Zeitschrift: Archives des sciences et compte rendu des séances de la Société
Herausgeber: Société de Physique et d'Histoire Naturelle de Genève
Band: 49 (1996)
Heft: 1: Archives des Sciences

Artikel: NMR studies of the physiological states in cancer cells and tissues
Autor: Lenk, Rudolf
DOI: https://doi.org/10.5169/seals-740411

Archs Sci. Genève	Vol. 49	Fasc. 1	pp. 51-57	Avril 1996

Communication présentée à la séance du 2 novembre 1995

NMR STUDIES OF THE PHYSIOLOGICAL STATES IN CANCER CELLS AND TISSUES

BY

Rudolf LENK*

ABSTRACT

NMR studies of the physiological states in cancer cells and tissues. - The physiological states are defined in terms of Statistical Thermodynamics and Brownian Motion theory. It is shown that the physiological transitions can be indicated by the nuclear spin-relaxation times and the NMR spectral-line widths. This yields the corresponding correlation times, microscopic thermodynamic functions and metastatic potentials.

Key-words: NMR, Correlation time, Entropy, Physiological states, Cancer cells and tissues.

INTRODUCTION

In spite of a great number of studies on Nuclear Magnetic Resonance (NMR) in Cancer Biology, relatively little emphasis has been placed to the physico-chemical aspects of this domain. This paper will attempt to contribute to this problem.

Living systems are characterized by a great number of molecular configurations, high entropy and significant Brownian (random) motion. Because of absence of symmetry and regularity of these systems, molecular motion yields the most important information of their physiological state (LENK, 1979, 1984, 1993).

The fast Brownian dynamics contributes to internal energy and entropy of the sample. *Biodynamics* investigates the microscopic states in Biology by the studies of random movements (LENK, 1984, 1993).

Microscopic entropy, given by the von Neumann relation, $S = - k \sum p_i \ln p_i$, can be interpreted as a mesure of the lack of knowledge of the system (LENK *et al.*, 1987).

Spectroscopically, this can be studied by NMR and by Spin Relaxation method (LENK, 1979). In some cases, the physiological and morphological transformations give rise to the changes of the state. A number of these transitions is known in Plant Physiology (GRANGE *et al.*, 1980; LENK *et al.*, 1981) and Tumour Biology (DAMADIAN, 1971; DAMADIAN *et al.*, 1974; BEALL *et al.*, 1981a).

* Laboratoire de Biochimie et Physiologie Végétales, Pavillon des Isotopes, 20, bv. d'Yvoy, CH-1211 Genève 4.

Over the last 25 years, Nuclear Magnetic Resonance (NMR) has been used extensively for the measurements of spectra, spin-lattice (T_1) and spin-spin (T_2) relaxation times and diffusion coefficients (D) of water molecules in healthy and tumoural tissues. The relaxation times of hydrogen protons of water molecules are reflecting the interactions of water with protoplastic macromolecules. In pure liquid water, the T_1 value for protons is ~ 3 sec, indicating rapid motion, with a correlation time $\tau_c \sim 10^{-11}$ sec. In the majority of biological systems, the T_1 values are in the range of 200 to 1000 msec, suggesting a slower average motion for water molecules in protoplasm. It has been postulated that this slower average motion of water in cells is due to long or short range interactions between water molecules and macromolecular surfaces (PACKER, 1977).

In his pioneer work, DAMADIAN (1971) reported that in tumoural tissues of rats, the NMR relaxation times are *longer* than in the corresponding normal tissues. This observation has been confirmed by other authors. It acquired the name the "*systematic effect*" (BEALL *et al.*, 1981a).

THEORETICAL BACKGROUNDS

The most significant statistical variable of the Brownian dynamics is the correlation time τ_c. It can be described by an integral over the related time-correlation function

$$\tau_c = \int < f(0) \cdot f(t) > dt \qquad (1)$$

In NMR the spin-lattice relaxation rate, $1/T_1$, is proportional, in the case of Brownian rotation, to the sum of the Debye-Bloembergen spectral densities

$$1/T_1 = k\{\tau_c/(1 + \omega^2\tau_c^2) + 4\tau_c/(1 + 4\omega^2\tau_c^2)\} \qquad (2)$$

This gives one the possibility to determine the correlation time from the T_1 relaxation experiment. At the room temperatures, for $\omega^2\tau_c^2 << 1$, eq. (2) simplifies to

$$T_1 = \text{const}/\tau_c \qquad (3)$$

Relation (3) can by modified in terms of the "generalized spectral densities" as follows

$$T_1 = k\,\omega^m\tau_c^{(m-1)} \qquad (4)$$

The exponent "m" has the values $0 < m < 0.5$. For $m = 0$, one has Brownian isotropic rotation. In this case the spin-lattice relaxation is frequency-independent. For $m = 0.5$ one has the cases of either isotropic translation or "elastic fluctuations" of the local anisotropy in the nematic phase (CABANE, 1972).

In water and physiological fluids one has isotropic rotation with m = 0. In cells and tissues the bound water is organized, due to the presence of proteins and lipids, into the "molecular aggregates" with the similar physico-chemical properties as the nematic mesophase. The similar properties have the lipids. Practically, in the ordered molecular systems one has: $0.4 < m < 0.5$.

The physiological state is given particularly by the fast Brownian motion. A phase transition can be defined by the ratio of the related correlation times

$$r = \tau_{cA}/\tau_{cB} = \{T_{1A}/T_{1B}\}^{1/(m-1)} = R^{1/(m-1)} \quad (5)$$

Entropy can be related with the correlation time, τ_c, as follows (LENK, 1984)

$$S \alpha 1/\tau_c \quad (6)$$

Consequently, one has

$$S_A/S_B = \tau_{cB}/\tau_{cA} = 1/r \quad (7)$$

Combining eqs . (5) and (7) yields

$$S_A/S_B = R^{1/(1-m)} \quad (8)$$

For $m \sim 0.45$, one has

$$S_A/S_B \sim R^{1.8} \quad (8b)$$

The entropy ratio can also be obtained from the related spectral line-widths, Δ, (LENK, 1979, 1984)

$$S_A/S_B = \Delta_B/\Delta_A \quad (9)$$

Equation (9) shows that by the increased entropy the related NMR spectral lines are narrowed.

SELECTED APPLICATIONS

A) *Spin-lattice relaxation.* — The Damadian's results (DAMADIAN *et al.*, 1974), at 24 MHz, yield the following ratios of the related relaxation times ($R = T_{1A}/T_{1B}$): R(breast) = 2.9; R(bone) = 1.85; R(skin) = 1.69 and R(spleen) = 1.60, etc. In all cases, $R > 1$. The similar results were obtained by the studies of cancer *in-vivo* by spin-lattice relaxation of normal mouse tail tissue and malignant transplanted tissue, located on the tail (WEISSMAN *et al.*, 1972). The spin-lattice relaxation results at 18 MHz are T_1(tumour) $\approx$ 0.7 sec, T_1(normal) $\approx$ 0.3 sec and $R \sim 2.33$. ZANER & DAMADIAN (1975)

found by the NMR studies of phosphorus -31 that T_1 is was much longer for malignant than for normal tissues. It was also found that the NMR relaxation times of the phosphorus were longer than those of the protons, both in malignant and normal tissues.

In some cases the measurement of T_1 relaxation time can be helpful in diagnosing benign thyroid tumours, as it was reported by SINADINOVIC *et al.* (1977). Following BEALL *et al.* (1981b), the difference in T_1 and T_2 between normal, preneoplastic and neoplastic cells remain even though there is no difference in hydration among these cell groups.

The changes in T_1 relaxation reflect the differences in mobility of all molecular components of tissue (water, macromolecules and lipids).

B) *High-Resolution (HR) NMR spectra.* — The recent progress of the NMR techniques, particularly the water-signal suppression facility and the two-dimension (2D) spectroscopy, gives one the possibility to study the non-water proton signals by the HR-NMR (BLOCK *et al.*, 1977; VAN HAFTEN-DAY *et al.*, 1988; SMITH *et al.*, 1990; LEAN *et al.*, 1993; MOUNTFORD *et al.*, 1993). The NMR studies of non-water protons confirms, at least qualitatively, the results obtained with water protons.

Surprisingly enough, the H-1 NMR spectrum of an excised distinct solid tumour is remarkably similar to that obtained from a suspension of the same type of cells, grown in culture (MOUNTFORD *et al.*, 1984). The origin of these spectra is given by rapidly tumbling lipid molecules.

The neoplastic process in tissues involves the narrowing of the NMR spectral lines. For example, the H-1 NMR spectra at 400 MHz of normal post-menopausal ovarian tissue, suspended in PBS/D2O, are narrowed by the action of the potent tumour promoter, TPA, (12-0-tetra-decanoylphorbol-13-acetate), as reported by VAN HAFTEN-DAY *et al.*, (1988). In the similar way, the 360 MHz, H-1 NMR spectra of human colorectal carcinoma exibit a neat NMR line-narrowing in comparison with normal colorectal mucosa (LEAN *et al.*, 1993). Following these results, the spectral-line width of the CH_2 resonance at 1.3 ppm is 78 ± 22 Hz for the normal mucosa, while this line-width is 46 ± 9 Hz in the case of carcinoma. In this case the proton resonance identifies abnormal colorectal mucosa, which is not morphologically manifested. Further, one-dimensional NMR spectra of axillary lymph nodes from tumour-bearing rats display resonances with considerably narrower line display resonances than those in the spectra of lymph nodes from healthy control or immunostimulated animals (MOUNTFORD *et al.*, 1993).

C) *Decrease of the molecular order by the tumour promoter action.* — The effect of the phorbol ester, TPA, on the mouse melanoma cells was studied by the deuterium NMR (SUGIMOTO *et al.*, 1986). Using an H-2 probe, the reduced splitting of the deuterium line shows that the TPA treatment implies the decrease of the molecular order in the investigated cells.

D) *The metastatic potential.* — Another task of the NMR studies of cancer is to indicate the metastatic potential of various cancer lines. Metastasis is a shifting of disease from one part of body to another. This process, progressing by a sequence of inter-related steps, allows cells to escape from a primary site and move around the body and then lodge and multiply in another site.

"How the physico-chemical properties of metastasis can be studied by NMR?" As shown by MOUNTFORD *et al.* (1986), the metastatic cells from the human colon exhibit the elevated spin-spin (T_2) relaxation times of 400 msec or above, for the peak at 1.3 ppm, in the cell line having a metastatic potential. These long spin-spin relaxation times indicate the presence of fast Brownian dynamics with high entropy and short correlation time, τ_c.

The relaxing proton NMR signals from cancer cells correlate with their metastatic ability and drug sensitivity. We wonder if this effect is reversible. Following the results reported by HOLMES *et al.* (1986), the long T_2 relaxation times (> 350 msec), observed in the plasma membranes of metastatic cells are decreased after an enzyme (trypsin/EDTA) treatment. Further, the 2D scalar-correlated (COSY) NMR spectra of these trypsin treated cells show that a cross peak, normally associated with malignancy and metastatic disease, is markedly reduced. The correlation between the absence of a long T_2 relaxation value and the diminished number of metastases in animals suggests that the plasma membrane particles are involved in the metastatic process.

Furthermore, the long T_2 relaxation times (500–800 msec) observed in the plasma membranes of metastatic rat mammary adenocarcinoma cells can be reduced by treatment with fucosidase (WRIGHT *et al.*, 1988). The fact that a cell surface metastasis-marker has an NMR signal with a long relaxation value has important consequences for the future use on NMR spectroscopy and imaging in the cancer clinic.

DISCUSSION

Following eqs. (8) and (9), the increase of the spin relaxation times and the narrowing of the H-1 NMR spectral lines in tumoural tissues and cells indicate the increase of molecular dynamics and entropy in the tumours. This is in general agreement with the assertion of SZENT-GYÖRGYI (1957) that a tumoural tissue has a lower degree of organization and less water structure than a normal tissue and also with the Ling's "*association-induction hypothesis*" (LING, 1965; LING & TUCKER, 1980) that the bulk of cell water is in a physical state of *polarized multilayers*, adsorbed onto cell proteins. The neoplastic cells and tissues are provided with a selective growth advantage over adjacent normal cells and the molecular order of the polarized multilayers is partially removed. This accelerates the molecular movements. The sudden increase of disorder and of molecular mobility of the water aggregates destabilizes the physiological regulation and inhibits the function of the living system.

The fact that the malignant cells show surface properties differing from those of normal cells is well established. Changes of properties of the plasma membrane surface can have cytopathological incidences, such as a loss of "contact inhibition" and a decrease of the potential barrier, limiting the space of cells transformed to malignancy (TURIAN, 1994). This superimposes to the malignant cells the necessary mobility to escape from their sites.

The spin relaxation properties of the methylene resonances in plasma membranes, at 1.3 ppm, can be used as an indicator of the metastatic potential of various cell lines. The long T_2 relaxation indicates the existence of the short correlation times, tc, and consequently the increased kinetic energy, ϵ, and entropy, S, in the plasma membranes.

Consequently, we can write the following inegalities: $\tau_c(N) > \tau_c(T) > \tau_c(M)$; $\epsilon(N) < \epsilon(T) < \epsilon(M)$ and $S(N) < S(T) < S(M)$ (ϵ is molecular energy, S is entropy and (N), (T), (M) specify the normal state, the tumoral state and the metastatic state, respectively).

It seems that the metastatic activity has the origin in the Brownian dynamics of the "hot" plasma membranes and that this phenomenon is reversible, as the enzyme treatments affect the dynamic but not the tumorigenic properties of metastatic cells.

REFERENCES

BEALL, P.T., MEDINA, D. & HAZLEWOOD, C.F. 1981a. Systematic effect of elevated tissue and serum relaxation times for water in animals and humans with cancer. *In: NMR, Basic Principl. Progr.*, 19: 39–57.

BEALL, P.T., ASCH, B.B., MEDINA, D. & HAZLEWOOD, C.F. 1981b. Distinction of normal, preneoplastic and neoplastic mouse mammary cells and tissues by NMR techniques. *In: The Transformed Cells.* 293–325, Acad. Press, New York 1981.

BLOCK, R.E., MAXWELL, G.P., PRUDHOMME, D.L. & HUDSON, J.L. 1977, High-Resolution Proton Magnetic Resonance spectral characteristics of water, lipid and protein signals from three mouse cell populations. *J. Natl. Cancer Inst.* 58: 151–156.

CABANE, B. 1972. Nuclear relaxation in liquid crystals. *Advan. Mol. Relax. Processes*, 3: 341–353.

DAMADIAN, R. 1971. Tumor detection by Nuclear Magnetic Resonance. *Science*, 171: 1151–1153.

DAMADIAN, R., ZANER, K., HOR, D. & DIMAIO, T. 1974. Human tumors detected by NMR. *Proc. Nat. Acad. Sci., USA*, 71: 1471–1473.

GRANGE, A., DUPANLOUP, A., DESCOUTS, P. & BENE, G. 1980. Evolution du temps de relaxation spin-réseau T1 des protons de l'eau biologique au cours de la maturation des graines de Haricot. *CR Acad. Sci., Paris*, B291: 307–309.

VAN HAFTEN-DAY, C., HOLMES, K.T., MAY, G.L., WRIGHT, L.C. & MOUNTFORD, C.E. 1988. Magnetic Resonance Spectroscopic studies of a human diploid ovarian tumour line treated with 12-0-tetra-decanoylphorbol-13-acetate. *Magn. Reson. Med. Biol.*, 1: 177–186.

HOLMES, K.T., WILLIAMS, P.G., MAY, G.L., GREGORY, P., WRIGHT, L.C., DYNE, M. & MOUNTFORD, C.E. 1986. Cell surface involving in cancer metastasis: An NMR study. *FEBS Lett.*, 202: 122–126.

LEAN, C.L., NEWLAND, R.C., ENDE, D.A., BOKEY, E.L., SMITH, I.C.P. & MOUNTFORD, C.E. 1993. Assessement of human colorectal biopsies by 1H MRS: Correlation with histopathology. *Magn. Reson. Med.*, 30: 525–533.

LENK, R. 1979. Time-evolution of entropy of fluctuations in some biological systems as studied by NMR. *Chem. Phys. Lett.*, 62: 399–401.

LENK, R., BONZON, M. & GREPPIN, H. 1981. Irreversible thermodynamics and biological evolution in Spinach leaves, as studied by NMR. *Z. Pflanzenphysiol.*, 101: 107–118.

LENK, R. 1984. Biodynamics and NMR. *Progr. Nucl. Med.*, 8: 55–61.

LENK, R., CRESPI, P. & GREPPIN, H. 1987. Evolution de l'entropie et de la néguentropie en biologie. *Archs Sci.* 40: 351–362.

LENK, R. 1993. Biophysical Application of NMR. Verlag Dr Kovac, Hamburg.

LING, G.N. 1965. The physical state of water in living cells and model systems. *Annal. N.Y. Acad. Sci.*, 125: 401–405.

LING, G.N. & TUCKER, M. 1980. NMR relaxation and water contents in normal mouse and rat tissues and in cancer cells. *J. Natl. Cancer Inst.*, 64: 1199–1207.

MOUNTFORD, C.E., MACKINNON, W.B., BURNELL, E.E., BLOOM, M. & SMITH, I.C.P. 1984. NMR methods for characterizing the state of the surface of complex mammalian cells. *J. Biochem. Biophys. Methods*, 9: 323–330.

MOUNTFORD, C.E., HOLMES, K.T. & SMITH, I.C.P. 1986. NMR analysis of cancer cells. *Progr. Clinical Biochem. Med.*, 3: 73–112.

MOUNTFORD, C.E., LEAN, C.L., HANCOCK, R. DOWD, S., MACKINNON, W.B., TATTERSALL, M.H.N. & RUSSELL, P. 1993. Magnetic Resonance Spectroscopy detects cancer in draining lymph nodes. *Invasion & Metastasis, Basel*, 13: 57–71.

PACKER, K.J. 1977. The dynamics of water in heterogeneous systems. *Phil. Trans. Roy. Soc. London B*, 278: 59–86.

SINADINOVIC, J., RATKOVIC, S. & KRAINCANIC, M. 1977. Relationship of biochemical and morphological changes in rat thyroid and proton spin relaxation of the tissue water. *Endocrinologie*, 69: 55–66.

SMITH, I.C.P., PRINCZ, E. & SAUNDERS, J.K. 1990. Magnetic resonance spectroscopy in cancer research. *J. Canad. Assoc. Radiol.*, 41: 32–38.

SUGIMOTO, Y., SAITO, H., TABETA, R. & KODAMA, M. 1986. A 2H NMR study on alteration of the membrane organization of mouse B16 melanoma cells treated with 12-0-tetradecanoylphorbol-13-acetate. *J. Biochem.*, 100: 867–874.

SZENT-GYÖRGYI, A. 1957. *Bioenergetics*, Acad. Press, New York.

TURIAN, G. 1994. *Polarity. From Electromagnetic Origins to Biological Take-Over*. Verlag Dr Kovac, Hamburg.

WEISSMAN, I.D., BENNETT, L.H, MAXWELL, L.R., WOODS, M.W. & BURK, D. 1972. Recognition of cancer in vivo by NMR. *Science*, 178: 1288–1290.

WRIGHT, L.C., MAY, G.L., GREGORY, P., DYNE, M., HOLMES, K.T., WILLIAMS, P.G. & MOUNTFORD, C.E. 1988. Inhibition of metastatic potential by fucosidase: An NMR study identifies a cell surface metastasis marker. *J. Cellular Biochem.*, 37: 49–59.

ZANER, K.S. & DAMADIAN, R. 1975. Phosphorus-31 as a nuclear probe for malignant tumors. *Science*, 189: 729–731.