IN VIVO 19F NMR MEASUREMENTS OF ENHANCED CONVERSION OF 5FU TO FLUORONUCLEOTIDES AFTER UPRT GENE TRANSDUCTION

JA Koutcher, C. Matei, M. Dubrovin, K. Zakian, M. Sadelain and J. Tjuvajev.
Memorial Sloan-Kettering Cancer Center ,New York, NY 10021.

Introduction

5-Fluorouracil (5FU) is a widely used anti-metabolite that has activity against a wide range of cancers. It is metabolized by different pathways to one of several fluoronucleotides (Fnuc) and then incorporated into DNA or RNA. One of the metabolic pathways is via uracil phosphoribosyl transferase (UPRT) which metabolizes 5FU directly to fluorouridine monophosphate (FUMP). Since mammalian cells do not express UPRT, we have investigated the effect of transducing tumor cells with bacterial UPRT in order to alter 5FU kinetics and tumor response by increased conversion of 5FU to Fnuc. We have used 19F NMR spectroscopy to monitor 5FU kinetics to determine whether we can non-invasively assess UPRT expression in Walker 256 cells that were retrovirally transduced with the *H.influenza* UPRT gene (W256UPRT$^+$).

Methods

Nude mice bearing s.c. tumors derived from the wild-type W256 cells or W256UPRT$^+$ cells were studied. Mice were injected with 5FU (150mg/kg i.v.) and studied by 19F NMR spectroscopy to monitor drug metabolism. The dose of 5FU was based on previous studies suggesting that this was the maximum tolerated dose. Spectra were obtained on a 4.7T NMR system with a 2-turn solenoidal coil. After the drug was injected, the mice were placed in the coil, the tune and match of the coil checked, and the magnet shimmed using the 1H NMR signal of the water. This typically required approximately 15 minutes. Experimental parameters included a repeat interval of 2 seconds, tip angle of 45°, block size of 1024 data points and a spectral width of 10,000 points. This resulted in good signal to noise with 17 minute temporal resolution.

Results and Discussion

Spectra obtained on wild type tumors (~120 mm^3; n=5) showed 5FU without any detectable Fnuc (Fig. 1). The 5FU peak decreased slowly over time and was detectable for approximately 2 hours with good signal to noise. In W256UPRT$^+$ tumors (n=5, Fig. 2), the 5FU signal decreased at a similar rate compared to the wild type tumors but the peak arising from Fnuc was readily detected. The Fnuc peak was detected at 15-32 min (initial spectrum) in 3 out of 5 mice studied. In all five mice the Fnuc peak was detected with excellent signal to noise and increased over time. By 66-83 min, the Fnuc peak was the dominant peak in the spectrum of W256UPRT$^+$ tumors, and was often detectable for more than four hours.

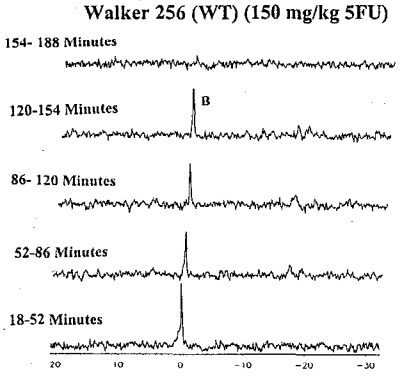

Fig. 1 19F NMR spectra obtained on Walker 256 (WT) after 150mg/kg of 5FU. Peak B= 5FU. 34 minute accumulation is shown to increase SNR

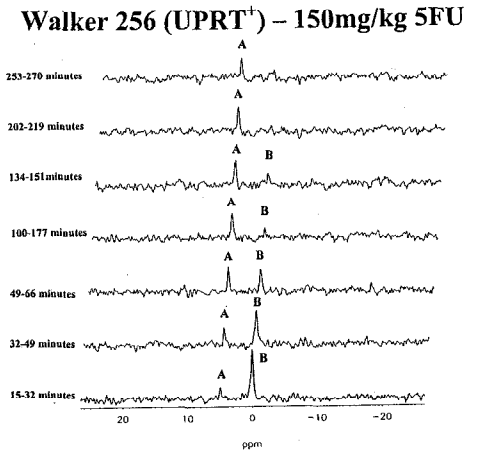

Fig. 2. 19F NMR spectra obtained on W256UPRT$^+$ after 150mg/kg of 5FU. Peak A= Fnuc, B= 5FU. Spectra obtained with 17 minute resolution

Fig. 3 shows the rate of disappearance of the 5FU peak and the rate that the Fnuc peak appears. A similar rate of decay of 5FU was noted for both wild-type (not shown) and W256UPRT$^+$ tumors. Fnuc was virtually never seen in the wild-type tumors. Tumor growth delay studies showed that the wild-type W256 tumor has poor sensitivity to 5FU. In contrast, the W256UPRT$^+$ cells showed increased sensitivity to 5FU.

In previous studies, it has been shown that enhanced conversion of 5FU to Fnuc (1-3) is associated with enhanced tumor response to 5FU. We hypothesize that gene transduction with UPRT in Walker 256 has led to greater activation of 5FU from its prodrug form (5FU) to Fnuc which has induced greater antitumor effect.

Conclusion

We conclude that the UPRT gene expression can be effectively monitored by NMR spectroscopy. NMR spectroscopy could be used to assess and predict the efficacy of UPRT/5FU prodrug activation therapy by measuring intratumoral levels of Fnuc.

References

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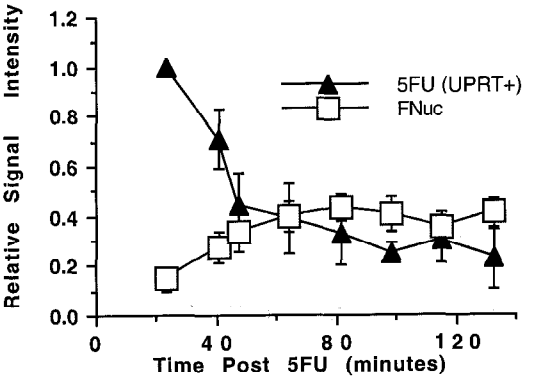

Fig. 3. Rate of decay of 5FU and rate of appearance of Fnuc in W256UPRT$^+$ tumors.