of view), and the two images were superimposed as described above. Maximum voxel SNR was calculated from the unprocessed ^{19}F -MR images generated by each scan.

Evaluation of tissue damage potential

A. Histology—In addition to the imaged tissue section, additional liver sections were obtained as described above (Liver procurement and treatment) and a single dose of TFA (100 μ L) in one of four concentrations (0.25, 0.5, 1.0, or 2.0M) was injected into eight additional tissue sections (two sections per tested concentration). Liver sections were bisected and a portion was fixed in 10% neutral buffered formalin. Samples were processed routinely, embedded in paraffin, sectioned at 4- μ m thickness, stained with hematoxylin and eosin, and examined by a board-certified veterinary pathologist. Phalloidin and 4',6-diamidino-2-phenylindole (DAPI) staining were also used to highlight cytoskeletal and nuclear structures, respectively (CytoPainter Phalloidin-iFluor 555 [ab176756] and Fluoroshield Mounting Medium with DAPI [ab104139] from Abcam plc, Cambridge, UK).

Statistical analysis

Average values are reported as the mean value with standard deviation. Differences were determined using Welch's *t*-test and considered significant if $p < 0.05$. All analyses were performed with GraphPad Prism (v7; GraphPad Software, La Jolla, CA, USA) and all fits used least-squares weighted values.

Results

Evaluation of imaging potential

A. TFA phantom imaging—The TFA in the phantom was successfully imaged at all concentrations. The optimal RARE TR (Fig. 1A) and ETL were determined to be 3 s and 32, respectively. The optimal bSSFP TR was found to be 2.5 ms, and the optimal flip angle was 70°. The optimal bSSFP sequence yielded 1.45 times the SNR efficiency of the optimal RARE sequence. The minimum imageable TFA concentration was determined to be 6.7 $\pm$ 0.5mM per minute of scan time (Fig. 1C). These results indicate that injected 1M TFA solution could be imaged with a temporal resolution of 1 s at a spatial resolution of 2 $\times$ 2 $\times$ 2 mm. The injection of 2M TFA into a tube was successfully visualized with a temporal resolution of 10 frames/s (Fig. 2).

B. Ex vivo MRI—TFA was successfully imaged with sufficient SNR during injection and at all acquisition times post-injection (Fig. 3). Maximum voxel SNR for the post-injection 0.73, 21.8, and 43.7 s scan times were 14.8, 41.5, and 52.7, respectively.

Evaluation of tissue damage potential

A. Histology—Within each section of liver, a well-demarcated focus of injury at the injection site was seen on gross photography and histologic examination (Figs. 4 and 5A). Histologic examination showed a gradient of severe to mild lesions extending outward from the center of each injection. Severe damage was observed in samples treated with 0.5M or greater TFA. Centrally, hepatic lobular architecture and hepatic cords were mildly disrupted. In liver samples treated with 0.25M TFA, hepatocyte cytoplasm was moderately vacuolated. In samples treated with 0.5M or greater TFA, hepatocytes were shrunken and had hypereosinophilic and vacuolated cytoplasm (Figs. 5B, 5C). In many regions, the perisinusoidal space was increased. Nuclear chromatin in hepatocytes was clumped. In more

severely affected regions in the 0.5M and greater samples, nuclear chromatin was margined and an eosinophilic, inclusion-like body (most likely aggregated nuclear proteins) was present centrally within the nucleus. In phalloidin-DAPI-stained sections, hepatocyte cytoskeletal collapse was present in severely affected regions, with markedly reduced binding of DAPI by local nuclei (Fig. 5D).

Discussion

Ablation therapies are a treatment strategy for hepatocellular carcinoma, but complete tumor eradication remains an issue, especially as tumor size increases over 3 cm. As noted earlier, incomplete ablation of liver tumors can provoke an aggressive and detrimental tumor response (5). In terms of disease burden worldwide, chemical methods remain the most cost-effective and accessible in resource-limited countries where the burden is greatest. That being said, even in more developed countries the use of specialized MR methods for CA is not expected to become commonplace. Research using these MR methods as tools to further understanding of an ablative agent's distribution and effects in tissues is expected to be beneficial and necessary to advancing chemical ablation. Additionally, real-time monitoring of chemical ablative agent delivery could inform techniques to enable safe administration and minimize treatment to undesired areas. In this study, TFA was demonstrated as an effective, imageable therapeutic agent for the ablation of solid tissue.

Initial experiments focused on optimizing ^{19}F -MRI for imaging TFA using a phantom with five concentrations produced by serial dilution. Image SNR was found to have a linear relationship with concentration, as expected. Both RARE and bSSFP sequences were successfully optimized. bSSFP yielded higher SNR efficiency than did RARE, in agreement with previous work (17). These results indicate that sensitivity was excellent for both RARE and bSSFP sequences, thus permitting short imaging times and image guidance of TFA ablation therapy. Cine ^{19}F -MRI confirmed that real-time image guidance of TFA ablation therapy is feasible.

Successful imaging of TFA phantoms led to imaging of an ex vivo liver section treated with TFA. In the liver sections, TFA distribution could not be visualized with standard proton MRI but was successfully imaged with ^{19}F -MRI both during injection and post-injection. Maximum ^{19}F -MRI SNR increased with increasing scan time as expected.

Results of histologic examination of liver sections treated with TFA ex vivo were consistent with regional hepatic injury. While some features were consistent with reversible cell injury, such as cytoplasmic vacuolation, the degree of architectural, cytoplasmic, and nuclear alteration in sections treated with 0.5M or greater TFA suggests that these changes would result in coagulative necrosis in vivo. In severely affected hepatocytes in these samples, nuclear binding of DAPI (used in vitro to identify nuclei in fluorescent staining protocols) was markedly reduced. Binding between DAPI and DNA occurs in the DNA minor groove and by intercalation between bases (18,19). The reduction in nuclear binding by DAPI was theorized to be due to DNA hydrolysis by TFA, which may have occurred through the destruction of phosphodiester and glycosidic bonds between bases (20,21).

This study was a preliminary investigation and, as such, included a number of limitations. This study utilized porcine liver tissue available from another study, allowing us to reduce the number of research animals used (a central tenet of animal welfare). Use of hepatocellular carcinoma tissue would be more applicable to the clinical applications, but its use is not expected to alter the MR sensitivity or damage potential of TFA significantly. Further, this simple model based on ex vivo tissue did not include perfusion, though blood flow would also not be expected to alter the findings of this study. Additionally, ^{19}F -MRI was optimized for this particular experiment, and TFA relaxation times (and, therefore, optimal imaging sequence parameters) are expected to change with variations in static magnetic field strength (such as those used in clinical systems), oxygen concentration, and temperature (22).

The theranostic efficacy of TFA could be further enhanced using various acceleration techniques that undersample k -space (e.g. parallel imaging, partial-Fourier, etc.), which would be expected to reduce the acquisition time of individual scans, thus permitting additional signal averaging and increasing the sensitivity of ^{19}F -MRI. This technique may also be applicable to other methods of ablation, such as thermochemical ablation (23–26).

In conclusion, ^{19}F -MRI was successfully optimized to image TFA with excellent sensitivity and temporal resolution. Ex vivo tissue ablation demonstrated TFA to be both effective and imageable, even at low concentrations. ^{19}F -MRI is a promising strategy for study of chemical ablation of solid tissue and additional applications potentially could be developed.

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Figure 1:

Results of fluorine-19 magnetic resonance imaging optimization using a phantom consisting of serial dilutions of trifluoroacetic acid. A, Plotting the signal-to-noise ratio (SNR) efficiency as a function of repetition time (TR; red circles) and echo train length (blue squares) of a rapid acquisition with relaxation enhancement sequence determined optimal sequence parameters. B, Plotting the SNR efficiency as a function of TR (red circles) and flip angle (blue squares) for a balanced steady-state free precession sequence yielded optimal parameters for that sequence. C, SNR of the various dilutions had a linear relationship with concentration, permitting an accurate assessment of the sensitivity limit of the technique.

Figure 2:

Images from cine fluorine-19 balanced steady-state free precession magnetic resonance imaging of injection of 2M trifluoroacetic acid (TFA) into a tube with 3.2 mm inner diameter. Ten fluorine images were acquired per second and representative images (every fifth image) are shown above superimposed on a proton landmark image. These results demonstrate the feasibility of real-time image guidance of TFA ablation therapy.

Figure 3:

Representative magnetic resonance imaging (MRI) results of an ex vivo liver section ablated with local injection of 700 μ L of trifluoroacetic acid (2.0M). Ablative agent distribution could not be visualized on the standard proton MRI, but was successfully imaged with fluorine-19 MRI (color images; superimposed on the grayscale proton MRI for anatomic reference). The section was imaged during injection (A) and post-injection with acquisition times of 0.73 (B), 21.8 (C), and 43.7 s (D). A gross photograph (E) and histologic section (F) are provided for reference.

Figure 4:

Representative gross photographs showing damage to ex vivo liver sections treated with 100 μ L of 0.25, 0.5, 1.0, and 2.0M trifluoroacetic acid. There was well-demarcated localization of coagulation.

Figure 5:

Histologic examination elucidated mechanisms of damage in porcine liver sections treated ex vivo with 0.5M trifluoroacetic acid. A, The lesion encompassed several hepatic lobules (arrow). B, In severely affected regions in the center of the lesion, hepatocytes (H) were shrunken, hypereosinophilic, and possessed clumped and margined nuclear chromatin. Sinusoids (S) and perisinusoidal spaces (arrow and P) were enlarged. C and D, In a satellite lesion affecting a lobule adjacent to the main lesion, hepatocyte damage was progressively more severe at the center of the lobule, with cytoskeletal collapse demonstrated by increased intensity of phalloidin binding and significantly decreased 4',6-diamidino-2-phenylindole binding, possibly resulting from acid-mediated DNA hydrolysis.