BRIEF COMMUNICATION

PROTON T_1 STUDY OF COVERAGE PARAMETER CHANGES IN TISSUES FROM TUMOR-BEARING MICE

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ABSTRACT Measurements of water proton spin-lattice relaxation time, T_1, at 20 and -15°C have been performed in spleen, kidney, liver, and muscle tissues from tumor-bearing mice, as well as in tumors grown in their dorsal subcutaneous tissues. All mice used were either from the C3H/HeJ or BALB/c strain. At -15°C the T_1's of tissues of a given type from tumor-bearing and healthy mice are essentially the same. It is shown that in spleen the increased T_1 from tumor-bearing mice can only be explained in terms of a large change in the water coverage parameter of macromolecules. In liver, muscle, and tumors the increased water content accounts for the changes in T_1, while kidney represents an intermediate case.

INTRODUCTION

It has been shown that there is an increase in the proton spin-lattice relaxation time, T_1, of water protons in spleen, kidney, and liver tissues from mice with transplanted tumors compared to similar tissues from mice without tumors (1–6). It also has been reported that in these tissues the relative amount of water is slightly increased (7–10). This observation may lead to the conclusion that the T_1 increase results solely from more water in tissue. Experiments on frozen tissues with tumors (11) have shown that in tumors the increased T_1 values are due to fewer water molecules in the hydration layer. In this communication we show quantitatively that, to explain the T_1 increase in the uninvolved spleen of mice with a large fast-growing tumor, a considerable change in the water coverage parameter of large molecules has to occur. In muscle and liver tissues such a change is not evident, while kidney tissue is an intermediate case.

RESULTS AND DISCUSSION

The double-blind experiments at room temperature were performed as reported previously (3). The animal-tumor models used were various transplanted and chemically

induced carcinomas growing in the dorsal subcutaneous tissues of 10–12-wk-old C3H/HeJ mice and the EMT6 fibrosarcoma growing in similar-aged BALB/c mice. The proton T_1 in the frozen tissues at $-15°C$ was measured with the same pulse sequence as at room temperature. Also, the magnetization readings were taken at the same 200-μs time window after the 90° excitation pulse. Since at $-15°C$ most water is frozen and the ice protons signal dephases in ~ 10 μs, only the nonfreezable water proton and some protons on large molecules contribute to the free induction decay at 200 μs. A quantitative analysis of the free induction decay amplitudes in natural and fully deuterated mouse muscle has shown that at $-15°C$ and at the 200-μs window, the protons on large molecules contribute 14 ± 6% to the signal and water protons 86%.

It should be noted that at $-15°$, the T_1's of all tissues of one type are essentially the same, although they may be significantly different at room temperature (Tables I–IV). This indicates that the average relaxation rate of protons of nonfreezable water is essentially the same in tissues of healthy mice and of mice with large, developed, fast-growing tumors. Since nonfreezable water and bonded water cannot be distinguished, the T_1 difference observed at room temperature (Tables I–III) could only result from a change in the relative amount of the free and bonded water. A similar conclusion has also been reached for muscle tissue with a tumor (11).

These observations could be explained quantitatively as follows. The water proton

TABLE I

SPLEEN SPIN RELAXATION RESULTS

	T_1^{-1} at $T = 20°C$	W	$(1-W)/W$	p_T/p_H	b_T/b_H	T_1^{-1} at $T = -15°C$
	s^{-1}					s^{-1}
BALB/c normal	2.00	0.733	0.364			18.0
(n = 7)	± 0.08	± 0.011				± 0.8
BALB/c + EMT6	1.60	0.760	0.316	0.69	0.60	16.0
(n = 12)	± 0.08	± 0.002				± 0.4
C3H/HeJ normal	2.00	0.738	0.355			15.0
(n = 17)	± 0.03	± 0.006				± 0.9
C3H/HeJ + BA	1.70	0.764	0.309	0.80	0.70	15.0
(n = 9)	± 0.12	± 0.003				± 0.6
C3H/HeJ + MCO	1.70	0.744	0.344	0.72	0.70	14.0
(n = 6)	± 0.07	± 0.011				± 0.5
C3H/HeJ + MCI	1.60	0.748	0.337	0.63	0.60	
(n = 5)	± 0.05	± 0.010				
C3H/HeJ + spontaneous mammary (n = 4)	2.00 ± 0.10	0.744 ± 0.011	0.344	1.03	1.00	17.0 ± 1.0
C3H/HeJ + C3HI	1.80	0.736	0.359	0.79	0.80	16.0
(n = 8)	± 0.10	± 0.016				± 0.4

$T_{1f}^{-1} = 1.0\,s^{-1}$. The fraction of water in the tissue, W, is given in grams H_2O per total tissue weight in grams. Each uncertainty shown represents the standard deviation. n is the sampling size. The subscripts H and T refer to healthy and tumor-bearing mice, respectively.

TABLE II

KIDNEY SPIN RELAXATION RESULTS

	T_1^{-1} at $T = 20°C$	W	$(1 - W)/W$	p_T/p_H	b_T/b_H	T_1^{-1} at $T = -15°C$
	s^{-1}					s^{-1}
BALB/c normal	2.10	0.731	0.368			17.0
($n = 7$)	± 0.05	± 0.006				± 0.8
BALB/c + EMT6	1.80	0.757	0.321	0.83	0.72	15.0
($n = 12$)	± 0.06	± 0.004				± 0.6
C3H/HeJ normal	2.20	0.737	0.357			17.0
($n = 17$)	± 0.05	± 0.004				± 0.7
C3H/HeJ + BA	2.00	0.755	0.325	0.92	0.84	16.0
($n = 9$)	± 0.06	± 0.008				± 0.7
C3H/HeJ + MCO	2.10	0.726	0.377	0.87	0.92	16.0
($n = 6$)	± 0.12	± 0.010				± 0.7
C3H/HeJ + MCI	2.00	0.757	0.321	0.93	0.84	16.0
($n = 5$)	± 0.06	± 0.005				± 0.8
C3H/HeJ + spontaneous mammary ($n = 4$)	2.20 ± 0.01	0.758 ± 0.006	0.319	1.12	1.00	16.0 ± 0.5
C3H/HeJ + C3HI	2.00	0.746	0.340	0.88	0.84	17.0
($n = 8$)	± 0.07	± 0.008				± 0.8

$T_{1f}^{-1} = 1.0 s^{-1}$. The fraction of water in the tissue, W, is given in grams H_2O per total tissue weight in grams. Each uncertainty shown represents the standard deviation. Abbreviations are as in Table I.

TABLE III

LIVER SPIN RELAXATION RESULTS

	T_1^{-1} at $T = 20°C$	W	$(1 - W)/W$	p_T/p_H	b_T/b_H	T_1^{-1} at $T = -15°C$
	s^{-1}					s^{-1}
BALB/c normal	3.00	0.652	0.534			18.0
($n = 7$)	± 0.10	± 0.004				± 0.4
BALB/c + EMT6	2.60	0.699	0.431	0.99	0.80	19.0
($n = 12$)	± 0.11	± 0.004				± 1.2
C3H/HeJ normal	3.30	0.658	0.520			18.0
($n = 17$)	± 0.10	± 0.004				± 0.5
C3H/HeJ + BA	2.90	0.679	0.473	0.91	0.83	17.0
($n = 9$)	± 0.17	± 0.011				± 0.6
C3H/HeJ + MCO	3.20	0.676	0.479	1.04	0.96	18.0
($n = 6$)	± 0.19	± 0.007				± 0.7
C3H/HeJ + MCI	3.20	0.687	0.456	1.09	0.96	17.0
($n = 5$)	± 0.13	± 0.009				± 0.7
C3H/HeJ + spontaneous mammary ($n = 4$)	3.10 ± 0.23	0.681 ±0.010	0.468	1.01	0.91	16.0 ± 0.7
C3H/HeJ + C3HI	3.10	0.676	0.479	0.99	0.91	19.0
($n = 8$)	± 0.17	± 0.007				± 0.7

$T_{1f}^{-1} = 1.0 s^{-1}$. The fraction of water in the tissue, W, is given in grams H_2O per total tissue weight in grams. Each uncertainty shown represents the standard deviation. Abbreviations are as in Table I.

TABLE IV
MUSCLE SPIN RELAXATION RESULTS

	T_1^{-1} at $T = 20°C$	W	$(1 - W)/W$	P_T/p_H	b_T/b_H	T_1^{-1} at $T = -15°C$
	s^{-1}					s^{-1}
BALB/c normal	1.80	0.714	0.401			17.0
(n = 7)	± 0.14	± 0.011				± 0.3
BALB/c + EMT6	1.70	0.741	0.350	1.00	0.87	15.0
(n = 12)	± 0.09	± 0.005				± 0.4
C3H/HeJ normal	1.60	0.714	0.401			14.0
(n = 17)	± 0.03	± 0.009				± 0.5
C3H/HeJ + BA	1.50	0.728	0.374	0.89	0.83	14.0
(n = 9)	± 0.01	± 0.005				± 0.8
C3H/HeJ + MCO	1.60	0.706	0.416	1.04	1.08	14.0
(n = 6)	± 0.17	± 0.009				± 0.3
C3H/HeJ + MCI	1.60	0.720	0.389	1.03	1.00	14.0
(n = 5)	± 0.04	± 0.005				± 0.7
C3H/HeJ + spontaneous mammary (n = 4)	1.60 ± 0.05	0.716 ± 0.014	0.397	1.01	1.00	14.0 ± 0.8
C3H/HeJ + C3HI	1.60	0.716	0.397	1.01	1.00	14.0
(n = 8)	± 0.04	± 0.004				± 0.3

T_{1f}^{-1} = 1.0 s^{-1}. The fraction of water in the tissue, W, is given in grams H_2O per total tissue weight in grams. Each uncertainty shown represents the standard deviation. Abbreviations and subscripts are as in Table 1.

spin-lattice relaxation can be described by the fast-exchange two-state (FETS) model (12–19)

$$T_1^{-1} = bT_{1b}^{-1} + (1 - b)T_{1f}^{-1}, \tag{1}$$

where the subscripts b and f refer to the bound and free state, respectively. Furthermore, the fraction of the bonded water, b, is proportional to the relative weight of large molecules (16):

$$b = p(1 - w)/w. \tag{2}$$

where w is the fraction of water in the tissue and p is a coverage parameter (16). The subscripts H and T (in tables) refer to healthy and tumor-bearing mice, respectively. The fraction of water measured in each tissue is indicated in the tables. If the order of magnitude of the quantities in Eq. 1 is taken to be $b \sim 0.1$, $T_{1b}^{-1} \sim 20$ s^{-1}, and $T_{1f}^{-1} \sim 0.75$ s^{-1}, it can be concluded that an increase in the amount of water of $\lesssim 3\%$, which will cause a variation of b by $\lesssim 10\%$, from 0.1 to 0.09, results in a T_1 increase of only $\lesssim 8\%$. It should be noted that the experimental T_1 increases for spleen are 16%. Thus, in spleen, within the framework of the FETS model, it has to be concluded that b decreases also because of a considerable decrease of the water coverage parameter (coordination shell) of large molecules. Note that the analysis presented below depends on the rate of the free water. We have taken as a representative rate $1/T_{1f} = 1$ s^{-1}; see Tables I–IV.

The relative changes of the coordination shell necessary to account for the T_1 increases were calculated; see Table I–IV. The relaxation rate for the bound state is a local property, sensitive to molecular parameters within a very small volume (10^3 Å^3). As such it does not depend on the state of the tissue. We have thus set the bonded rate to be constant. To explain the T_1 increases the parameter p has to decrease in spleen tissue of tumor-bearing mice by $\sim 27\%$. It is noticeable that the parameter p remains approximately the same in liver and muscle tissues. This means that in these tissues the decrease in b is brought about by an increase in the fraction of the free water only. Kidney tissue is intermediate with a decrease in p equal to 17%. Considering that in spleen several types of large molecules will not change their coordination shell to any appreciable extent, it must be that some large molecules change their coordination shell drastically. To account for an average decrease in p of $\approx 20\%$, some large molecules must reduce their coordination shell by more than 20%. This means that these molecules become dehydrated, relative to their normal state of hydration, in the presence of a developed, large, fast-growing tumor in the body.

This conclusion is based on the FETS model. In addition, it is assumed that the distribution of molecules, although with altered configurations, does not change strongly in tissues during the tumor growth.

It has been pointed out that the FETS model applies extremely well to relaxation phenomena at high fields (T_1), since the mixing time of $\sim 10^{-5}$ is much shorter than the water proton spin relaxation time in either bonded state (~ 50 ms) or free state (~ 1 s). While the validity of the FETS model has been borne out by substantial experimental evidence (12–19), the second of the above assumptions may appear to be too weak. It can be shown that this requirement is adequate.

The proton spin relaxation is the result of scattering of free state H_2O on large molecules. During the contact with the scatterer the water molecule is caught in a hydration layer, where it reorients anisotropically at a fast rate. It may also diffuse within the layer a few times before it escapes. In this process each water molecule senses only a small "compartment" with volume $\sim 10^3$ Å^3. Thus, the water spin relaxation depends only on the composition of the particular compartment the water molecule senses. In each compartment there are only a few small parts of large molecules. Since T_1 depends on the distribution of compartments in the sample, it thus depends on the distribution of small parts of large molecules rather than on the distribution of whole large molecules. Since most large molecules are made of similar parts, the distribution of small parts of large molecules remains essentially unchanged in tissues, even if the distribution of large molecules changes during the growth of the tumor. If it is considered that a compartment is smaller than a typical amino acid, this requirement should hold quite well in most cases.

Abscopal effects of a tumor growing in a host animal are well documented (20); amino acids, proteins, carbohydrates, lipids, and metallic ions are changed in the nonmalignant tissues of the host. It should be noted that small changes in ion concentrations may lead to large changes of the coverage parameter, which result in different T_1. In addition, ion concentration changes may influence directly the free water content; this could be organ-specific or a generalized systemic edema. The spleens of tumor-bearing mice are more than twice the weight of spleens from normal animals;

they become hyperplastic and the ratio of white pulp to red pulp increases directly with the rate of growth of the tumor. Slow-growing and spontaneous tumors also show these changes, but to a lesser extent. Since small ionic changes in tissues can be monitored, it should be possible to alter the hydration of some large molecules in tissues in a controlled way. We propose to investigate this in the future.

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