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Nuclear Magnetic Resonance Studies of Several Experimental and Human Malignant Tumors

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SUMMARY

Several animal and human malignant tumors were examined by nuclear magnetic resonance spectrometry. When compared with normal tissue, the spin-lattice (T_1) magnetic relaxation times were longer for all tumors except one human sample. Comparable results were obtained for T_1 measurements of cultured tumor cells. Possible mechanisms for this effect are reviewed briefly and its potential in cancer diagnosis is discussed.

INTRODUCTION

Measurements of spin-lattice (T_1) and spin-spin (T_2) magnetic relaxation times of the protons of tissue water were reported by Damadian (4) and subsequently by others (7, 10, 15) to discriminate between normal and malignant tissue. The relaxation times T_1 and T_2 are time constants that describe the exponential return to equilibrium of the nuclear magnetism of the water protons in directions parallel or perpendicular, respectively, to the applied magnetic field (12).

When compared to normal tissues, malignant tumors have longer relaxation times. The physical nature of this phenomenon and its biological significance are poorly understood.

This report presents spin-lattice (T_1) relaxation times for 9 mouse tumors, 4 human malignant tumors, and assorted normal tissues. It is the most extensive study to date of animal experimental tumors. Measurements were also obtained for malignant cells maintained in culture. Possible clinical applications of this phenomenon and its underlying physical mechanism are discussed.

MATERIALS AND METHODS

Eight mouse tumors obtained from The Jackson Laboratory, Bar Harbour, Maine, and a methylcholanthrene-induced fibrosarcoma produced in this laboratory were studied. All were transplantable solid tumors produced by s.c. trocar injection. Control tissues were obtained from normal animals of each of the mouse strains used. Four human malignant tumors, 3 with normal tissue controls, were obtained from the Division of Surgical Pathology. They include an adenocarcinoma of the lung, a squamous carcinoma of the renal pelvis, an adenocarcinoma of the colon, and stromatosis involving pelvic soft tissue. Samples of these tissues were excised from the surgically resected specimens and gently tamped into cellulose nitrate sample tubes.

Four mouse tumors, maintained in standard culture media (minimal essential medium, 20% fetal calf sera, antibiotics, North American Biologicals, Winnipeg, Manitoba, Canada), were also examined. Control tissues were not available.

Specimens were packed into Beckman cellulose nitrate centrifuge tubes 1/6 inch in diameter and 1-3/8 inch long for T_1 measurement. Spin-lattice (T_1) magnetic relaxation times were measured using the null method (2) on a pulsed NMR[4] spectrometer operating at 24 MHz. Replicate measurements were made as indicated in the tables. On a single specimen the T_1 values were reproducible to about ±5%. Readings were made at ambient temperature.

Measurements were unaffected when tissues were kept in airtight plastic tubes at room temperature for up to 12 hr. In instances when measurements were not made immediately after excision, the samples were kept in airtight tubes on ice for less than 4 hr. Samples kept cold in this way before measurement were allowed to equilibrate to room temperature before being placed in the spectrometer. Relaxation times did not vary with sample packing or orientation of the sample in the probe.

Representative samples of all tumors and the human control tissues were fixed in 10% formalin and imbedded in paraffin. Hematoxylin- and eosin-stained sections, cut at 6 μm were examined by light microscopy.

RESULTS

T_1 relaxation times for the transplantable mouse tumors and control tissues are presented in Tables 1 to 3. The T_1's

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[4] The abbreviation used is: NMR, nuclear magnetic resonance.

Table 1

Spin-lattice relaxation times for transplantable mouse tumors

From each of 3 mice, as many as 3 different samples of each tumor type were measured, depending on the amount of tumor tissue available.

Tumor type	Mouse strain	Jackson Laboratories Code no.	T_1 (msec) Mouse I Sample no. 1	Mouse I 2	Mouse I 3	Mouse II 1	Mouse II 2	Mouse II 3	Mouse III 1	Mouse III 2	Mouse III 3
Adenocarcinoma	C57BL/6J	BW10232	822	815	808	791	772	808	805	778	787
Anaplastic carcinoma	A/J	15091 A	670	673	661	637			662	654	
Fibrosarcoma	C57BL/6J	Our laboratory	621	625	640		596	605	731	725	749
Lymphosarcoma	C3H/HEJ	6C3HED	803	815	847						
Melanoma	C57BL/6J	B16	695	690	741	746	730	760	660	681	668
Prepuptial gland tumor	C57BL/6J	ESR586	758			675					
Rhabdomyosarcoma	CE/J	BW10139	705	702	693	720	693	679	738	745	749
Round cell tumor	A/J	C1300	593	635	696	681	633	627			
Spindle cell carcinoma	A/J	Sal	681	628	649	681	660	682	687	685	693

Table 2

Spin-lattice relaxation times for normal mouse tissue, strain C57BL/6J

Two samples of liver and 1 sample each of 4 other tissues are presented.

Mouse	T_1 (msec) Liver	Kidney	Spleen	Skeletal muscle	Brain
1	292, 291	351	444	454	483
2	229, 238	284	333	386	465
3	259, 261	286	312	378	456
4	251, 269	284	337	352	480
5	246, 252	284	316	396	461
6	285, 277	242	416	415	478
7	239, 252	259		358	513
8	295, 307	319	338	448	506
9	287, 287	318	368	464	470
10	301, 301	316	432	454	520
Av.	271 ± 24	294 ± 30	366 ± 48	411 ± 40	483 ± 22

Table 3

Spin-lattice relaxation times for normal mouse tissue

Tissue type	T_1 (msec) C57BL/6J mouse	A/J mouse	CE/J mouse
Brain	526	508	518
Lung	491	500	482
Heart	490	476	482
Liver	263	283	296
Spleen	458	433	399
Kidney	370	314	286
Skeletal muscle	471	415	372
Small intestine	255		357
Tail	218	207	216
Skin	199	186	194
Ribs and muscle	421		390
Testis			205

ranged from 593 to 847 msec for the 9 tumor types studied and from 186 to 526 msec. for the various control tissues. For the tumor data (Table 1), as many as 3 different samples of each tumor type from each of 3 mice were measured, except in cases where the tumor size was too small to provide 3 specimens. In Table 2, a study of 10 control mice of the C57BL/6J strain showed that for 60 normal tissue samples no control T_1 overlapped with any tumor T_1. In Table 3, it is shown that individual normal specimens from other mouse strains have values comparable to those for the C57BL/6J mice. The data seem sufficient to support the principal conclusion that the T_1 of the various normal mouse tissues examined do not overlap with the values for any of the tumors examined. The difference between relaxation times of normal and malignant tissues holds on a statistical as well as an individual basis. For comparison, T_1 values for 4 mouse tumors grown in tissue culture were obtained as shown in Table 4. That the phenomenon of T_1 elevation for tumors is a characteristic of the cancer cell and not merely a function of cell packing or extracellular material is suggested by the observation (Table 4) of elevated T_1 values for malignant cells maintained in tissue culture. Control cultured cells were not available. The T_1 values observed for the cultured tumor cells are above the range observed for normal tissues and are comparable to the corresponding *in vivo* tumors.

A spleen from a lymphosarcoma-bearing mouse, grossly thought to be normal, registered a high T_1 of 612 msec. Subsequent microscopic examination revealed partial involvement of the splenic tissue by lymphosarcoma cells as well as scattered large bizarre cells interpreted as atypical megakaryocytes.

Histological examination of representative samples of the 9 mouse tumors used in this study was performed in an attempt to correlate T_1 values with morphological features. The percentage of each tissue sample uninvolved by tumor was noted, as was the extent of necrosis within each tumor. In addition, semiquantitative comparisons of cell size, nuclear-cytoplasmic ratio and number of mitotic figures were made, as well as a subjective determination of the degree of cytoplasmic differentiation of each tumor.

None of the features evaluated could be correlated with the degree of elevation of the T_1 values. However, the T_1 determinations and the morphological evaluations for each tumor were performed on adjacent samples of tissue, not on

Table 4
Spin-lattice relaxation times of mouse tumors maintained in standard culture media

Cultured tissue sample (pellet)	T_1 (msec)		
	Mouse	Code no.	T_1 (msec)
Hepatoma	C57L/J	BW7756	600
Melanoma	C57BL/6J	B16	620
Fibrosarcoma	C57BL/6J	Our laboratory	619
Anaplastic carcinoma	A/J	15091 A	697

the same piece of tissue. Furthermore, all of the tumors demonstrated a poor or at best only a moderate degree of cytoplasmic differentiation and represented a wide variety of presumed cells of origin. Thus the possibility remains that within a group of similar tumors the degree of elevation of T_1 values may reflect variations in these or similar morphological features and/or in the biological properties of the tumors, as was found for 2 Morris hepatomas which we examined earlier (10).

Four human malignant tumors were studied (Table 5). Three of these cancers had T_1 values clearly elevated above the normal range. The lung adenocarcinoma sample could not be discriminated from adjacent uninvolved lung.

Histologically, the control lung showed focal atelectasis and inflammation, anthracosis, numerous hemosiderin-laden macrophages, and a small, partially calcified granuloma. Although the tumor sample showed a noteworthy amount of fibrous tissue, there was abundant poorly differentiated adenocarcinoma in the tissue.

DISCUSSION

Cancer Diagnosis. These findings confirm earlier reports of elevated relaxation times for water in malignant tissues of small animals (4, 7, 10). Earlier work has dealt with a total of 5 types of animal malignant tumors. In the present study T_1 measurements on 9 additional murine tumors are reported as well as data on a more extensive selection of normal tissues than have been reported previously. The results confirm and extend the earlier studies and support the suspicion that this phenomenon is general for malignant tissues at least in small animals. It was suggested by Damadian (4) that this phenomenon might be useful as a cancer diagnostic tool and it was our interest in this aspect which prompted the present study. From Tables 1 to 3, it is evident that although a range of T_1 values is observed for both normal and malignant tissues, there is no overlap between any normal tissue and any of the 9 malignant specimens studied. This further validates the possibility of diagnostic use of NMR measurements.

The results on the 4 human cancers which we have examined suggest that ambiguities may be encountered in some human tumors. In contrast to the other cancers examined, the adenocarcinoma of the lung was not significantly different in its T_1 value from adjacent uninvolved lung. Damadian *et al.* (5) recently reported mean values of T_1 for several types of human cancers and their host tissues. They reported mean values for carcinomas of the lung of 1.165 sec compared to 0.776 sec for normal lung. Details of the individual measurements are not available to us at present. It may be significant, however, that the uninvolved host lung that we examined could not be considered as normal and showed focal atelectasis and inflammation as well as other abnormalities as described in "Results." This raises the possibility that abnormal states other than cancer might produce elevated T_1 values, an eventuality which could have profound consequences in any serious consideration of NMR as a diagnostic tool.

It would be premature at this point to compare NMR with alternative methods of cancer detection such as radiography or thermography because to date NMR has been studied only in situations in which there is very obvious difference between malignant tumors and normal tissues. [Two exceptions are a single benign rat tumor (4) and a precancerous nodule in murine mammary glands (7). Our observation on the uninvolved lung reported here is a 3rd example.] This is not a rigorous test for any cancer detection method and until NMR has been tested in a variety of abnormal but nonmalignant conditions such comparisons are not meaningful. It might be of value, however, to lay down certain criteria which NMR must be shown to fulfill if it is to replace the currently most reliable method of cancer detection, namely, microscopic examination of resected tissue. Using frozen section this can be done in minutes with very high accuracy. For replacement of frozen section, NMR would need to be more reliable, more economical, or easier to use. Interpretation of histological material requires considerable training and experience and NMR may have a potential advantage in this sense. Avoidance by NMR studies of surgical removal of tissue from the patient would constitute a clear advantage. Possible elimination of sampling error which is present in frozen section could theoretically be eliminated if NMR were sufficiently sensitive to detect small amounts of tumor imbedded in uninvolved tissue. NMR might also have an advantage if it proved capable of predicting the behavior of certain tumors, *e.g.*, hemangiopericytomas, the malignant properties, of which *i.e.*, metastasis, cannot be accurately predicted morphologically.

Our experience with a grossly normal mouse spleen that registered a high T_1 and proved microscopically to contain malignant cells is of interest here. In all other cases

Table 5
Spin-lattice relaxation times of human normal and malignant tissue
The data refer to specimens obtained from 4 different patients.

Human tissue type	T_1 (msec)	
	Sample I	Sample II
Lung		
Adenocarcinoma	428	412
Normal	395	412
Kidney		
Squamous carcinoma	685	591
Normal	464	454
Colon		
Adenocarcinoma	535	570
Normal	475	
Stromatosis	580	552

described here and in the literature, the comparison has been between obviously normal and obviously malignant specimens. If NMR is to prove practical in diagnosis its ability to detect malignant cells admixed with normal cells is important. The use of our very simple null method gives a weighted average T_1 for all types of tissues present in the specimen (15). Separate T_1's for normal tissue and melanoma were observed in a mouse tail by Weisman *et al.* (15) using the more time-consuming partial relaxation technique. This experiment differs from ours in that the melanoma was evident by visual inspection.

Several avenues may be open to the improvement of the sensitivity of NMR to malignant tissue. The observed relaxation times are frequency dependent according to preliminary observations in this laboratory and they may be temperature sensitive as well. The frequency dependence is expected when the observed relaxation rate is determined by Equation 1, *i.e.*, by T_m, rather than by the lifetime of the water protons in the rapidly relaxing phase (11, 12). Optimization of these factors may improve the sensitivity. Use of Fourier transform techniques and determination of T_1 by the technique of partial relaxation would also be of considerable advantage in this respect.

Relaxation Mechanism. Earlier workers assumed that the shorter relaxation times of cell water relative to distilled water are the result of restricted molecular motion of the cell water caused by its binding to membranes and macromolecules (1, 3, 8, 9). Evidence purported to support the idea of ordered water as the major cause of enhanced relaxation rates of cell water protons has been indirect in the sense that efforts were made to eliminate other possible relaxation mechanisms such as (*a*) interaction of water with paramagnetic centers, (*b*) the effect of magnetic anisotropy of aromatic groups of DNA and other macromolecules, (*c*) magnetic field inhomogeneities caused by small liquid compartments separated by membranous walls. Cope (3) attempted to eliminate the possibility of interaction with paramagnetic ion by demonstrating that while 0.07 M Mn^{+2} in distilled water was required to produce the T_1 value observed in the major phase of muscle water only about 10^{-4} M total of Mn^{+2}, Cu^{+2}, and Fe^{+3} are present in muscle. This analysis is incomplete because it fails to consider motional effects. The relaxation rate of a proton in a water molecule bound in the 1st coordination sphere of a paramagnetic ion is given by the Solomon-Bloembergen equation:

$$\frac{1}{T_{1m}} = \frac{2}{15}\frac{S(S+1)\gamma_1^2 g^2 \beta^2}{r^6}\left(\frac{3\tau_c}{1+\omega_1^2\tau_c^2} + \frac{7\tau_c}{1+\omega_S^2\tau_c^2}\right) + \frac{2}{3}\frac{S(S+1)A^2}{\hbar^2}\left(\frac{\tau_e}{1+\omega_S^2\tau_e^2}\right)$$

where S is the electron spin quantum number; γ_1 is the nuclear magnetogyric ration; r is the ion-proton internuclear distance; g is electronic "g" factor; β is the Bohr magneton; ω_1 and ω_S are the Larmor angular precession frequency for the nuclear and electron spins, respectively; A is the hyperfine coupling constant, and τ_e is the correlation time for the scalar interaction. The 1st term in Equation 1 represents the dipolar interaction while the 2nd term represents the contribution from scalar coupling between the paramagnetic ion and the protons of a water molecule in the 1st coordination sphere. In studies involving Mn^{+2} bound to enzymes for example the 2nd term has usually been neglected since τ_e is sufficiently long that $\omega_S^2\,\tau_c^2 \ll 1$. The relevant correlation time τ_c for the dipolar interaction of the cell water with the Mn^{+2} ion may well be far greater than that of free Mn^{+2} with water because of the binding of the Mn^{+2} to macromolecules or to membranes resulting in much more efficient relaxation. Since

$$1/\tau_c = 1/\tau_r + 1/\tau_S + 1/\tau_m$$

where τ_r, τ_s, and τ_m are correlation times for rotation, electron spin-lattice relaxation and chemical exchange, respectively, any one of these may dominate the relaxation rate or any or all may contribute significantly. Furthermore, any one or all of these correlation times could change when the Mn^{+2} is bound to a macromolecule or membrane. Furthermore, there is no guarantee that the scalar coupling term will be negligible for Mn^{+2} as it occurs in the cell. A detailed discussion of these well-known effects is given in "References (12, 14).

Hazelwood *et al.* (8) have concluded that the irreversible narrowing of the NMR signal of muscle water upon heating sufficient to denature the proteins eliminates the possibility of a significant contribution of paramagnetic ions to the relaxation rate. This argument has the shortcoming that any relaxation mechanism requiring the participation of native proteins or intact membranes could be distrupted by denaturing the protein. This would include the binding of paramagnetic ions to proteins or membranes. Failure by these workers to observe relaxation due to soluble paramagnetic ions in an extraction of such denatured muscle by D_2O can be interpreted as being caused by occlusion of a portion of the bound ions with the denatured protein and the deenhancement of the relaxation rate when the Mn^{+2} is unbound. In a recent study, Outhred and George (13) concluded that relaxation by paramagnetic centers could not be excluded by a study of the frequency dependence of the relaxation times of muscle water. In fact, these workers conclude from a comparison of D_2O and H_2O relaxation at the same frequency that a significant contribution from causes other than spin-diffusion, translational restrictions and paramagnetic ions have a major role in water proton relaxation in muscle but that any or all of these 3 factors may also play a significant role.

Cope (3) also states that relaxation caused by magnetic anisotropy of aromatic groups in DNA or other macromolecules can be eliminated since they cannot affect T_1. However, he considered only static anisotropy, whereas magnetic anisotropy coupled with molecular motion is well known as a T_1 relaxation mechanism (6).

No attempts appear to have been made to eliminate wall effects caused by compartmentation of cellular water. Hazlewood *et al.* (9) have stated the opinion that both ordered water and compartmentation effects are operative.

It thus appears fair to conclude that none of the relaxation mechanisms so far considered in the literature have been ruled out and that the observed increase in relaxation rates in malignant tissues as compared to normal tissues could be caused by changes in any or all of the possible factors considered so far.

Assuming that a different degree of water ordering between normal and malignant tissue does make a significant contribution to the change in T_1, the question arises as to what fraction of the cell water is involved and how great is the change in motional freedom of this fraction. Cope (3) has claimed that low values of NMR relaxation times in tissue water indicate "crystallinity," and Damadian (4) has suggested that "dedifferention and anaplasia commonly equated with neoplasia may be associated with profound changes in endosolvent structure." Neither of these workers attempted any quantitiative analysis of the degree of modification which the data might imply. Outhred and George (13), however, have recently shown that not more than 2% of the NMR-visible protons in toad muscle water must have correlation times more than 100 times larger than normal water (*i.e.*, $\geq 10^{-9}$ sec) to account for the T_1 and T_2 values found for muscle. These authors assumed that the water relaxation was dominated by proton-proton dipolar interactions. Should other factors, as discussed above, also contribute significantly to the relaxation rate, the calculated fraction of motionally hindered protons could only diminish (13). Thus a relatively minor change in a small fraction of the water could account for the NMR results.

Regardless of what mechanism or mechanisms are responsible for the enhanced relaxation rate of some fraction of the cell water protons, the influx of additional water as in edema would increase the relaxation rate provided that the additional water protons were in rapid exchange with the rapidly relaxing fraction. Only Weisman *et al.* (15) seem to have considered this possibility, and, to date, apparently no effort has been made to assess its importance.

We conclude that the present state of knowledge about the relaxation mechanism or mechanisms of the protons of cell water is not sufficient to eliminate any of the possible mechanisms thus far suggested in the literature. As a corollary, the relatively small differences in relaxation rate between normal and malignant cells cannot presently be assigned with any certainty to a change related to any particular mechanism.

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