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Application of ^{1}H and ^{23}Na magic angle spinning NMR spectroscopy to define the HRBC up-taking of MRI contrast agents

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Abstract

The up-take of Gd(III) complexes of BOPTA, DTPA, DOTA, EDTP, HPDO3A, and DOTP in HRBC has been evaluated by measuring the lanthanide induced shift (LIS) produced by the corresponding dysprosium complexes (DC) on the MAS-NMR resonances of water protons and free sodium ions. These complexes are important in their use as MRI contrast agents (MRI-CA) in diagnostics. ^{1}H and ^{23}Na MAS-NMR spectra of HRBC suspension, collected at 9.395 T, show only one signal due to extra- and intra-cellular water (or sodium). In MAS spectra, the presence of DC in a cellular compartment produces the LIS of only the nuclei (water proton or sodium) in that cellular compartment and this LIS can be related to the DC concentrations (by the experimental curves of LIS vs. DC concentrations) collected in the physiological solution. To obtain correct results about LIS, the use of MAS technique is mandatory, because it guarantees the only the nuclei staying in the same cellular compartment where the LC is present show the LIS. In all the cases considered, the addition of the DC to HRBC (100% hematocrit) produced a shift of only the extra-cellular water (or sodium) signal and the gradient of concentration (G_C) between extra- and intra-cellular compartments resulted greater than 100:1, when calculated by means of sodium signals. These high values of G_C are direct proofs that none of the tested dysprosium complexes crosses the HRBC membrane. Since the DC are iso-structural to the gadolinium complexes the corresponding gadolinium ones (MRI-CA) do not cross the HRBC membrane and, consequently, they are not up-taken in HRBC. The G_C values calculated by means of water proton signals resulted much lower than those obtained by sodium signals. This proves that the choice of the isotope is a crucial step in order to use this method in the best way. In fact, G_C value depends on the lowest detectable LIS which, in turn, depends on the nature of the LC (lanthanide complex) and the observed isotopes.

Keywords: Cell membrane; Magic angle spinning; MRI; Contrast agents; Water; Sodium; NMR; Organ-selectivity

1. Introduction

Organ-selective contrast agents (CAs), able to exhibit different cellular up-take depending on the nature and pathological state of the cells, represent one of the most relevant goals of research in the field of clinical magnetic resonance imaging (MRI). These MRI-CAs, in fact, could mean lower dosages and a greater differentiation of disparate pathologies.

A relevant aspect of the developmental stages of these MRI-CAs concerns the cellular up-take data collected on in vitro samples. Regarding this topic, a new method for determining the concentration of gadolinium-based MRI-CAs in the intra- and extra-cellular compartments of human red and white blood cells (HRBC and HWBC) has recently been reported [1]. It is based on the proton high resolution magic angle spinning (^{1}H HR-MAS) NMR spectroscopy and on the utilisation of lanthanide complexes (LCs), iso-structural to the CAs but able to produce a clearly detectable lanthanide induced shift (LIS) and a very weak relaxation effect (line broadening) on water proton signals.

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Employing MAS technique, the shifts due to the chemical shift anisotropy (CSA), the dipole–dipole interactions and the anisotropic component of bulk magnetic susceptibility (BMS) [2–5] are cancelled. On the contrary, the shifts due to the isotropic term of BMS, the formation of the complex, the hyperfine contact shift, and the hyperfine dipolar shift remain active. All this produces two relevant effects on the proton spectra of cellular samples: (i) the resulting water signals are much more sharp then those obtained by standard probes for liquid samples [6]; (ii) only the nuclei staying in the same cellular compartment where the LC is present show the LIS.

The utilisation of probes for HR-MAS further narrows the lineshape. In this state, the intra- and extra-cellular water proton signals of HRBC and HWBC are distinct and their chemical shifts can be directly correlated to the concentration of the LC in the two cellular compartments. Gd-complexes could be directly used instead of the corresponding iso-structural LC but, as proved [1], the LIS is much easier to measure than the line broadening, especially when a partial CA penetration in an intra-cellular compartment entails the broadening of both intra- and extra-cellular water signals.

The method described above requires very soft sample treatments, the LIS can be directly correlated to the concentration of LC and the measurements are very fast and easy to make. It is to be noted that the other methods currently in use supply data whose reliability is often uncertain because of the heavy treatment of the sample and/or because the measured parameter is indirectly linked to cellular up-take [1]. In particular, most of these methods require a rigorous separation of cells from extra-cellular fluids which can modify the concentrations of the species at equilibrium and cause the breaking of part of the cells, with consequent perfusion in the extra-cellular compartment of part of the intracellular fluid.

An important aspect of the method, described above, is the minimum concentration of LC (ρ_{min}) measurable by the LIS, because it fixes the sensitivity threshold of the method. The sensitivity threshold depends on the minimum detectable LIS (LIS_{MIN}) which, in turn, is a function of: the linewidth of the signals used as probes; the availability of two separate signals for intra- and extra-cellular water protons in absence of the LC; the nature of the LC; the nature of the chemical species used as probe. The last two aspects determine the LIS magnitude vs. the LC concentration. In principle, the NMR signals of any substance distributed between the intra- and the extra-cellular compartments can be used as probe if the signals are affected by the LIS in presence of a LC, but their real utility depends on the lowest detectable LIS the signals permit.

In the present study, the method based on ^{1}H HR-MAS NMR spectroscopy has been further investigated, with the aim to recognise a more general behaviour of the MRI-CA towards HRBC and to investigate thoroughly how the parameters determining the sensitivity threshold of the method can act. For this purpose, the following MRI-CAs were considered: Gd-BOPTA, Gd-DTPA, Gd-DOTA, Gd-EDTP, Gd-DOTP, and Gd-HPDO3A.[1] These Gd-complexes cover a rather wide range of MRI-CAs because Gd-BOPTA, Gd-DTPA, Gd-DOTA, Gd-EDTP, and Gd-DOTP are ionic (negatively charged) while Gd-HPDO3A is neutral. Moreover, Gd-BOPTA, Gd-DTPA, and Gd-EDTP have linear ligands while Gd-DOTA, Gd-DOTP, and Gd-HPDO3A have cyclic ones. In all cases, the corresponding iso-structural dysprosium complexes (as methylglucamine salts—meg$^+$—with the exception of HPDO3A, which is neutral) were employed, because LIS is much easier to measure than the line broadening. Dy(III) was preferred with respect to other lanthanides because, taking into consideration the ligands used, it produces the greatest LIS. Iso-structural relationship between Dy(III) and Gd(III) complexes has been previously established.

For the purposes of the present study, the LIS of two different isotopes were observed: proton and sodium. In particular, ^{23}Na NMR spectra were employed because, as will be proven herewith, some LC produce a ^{23}Na LIS which is more intense then the ^{1}H LIS, allowing lower detection limits of the cellular up-take, which is a crucial aspect of the cellular up-take measurements, independently of the utilised method.

The investigation has also concerned the MAS probe. Since some commercially available HR-MAS probes do not allow a broad band (BB) detection and BB-CP-MAS probes are more frequently present in NMR Labs than HR-MAS probes, in the present study a BB-CP-MAS probe without lock channel was used to recognise the limits, if they indeed exist, due to the use of these probes instead of HR-MAS ones.

HRBC were chosen in this study because of their importance in MRI applications—mainly MRI-angiography [7]—and the simplicity in handling them during the tuning of the method.

2. Experimental

Centrifugation. The employed centrifuge was HERAEUS SEPATECH OMNIFUGE 2 ORS, rotor

[1] BOPTA, (4*RS*)-[4-carboxy-5,8,11-tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecan-13-oic acid]; DTPA, diethylenetriamine pentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; EDTP, ethylenediamino-*N*,*N*′-tetra(methylenephosphonic)acid; DOTP, 1,4,7,10-tetraazacyclo dodecane-*N*,*N*″,*N*″,*N*‴-tetrakis(methylenephosphonic)acid; HPDO3A, 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1.4.7-triacetic acid; methylglucamine, 1-deoxy-1-(methylamino)-D-glucitol.

model 3360; centrifugation was done at 2109*g* (equivalent to 3500 rpm) at 4 °C for 15 min.

Living HRBC preparation. Human blood (to which sodium citrate as an anticoagulant is added) was centrifuged. After that, HRBC pellets were separated from serum and white cell interface, being careful to obtain a solution of red cells free of white cells; accordingly, red cells with 100% hematocrit were obtained.

Lanthanide complexes. Lanthanide complexes were prepared according to [13] and related patents.

Samples containing lanthanide complexes. All the samples were obtained by adding of definite amounts of 0.1 M stock solution of lanthanide complexes to 1 ml of HRBC sample, prepared as previously described. All the concentrations reported in the text refer to the whole sample (i.e., a volume equal to 1ml plus the micro-liters of added stock solution).

Calculation of the concentration gradient. The concentration gradient G_C of LC between extra- and intracellular compartments can be calculated by the following equation:

$$G_C = \frac{\left(V_A M - V_I \rho_{MN}^{HRBC}\right)}{(V_A + V_E)} \times \frac{1000}{\rho_{MN}^{HRBC}}, \quad (1)$$

where: V_A, μl of the added lanthanide complex; V_E, μl of *extra*-cellular fluid; V_I, volume of *intra*-cellular fluid; and M, molarity of added LC stock solution.

Since the hematocrit was 100%, V_E is fixed equal to zero. Example: if $\rho_{MIN}^{HRBC} = 2$ mM, $V_A = 320$ μl, $M = 0.1$ then added lanthanide complex = 320 μl × 0.1 M = 32 μmol; lanthanide complex in the *intra*-cellular = 2 mM × 1 ml = 2 μmol; lanthanide complex in the *extra*-cellular = 32 μmol − 2 μmol = 30 μmol; concentration of lanthanide complex in the *extra*-cellular = 30 μmol/320 μl = 0.0935 M; concentration gradient: 93.5 mM/2 mM = 46.9:1.

NMR spectra. ^{1}H and ^{23}Na MAS NMR spectra were acquired on a Bruker AMX 400 WB spectrometer operating at the frequency, respectively, of 400.13 and 105.84 MHz. A commercially available multinuclear double bearing CP-MAS probehead (without lock channel) were employed with a 4 mm rotor with an inner 2.5 mm diameter spherical chamber. Experimental conditions were: ^{1}H spectra) spectral width = 20,000 Hz (ca. 50 ppm); time domain size = 32K data points; number of scans = 32; pulse length = 6 μs; recycle delay = 1 s; spinning rate = 3500 Hz; Fourier Transform without application of window functions on the FID; ^{23}Na spectra spectral width = 16,000 Hz (ca. 150 ppm); time domain size = 16K data points; number of scans = 1024; pulse length = 15 μs; recycle delay = 1 s; spinning rate = 3500 Hz; Fourier Transform after application of 10 Hz line broadening enhancement multiplication on FID. All spectra were acquired at 25 °C.

3. Results

Fig. 1 (trace a) shows the region of 7 ppm around the ^{23}Na MAS NMR signal of packed HRBC (100% hematocrit). Only one resonance appears: being the intra- and extra-cellular ^{23}Na isochronous resonances. Fig. 1 (traces b–d) shows the same sample of trace a, after the addition of 80, 160, and 320 μl (respectively) of Dy-BOPTA 0.1 M stock solution to 1 ml of packed HRBC. Two signals appear: one at the same chemical shift as trace a, due to the intra-cellular sodium (Na_i); and the other one at higher field, due to the extra-cellular sodium (Na_o). The latter increases its shift as the amount of Dy-BOPTA increases. The addition of Dy-BOPTA also modifies the area of the Na_o signal towards the Na_i area but the sum of the areas of the two signals does not change with respect to that shown in trace a. This is due to a sodium ions crossing through the cellular membrane from the intra- to the extra-cellular compartment, that equilibrates the Na_o and Na_i concentration (the Dy-BOPTA stock solution does not contain sodium ions). This equilibrium was reached in the few minutes which elapse between the LC stock solution addition and the spectrum acquisition. Moreover, LISs did not

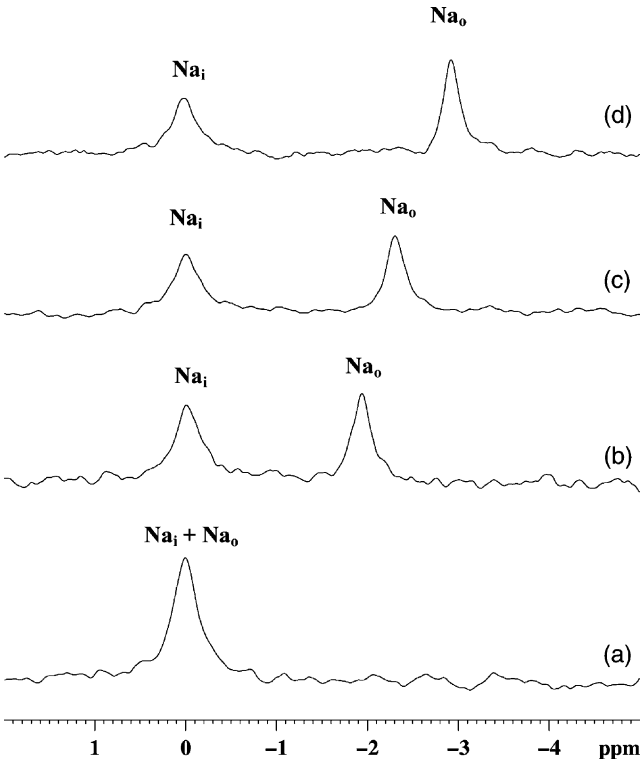

Fig. 1. (a) The 105.84 MHz ^{23}Na MAS NMR spectra of packed human blood cell suspension in serum (100% hematocrit); (b) Same sample of trace (a) but after the addition of 80 μl of Dy-BOPTA 0.1 M stock solution; (c) Same sample as (a), but after the addition of 160 μl of Dy-BOPTA 0.1 M stock solution; (d) Same sample as (a), but after the addition of 320 μl of Dy-BOPTA 0.1 M stock solution.

change in time, as tests performed several hours after the first spectrum proved.

Fig. 2 shows the ^{1}H MAS NMR spectra of the same HRBC sample used for ^{23}Na NMR spectra in the region of about 3 ppm around the water signal. The behaviour is similar to that observed for sodium spectra. The intracellular water (W_i) signal does not shift, while the extracellular water (W_o) signal increases its shift toward high field as the amount of Dy-BOPTA is increased. The increase of the W_o signal area with respect to the W_i signal area is a result of the increment of water, due to the addition of the Dy-BOPTA stock solution. The same behaviour was observed for all the other tested Dy-complexes, i.e., the LISs of Na_i and W_i signal was always zero.

Figs. 3 and 4 show, respectively, the observed sodium and water proton sodium LIS vs. the concentration ρ (expressed as mM) of the employed Dy-complexes dissolved in physiological solution (H_2O/NaCl 0.9%). These graphs allow to directly link the observed LIS in cells to the ρ in the different cellular compartments.

The ^{1}H and ^{23}Na MAS NMR spectra of packed HRBC (100% hematocrit), plotted three times by applying a shift of 5 Hz between adjacent traces (data not shown), reveal that the minimum detectable shift (LIS_{MIN}) is lower than 5 Hz for both nuclei. Therefore, a value of 5 Hz can be fixed as the LIS_{MIN} in HRBC. Using data reported in Figs. 3 and 4, it is possible to calculate the concentration of Dy-complex (ρ_{MIN}) corresponding to the LIS_{MIN} for all the tested Dy-complexes. These data are reported in Table 1.

The LIS depends on the stoichiometric molar ratio ρ^* between LC and the chemical species which undergoes the shift. For water, ρ and ρ^* have equivalent meaning and equal value; but for sodium, ρ and ρ^* are different. The ρ^* values ([LC]/[Na^+]) which correspond to the ρ_{MIN} values can be calculated considering the sodium concentration is 154 mM in physiological solution. Results are reported in Table 2, as ρ^*_{MIN}.

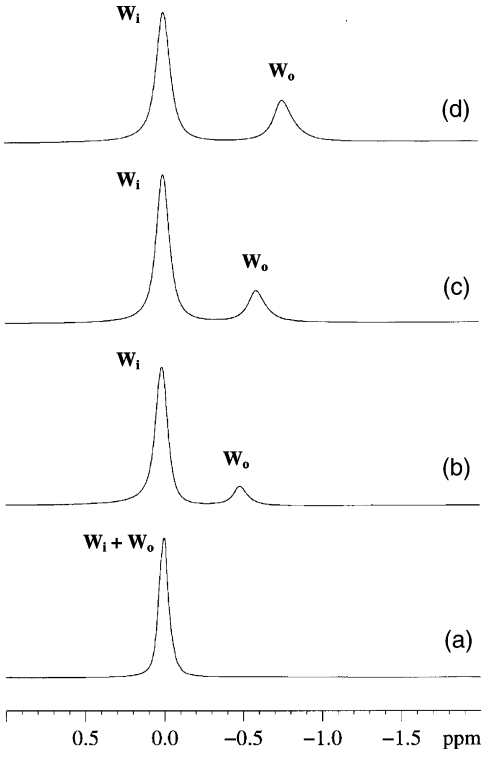

Fig. 2. (a) The 400.13 MHz ^{1}H MAS NMR spectra of packed human blood cell suspension in serum (100% hematocrit); (b) Same sample of trace (a) but after the addition of 80 μl of Dy-BOPTA 0.1 M stock solution; (c) Same sample as (a), but after the addition of 160 μl of Dy-BOPTA 0.1 M stock solution; (d) Same sample as (a), but after the addition of 320 μl of Dy-BOPTA 0.1 M stock solution.

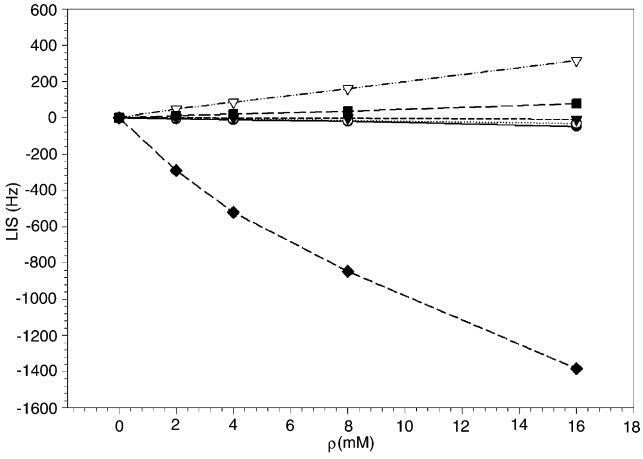

Fig. 3. Plots of observed LIS in physiological solution of sodium signal vs. concentration ρ (mM) of added paramagnetic complex. Spectra were acquired at 105.84 MHz, using MAS technique. (●) Dy-BOPTA, (○) Dy-DTPA, (▼) Dy-DOTA, (▽) Dy-EDTP, (■) Dy-HPDO3A, and (□) Dy-DOTP.

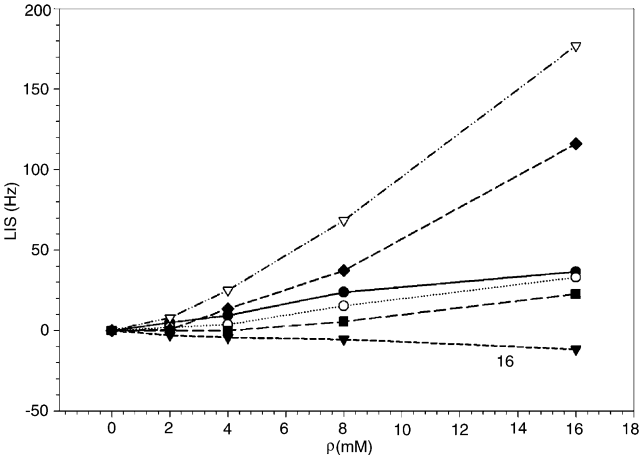

Fig. 4. Plots of observed LIS in physiological solution of water proton signal vs. concentration ρ (mM) of added paramagnetic complex. Spectra were acquired at 400.13 MHz, using MAS technique. (●) Dy-BOPTA, (○) Dy-DTPA, (▼) Dy-DOTA, (▽) Dy-EDTP, (■) Dy-HPDO3A, and (□) Dy-DOTP.

Table 1
Minimum detectable concentration ρ (mM) and lowest stoichiometric molar ratio ρ^*_{MIN} of added dysprosium complex in physiological solution, relative to water proton and sodium signals, calculated from lowest LIS in MAS NMR spectra

	ρ_{MIN} (mM)		ρ^*_{MIN}	
	^{1}H water signal	$^{23}Na^+$ signal	^{1}H water signal	$^{23}Na^+$ signal
Dy-BOPTA	2	2	2	0.013
Dy- DTPA	6	4	6	0.026
Dy-DOTA	8	12	8	0.078
Dy-EDTP	2	<0.5	2	<0.003
Dy-HPDO3A	8	1	8	0.006
Dy-DOTP	3	<0.5	3	<0.003

Table 2
Minimum concentration ρ^{HRBC}_{MIN} (mM) of added dysprosium complex, relative to water proton and sodium signals, detectable from MAS NMR spectra of HRBC (100% hematocrit)

	ρ^{HRBC}_{MIN} (mM)	
	^{1}H water signal	$^{23}Na^+$ signal
Dy-BOPTA	2.0	0.29
Dy- DTPA	6.0	0.67
Dy-DOTA	8.0	1.71
Dy-EDTP	2.0	<0.07
Dy-HPDO3A	8.0	0.14
Dy-DOTP	3.0	<0.07

Values reported in Table 1 refer to chemical shifts collected in physiological solution considering the LIS_{MIN} in HRBC. Previously [1] it was proven that the water proton LIS in the extra- and intra-HRBC compartments and in physiological solution is very similar, especially at low LC concentrations. Consequently, the reported values in Table 1 can also be considered correct for water proton in the extra- and intra-HRBC compartments. All this should be reasonably true for the sodium ion as well. With regard to sodium ion, the concentration difference between physiological solution and intra-cellular HRBC compartment must be considered. Values of the HRBC intra-cellular sodium concentrations in the range of 15 and 22 mM are reported in literature [8]. Considering a value of 22 mM, the overestimated minimum concentration of detectable LC (ρ_{MIN}) for HRBC ^{23}Na spectra (indicated as ρ^{HRBC}_{MIN}, in Table 2) can be calculated from the corresponding ρ^*_{MIN} values. Literature also reports proofs of the complete visibility of all the sodium in RBC [9], which is a necessary requirement for this calculation. For water proton signal, $\rho^{HRBC}_{MIN} = \rho^*_{MIN}$.

In absence of a detectable LIS of the intra-cellular water proton and sodium signals, the ρ^{HRBC}_{MIN} values can be considered as the highest possible concentrations of LC in the intra-HRBC compartment. Assuming the intra-cellular fluid volume is 1 ml (100% hematocrit), the underestimated concentration gradient (G_C), that is the ratio between the LC concentration in the extra- and intra-cellular compartment, can be easily calculated (see Section 2). Results are reported in Table 3.

Table 3
Gradient of concentration G_C between extra- and intra-cellular compartments of HRBC, calculated by using 0.1 and 0.2 M

	Gradient of concentration	
	^{1}H water signal	$^{23}Na^+$ signal
Dy-BOPTA	46.9:1	341.7:1
Dy- DTPA	13.5:1	146.1:1
Dy-DOTA	9.4:1	113.8:1$^{(*)}$
Dy-EDTP	46.9:1	>1425:1
Dy-HPDO3A	9.4:1	711.2:1
Dy-DOTP	30.2:1	>1425:1

$^{(*)}$Dy-complex stock solutions.

4. Discussion

Spectra reported in Figs. 1 and 2 furnish a clear picture of the method, which is based on the discrimination and identification of extra- and intra-cellular NMR signals and on the measurement of the LIS. The LIS is directly linked to permeability, therefore the method supplies direct information about the cellular membrane permeability towards LCs.

For all the Dy-complexes tested herewith, the LIS of intra-cellular signals was found to be zero, thus concluding that none of the tested Dy-complexes was able to penetrate the HRBC membrane. In order to have reliable results, the sensitivity of the measurement (i.e., the minimum detectable concentration ρ^{HRBC}_{MIN}) and the values of G_C must be considered. Moreover, it is necessary to fix a G_C value, over which, the membrane could be considered not permeated. This value has been fixed at 100:1 because, in case of permeability, a much lower value is expected [1].

The G_C values in Table 3 are underestimated because ρ^{HRBC}_{MIN} values are an overestimation of the intra-cellular Dy-complexes concentrations. Moreover, after the addition of the Dy-complex stock solution, $^{23}Na_i$ partially crosses the cellular membrane to equilibrate the intra- and extra-cellular sodium concentration (Fig. 1). This means that the Na_i concentration decreases with respect to the original one. Consequently, the real sodium ρ^{HRBC}_{MIN} values, which were obtained considering a 22 mM intra-cellular sodium concentration, are lower than those reported in Table 3. This determines a further underestimation of the calculated G_C.

Water G_C values reported in Table 3 are lower than 100:1. Although G_C are underestimated, these values are too small to establish the condition of non-permeability. In particular, the G_C values of Dy-DOTA and Dy-HPDO3A are equal to 9.4, a value which could lead to a

reasonable hypothesis of partial penetration of these complexes inside the HRBC. These results seem to be in contradiction with the absence of a shift of the intracellular water proton resonance (as observed by the spectra) but, as discussed above, considering LIS_{MIN}, and consequently ρ_{MIN}^{HRBC} and G_C is necessary to obtain reliable results.

Further proofs would require the use of more concentrated Dy-complexes stock solutions, to increase the Dy-complex concentration in the extra-cellular compartment and the value of G_C. Especially for Dy-DOTA and Dy- HPDO3A, the stock solutions should have concentrations of 0.825 M to reach a G_C of 100:1. In general high concentrations would not be compatible with the LC solubility and could cause significant alterations of the extra-cellular fluid and, eventually, destruction of the HRBC integrity. It is, therefore, better to use LC at low concentration. In a previous paper [1] we reported G_C of 197:1 for Dy-BOPTA instead of 46.9:1, as it is reported in Table 3, despite the same experimental conditions, i.e., 320 μl of 0.1 M stock solution and 1 ml of HRBC (100% hematocrit). This significant difference is a result of the different value of ρ_{MIN}^{HRBC} which is 2 mM in this study (Table 2) and 0.5 mM in the previous one. This difference is due to the different linewidths obtained by CP-BB-MAS probe in the present paper and by ^{1}HR-MAS probe in the previous one resulting in a minimum detectable LIS of only 2 Hz.

All the sodium G_C values are higher than 100:1, except the G_C corresponding to Dy-DOTA whose value is 55.4:1. For this complex, measurements were repeated by using 320 μl of stock solution 0.2 M. In this experimental condition no shift of the intra-cellular sodium resonance was detected and the resulting G_C was 113.8:1.

These results demonstrate that all the Dy-complexes considered in this paper do not cross the cellular membrane of HRBC and, due to iso-structural relationships, this is also true for the corresponding gadolinium complexes. This confirms results already obtained for BOPTA, DTPA, and DOTA complexes, by means of proton NMR data [1], and supplies new information about the features of EDTP, HPDO3A, and DOTP complexes. Moreover, this confirms results previously obtained on the base of imaging data (indirectly linked to cellular up-take) and by traditional chemical tests, which require a strong manipulation of the sample. The advantage of the method here presented is evident because data are quantitative, a sensitivity threshold can be evaluated, the measured parameters (peak areas) are directly linked to cellular up-take, and sample does not undergo to manipulations can modify the concentrations of the species at equilibrium. Moreover, measurements are easy and fast.

Data reported herewith also confirm the feasibility of the method, already demonstrated in a previous paper [1], and highlight some other aspects. Firstly, the difference between G_C values obtained by using water proton as opposed to sodium resonances, is noticeable. Determining the HRBC permeability on the basis of only proton spectra should have been very difficult: it should have required the use of stock solutions at a very high concentration (about 0.8 M in some cases), which could generate problems regarding the reliability of the results for reasons discussed above. This illustrates the strong dependence of the sensitivity of the method, i.e., ρ_{MIN}^{HRBC}, on the observed chemical species and the usefullness of employing isotopes different from proton: all the sodium ρ_{MIN}^{HRBC} resulted greater than proton ρ_{MIN}^{HRBC}. In addition, the complexes with the higher negative charge, as Dy-DOTP and Dy-EDTP, show the lowest values of ρ_{MIN}^{HRBC}, in agreement with the stronger electrostatic interaction with sodium ions. Secondly, the strong dependence of ρ_{MIN}^{HRBC} on the probes utilised for measurements, as demonstrated by comparing ρ_{MIN}^{HRBC} relative to water proton resonance of Dy-BOPTA, is apparent. The advantages of HR-MAS probes with respect to BB-CP-MAS probes is evident, especially in the cases which can be studied only by the proton spectra. All this suggests the need to use HR-MAS technique, affording the possibility to observe nuclei on a large range of frequency (i.e., broad band HR-MAS probe).

Lastly, it is to be noted that, in principle, standard probes for liquids (and cylindrical tubes) could be used by calculating the BMS contribution but, in our opinion, the procedures proposed for this aim [10–12] are complex and not completely satisfying, especially when small shifts must be detected. Spherical tubes could also be used but, in our experience, lineshape is not sharp enough to allow the distinction between the signals belonging to intra- and extra-cellular water.

5. Conclusion

The results here reported permit to assert that gadolinium complexes of BOPTA, DTPA, DOTA, DTPA, EDTP, HPDO3A, and DOTP do not cross the cellular membrane of HRBC and, consequently, their cellular up-take in these cells does not take place. This confirms prevoiusly obtained results for BOPTA, DTPA, and DOTA lanthanide complexes [1] and supplies new information about analogous complexes with EDTP, HPDO3A, and DOTP.

The feasibility of the method for the quantitative determination of the cellular up-taking of MRI-CAs has also been confirmed and proofs about the high sensitivity of the measurements have been given.

All our results confirm that this method can be a useful "tool" for the study of the organ-selectivity mechanism of the MRI-CAs and can be an alternative to the methods currently used, seeing as it seems to be

applicable to a wide typology of cells and tissues because of the ubiquity of water and sodium in living systems. Moreover, the measurements are easy, fast and do not require cell treatment, an aspect that cuts off any possible cause for invalidation of the results due to an alteration of the sample.

Regarding the feasibility of the measurements and the possibility of studying the permeability of a large number of cells by various MRI-CA, further studies are in progress on other types of cells and MRI-CA.

Acknowledgments

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