Proton Relaxation Rates of Water in Brain and Brain Tumors

Abstract. *The distribution of relaxation rates of water in "normal" (autopsy) samples of canine and human brain shows considerable overlap with that for brain tumor samples. The following ranges of values for the spin-spin relaxation rates were observed: for normal brain gray matter, 8.6 to 11.3 sec^{-1} (mean, 9.5 sec^{-1}); for normal brain white matter, 13.3 to 15.7 sec^{-1} (mean, 15.5 sec^{-1}); for six types of malignant tumor, 4.8 to 13.4 sec^{-1} (mean, 9.3 sec^{-1}); for five types of benign tumor, 7.1 to 16.4 sec^{-1} (mean, 11.5 sec^{-1}). Spin-lattice relaxation rates showed a similar pattern. At least two nonexchanging water components with different relaxation rates were indicated by the analysis of the spin-spin relaxation measurements for the white matter samples.*

Damadian (*1*), Weisman *et al.* (*2*), Chang *et al.* (*3*), and Carver (*4*) have suggested that normal and neoplastic tissue may be distinguished by differences in the proton relaxation times of cell water measured by nuclear magnetic resonance (NMR) techniques. The longer proton relaxation times for malignant tumors, compared to those for normal tissue and for benign tumors, were attributed to the "lower degree of organization" and "decreased water structure" in the malignant cell (*1*). Longer proton relaxation times have also been measured in samples of normal tissue from young animals (*4, 5*), particularly in tissue types where the cells are expected to proliferate more rapidly than they would in mature animals (*4*). An assessment of the information about cell structure that can be revealed by such NMR studies and of the applicability of these techniques to clinical situations requires proton relaxation times for a wide variety of tissue and tumor types. If the tissue is highly heterogeneous or an appreciable fraction of tissue water is extracellular, the interpretation of relaxation time data may not be straightforward. Proton relaxation time studies of brain tissue water are complicated by both of these factors and thus might provide a test of whether NMR data can be used to distinguish between normal and neoplastic tissue. We report here preliminary results of NMR studies of proton relaxation times for brain cortex and brain tumors.

Proton relaxation rates, R_1 (the inverse of the spin-lattice relaxation time, T_1) and R_2 (the inverse of the spin-spin relaxation time, T_2), were measured at 60 Mhz (*6*) for tissue samples taken from brain tumors and normal brain. Measurements of R_2 were carried out with the Carr-Purcell pulse sequence, as modified by Meiboom and Gill (*7*). The triplet pulse sequence, $(\pi/2,\Delta t,\pi,\Delta t,\pi/2,t)_n$, was used to determine R_1 (*8*). The average precision of a relaxation rate measurement was better than 3 percent for a specimen. In order to determine whether several nonexchanging water components with different relaxation rates were present, the Carr-Purcell data were analyzed as follows (*9*). For the case of two components, the magnetization decay curve, $y(t)$, has the form

$$y(t) = C\{x_a \exp[-R_{(2)(a)}t] + x_b \exp[-R_{(2)(b)}t]\}$$

x_a and x_b are the fractions of components A and B, respectively, and $R_{(2)(a)}$, $R_{(2)(b)}$ are the relaxation rates of components A and B. A least squares fit of ln $y(t)$ to the quadratic equation $f(t) = B_0 + B_1 t + B_2 t^2$ gives the following expressions for the coefficients:

$$B_0 = \ln C$$

$$B_1 = x_a R_{(2)(a)} + x_b R_{(2)(b)} = \bar{R}_2$$

$$B_2 = \frac{[R_{(2)(b)} - R_{(2)(a)}]^2}{2} x_a x_b$$

Thus, if only a single water component is present, the coefficient B_2 is zero within the appropriate error limits (*10*); if two components are present, B_2 is nonzero and B_1 is the mean relaxation rate, $\bar{R}_2$.

Samples were prepared as follows: Tumor samples were taken during surgery and placed in a closed vial on ice; the usual procedures for obtaining pathological samples were employed except that saline solution and fixative were not used. The autopsy samples of human cortex were taken from the right frontal lobe before the brain was treated with preservative. For NMR measurements, cylindrical specimens (3 to 5 mm long and 3 mm in diameter) were dissected from the tumor and autopsy samples, blotted dry, and enclosed in a thin-walled Pyrex tube. These specimens were kept on ice except for the time required for samples to reach ambient temperature (32°C) before each measurement and the time required for the measurement. The time between death and the first NMR measurement was less than 36 hours for each autopsy sample and less than 24 hours for three samples. The NMR measurements of specimens from the tumor samples were made on the same day as the surgical removal of the tumor and on subsequent days. Changes in relaxation rates during a 48-hour period were less than the standard deviation of the NMR measurement for each specimen.

In order to establish that the autopsy samples were a valid control, the following experiments were carried out: An 18-kg male dog was anesthetized with sodium pentobarbital; "surgical" brain samples were dissected from the left cerebral hemisphere and placed in glass vials on ice. The dog was then killed with an overdose of anesthesia, and the incision was closed. The carcass was refrigerated overnight (*11*), and "autopsy" samples were taken in the same way from the opposite hemisphere. (Samples were not taken from edematous regions.) The relaxation

Table 1. Proton relaxation rates for brain tumors.

Tumor No.	Pathological diagnosis	Specimens (No.)	R_1 (sec^{-1})	R_2 (sec^{-1})	B_2 (sec^{-2})
4	Mild reactive gliosis	3		16.4 ± 0.2	12.5
9	Neurofibroma	2	2.0 ± 0.6	14.0 ± 0.6	43.9
10	Glioblastoma multiforme or astrocytoma grade 3 or 4*	2	2.0 ± 0.2	13.4 ± 0.2	37.0†
5	Glioblastoma multiforme*	3		10.3 ± 0.2	14.8†
1	Ependymona	3		10.1 ± 0.1	6.9†
6	Glioblastoma multiforme*	3		9.9 ± 0.1	3.5
2	Transitional meningioma of the olfactory groove	2		9.9 ± 0.1	1.5
8	Malignant mixed glioma*	2	1.7 ± 0.1	9.0 ± 0.1	9.5†
7	Malignant mixed glioma*	2	1.4 ± 0.7	9.0 ± 0.1	4.0†
11	Astrocytoma grade 1 or 2	2	1.2 ± 0.1	7.1 ± 0.3	6.8
3	C_2 Chordoma	2		4.75 ± 0.06	4.0†
	Mean for malignant tumors		1.7	10.4	12.6
	Mean for benign tumors		1.9	10.4	15.7

* Classified as malignant by histological criteria. † Significant at the 95 percent level or better, on the basis of *F*-tests of the variance.

rates for 15 surgical and 15 autopsy brain cortex (gray matter) specimens were measured. In order to determine the average relaxation rate, R_2, for a group of specimens, the data pooled from a type of specimen (either surgical or autopsy) measured on a particular day were fitted to a quadratic regression equation as a function of time. The linear coefficient obtained from this equation gives R_2 for the specimens. The values of R_2 follow: (i) 10.9 ± 1.0 sec^{-1} for surgical specimens on the day of surgery; (ii) 11.3 ± 0.9 sec^{-1} for surgical specimens 1 day after surgery; (iii) 11.3 ± 0.9 sec^{-1} for autopsy samples 1 day after surgery; (iv) 11.8 ± 1.0 sec^{-1} for autopsy samples 2 days after surgery. The error limits reflect a variation in relaxation rates for samples taken from different brain areas. No pattern in the distribution of relaxation rates for different brain areas could be discerned except that relaxation rates for white matter (which data are not given) were 30 to 40 percent greater than those for gray matter. Since the difference between relaxation rates of the same type of specimen measured on different days and the difference between surgical and autopsy specimens measured on the same day is less than the differences due to brain area, we can conclude that autopsy samples provide a useful (although not necessarily ideal) control for these studies.

There are large differences between values of R_2 for gray and white matter samples and nonzero values of B_2 for the white matter samples. Relaxation rate measurements of specimens from human autopsy samples gave the following results: (i) for ten gray matter samples, the average $R_1 = 2.3$ sec^{-1} (range: 1.8 to 2.7 sec^{-1}) and the average $R_2 = 9.5$ sec^{-1} (range: 8.6 to 11.3 sec^{-1}); (ii) for seven white matter samples, the average $R_2 = 15.5$ sec^{-1} (range: 13.3 to 15.7 sec^{-1}). Values for R_2 were obtained from the linear coefficients, B_1, in the quadratic regression equation of the Carr-Purcell decay curve. The values of the quadratic coefficients, B_2, obtained from this analysis follow: (i) for ten gray matter samples, the average $B_2 = 4.5$ sec^{-2} (range: 0.0 to 13.7 sec^{-2}) and for seven white matter samples, the average $B_2 = 20.3$ sec^{-2} (range: 18.3 to 22.8 sec^{-2}). All of the values of B_2 for white matter samples are statistically significant at the 95 percent confidence level, on the basis of F-tests of the variance; only four of the gray matter samples have B_2 values (13.7, 5.1, 4.3, and 4.2) that were significant at this level.

The relaxation rates for the tumor samples are given in Table 1. Tumor type was determined by pathological analysis. The range of patient ages for the tumor samples was 2.5 months to 35 years; for the group of autopsy samples, the range of patient ages was 48 to 84 years. No relation between age and relaxation rate was apparent for the autopsy samples. A comparison of the autopsy and tumor data indicates that the ranges of relaxation rates for the tumor samples are greater, that the distributions of autopsy and tumor relaxation rates overlap considerably, and that the distributions of relaxation rates for the tumor samples are displaced toward smaller values, that is, longer relaxation times. There is little correlation between the degree of tumor malignancy and the relaxation rate. The relaxation rates for two benign tumors (3 and 11) are much smaller than the smallest of those for the autopsy samples; six malignant tumor samples (1, 5, 6, 7, 8, and 10) have relaxation rates within the range for gray and white matter autopsy samples; two benign tumors (4 and 9) have relaxation rates similar to those of white matter autopsy samples. The most consistent difference between the benign and malignant tumor samples is the value of the quadratic coefficient, B_2; all but one of the values of B_2 for the malignant tumor samples are statistically significant at the 95 percent confidence level on the basis of F-tests of the variance, while only one of the benign tumors (3) showed a statistically significant value for B_2.

The relaxation rates and B_2 values for the white matter autopsy samples are the most unambiguous results of these experiments. The B_2 values indicate that at least two nonexchanging water components may be present in white matter. It may be that since the proportion of phospholipid is higher in white matter than in gray matter, the higher relaxation rates are due to adsorption of water on the polar groups of the phospholipids. The small values of B_2 for tumors 3 and 11 may not be entirely consistent with the small relaxation rates of these tumors being due to extracellular, nonexchangeable water. The difference between the relaxation rates of normal and neoplastic tissue found in this work is much less than that reported in earlier studies (*1–3*). The greater heterogeneity of brain tissue and "clinical" tumors, compared to normal and neoplastic tissue samples from laboratory animals and tissue cultures, may be a factor here. However, some caution in the interpretation of relaxation time results, either as an empirically justified clinical tool for the detection of malignant tumors or as a probe of cell structure in complex tissues, is clearly required.

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References and Notes

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6. A Bruker B-KR 321s pulsed spectrometer was used. The spectrometer was modified for field-frequency controlled operation. Details of this modification will appear elsewhere. We acknowledge the loan of a radio-frequency power amplifier from Bruker Magnetics, Inc.
7. S. Meiboom and D. Gill, *Rev. Sci. Instrum.* **29**, 688 (1958). The echo envelope was observed, as a function of π-pulse spacing, for a period of at least one relaxation time, and, in several cases where the signal-to-noise ratio was sufficiently large, for more than two relaxation times.
8. The triplet sequence, described in the Bruker Pulsed Spectrometer manual, was used; the data was analyzed (R.J.K. and R.G.P.) to account for spin-spin relaxation which occurred while the magnetization was in the (XY) observation plane. Description of this method of determining R_1, which gave results for test samples in agreement with the Carr-Purcell 180°-90° method, is in preparation. Also see R. L. Streever and J. Y. Carr, *Phys. Rev.* **121**, 20 (1961).
9. Although several methods exist for the analysis of superimposed exponential decays [for example, Prony's method as outlined by C. W. Clenshaw, in *Numerical Approximation to Functions and Data*, J. G. Hayes, Ed. (Athlone, London, 1970), pp. 10–11], they all require very high signal-to-noise ratios in order to extend the measurement time to the period of several relaxation times.
10. In order to determine the statistical error limits for the coefficient B_2 independently of those for the linear coefficient B_1, a quadratic regression equation in polynomials orthogonal over the time interval of interest was used; the coefficients for the regression in these Tschebyscheff polynomials were transformed to the coefficients B_0, B_1, and B_2. See, for example, R. L. Anderson and T. A. Bancroft, *Statistical Theory in Research* (McGraw-Hill, New York, 1952), chap. 16.
11. Refrigeration was 2° to 4°C; the temperature of the morgue from which autopsy samples were obtained varied from 0° to 6°C.
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