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PROTON MAGNETIC RELAXATION IN INTACT MICE LUNGS DURING OXYGEN EXPOSURE

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SUMMARY

Proton spin lattice relaxation times have been determined in mice lungs during exposure to hyperbaric oxygen. The water protons within the lungs of the oxygen-exposed mice relax slower than those in control animals. The rate of changes followed approximately the rate of water accumulation within the lungs. The results are interpreted in terms of a hypothesis that a minor fraction of the protons in the cell is distributed over states of low mobility and exchanges rapidly with a major fraction which exhibits shorter correlation time. The change in water relaxation rates in lungs following the exposure to oxygen is attributed to the difference in the distribution ratio of water between the hydration and free states.

INTRODUCTION

One of the most challenging problems in measuring the development of pulmonary edema is estimating the rate of water accumulation within the intact lung. In particular it is difficult to determine the increase in lung water in a non-invasive method in vivo. Nuclear magnetic resonance technique has the potential to examine the severity of pulmonary edema in isolated lungs as well as to determine the degree of immobilization of the water, either free or bound to structural entities within the cell (1). In principle the technique can also be used for studying the water content and properties in organs in vivo (2). We report here on a preliminary nuclear magnetic relaxation study of water protons in isolated lungs following exposure of mice to hyperbaric oxygen.

Pulmonary edema is a common pathological manifestation of oxygen toxicity (3-5). Perivascular, interstitial and intracellular accumulation of water appears relatively early in the poisoning process (4, 6). A quantitative

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determination of water accumulation by the NMR method may indicate the severity of lung damage and can give an objective criteria for the development of pulmonary oxygen toxicity.

MATERIALS AND METHODS

Female C-57 black mice were exposed to 1 atmosphere of 100% O_2 for varying periods of time in a perspex chamber. The chamber was continuously flushed with oxygen at a flow rate of 3 liter/min. Oxygen concentration was monitored continuously. The partial pressure of CO_2 in the chamber was less than 1.5%. Control mice were exposed to air. Food and water were provided ad libitum. Animals were sacrificed within 1 h after the end of the O_2 exposure. The lungs were removed and inserted into the bottom of a 10 mm OD NMR tube and placed in the spectrometer.

Measurements of T_1, the spin lattice relaxation time, were made at 60 MHz on a Brucker BKR 322S pulsed spectrometer. T_1 values were determined after 180°-τ-90° pulses from the slope of a semilog plot of $A_\infty - A_\tau$ versus τ following eq. 1:

$$\log (A_o - A_\tau) = \frac{-\tau}{T_1} + \text{const.} \quad (1)$$

A_τ is the initial amplitude following the 90° pulse which occurs at time τ after the 180° pulse. A_o is the limiting value of A_τ for a very long interval between the 180° and 90° pulses. The pulse width for a 180° pulse was 5 μsec: Sample temperature was held constant at 25°C ± 1°. The uncertainty of individual T_1 measurements is less than 5%.

RESULTS

Figure 1 presents the recovery of magnetization signals of water protons after a 180° pulse. To illustrate the rate of recovery of the magnetization, a sequence of $(t - 180° - \tau - 90°)_n$ pulses has been applied. In this sequence $t \approx 10\ T_1$ and τ was varied by steps of 40 msec. Figure 1 shows clearly that the water within the untreated control lung relaxes faster than the water in lungs of mice that have been exposed to oxygen for 95 hours. In Figure 2 the amplitudes of the free induction decay are plotted vs time in a semilog fashion for the evaluation of T_1. The straight lines thus obtained indicate exponential free induction decays. It should be noted, however, that although the magnetization plot for all protons in the whole lung is exponential, the T_1 values obtained are at best a representative of an overall quantity for superimposed signals.

The spin lattice relaxation times in lungs of mice exposed to oxygen for various periods of time are given in Figure 3. Each point represents an ave-

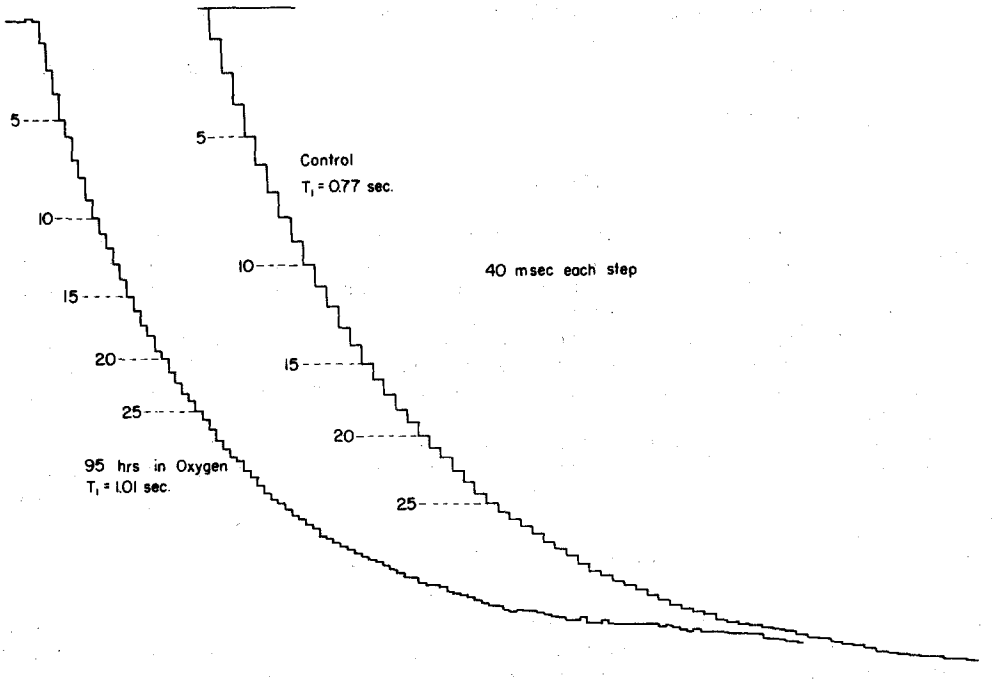

Figure 1. Decay of magnetization in a 180°-τ-90° sequence vs time at 25°C for water in mice lungs.

rage of 3 mice. It seems that T_1 does not change in the first forty hours of exposure. Then, T_1 becomes longer monotonically until death of the animal occurs, about 105 hours after 0_2 exposure. At that stage T_1 values are 40% longer than control values.

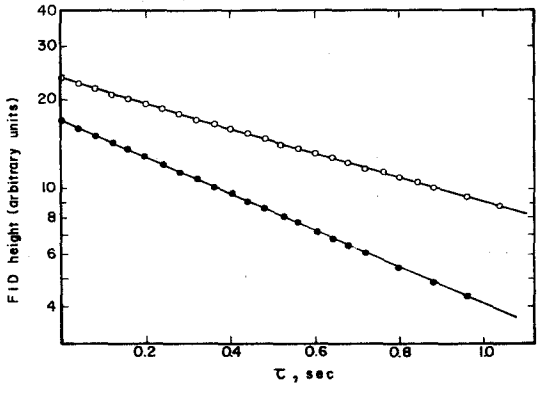

Figure 2. The intensity of the free induction decay of protons for H_2O in control mice lung (closed circles) and after 95 hours of exposure to oxygen (open circles).

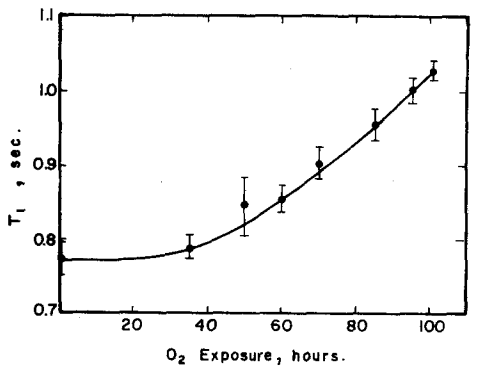

Figure 3. Spin-lattice relaxation times of water protons in mice lungs exposed to 100% oxygen.

DISCUSSION

The value of protons T_1 and the line width of water in biological tissue are directly related to the total water content in the tissue (7, 8). Our results clearly show longer T_1 values after oxygen exposure, concomitantly with the development of pathological damage of the lung.

The results can be explained by adapting a two-phase model for water mobility in tissues (8-10). According to this model, a small fraction of the water within the lung (x) could form an immobilized hydration layer around macromolecules within the cytoplasm as well as on the cell surface. Those water molecules present a relatively long rotational correlation time. In fact, they exhibit a distribution of correlation times in the form of a log-Gaussian function. This is because T_1 values for water in different parts of the cell might differ. The relaxation time of the "bound" water can be represented by a single value, T_{1b}. The rest of the water molecules in the lungs (1-x) have a shorter and probably a single relaxation time, T_{1f}. The observed relaxation rate in the tissue is believed to be a weighted average of the two types of water, i.e., the magnetic relaxation is caused by a rapid exchange of water between the fast-relaxing fraction bound to macromolecules and a slowly-relaxing solvent fraction

$$\frac{1}{T_{1\ \mathrm{obsd}}} = \frac{x}{T_{1b} + \tau_b} + \frac{(1 - x)}{T_{1f}} \qquad (2)$$

In eq. 2, τ_b is the residence lifetime of a water molecule in the fast relaxing state. With the accumulation of water into the lung, during the exposure to oxygen, the fraction of the free water (1-x) increases markedly. The contribution of the fast relaxing term in eq. 2 decreases and T_1 becomes longer. In such an explanation we assume that the relaxation behavior of either type of water molecules does not change during the pulmonary oxygen toxicity process. Also, the absolute amount of water in the hydration layer does not seem to change. The only difference is that the fraction of water in the hydration layer becomes smaller. Although this may be an oversimplification of the situation (11), it should be noted that the same explanation was offered for proton magnetic relaxation studies in cancerous cells (12). It is now well recognized that the spin-lattice relaxation time of the water protons in a tissue infected with a tumor is in general longer than that of a normal tissue of the same kind. This fact was explained simply by assuming a decrease in the value of x in eq. 2 Indeed, it was found that the water content of the tumor cell studied is significantly larger than that for normal tissue (12).

In normal tissues the fraction of water in the hydration layer is not more than 15%, but due to the very long τ_c ($\tau_c > 10^{-8}$ sec) contributes much to the observed T_1. Taking $T_{1f} = 2.5$ sec, $x = 0.15$ and assuming that the fast exchange limit prevails for the protons in the hydration layer (i.e., $\tau_{1b} \gg \tau_b$), T_{1b} can be calculated as 0.155 sec. Reinserting this value into eq. 2 and taking T_1 observed after 95 hours of exposure to oxygen as 1.05 sec (see Figure 3), the calculated fraction of the bound water molecules is reduced to 9% of the total water content in the lungs. Although roughly estimated, this calculation gives us an idea of the water accumulation in the cells during exposure to hyperbaric oxygen.

The observed decrease in the relaxation rate started about 40 hours after exposure to oxygen, and then proceeded monotonically. This reflects the development of lung injury as observed also in ultrastructural studies (4). It seems that the NMR technique may offer a unique tool for studying edematous

processes in the lung. Although this study is conducted on excised lungs, the magnetic resonance techniques may be used for studying organs and tissues in vivo. NMR imaging and surface coil techniques have been recently proposed and demonstrated (13, 14). Their application to the investigation of tissues of whole organisms is of great potential value since the techniques are non-invasive, have no known hazard and can provide a powerful measure of molecular level structure and motion.

Our work is preliminary in nature. Since the bound water exhibits a long rotational correlation time, T_{1b} should be frequency-dependent. On the other hand, the longer T_{1f} is almost frequency-independent. Hence, the frequency-dependence studies of the relaxation should give further insight into the spectrum of proton mobilities in the hydrated system. Such a work, as well as relaxation studies in rat lungs, is now under way.

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