Spectroscopy of Intracellularly Located ^{133}Cs Has Been Used to Monitor the Uptake of the Isolated Rat Liver

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MR spectroscopy of intracellularly located ^{133}Cs has been used to monitor the uptake of Gd-EOB-DTPA by the isolated rat liver. As shown by ^{31}P spectroscopy, accumulation of ^{133}Cs ions in hepatocytes does not produce detectable effects on the metabolism. The hepatic internalization of Gd-EOB-DTPA was followed by the paramagnetic relaxation enhancement of the intracellular ^{133}Cs ions, and confirmed by parallel quantitations of Gd and Cs run by inductively coupled plasma analysis of liver samples and aliquots of perfusate. Two peaks are observed at -22.0 and -23.5 ppm, with respect to the line of the external reference arbitarily set to 0 ppm. Upon rinsing of the extracellular compartment with regular K-H free of CsCl, the high-field resonance disappears within 20 min. The intracellular concentration was confirmed by ICP, which gives a Cs^+ content of 22.0±3.5 mM. The relaxation data significantly underestimate the Gd content, suggesting a potential compartmentation of Cs^+ and the contrast agent.

Key Words: Magnetic Resonance(MR) spectroscopy, Intracellular, Extracellular

INTRODUCTION

The evaluation of a new specific magnetic resonance image (MRI) contrast agent encompasses the quantitative monitoring of its cellular uptake. Chemical methods such as inductively coupled plasma or high performance liquid chromatography are usually utilized, but they often lead to a complete destruction of the tissue, thus requiring a large number of experiments and, therefore, of animals. In addition, these procedures are unable to identify the compartments containing the agents, magnetic resonance relaxometry, which may allow a quasi real time, noninvasive monitering, appears to be a potentially valuable method for in vivo quantitation-provided a reporter of the uptake is available. Because of the ubipuitous distribution of water, proton relaxometry does not bring specific information about the intra-or extracellular location of the paramagnetic molecule. Additionally, the possible variation of the in vivo relaxivity of the contrast agent prevents quantitation. Other endogenous sensors could be proposed, but most of them present major drawbacks. Sodium-23 has a low cytoplasmic concentration and, because of the small intrinsic difference between the chemical shifts of the intra-and extracellular locations, the use of shifts reagents or multiple quantum filtering techniques is required to descriminate between the compartments (Bansal et al., 1993). In addition to these same problems, potassium-39 suffers from a very poor sensitivity.

Cesium-133 (^{133}Cs) has been shown to accumulate in various cell types (such as myocytes and cardiocytes) through Na^+/K^+-ATPase pumps and K^+-channels (Davis et al., 1988; Murphy et al., 1997). With a 100% natural abundance, and in spite of a spin number of 7/2, this ion is fully detectable in biological tissues. Relatively low electric quadrupole moment and electric field gradient lead to long quadrupolar relaxation times (Wehrli, 1978). The extra-and intracellular locations are characterized by naturally resolved resonances. This ion is therefore a very attractive probe for the in situ-monitoring of the uptake of paramagnetic systems.

We report here prelimonary results prefacing the realization of an original protovol based on ^{133}Cs MR spectroscopy for the monitoring of the cellular uptake of organ-oriented contrast agents for MRI (Clement et al., 1992;

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Schuhmann et al., 1992).

MATERIALS AND METHODS

1. Chemicals

Chemical products were obtained from Sigma (St. Louis, MO) and Acros (Geel, Belgium). Eovist® (Gd-EOB-DTPA) was provided by Schering AG (Berlin, Germany) as a 250 mM aqueous solution. Wistar male rats (N=10) weighing approximately 200 g were anesthetized with 50 mg Nembutal i.p. and injected with 500 U.I. of heparin into the vena cava. The livers (average wet weight = 7.0 g) were isolated as described (Colet et al., 1996), and perfused at a contrant flow rate (3~4 ml g^{-1} of liver. min^{-1}) through the portal vein with a recirculating Krebs-Henseleit buffer (K-H, mean vol = 182.5 ml) kept at 37℃. This solution contains (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 3.0, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, and D-glucose 5.5.

2. MR measurements

^{133}Cs and phosphorus-31 (^{31}P) MR spectra were recorded on a 4.7T MSL-200 spectrometer (Bruker, Karlsruhe, Germany) at 26.13 and 81 MHz, respectively. Two probeheads were used: a 10 mm broadband coil and a 25 mm probe initially dedicated to ^{23}Na/^{31}P/^{1}H and tuned to the ^{133}Cs Larmor frequency. Temperature regulation (37℃) was ensured by a BVT 100 unit.

3. Cs^+ accumulation and washout

Three livers were perfused with the modified K-H solution (4.7 mM of CsCl) for 60 min, followed by a 60 min washout period with regular K-H in order to eliminate the Cs^+ ions remaining in the extracellular space. Throughout the experiment, ^{133}Cs spectra were acquired to check the accumulation and the persistence of Cs^+ in the IC compartment (25 mm coil, 90° pulse: 275 μs. TR = 0.2 sec. NS = 1500, and Θ = 90°. An external reference containing 9 μm CsCl, 100 μm KCI, and 1 μm $GdCl_3$, in 400 μl H_2O was placed in the MR tube beside the liver for calibration. At the end of the experiment, the organs were weighed and dried overnight at 60℃ for water content determination. For each liver. three pieces of dried tissue (0.4 g) were subsequently digested in acidic conditions (3 ml HNO_3 + 1 ml H_2O_2) and homogenized with microwaves (Milestone MLS-1200, Sorisole. Italy) for ICP measurement of cesium content.

4. Paramagnetic calibration relaxation

Based on our Cs^+ accumulation and washout data indicating intracellular Cs^+ concentration of about 24.5 mM, solutions reconstituted from 5 g of rat liver acetone powder (L-1380; Sigma, St. Louis, MO) and 12.5 ml of distilled water, adjusted to a concentration of 25 mM of CsCl and 0.1~2.0 mM of Eovist®, were prepared. A second intracellular-like (IC) series of solutions was prepared from rat livers flushed with K-H and homogenized (24,000 rpm, Ultra-Turrax; Janke and Kunkel, Staufen. Germany). The CsCl concentration of this series of IC media was fixed to 40 mM, a value intermediate between our measurements and those reported (Shehan et al., 1993). The Gd-EOB-DTPA concentration was varied from 0.1 to 1.6 mM.

R1, ($1/T_1$) was measured at 37℃ in these two sets of calibration solutions using a 10 mm BB coil and an inversion recovery pulse sequence (180° pulse of 42 μs, eight scans, and eight recovery delays (τ) varying front 1~100 sec (TR = 100 sec) for the reconstituted solutions, and from 0.05~6.4 sec (TR = 10 sec) for the IC solutions). The data were fitted by a monoexponential function using the spectrometer routine. The paramagnetic longitudinal relaxation rate $R_{1,P}$ was calculated as the differented between the measured rate in the presence of Gd-EOB-DTPA and the diamagnetic rate $R_{1,D}$ measured in the absence of gadolinium complex.

5. Distribution of Gd contrast agents

The internalization of Gd-EOB-DTPA was follolwed in situ through the paramagnetic enhancement of the Cs^+ longitudinal relaxation rate determined from the evolution of the peak intensity recorded in the saturation regime. The calculation is based on the Eqs. [1] and [2]:

$$S_0 = S_1 \frac{1}{1-e^{-TR/T_1}} \qquad [1]$$

$$C = \frac{1}{r_{1Cs}} - \left(\frac{1}{TR} \ln \frac{S_0 - S_Z}{S_0}\right) - R_{1.D} \qquad [2]$$

where S_0 and S_1 are the unsaturated and saturated signal intensities, respectively, in the absence of paramagnetic agent. S_2 is the area of the measured peak in the presence of the

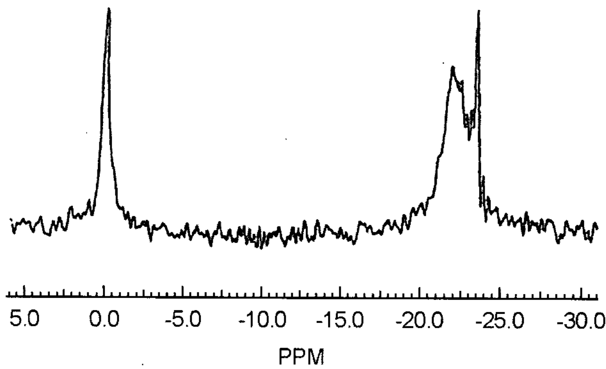

Fig. 1. MR spectrum of ^{133}Cs an isolated rat liver perfused for 60 min with a modified Krebs-Henseleit solution containing 4.7 mM of CsCl instead of KCl. Reference peak (400 μl og H_2O with 22.5 mM of CsCl, 2.5 mM $GdCl_3$, and 250 mM of KCl) at 0 ppm, intracellular ^{133}Cs resonance at -22.0 ppm, and extracellular ^{133}Cs resonance at -23.5 ppm.

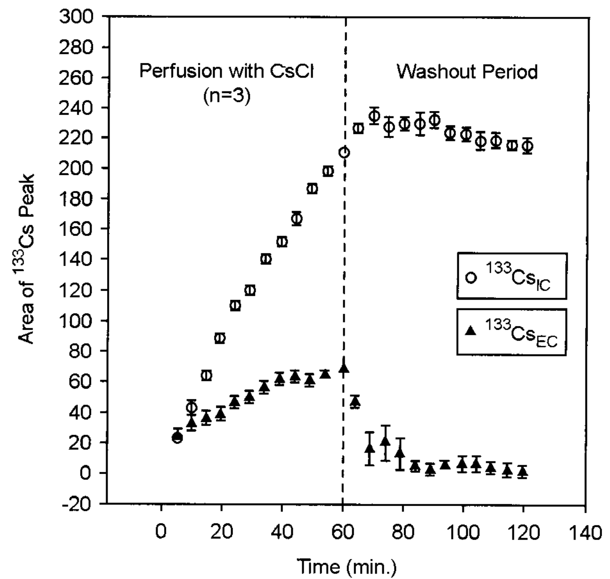

Fig. 2. Evolution of the intra- and extracellular ^{133}Cs signals during the perfusion of rat livers with modified buffer (CsCl = 4.7 mM) and during the subsequent washout of the extracellular compartment with Cs-free buffer.

contrast agent. C is the concentration of the contrast agent, TR the repetition delay, and r_1Cs is the intracellular relaxivity of the Gd-EOB-DTPA with respect to the cesium ion.

Three livers were perfused for 60 min with modified K-H (4.7 mM of CsCl) and immediately rinsed with regular K-H for 10 min. The renewal of the perfusate in the MR tube was accelerated by sucking up the liquid surrounding the liver through a second line. Gd-EOB-DTPA (50 μl from the 250-mM stock solution) was then delivererd in a mean volume of 182.5 ml of regular K-H for 30 min. Perfusate samples (1 ml) were collected every 5 min during the perfusion with the contrast agent and submitted to ICP analysis for Gd content. At the end of the experiment, livers were flushed with fresh K-H for 10 min to remove all extracellular contrast agent, and dried overnight at 60℃ for water content and Gd (ICP) quantitation, as previously described.

To prove the inability of the extracellular fraction of the paramagnetic compound to enhance the relaxation of intracellularly located ^{133}Cs ions, the same experiment was reproduced on two livers perfused with a purely extracellular contrast agent Gd-DTPA (Magnevist®, Schering, Germany).

6. Statistical evaluation

The results are expressed as means ± SEM (standard error of the mean). The variations between groups are compared using the bilateral Student's t-test and the Fisher test for variances. Results are assumed to be significant for $P \leq 0.05$ and highly significant for $P \leq 0.01$.

RESULTS AND DISCUSSION

1. Cs^+ accumulation and washout

^{133}Cs spectrum of the perfused liver acquired at the end of the perfusion with CsCl (Fig. 1). Two peaks are observed at -22.0 and -23.5 ppm, with respect to the line of the external reference arbitarily set to 0 ppm. Upon rinsing of the extracellular compartment with regular K-H free of CsCl, the high-field resonance disappears within 20 min (Fig. 2), suggesting that this peak globally represents the extracellular ^{133}Cs ions (i.e., the Cs ions contained in the vascular compartment, the space of Disse, and in the liquid bathing the liver in the MR tube). During the washout period, the low-field resonance remains at a level of about 24.5±0.9 mM, as determined from the area of the reference. This confirms that the low-field resonance arises from intracellular ^{133}Cs ions, as previously observed by Wittenkeller et al., 1992, for the erythrocytes.

The intracellular concentration was confirmed by ICP, which gives a Cs^+ content of 22.0±3.5 mM. The stability of the IC content and the absence of EC Cs^+ ions after rinsing indicate that the efflux, if any, of Cs^+ from the cells is very slow. This observation differs from that of Shornack et al., 1997, who observed a net efflux of Cs^+ through bidirectional K^+-channels in the rat heart.

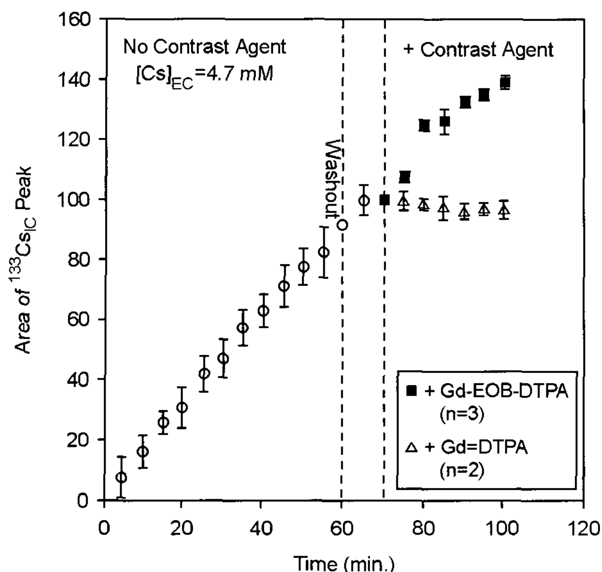

Fig. 3. Effects of extracellular (Gd-DTPA) and intracellular (Gd-EOB-DTPA) contrast agents on the ^{133}CsIC signal (TR = 200 msec, NS = 1500).

2. Paramagnetic relaxation calibrations

An $R_{1,D}$ of 0.08 sec^{-1} for ^{133}Cs in the perfusion medium was measured. The IC medium induces a significant increase of the relaxation rate (R_1 = 0.65 sec^{-1}). Although the relaxation process for ^{133}Cs (I = 7/2) is expected to be multiexponential, the experimental data were fairly well-fitted with a monoexponential function.

In the range of Gd-EOB-DTPA concentrations used (0.1^{-1}.6 mM), the $R_{1,p}$ of ^{133}Cs linearly increases with the amount of contrast agent. The calibration curve is not critically dependent on the types of IC-like media or on the Cs concentration. The linear regression of these experimental data gives the following relationship: $R_{1,p}$ = A * [Gd-EOB-DTPA] (mM) - 0.01 with A = 1.07 and 1.02 $sec^{-1}mM^{-1}$ for the homogenate and the rehydrated powder, respectively.

3. Distribution of Gd contrast agents

The evolution of the intracellular ^{133}Cs resonance acquired with a saturation-recovery pulse sequence (TR = 200 msec) (Fig. 3). As expected, the area of the partially saturated peak increases during the perfusion with CsCl due to the cellular uptake of Cs^+ ions. At the end of a 10-min washout period, a duration sufficient to eliminate most of the extracellular Cs^+, Gd-EOB-DTPA was added to the perfusion fluid (three livers). Although the level of intracellular ^{133}Cs is known to be stable, as shown by the previous experiments, the area of the peak further increases due to the paramagnetic relaxation enhancement provided by the internalized Gd-EOB-DTPA. The effect if essentially attributable to the intracellular Gd-EOB-DTPA since, as shown in another series of experiments (N = 2), Gd-DTPA (an extracellular compound) does not influence the amplitude of the partly saturated ^{133}CsIC resonance. The increase of the Csin resonance cannot be attributed to further internalization of the ion during the washout period since such an effect should have been noticed in the presence of Gd-DTPA.

Table 1. Evolutions of Gd-EOB-DTPA concentrations in the perfusion fluid and in the liver cells

Time (min)	Gd-EOB-DTPA in perfusion fluid (μM)	Gd-EOB-DTPA in liver fluid (mM)
0	73.4±5.2	0.00
5	62.5±3.5	0.49
10	53.8±4.5	0.89
15	48.8±4.8	1.08
20	46.3±5.0	1.23
25	44.0±5.1	1.33
30	42.5±5.0	1.39

The amount of contrast agent taken up by the liver cells was determined by ICP analysis of aliquots of perfusion fluid collected during the administration of Gd-EOB-DTPA (Table 1). The concentration of the compound in the perfusate, initially 73.4±5.2 μM, rapidly decreases due to the uptake by liver cells. After 30 min, it reaches 42.5±5.0 μM. The concomitant cellular concentrations of contrast agent were calculated from these data, knowing the water volume of the tissue (2.5 ml/g of dry weight) and the average liver dry weight of 2.0 g. and assuming that all Gd-EOB-DTPA leaving the recirculating perfusate (182.5 ml) is incorporated in cells. After 30 min of perfusion with the contrast agent, an amount of 1.12±0.04 mmole of Gd per liter of tissue water was calculated. Considering that the intracellular volume represents 80% of the total liver volume, an intracellular Gd concentration of 1.4 mM can be estimated. The concentration determined by ICP on livers dried and homogenized at the end of the experiment was 0.94±0.16 mmol/L of tissue water, giving an intracellular concentration of 1.2 mM, which confirms the previous analysis.

The concentration of Gd-EOB-DTPA in liver cells was then evaluated from the longitudinal relaxation of intracell-

ular ^{133}Cs, as described earlier. Thirty minutes after the delivery of Gd-EOB-DTPA through the perfusion medium, the resonance of intracellular ^{133}Cs increased by 38.3±2.2% as compared to its value recorded immediately before the administration of the agent. From the calibration curve, this enhancement would correspond to a concentration of 0.26 mmol of Gd per liter of intracellular medium. This method of dermining concentration significantly underestimates the Gd concentration relative to ICP. This discrepancy may suggest that either the relaxivity of Gd-EOB-DTPA with respect to Cs is lower than in the model systems, or about 80% of the intracellular contrast agent (1.3~0.3 = 1.0 mM) is not exposed to the Cs^+ ions. Indeed, a portion of Gd-EOB-DTPA could be sequestered in intracellular vesicles, reducing its interaction with cytoplasmic ^{133}Cs ions.

In conclusion, The present work has demonstrated the potential of ^{133}Cs MR spectroscopy, based on the use of intracellularly located Cs^+ ions, to follow in quasi real time the the liver uptake of Eovist®. The evolution of the intensity of the partially saturated cesium peak, related to the paramagnetic relaxation enhancement brought by Gd-EOB-DTPA, cleary reflects the time course of its internalization and demonstrates the potential of the method. Quantiative analysis based on this paramagnetic relaxation enhancement seems to significantly underestimate the cellular concentration of the contrast agent as compared to the chemical methods of analysis. The hypothesis of a sequestration of Eovist® and/or of Cs^+ ions in different compartments, as well as a reduced relaxivity of the gadolinium comples with respect to intracellular cesium, have to be assessed by further experiments.

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