Study of Cell Viability in MR Imaged Focused Ultrasound Lesion In Vivo in Rabbit Brain

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Introduction On T2-weighted MRI, focused ultrasound lesions (FUS) are characterized by an hyperintense central area of coagulation necrosis and an hyperintense rim of edema. Within the bright rim, the tissue is damaged, although the extent of damage could not be determined by either MRI or on histologic sections stained with hematoxylin and eosin (H&E) in our previous study [1]. The purpose of this study was to investigate the cell viability in MR imaged FUS lesions using the Triphenyl Tetrazolium Chloride (TTC) cell viability staining technique and electron microscopy (EM) ultrastructure. Lesion sizes measured on TTC stained specimens were compared to MRI and histology stained with H&E.

Methods Ten paired ultrasonic lesions were created in 5 rabbit brains in vivo. Before FUS treatment, each animal underwent general anesthesia. A piece of skull was surgically removed. The animal was mounted on the top of the water tank in the supine position. The animal was placed in the bore of a 1.5 T MR scanner (GE Systems). Two separate lesions were created in the brain on the MR sagittal plane. The animals were sacrificed immediately after imaging 4 h after FUS treatment. The brain was removed, and the cerebral hemispheres were cut through the middle of the lesions at a distance from the longitudinal fissure measured on the coronal images. The specimens were immediately incubated in a 1% solution of TTC. Within the 20 minutes incubation, the FUS lesion could be clearly distinguished as a gray-white color surrounded by normal tissue stained deep-red. The maximum lesion diameters were measured with a vernier caliper. After lesion measurement, the specimens were prepared for histological study. Ten-μm thick sections taken at 500-μm intervals, perpendicular to the lesion length were stained with H&E.

Three samples measuring approximately 1 mm³ were taken from TTC stained specimen for study of the ultrastructure changes in cells by EM. The first sample was taken from the central lesion. The second sample was taken immediately inside of the boundary between TTC stained and unstained tissue and the third sample was taken from the normal tissue.

Two measurements were made on H&E sections. The first was the diameter of the cutting edges for comparison with the diameter measured on TTC. The second was the maximum lesion diameter for comparison with the maximum lesion diameter found on the MRI. The measured values on the cutting edges for four of the lesions were smaller than the maximum lesion diameter on histological sections. As a result, direct comparison between TTC and MRI measurements were made in only 6 of the 10 lesions.

Tissue shrinkage on TTC and H&E were corrected by using correction factors. The correction factors for the TTC specimens were obtained from the ratio of the distance from two lesion centers in MRI to the distance from the two lesion centers on the TTC stained specimens. To correct for tissue shrinkage on H&E sections, the correction factors were obtained from the ratio of the distance between two lesion centers on the MR images to the distance between two lesion centers on H&E sections.

Results Lesion sizes measured from TTC staining versus those measured from the cutting edges on H&E are plotted in figure 1. On average the measured values on MRI were slightly larger than that on H&E. The difference in lesion sizes were 0.1-0.6 mm.

The lesion diameters measured by T2-weighted MRI versus those measured by H&E are plotted in figure 2. The difference in lesion diameters measured from MRI and H&E were 0.1-0.7 mm. On average the lesion measurements on MRI are slightly larger than those measured on H&E.

Lesion diameters for the 6 lesions that allowed direct comparison between TTC and MRI are given in Fig. 3. The differences in lesion sizes between TTC and MRI were 0.1-0.7 mm. For 5 of the 6 lesions the measured values on MRI were slightly larger than that on TTC measurement.

Electron microscopy showed that within both the central zone and just within the TTC border only the nuclear and cytoplasmic ultrastructure had been disrupted, whereas in the sharply demarcated surrounding normal tissue, there was preservation of neuronal ultrastructure.

Summary The results of our study indicate that lesion sizes compare well between H&E and MRI. However both slightly overestimate the lesion size found on TTC stained specimens. The difference is only one MR imaging pixel value (0.31 mm) except for one lesion (0.1 mm).

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References Chen et al. JMRI, 10:146-153, 1999.