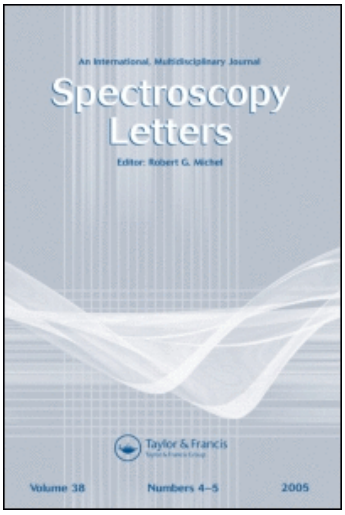

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Frequency Dependence of T^1 and T^2 Relaxation Times of Water in Normal and Tumoral Lung Tissues. T^2 Relaxation Time Evidence of Water Different Chemical Shifts and Exchange Rates.

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FREQUENCY DEPENDENCE OF T_1 AND T_2 RELAXATION TIMES OF WATER IN NORMAL AND TUMORAL LUNG TISSUES. T_2 RELAXATION TIME EVIDENCE OF WATER DIFFERENT CHEMICAL SHIFTS AND EXCHANGE RATES.

Key words: lung tumours; water relaxation times; frequency dependence; water exchange rates in lung

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ABSTRACT

Water ^1H relaxation times, T_1 and T_2, have been measured at different magnetic fields for a total of 22 samples of normal and tumoral lung tissues taken away just after surgery.

Results indicate that the average T_1 values increase continuously with the inverse of the square root of the resonance frequency.

The opposite trend is manifested for the average T_2 values, which decrease continuously with the square of the frequency.

We interpret the anomalous frequency dependence of T_2 by introducing in the relationship a field dependent term.

This term is indicative of the presence of a minimum of two kinds of water molecular surroundings in both normal and neoplastic lung tissues. The upper limits of the exchange rates are derived in this way. The values of these exchange rates are different for normal and neoplastic tissue.

INTRODUCTION

Since from the early applications of Nuclear Magnetic Resonance to living tissues, the determination of the water protons relaxation times T_1, T_2 and T_1^* in normal and pathologic samples has been the object of a widespread interest (1-4).

Particularly important is the determination of the dependence of the relaxation rates from the intensity of the applied main magnetic field,

to give insight to the details of the dynamic properties of the tissutal water.

Using this technique French authors have shown how the principal contribution to the T_1 relaxation times in mouse tissues was due to dipolar interactions between the water protons and the hydrogens of protein at the tissue interface, thus explaining the continuous increase of T_1 between 6.5 and 90 MHz.

In this work we have measured the T_1 and T_2 relaxation times at frequencies ranging from 20 to 90 MHz for 22 samples of freshly removed lung tissue at frequencies ranging from 20 to 90 MHz. Then we have combined our results with those already in the literature (1,5,6) deriving the functional dependance of the relaxation rates from the frequency. Further analysis of this functional dependence gives more details on the dynamic properties of the water molecules in normal and neoplastic lung tissues, which can be used in the medical application of N.M.R. to the lung tumoral pathology in relation to pulmonar Magnetic Resonance Imaging.

MATERIAL AND METHODS

22 samples of lung tissue affected from different types of tumours are obtained from freshly surgered patients. Adiacent normal lung parenchima are also collected.

The samples are gently cutted in tiny slices to fill glass capillaries (2 mm in diameter) which are inserted, with Teflon fitters, in standard 5

mm NMR tubes. The space between the concentric glasses is filled with CD_3OD to provide the spectrometer lock.

We have used three different Bruker spectrometer working respectively at 40, 80.13 and 90 MHz.

For T_1 measurements the Inversion Recovery sequence is used with delays ranging between 0.18 and 6.38 s.. T_2 was measured with the Carr Purcell Spin Echo sequence, modified by Meiboom and Gill, using a delay of 1 ms. to minimize the molecular diffusion and repeating the 180 focusing pulses to obtain echoes F.I.D. between 2 and 1024 ms..

For both relaxation times, waiting times of 20 s. allow the complete recover of magnetization between successive measurements.

In the range of the delay times that we used, the lung water relaxes with a single exponential. High resolution spectra of water are obtained at 80.13 and 90 MHz to draw out the halfheight width of the resonance peak, with a digital resolution of 0.063 Hz/Pn. All measurements were performed at the probe temperature of 25°C. The characteristics of the normal lung tissues and the type of tumours are obtained with histologic analysis.

RESULTS AND DISCUSSION

The tables 1A and 1B report the relaxation parameters of the lung water averaged over the number of samples with the same pathology and the halfheight linewidths obtained from high resolution spectra,

TABLE 1A

Water $T_1^{a,b}$, $T_2^{a,b}$ average relaxation times, halfheight linewidths $\Delta\nu^{a,b}$ in normal lung tissues at different frequencies and at 25°C.

		40 MHz			80.13 MHz			90 MHz		
No. samples	Histology	T_1 (s)	T_2 (s)	$\Delta\nu$ (Hz)	T_1 (s)	T_2 (s)	$\Delta\nu$ (Hz)	T_1 (s)	T_2 (s)	$\Delta\nu$ (Hz)
3	EPIDERMOID CA. G1	0.574	0.082	/	0.600	0.067	40	0.700	0.070	35
6	EPIDERMOID CA. G2	0.531	0.069	/	0.610	0.080	47	0.548	0.060	30
3	EPIDERMOID CA. G3	0.540	0.060	/	0.649	0.084	/	0.800	0.073	30
3	ADENO CA. G3	0.592	0.086	/	0.634	0.059	38	0.693	0.060	29
3	BIG CELLS CA. G3	0.561	0.085	/	0.670	0.072	17	0.700	0.065	27
2	CLEAR CELLS MICROCITOMA	0.610	0.066	/	0.689	0.083	28	0.540	0.060	32
1	ATIPICAL CARCINOID	0.557	0.074	/	0.630	0.064	30	/	/	/
1	FIBROTIC TISSUE	/	/	/	/	/	/	0.490	/	106
Average values over all samples		0.568 ±0.043	0.074 ±0.009	/	0.640 ±0.050	0.072 ±0.009	32 ±11	0.664 ±0.064	0.068 ±0.009	31 ±2.5

[a] = standard deviation over a single measurement: ±10%

[b] = average dispersion in each set of measurements: T_1=±8%, T_2=±14%, $\Delta\nu$=±11%

TABLE 1B

Water $T_1^{a,b}$, $T_2^{a,b}$ average relaxation times, halfheight linewidths $\Delta\nu^{a,b}$ in tumoral lung tissues at different frequencies and at 25°C.

		40 MHz			80.13 MHz			90 MHz		
No. samples	Histology	T_1 (s)	T_2 (s)	$\Delta\nu$ (Hz)	T_1 (s)	T_2 (s)	$\Delta\nu$ (Hz)	T_1 (s)	T_2 (s)	$\Delta\nu$ (Hz)
3	EPIDERMOID CA. G1	0.775	0.086	/	0.780	0.048	20	0.858	0.037	14
6	EPIDERMOID CA. G2	0.927	0.126	/	0.868	0.079	22	0.685	0.040	12
3	EPIDERMOID CA. G3	0.696	0.077	/	0.756	0.054	20	0.900	0.045	12
3	ADENO CA. G3	0.775	0.086	/	1.038	0.044	23	0.900	0.038	13
3	BIG CELLS CA. G3	0.651	0.070	/	0.944	0.054	20	0.950	0.050	32
2	CLEAR CELLS MICROCITOMA	0.843	0.123	/	0.729	0.077	30	1.010	0.035	25
1	ATIPICAL CARCINOID	0.676	0.107	/	0.757	0.067	25	/	/	/
1	FIBROTIC TISSUE	/	/	/	/	/	/	1.153	/	23
Average values over all samples		0.751	0.099	/	0.838 ±0.107	0.060 ±0.013	23 ±3.5	0.884 ±0.100	0.040 ±0.005	18 ±8

[a] = standard deviation over a single measurement: ±10%

[b] = average dispersion in each set of measurements: T_1=±10%, T_2=±20%, $\Delta\nu$=±18%

at different frequencies. The average values of T_1 are greater for the tumoral samples than in the normal ones as already found (2), while the T_2 average values and the overall line widths present opposite behaviour. The ratio T_1 tumoral/T_1 normal are respectively 1.32, 1.31, 1.33 at the frequency of 40, 80.13, 90 MHz and are consistent with the values reported at 22.5 MHz (1) between 1.24 up to 1.36. This invariance means that if also the values of T_1 for both lung tissues are field dependent, their ratio, with good approximation, is independent from the applied field.

On the contrary, the T_2 average values in normal and tumoral lung tissues, at each frequency, behave in a way which is opposite to the parallelism with T_1, largely quoted in literature (1,2). We found that T_2 in normal tissues is greater than the corresponding value in the tumoral ones.

The table 2 shows the dependence of the average relaxation rates from the intensity of the magnetic field applied. The data in the first row are taken from the work of Goldsmith et al. (1) on the same lung tissue pathology.

A look at the table 2 evidences that the longitudinal relaxation rates R_1 decrease with the increasing of magnetic field, as expected from dipolar nuclear interactions (7,8).

For the transversal relaxation rates R_2 in normal and tumoral lung tissue, we found that T_2 decreases with the frequency, in disagreement with the dipolar theory (7). The correct behaviour has

TABLE 2

Magnetic field dependence of average relaxation rates of water in lung tissues.

	NORMAL TISSUE		TUMORAL TISSUE	
ν(MHz)	$R_1(s^{-1})$	$R_2(s^{-1})$	$R_1(s^{-1})$	$R_2(s^{-1})$
22.50	2.045	13.514	1.508	8.620
40.00	1.762	13.585	1.330	10.117
80.13	1.563	13.889	1.193	16.667
90.00	1.506	14.710	1.331	25.000

been observed for both T_1 and T_2 in human blood and other tissues (7).

According to our observation, R_1 is linear with $\nu^{-1/2}$, as already reported by Escanje et al (5,6) for different mouse tissues. These authors explain this behaviour on the basis of equations which describe the intermolecular water-proteins hydrogen atoms dipolar relaxation in the region of slow and intermediate motional narrowing (7,8).

In a previous work (9) we have reported the fractional populations of the easely removable water in normal and tumoral lung tissues. Combining the values of the populations with the values of the intercept B of the linear plots, we obtain the relaxation times T_1 for

bulk water in both kinds of lung tissues. The values are 0.223 s. and 1.174 s. respectively for normal and tumoral tissues. The former value for free water is too low with respect to 1.75 s. previously reported for normal mouse tissues (5).

The ratio of the bulk water T_1 in tumoral (t) and normal (n) lung tissues amounts to 5.25. We suggest that this relatively high ratio origins from the lower fractional population for free water in normal with respect to tumoral tissues, and differences in water translational self diffusion coefficents. In the limit of strongly motional narrowing of dipolar interaction (10), the absolute free water longitudinal relaxation rate, summing the rotational and translational contributions, is given by:

$$\frac{1}{T_1} = \frac{a^2}{b^2} \cdot \frac{g^4 \hbar^2}{3Db^4} \left\{ 1 + \frac{3p}{5} \cdot N_2 \cdot \frac{b^6}{a^3} \right\} \qquad [1]$$

where D is the translational self-diffusion coefficient of free water, which amount to $1.85 \cdot 10^{-5}$ cm²/s at 22°C.

From the relationship:

$$\left[\frac{T^t_{1\,\text{freewater}}}{T^n_{1\,\text{freewater}}} \right]_{\text{experimental}} = \frac{D^t_{\text{freewater}}}{D^n_{\text{freewater}}} \int \left(\frac{p^n_{\text{freewater}}}{p^t_{\text{freewater}}} \right) \qquad [2]$$

substituting in 1 the suitable values, we easely obtain the ratio of the free water diffusion constants for the lung tissue, which amounts to 6.58.

The same ratio has been evaluated (11) to be 2.3 in normal and tumoral mammarian tissue of mouse. The high ratio found is not

unexpected if we take in account the alveolar structure of lung tissue described by a compartmental model as developed by Durney (12) and Case (13). On the experimental ground they found that ratio of the F.I.D. decaying time constant T_2^* in collapsed and inflated lung tissue ranges between 7.5 and 5.

Furtherly, direct measurement of the water diffusion constant in normal lung tissue with pulsed gradient spin echo technique (14) founds a ratio between the pure water and lung water self diffusion constant which ranges between 4.5 and 13.9.

To conclude, the mechanism of intermolecular dipolar spin-spin interactions appears to be more efficient for the free water molecules in normal tissue than in tumoral one and seems to be mainly related to the water diffusive translational motions differences, also if the relative population are in the ratio of 1:4 (9).

The measurement of irreversible (10) [neat from reversible effects such as magnetic field inhomogeneity combined with the tissutal diamagnetic anisotropy effects (12)] T_2 with the method of spin echo in relation with the overall halfheigh linewidths, obtained from the high resolution ^1H NMR spectra of the lung water, allows us to determine the contribution of the heterogeneity effects due to the structure postulated by Case (13) and Durney (12) on the lung water NMR spectra.

In table 3 are reported the overall average halfheight linewidths, the contributions to the linewidths due to the heterogeneity of lung tissues (12,13), the extremely fast water exchange rates R_{2a}

TABLE 3

Experimental halfheight linewidths $\Delta\nu$[a]; C.P.M.G. transversal relaxation rates R2; heterogeneity halfheight linewidths $\Delta\nu_h$; extremely fast relaxation rates R_{2a}; frequency dependent relaxation rates R_{2b} in normal and tumoral lung tissues at 25°C.

	NORMAL TISSUES					TUMORAL TISSUES				
ν (MHz)	$\Delta\nu$ (Hz)	$\Delta\nu_h$[b] (Hz)	$R_2(s^{-1})$	$R_{2a}(s^{-1})$	$R_{2b}(s^{-1})$	$\Delta\nu$ (Hz)	$\Delta\nu_h$[b] (Hz)	$R_2(s^{-1})$	$R_{2a}(s^{-1})$	$R_{2b}(s^{-1})$
22.50	/	/	13.514	13.38	0.130	/	/	8.62	7.00	1.62
40.00	/	/	13.585	13.38	0.205	/	/	10.12	7.00	3.12
80.13	32	27.6	13.889	13.38	0.510	23	17.7	16.67	7.00	9.70
90.00	31	26.4	14.710	13.38	1.330	18	10.0	25.00	7.00	18.00

a) The field homogeneity, before and after every measure, checked on a test sample of distilled water, is found to be 0.5 MHz: negligible respect to the tissue linewidths.

b) Calculated as $\Delta\nu_h=\Delta\nu-R_2/\pi$

independent from the frequency and the frequency dependent term R_{2b} of the whole transversal relaxation rates R_2.

The plot of R_2 against the square of frequency fits the linear relationship:

$$R_2 = R_{2a} + B^2 \qquad [3]$$

where the intercept R_{2a} is equal to:

$$p_f^2 p_b^2 4^2 (d_f - d_b)^2 (t_{f\to b} + t_{b\to f}) 10^{-12}$$

These expressions are derived from the equation for the transversal relaxation rate in the case of the two sites exchange (7). It may be noted that the value of R_{2a} for the normal is greater than the

corresponding value for the tumoral lung tissue. Hence, in absence of any dependence from the frequency, T_{2a} for the tumoral tissue is greater than the corresponding value for the normal tissue, as generally found for living tissue (2).

The bestfit values of the slope B are 1.33×10^{-16} s. for the normal and 1.95×10^{-15} s. for the tumoral lung tissues. With the values p_f and p_b reported for the free and bound water (9), assuming as difference of the water chemical shifts $\delta_f - \delta_b$ the values obtained from the overall halfheights linewidths, surely an overstimation (15), and considering that at the thermodinamical equilibrium (16):

$$p_f t_{f \to b} = p_b t_{b \to f} \qquad [4]$$

we may obtain an extimation of the exchange lifetimes for the water in the free and bound phase (5,6). The upper limit values in seconds result:

$$t_{f \to b} = 6.44 \cdot 10^{-4} \qquad t_{b \to f} = 1.82 \cdot 10^{-4}$$

for the water in normal lung tissues. The corresponding values for the tumoral tissues are:

$$t_{f \to b} = 15.2 \cdot 10^{-3} \qquad t_{b \to f} = 202 \cdot 10^{-3}$$

The values reported are averaged over that at 80.13 and 90 MHz.

The values obtained for the water exchange lifetimes in the normal lung tissues are one order of magnitude smaller compared to the

water exchange lifetimes in blood red cells membranes (17).The converse is true for water lifetimes in tumoral lung tissues. It may be worthy to note that recently (18) a relationship similar to equation [3] has been invoked to explain the T_2 relaxation times of mobile protons in hystidil residues located on surface proteins in human hemoglobin of normal and sickle red cells.

CONCLUSION

The dynamic properties of the water in healthy lung are significatively different respect to the tumoral tissues. We have evidenced how this fact may be related to the subtle details of the lung water enviroment, such as the strenght of intermolecular water-proteins hydrogen bonds, cell membrane compositions, stability and permeability, factors known to influence deeply the self diffusion process (11,14). Further studies need to reveal quantitatively the motional details of this difference and may help to understand at molecular level the dynamic of a tumoral invasion and its aggressivity to perturb the cellular water equilibrium in the healthy lung tissue.

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