Paramagnetic metalloporphyrins as potential contrast agents in NMR imaging

Chi-wan Chen, Jack S. Cohen, Charles E. Myers and Miriam Sohn

Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205, USA

Received 16 January 1984

Several water-soluble paramagnetic metalloporphyrins were found to increase significantly the relaxation rate ($1/T_1$) of water. Such compounds are known to be selectively retained by tumors in animals, and consequently we identify them as potential contrast agents for tumors in NMR imaging.

Metalloporphyrin *NMR imaging* *Paramagnetic reagent* *NMR contrast agent*

1. INTRODUCTION

Nuclear magnetic resonance (NMR) imaging has become a significant tool in diagnostic medicine [1,2]. One major reason for its preference to X-ray computed axial tomography (CAT) scanning is the superior contrast observed in NMR images between soft tissues. This results largely from the differences in relaxation rates of the bulk water in different tissues, and between normal tissues and tumors [3]. However, the differences in relaxation rates between tissue often do not differ by more than a factor of 2–3 [4], and the spatial definition of a tumor and the distinction between malignant and benign growths in NMR images is still quite limited. Consequently, many paramagnetic reagents are currently being considered as potential contrast agents for NMR imaging [5–7]. Paramagnetic species by virtue of their unpaired electron spins are very efficient relaxation agents for protons. Therefore, if selective absorption of these agents by cancerous tissue could be obtained, then the increase in the relaxation rate of the bulk water would give a greater contrast in the NMR image between the tumor and the surrounding normal tissue. Among the paramagnetic reagents tested have been several 'bare' metal ions [8], which are quite toxic at low concentrations, and whose basis for selective uptake by tissues is uncertain. Various metal ion chelates have also been tried [9].

We have chosen to test water-soluble metalloporphyrins as NMR contrast agents for several reasons. Porphyrins are ubiquitous natural products, and many derivatives of hematoporphyrin (I) are well known to be selectively retained by tumors [10]. Water-soluble porphyrins such as the tetraphenylsulfonyl porphyrins ($TPPS_4$) have also been shown to be localized in tumors [11] and in cells [12]. These porphyrins form complexes with a wide variety of paramagnetic metal ions, and the metalloporphyrins formed from them are stable in vivo. Metalloporphyrins have been used as contrast agents in radiological imaging [11,13], and a great deal is known about their selective retention by different tissues, including tumors, in live animals. Particularly, it was found that the radioactive ^{109}Pd derivative of $TPPS_4$ (II), showed the greatest degree of selective retention in cancerous *vs* normal tissue in mice [14]. Consequently, we have evaluated several of these water-soluble metallo-$TPPS_4$ complexes as potential contrast agents in NMR imaging by measuring their effects on the spin lattice relaxation rate ($1/T_1$) of water.

Published by Elsevier Science Publishers B.V

2. EXPERIMENTAL

2.1. *Materials*

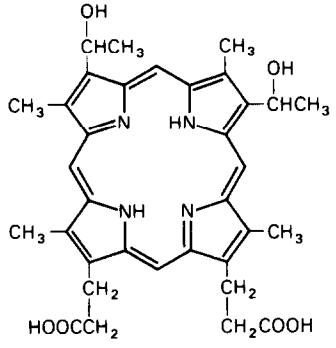
Reagent grade $CuCl_2 \cdot 2H_2O$ (Fisher), $CuSO_4 \cdot 5H_2O$ (Allied), $FeCl_3 \cdot 6H_2O$ (Baker), $MnCl_2 \cdot 4H_2O$ (Baker), and FeEDTA (EDTA, ferric-sodium salt with 1.5 equiv. H_2O; Sigma) were used without further purification. Practical grade $Mn(OAc)_3 \cdot H_2O$ (Fluka) was purified by filtration to remove some insoluble material. Cu(II)TPPS$_4$ (sodium salt, $C_{44}H_{24}N_4CuO_{12}Na_4S_4 \cdot 4HOAc \cdot H_2O$ [III]), Fe(III)TPPS$_4$ (acid form, $C_{44}H_{28}N_4FeO_{12}S_4 \cdot Cl \cdot 2H_2O$ [IV]), and Mn(III)TPPS$_4$ (acid form, $C_{44}H_{28}N_4MnO_{12}S_4 \cdot Cl \cdot 4H_2O$ [V]) were purchased from Porphyrin Products, Logan, UT. Cu(II)TPPS$_4$–CH_3 (sodium salt, $C_{45}H_{27}CuN_4O_{12}Na_4S_4 \cdot CF_3SO_3 \cdot 5H_2O$ [VI]) was supplied by the Drug Synthesis and Chemistry Branch, NCI. All metalloporphyrins were analyzed for C, H, Cl (when applicable), N, S, and the relevant metal, and were found to have chemical compositions consistent with these molecular formulae and solvents of crystallization. The oxidation state of the manganese in Mn(III)TPPS$_4$ was determined from its characteristic UV absorption in water by comparison with the spectrum of the corresponding Mn(II)TPPS$_4$, which was obtained by sodium hydrosulfide reduction of Mn(III)TPPS$_4$ in a sealed container. The absorption maxima for the Mn(III) derivative were 465, 561 and 593 nm, while those for the Mn(II) derivative were 434, 572, and 612 nm.

2.2. *Methods*

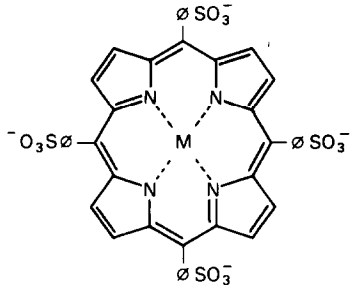
All T_1 and T_2 measurements were performed on an IBM PC-20 series NMR Analyzer (Minispec) at 20 MHz, with a 13 mm probe, and a microprocessor which provides automatic calculation of T_1 or T_2 (in s). All measurements were carried out at 37°C, determined with a copper-constantan thermocouple in a glass sleeve inserted into the sample tube prior to the measurement. The maximum temperature deviation measured was 0.4°C after 3–4 min, the time required for the longest experiment. A standard 10 mm round-bottomed NMR tube was placed inside a 13 mm flat-bottomed tube to reduce the sample volume to 2.0–2.2 ml. Aliquots of concentrated solutions were added stepwise to the sample tube to obtain the concentration of metalloporphyrin desired. In general, the T_1 data were reproducible in duplicate runs to within 1%, and were consistent with the values calculated in a different computer using the same intensity data and a standard equation of the form, $I(\tau) = A + B(\tau/T_1)$, where I is the intensity, τ the delay time between the two pulses, and A and B are constants.

3. RESULTS AND DISCUSSION

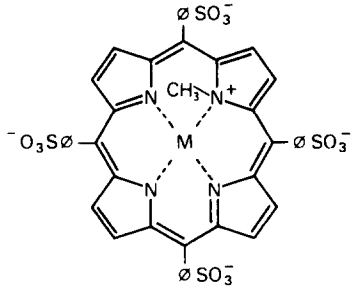
Four different paramagnetic metallo-TPPS$_4$ complexes were evaluated for their effect on the spin lattice relaxation rate ($1/T_1$) of water. The corresponding metal ions (as chloride, sulfate or acetate salts) were also tested for comparison. The results are presented in fig.1. The water relaxation rate increases linearly with concentration for all compounds studied (except for $FeCl_3$ below 1 mM). This was expected since it is known that



I



II M = Pd(II)
III M = Cu(II)
IV M = Fe(III)
V M = Mn(III)



VI M = Cu(II)

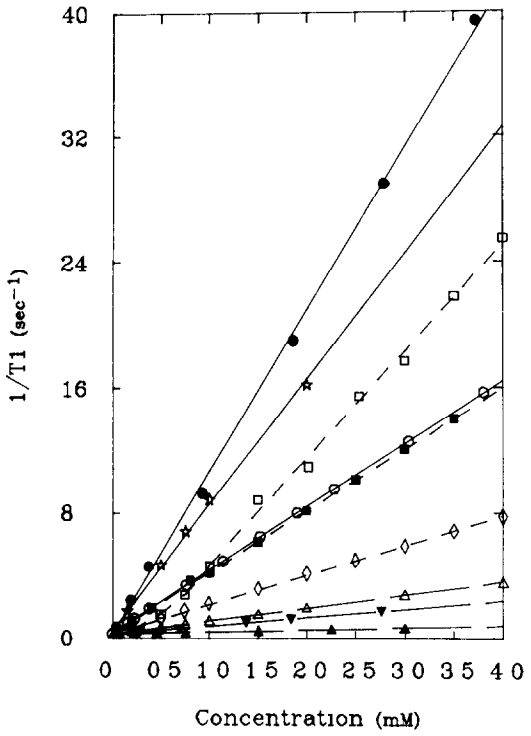


Fig.1. Spin lattice relaxation rate ($1/T_1$) of water as a function of concentration of metal ion or metallo-$TPPS_4$ complex. Lines are least-square fits: (——) Mn, (---) Fe, (– – –) Cu. Symbols are for experimental data: (●) Mn(III)$TPPS_4$, (○) $Mn(OAc)_3$, (☆) $MnCl_2$, (■) Fe(III)$TPPS_4$, (□) $FeCl_3$, (◇) FeEDTA, (▲) Cu(II)$TPPS_4$, (△) $CuCl_2$ or $CuSO_4$, (▼) Cu(II)$TPPS_4$–CH_3.

the water relaxation rate is proportional to the concentrations of paramagnetic ions present in solution [15], according to the equation,

$$(1/T_1) = 12\pi\gamma^2\eta\mu^2N/5kT$$

where γ is the gyromagnetic ratio of protons, η the solvent viscosity, μ the effective magnetic moment of the paramagnetic agent, k Boltzmann's constant, T the absolute temperature, and N the number of paramagnetic ions per unit volume. The slopes obtained by linear least-square fitting are given in table 1. The paramagnetic effect falls off rapidly with distance, i.e., $(1/T_1) \propto 1/r^6$, where r is the mean distance from the paramagnetic center to the water protons. Thus, an effective paramagnetic contrast agent should have a metal ion in a high-spin state (large magnetic moment) and have facile access to coordination sites by water molecules.

Among the 4 metallo-$TPPS_4$ complexes investigated Mn(III)$TPPS_4$ had the greatest effect, increasing the water relaxation rate with a slope of 10.4 $(mM \cdot s)^{-1}$, and Cu(II)$TPPS_4$ had the smallest effect with a slope of 0.14 $(mM \cdot s)^{-1}$. This large difference can be attributed to the fact that Mn(III) has 4 unpaired electron spins, while Cu(II) has only one unpaired spin, and C(II) porphyrins do not readily coordinate axial ligands. Cu(II)$TPPS_4$–CH_3, an *N*-methyl-substituted derivative [16], was more effective (slope 0.54 $(mM \cdot s)^{-1}$) than the non-methylated material in increasing the water relaxation rate, apparently due to its non-planar geometry affecting the electronic structure allowing binding of axial water ligands to the metal site. Although Fe(III)$TPPS_4$ with 5 unpaired electron spins was less effective (slope 3.9 $(mM \cdot s)^{-1}$) than Mn(III)$TPPS_4$, it was more than twice as effective in increasing the water relaxation rate than FeEDTA (slope 1.9 $(mM \cdot s)^{-1}$), which has been tested as a potential contrast agent in NMR imaging of rabbits [9]. This clearly shows the advantages in terms of relative efficacy of paramagnetic agents of having relatively labile axial ligands such as water in metalloporphyrins compared to the EDTA chelating functional

Table 1

The effect of metallo-$TPPS_4$ complexes on water relaxation rate $(1/T_1)$[a]

Metal ion or complex	Slope $(mM \cdot s)^{-1}$
Mn(III)$TPPS_4$	10.36 ± 0.09
$Mn(OAc)_3$	4.04 ± 0.01
$MnCl_2$	8 03 ± 0.12
Fe(III)$TPPS_4$	3.91 ± 0.02
$FeCl_3$[b]	6.82 ± 0.20
FeEDTA	1.87 ± 0.01
Cu(II)$TPPS_4$	0.139 ± 0.004
Cu(II)$TPPS_4CH_3$	0.538 ± 0.006
$CuCl_2$ or $CuSO_4$	0 836 ± 0.004

[a] Data from fig.1; $(1/T_1)$ intercepts were from 0.26–0.51, while the value for deionized water at 37°C was 0.26 s^{-1}

[b] The linear fit applies only when concentration is higher than 1.0 mM

groups. Both Cu(II)- and Fe(III)$TPPS_4$ were less effective than their corresponding free metal ions in increasing the water relaxation rate. By contrast, Mn(III)$TPPS_4$ had a greater effect than its corresponding free metal salt. It should be noted that free Mn(III) salts are relatively unstable in solution. However, Mn(III)$TPPS_4$ is completely stable while Mn(II)$TPPS_4$ is readily oxidized. Mn(III)$TPPS_4$ also had a greater effect than $Mn(II)Cl_2$, which has 5 unpaired electron spins. These differences depend upon the rate and the extent of ligand water exchange, and on the degree of delocalization of the unpaired electron spin in the porphyrin ring system.

To test for the effect of another ligand upon the water relaxation rates we carried out parallel experiments adding Mn(III)$TPPS_4$ and Fe(III)$TPPS_4$ to a solution of 0.2 M pyridine in water. The slopes of plots of $(1/T_1)$ *vs* concentration of complex obtained in these experiments were 7.6 and 0.2 $(mM \cdot s)^{-1}$, respectively. In other words the effectiveness of the Fe(III) complex is reduced by a factor of 20 while that of the Mn(III) complex is only reduced by about 25% by competition of pyridine for water. It has also been shown that the presence of pyridine in water significantly reduces the proportion of the high-spin form of Fe(III)-protoporphyrin [17]. A similar phenomenon may explain the dramatic reduction in the water relaxation rate due to Fe(III)$TPPS_4$ in the presence of pyridine.

The T_2 relaxation time of aqueous Mn(III)$TPPS_4$ was also measured as a function of concentration. It was found that the T_2 relaxation rate of water also increased linearly with concentration with a slope of 12.6 $(mM \cdot s)^{-1}$. In view of the shorter values of T_2 and the consequent greater scatter in the data, these values were not determined in the other cases.

From the above results it is apparent that several of the soluble metalloporphyrins, notably Mn(III)$TPPS_4$, show great potential as contrast agents in NMR imaging. However, aqueous solution is not the same as the mileau of the cell, and the relative differences in the amount of free water and in the intrinsic relaxation times of water in given tissues, will affect the efficacy of these agents in vivo. In addition, potential coordinating ligands other than water may also significantly reduce their effectiveness in vivo. This may not be a disadvantage since there may be different compartments with different coordinating ligands in different tissues such that the effectiveness of the paramagnetic contrast agents may vary although their concentrations may be the same. These considerations are in addition to the degree of selectivity of retention of metalloporphyrins in different tissues, notably cancerous tissue. By contrast, the order of magnitude of the effect of radiopharmaceuticals is solely dependent on their degree of localization, and not on the coordination chemistry of the metal. We are preparing to analyze the pharmacokinetics of metalloporphyrins, as well as to observe directly their effect on NMR images of live animals.

REFERENCES

[1] Margulis, A.R., Higgins, C.B., Kaufman, L. and Crooks, L.E. (1983) Clinical Magnetic Resonance Imaging, University of California, San Francisco.

[2] Cohen, J.S., Knop, R.H., Navon, G. and Foxall, D.H. (1983) NMR in Biology and Medicine, Life Chemistry Reports, Harwood Academic Press, London, pp.281–457.

[3] Straughan, K., Spencer, D.H. and Bydder, G.M. (1983) in: Nuclear Magnetic Resonance Imaging (Partain, C.L. et al. pp.195–206, Saunders, New York.

[4] Hutchinson, J.M.S. and Smith, F.W. (1983) in: Nuclear Magnetic Resonance Imaging (Partain, C.L. et al. eds) pp.231–249, Saunders, New York.

[5] Brasch, R.C. (1983) Radiology 147, 781–788.

[6] Proc. 2nd Conf. Soc. Mag. Res. Med. (1983) San Francisco.

[7] Runge, V.M., Stewart, R.G., Clanton, J.A., Jones, M.M., Lukehart, C.M., Partain, C.L. and James, A.E. jr (1983) Radiology 147, 789–791.

[8] Mendoca, D.M.H., Lauterbur, P.C. and Rudin, A.M. (1982) Proc. First Conf. Mag. Res. Med., Boston, pp.103–104.

[9] Carr, D.H., Brown, J., Pennock, J.M., Gadian, D.G., Young, I.R. and Steiner, R.E. (1983) Proc. Second Conf. Soc. Mag. Res. Med., pp.79–80.

[10] Kassel, D. and Chou, T.-H. (1983) Cancer Res. 43, 1994–1999.

[11] Zanelli, G.D. and Kaelin, A.C. (1981) Br. J. Radiol. 54, 403–407.

[12] Austen, J.D., McConnel, S., Carrano, C.J. and Minoru, T. (1978) Cancer Treat. Rep. 62, 511–518.

[13] Winkelmann, J., Slater, G. and Grossman, J. (1967) Cancer Res. 27, 2060–2064.

[14] Hambright, P (1975) Bioinorg. Chem. 5, 87–92.
[15] Bloembergen, N., Purcell, E.M. and Pound, R.V. (1948) Phys. Rev. 73, 679–712.
[16] Doi, J.D , Lavallee, D.K., Srivastava, S.C., Prach, T., Richards, P. and Fawwaz, R.A. (1981) Int J. Appl. Rad. Isotopes 32, 877–880.
[17] Degani, H.A. and Fiat, D. (1971) J. Am. Chem. Soc. 93, 4281–4289.