Evaluation of Anticancer-drug Efficacy in Tumor-bearing Rat by Using ^{19}F/^{1}H-MRI

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Introduction

^{19}F-MRI/MRS has been used in pharmacokinetic studies of ^{19}F-containing drugs [1-2]. We previously developed a ^{19}F receive coil with a ^{19}F/^{1}H transmit coil for monitoring of pharmacokinetics at 7-T and showed time-course images of 5-fluorouracil (5-FU), its active metabolites (fluorinated nucleosides and nucleotides, Fnuc), and its catabolites (α-fluoro-β-alanine) in a tumor-bearing rat [3]. To evaluate drug efficacy, not only pharmacokinetics but also pharmacodynamics of the drug must be understood. A k-means clustering algorithm was used to understand pharmacodynamics, which segments a tumor region into viable tissue and necrosis [4]. In this study, we developed the a method for evaluating drug efficacy on the basis of images of active metabolites obtained by ^{19}F-MRI and a k-means class map of tumor status obtained by ^{1}H-MRI. We demonstrated that the developed method can evaluate the drug efficacy of 5-FU in a tumor-bearing rat at 7 T.

Materials and Methods

Instruments & Animal: A 7-T animal MRI system (MRI System, Agilent, USA) was used. For ^{19}F imaging to obtain active metabolites, the ^{19}F/^{1}H RF coils was used. For ^{1}H imaging to evaluate tumor status, a QD birdcage coil (I.D. of 180 mm, Agilent) was used. All animal studies were conducted in accordance with guidelines for the care and use of laboratory animals. A female Wistar rat (body weight: 180 g) bearing Walker 256 tumors were used. The rat was fixed on a holder to keep the rat positioned between the two coils. The rat was anesthetized with 2-4% isoflurane administered in combination with 30% oxygen through a mask. ^{19}F and ^{1}H MRI images were obtained after intravenous bolus injection of 250 mg/kg of a 5-FU; Kyowa Hakko Kirin, Japan) into the rat. (5-FU is a metabolic antagonist that is converted into active metabolites in cells, especially in active cells.) The 5-FU and its active metabolites contain one fluorine atom in each molecule and have different chemical shifts.

Imaging of active metabolites: To obtain time-course images of Fnuc, ^{19}F images were obtained by using a fast-spin-echo (FSE) sequence with 3-ms Gaussian-shaped frequency-selective pulses. The scan time of ^{19}F imaging was ranged from 50 to 100 minutes after injection of 5-FU. The sequence parameters of the FSE are FOV of 384 × 96 mm, matrix size of 64 × 16 without slicing, TR/TE of 1000/7 ms, and ETL of 4. All ^{19}F images were obtained by reconstruction from consecutive 10-minute accumulated data sets.

Evaluation of tumor status: A k-means clustering algorithm (k=3) using the tumor's T_2 and ADC data set was employed to segment a tumor into three classes: viable tissue, necrosis 1, and necrosis 2. The necrosis-1 class indicates necrosis tissue with high ADC and short T_2 values, whereas the necrosis-2 class indicates necrosis tissue with high ADC and long T_2 values. T_2 maps were constructed from the T_2-weighted spin-echo data under the following condition: FOV of 64 × 64 mm, matrix size of 64 × 64, thickness of 1 mm, TR of 4000 ms, and TE of 7, 25, 46, and 67 ms. ADC maps were constructed from the diffusion-weighted spin-echo data under the following condition: FOV of 64 × 64 mm, matrix size of 64 × 64, thickness of 1 mm, TR of 4000 ms, TE of 20 ms, and b-values of 89, 208, 368, 594, 850, and 1165 s/mm^2. Additionally, to reduce motion artifacts, a fat-saturation pulse and spatial-saturation pulses were used in the imaging sequences.

Evaluation of drug efficacy: The binary classification was employed to segment the field of view (FOV) of Fnuc and the projection images of viable tumors into four classes: true-positive (TP), true-negative (TN), false-positive (FP), and false–negative (FN). TP means that Fnuc was detected in the existed area of viable tumor (viable-tumor').TN means that Fnuc was not detected in the area of non viable-tumor' tissue. FP means that Fnuc was detected in the area of non viable-tumor' tissue. FN means that Fnuc was not detected in the viable-tumor'. The accuracy (namely, the rate of true positive and negative) is given by (TP+TN)/(TP+TN+FP+FN).

Results and Discussion

Fnuc signals were obtained mainly in the tumor region (as shown in Fig. 1). The distributions of Fnuc vary with time (Fig. 1b). Accordingly, to evaluate the area of detected Fnuc, a prolonged scan is needed. The resulting T_2 and ADC maps for tumor segmentation that shows tissue information are shown respectively in Figs. 2a and 2b. The resulting k-means class map that shows the tissue segmentation is shown in Figure 2c. Cluster plots of ADC and the T_2 data set for the tumor are shown in Fig. 2d. The sum of class plots gives the volume of each tissue class (Table 1). The viable tissue was 76% of the total tumor volume. A 50-min accumulated image of Fnuc is shown in Fig. 3a. A projection image of the viable tissue of the tumor (derived from the k-means class map) is shown in Fig. 3b. The projection image has lost some thickness information, but it has a similar shape to the detected area of Fnuc (Fig. 3a). The results of binary classification calculated from an FOV image of the detected area of Fnuc and an FOV image of the area of the viable tumor are listed in Table 2. A binary classified map overlaid onto the ^{1}H image of the rat is shown in Figure 3c. This map shows the region of positive effect and negative effect of the 5-FU and has an accuracy of 0.91, which demonstrates the efficacy of 5-FU.

Conclusion

A method for evaluating drug efficacy—which uses images of active metabolites obtained by ^{19}F-MRI and a k-means class map of tumor status obtained by ^{1}H-MRI—was developed. The developed method will be a powerful tool for pharmacokinetics and pharmacodynamics research.

References

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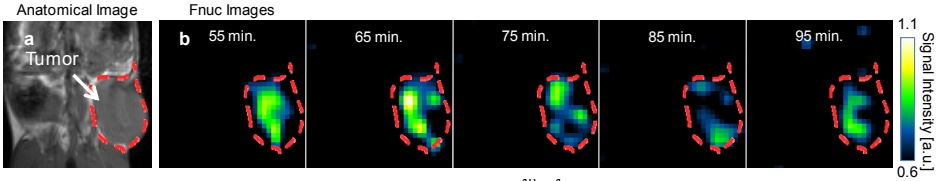

Fig. 1. Results of metabolite imaging using developed ^{19}F/^{1}H RF coils. **a**: Anatomical image of the rat. **b**:Time-course images of Fnuc distribution of the rat after bolus injection of 5-FU.

Table 1. Features of clustering

Tissue class	ADC [10^3 mm^2/s]*	T_2 [ms]*	Volume [mm^3]
Viable tumor	0.74±0.15	43±7.8	5800
Necrosis1	1.5±0.40	42±15	1613
Necrosis2	2.2±0.40	128±35	260

* mean±SD

Table 2. Results of binary classification

		^{1}H imaging	
		Viable-tumor'	Non viable-tumor'
^{19}F imaging	Fnuc positive	16%	8.2%
	Fnuc negative	1.1%	74%

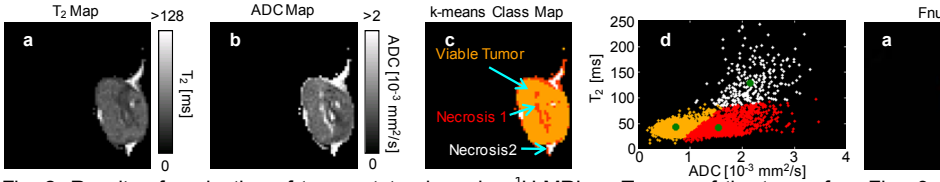

Fig. 2. Results of evaluation of tumor status by using ^{1}H-MRI. **a**: T_2 map of the tumor for segmentation. **b**: ADC map of the tumor for segmentation. **c**: k-means class map of the tumor segmented into viable tissue (orange), necrosis 1 (red), and necrosis 2 (white). **d**: Cluster plots of the parameters used in the classification. Plot colors are the same as those in Fig. 2**c**.

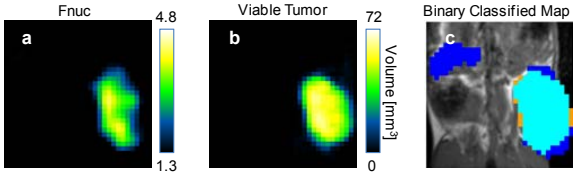

Fig. 3. Results of evaluation of drug efficacy **a**: 50-min accumulation image of Fnuc. **b**: projection image of viable tumor tissue. **c**: Binary-classified map overlaid onto the ^{1}H image of the rat. TP: aqua; TN: clear; FP: blue; FN: orange.