Examining the Source of T_2 Components Fit with NNLS

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Introduction

A well-known characteristic of malignant glioma is an increase in cell density with increasing malignancy of the tumor. In a previous study [1], we explored the relationship between multiexponential T_2 and cell density in a simple model of astrocytoma cells suspended in agarose at different cell densities. Using Non-Negative Least Squares (NNLS), we were able to fit two T_2 components to pure agarose and three T_2 components to samples with cells. The longest T_2 component significantly decreased with increasing cell density, but the associated percentage of signal did not have a significant relationship with cell density. Additionally, the component was sporadically fit amongst the different cell density samples and only 1/5 of the samples had a long component that contributed more than 10% of the signal. The coefficients of variance for the long component (0.28±0.26) were larger than that of the more stable medium (med) component (0.08±0.06) for four of the five cell densities. Because of the variability, we were not sure if the long component truly arose from a real compartment within the sample or if it was the result of measurement errors. In the current study, we investigated the reproducibility of measuring the long T_2 component and established criteria of acceptance for components fit with NNLS in these studies. We then used the criteria to verify the number of components associated with pure agar and pure cell samples. Further, we doped a pure cell sample with gadolinium to determine if any component was associated with the extracellular compartment.

Methods

SAMPLE PREP: Low melting point agarose (Sea Prep, Lonza, Basel, Switzerland) was made with D_2O based phosphate buffered saline (D-PBS) and TSP/D_2O to create 1.5%, 2.0%, 2.5%, and 3.0% (w/v) solutions. We performed NMR studies in triplicate to test the reproducibility of the T_2 fit. Cultured genetically engineered astrocytoma cells[2] were trypsinized, washed in D-PBS, and centrifuged at 400×g at 5°C. For the different gadolinium (Gd) concentrations (0.1 and 0.2mM), the cells were washed in gadopentetate dimeglumine (Berlex, Montville, NJ) diluted with D-PBS. Each sample type (cells or agarose) was transferred to a dual-open-ended 5mm NMR tube with susceptibility matched plugs (New Era, Vineland, NJ). Each agar sample was placed on ice and allowed to gel for at least 2 hours prior to being analyzed. **NMR MEASUREMENTS:** All NMR experiments were performed at 10°C on a 500MHz Varian spectrometer. CPMG experiments were executed using 64 TEs linearly spaced between 10ms and 1270ms, TR=10s, NEX=2. **DATA ANALYSIS:** The water peak integrals were measured at 4.9ppm using ACD (Toronto, Canada) and were used to construct T_2 decay curves. The resulting exponentials were analyzed using the Non-Negative Least Squares (NNLS) [3,4] algorithm in MATLAB (MathWorks, Natick, MA). The general fit equation was

$$S/S_0 = \sum_{a=1}^{n} f_a \exp(-TE/T2_a)$$

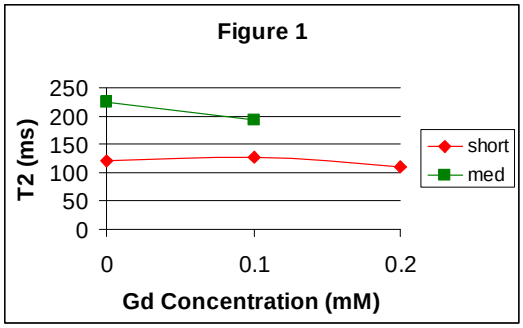
where S/S_0 is the normalized signal intensity, T_2 is the transverse relaxation, f_a is the fraction of signal associated with a particular T_2 assuming no exchange, TE is the echo time, and n is the number of components, which was one of the fit parameters for NNLS. Components that were too small (e.g.<25ms) to fit accurately or that were assigned to the maximum T_2 value (6000ms) were assumed to be noise.

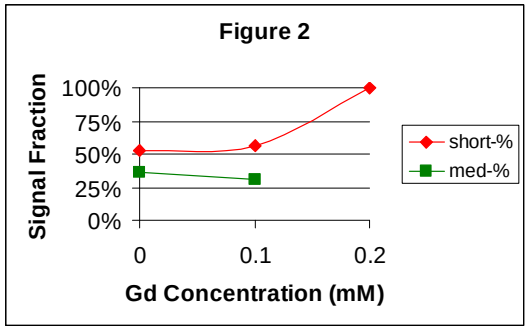
Table 1

Sample Type	short	short-%	med	med-%	long	long-%
1.5%			386	64%	2640	6%
1.5%			358	54%	1352	8%
1.5%			310	60%	1134	12%
2.0%			264	65%		
2.0%			279	81%	1061	9%
2.0%			266	87%	1235	8%
2.5%	179	9%	313	63%		
2.5%			224	61%	1517	7%
2.5%			264	56%		
3.0%			212	56%	1230	5%
3.0%			194	63%	1514	7%
3.0%			202	55%	837	5%

Results and Discussion

PURE AGAR: One or two components were identified for each sample of agarose and were grouped into three categories according to their T_2 values. The long T_2 value had higher coefficients of variance (CVs) than the medium T_2 value for the 1.5% (0.48 vs. 0.11) and 3.0% (0.29 vs. 0.04) samples. CVs were not calculated for 2.0% or 2.5% samples, because the long component had missing values. We interpreted both the higher variability and missing values as evidence that the long component was not reliably measurable. Therefore, we chose the maximum signal contribution of the long component (12%) as our threshold of measurability. Using that threshold we found only one component (Table 1: "med") associated with agarose, and it decreased significantly with an increase in agar concentration ($p<0.001$) as would be expected.





CELLS AND GADOLINIUM: Using our threshold, only 2 of the 3 components from our previous cell density study ("med" and "short") were fit for packed cell samples washed in D-PBS with no contrast agent or with 0.1mM Gd (Figures 1 and 2). For 0.2mM, only the short component was fit. The "med" T_2 (Figure 1) decreased with increasing Gd concentration and was not fit for the maximum Gd concentration. Additionally, the "med" T_2 signal fraction (Figure 2) also decreased with the addition of Gd. Taken together, these results suggest that the "med" component was exposed to the contrast agent in the extracellular compartment. The short T_2 value (Figure 1) was stable (119±8ms) amongst all concentrations of Gd. The signal fraction increases slightly with the addition of Gd and is responsible for the entire signal at 0.2mM. These results suggest that the short component was not exposed to the Gd in the extracellular compartment.

Conclusions

Using a threshold of 12% signal fraction from NNLS, we can reproducibly measure one component from agarose and two from cells. The longer ("med") component from the cells appears to interact with the extracellular compartment. This study shows the utility of identifying cellular compartments with unconstrained algorithms such as NNLS but underscores the necessity of determining thresholds of acceptance of the indentified components.

Acknowledgements: The authors would like to thank Dr. John Kurhanewicz and his lab for assistance with the data acquisition. This work was supported by a supplement to R01CA116041.

References

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