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Potential of ^{31}P Magnetic Resonance Spectroscopy in Monitoring the Viability of Human Renal Grafts Stored in Euro-Collins Perfusion Solution

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Summary

The relative concentrations of inorganic phosphate and phosphomonoesters (PME) of 18 human cadaveric kidneys stored in Euro-Collins perfusion solution were measured by ^{31}P MR spectroscopy. The signals of intracellular inorganic phosphate (P_{ii}) and inorganic phosphate contained in the perfusion solution (P_{ie}) were separated by the deconvolution technique. The ratio of the signal intensities of phosphomonoesters and intracellular inorganic phosphate (P_{ME}/P_{ii}) was used as a marker of kidney viability and correlated with kidney function after transplantation. Separation of the P_{ii} and P_{ie} signals in the measured spectra was successful in 72 % of kidneys. The results of MR analysis satisfactorily agree in 78 % with the post-transplant function of kidneys.

Key Words

Human kidney – Viability – ^{31}P MR spectroscopy – Euro-Collins solution

Introduction

The viability of a kidney after transplantation often correlates with renal ischaemic time. Different studies have demonstrated a direct relationship between the post-transplant viability of the kidney and its ability to regenerate the mechanism of ATP production. The most recent studies, based on ^{31}P MR analysis of the isolated kidney before transplantation, show a correlation between ATP production and the ratio of phosphomonoesters to intracellular inorganic phosphate (P_{ME}/P_{ii}) in the kidney obtained by ^{31}P MR spectroscopy (Bretan *et al.* 1986a,b, 1987, Shapiro *et al.* 1989, Shanley *et al.* 1988, Ratcliffe *et al.* 1988). The P_{ME}/P_{ii} can be measured from the ^{31}P MR spectrum of the isolated kidney stored in a solution not containing inorganic phosphate, e.g. the HKT (Kallerhoff *et al.* 1987) solution. These results have been verified by experiments performed in animal models (dog, rabbit and rat kidneys) and human grafts (Ross *et al.* 1985, Bretan *et al.* 1986, 1988, 1989a,b, Freeman *et al.* 1986, Matson *et al.* 1988, Ciancabilla *et al.* 1993, Moller *et al.* 1993, Vestring *et al.* 1994, Hene *et al.* 1994). If a perfusion solution contains inorganic phosphate (e.g. Euro-Collins, UW or Collins-2 solutions) a contribution of the inorganic phosphate (P_{ie}) from the solution can be seen in the ^{31}P MR spectrum. The P_{ii} signal overlaps the signal of P_{ie} or *vice versa*. A separation of signals is only possible by the deconvolution technique of spectral analysis. In the study of 40 cadaveric kidneys perfused prior to the transplantation with Collins-2 solution, Bretan *et al.* (1989a,b) noted two signals in the area of inorganic phosphate. The spectral analysis of these signals by the deconvolution technique led to the determination of intensities of intracellular P_{ii} and extracellular P_{ie} inorganic phosphate. The P_{ME}/P_{ii} obtained from the spectral analysis were correlated with the post-transplant kidney function. If the value of P_{ME}/P_{ii} was > 0.5, they found good post-transplant function and the patient did not require dialysis. The values $P_{ME}/P_{ii} < 0.5$ correlated with reduced graft function.

The aim of our study was to measure the ^{31}P MR spectra of the group of cadaveric kidneys obtained directly from our clinical practice (removed or transplanted in our institute) and to correlate the results of measurement with post-transplant renal function in routine clinical practice.

Material and Methods

A group of 18 human cadaveric kidneys was studied. Kidneys were preserved in Euro-Collins perfusion solution using the standard clinical technique (Morris 1988). The function of kidney after transplantation was evaluated according to the clinical stage of the patient by a five point scale:

1 point – diuresis < 500 ml/day,
2 points – diuresis > 500 ml/day, creatinin clearance < 0.1 ml/s,
3 points – diuresis, creatinin clearance 0.1–0.2 ml/s,
4 points – creatinin clearance 0.2 - 0.8 ml/s,
5 points – creatinin clearance > 0.8 ml/s.

The ^{31}P MR spectra were measured using a whole-body Magnetom imager (Siemens) with a magnetic field of 1.5 T and a home made surface coil of 12 cm in diameter. The kidneys were examined in the container specially developed for this purpose (Kurková and Hájek 1994). Total examination time was around 35 min.

The parameters of the measurement were as follow: flip angle 180° in the Center of the surface coil, number of accumulations NS = 64, pulse repetition time TR = 15 s, sweep width SW = 68 ppm.

Spectrum analysis was performed using the program SOFIS (Scientific Instrument, Brno 1992), FID was zero filled up to 4096 points and followed by the Fourier transformation. In some cases, the spectrum was smoothed using the method of linear averaging (3–5 points). The Gauss and/or Lorentz/Gauss technique of the deconvolution was employed to separate inorganic phosphate signals to obtain P_{ii} and P_{ie} signal intensities. The position of P_{ii} and P_{ie} signals (δP_{ii}, δP_{ie}) and their linewidth $\Delta\delta_{1/2}$ were optimized by this method. The same procedure was used for calculation of the intensity of the P_{ME} signal.

Saturation factor Sf was established using spectra measured with pulse repetition time TR = 2 s (NS = 256) and TR = 15 s (NS = 32), respectively. The value of Sf was found 1.2 ± 0.2 from 7 measurements.

Table 1
PME/P_{ii} values and post-transplant renal function.

No.	P_{ME}/P_{ii}	Function	P_i sign	$\Delta\delta$(ppm)	Time(hours)
1	1.3	xx	1	–	25
2	0.5	xx	1	–	2
3	0.6	xx	2	0.5	16
4	0.3	xx	2	0.4	12
5	0.3	xx	1	–	12
6	0.6	xx	2	0.3	19
7	0.4	xx	2	0.4–0.7	20
8	0.4	xx	2	0.3–0.7	
9	1.0	xx	2	0.5–1.0	3
10	1.1	1/1	2	0.7	5/21
11	1.0	4/5	2	0.3	12/20
12	1.0	4/4	2	0.2	12/18
13	0.7	4/5	2	0.3–0.7	14/23
14	0.6	4/4	2	0.3	5/33
15	0.4	1/1	1	–	13/32
16	0.4	1/4	2	0.2	7/21
17	0.4	1/3	2	0.7	14/20
18	0.3	4/4	1	–	14/21

Function: 1st day/7th day after transplantation.
1 – diuresis < 500 ml/day; 2 – diuresis > 500 ml/day, creatinin clearance < 0.1 ml/s)
3 – diuresis, creatinin clearance: 0.1–0.2 ml/s; 4 – creatinin clearance: 0.2–0.8 ml/s
5 – creatinin clearance > 0.8 ml/s; xx – post-transplant function not known
P_i sign: number of inorganic phosphate signals in spectrum; $\Delta\delta$: relative chemical shift of P_{ii} and P_{ie} signals
HW: half width of P_{ii} + P_{ie} signals; Time: time of measurement after removal/time of total ischaemia

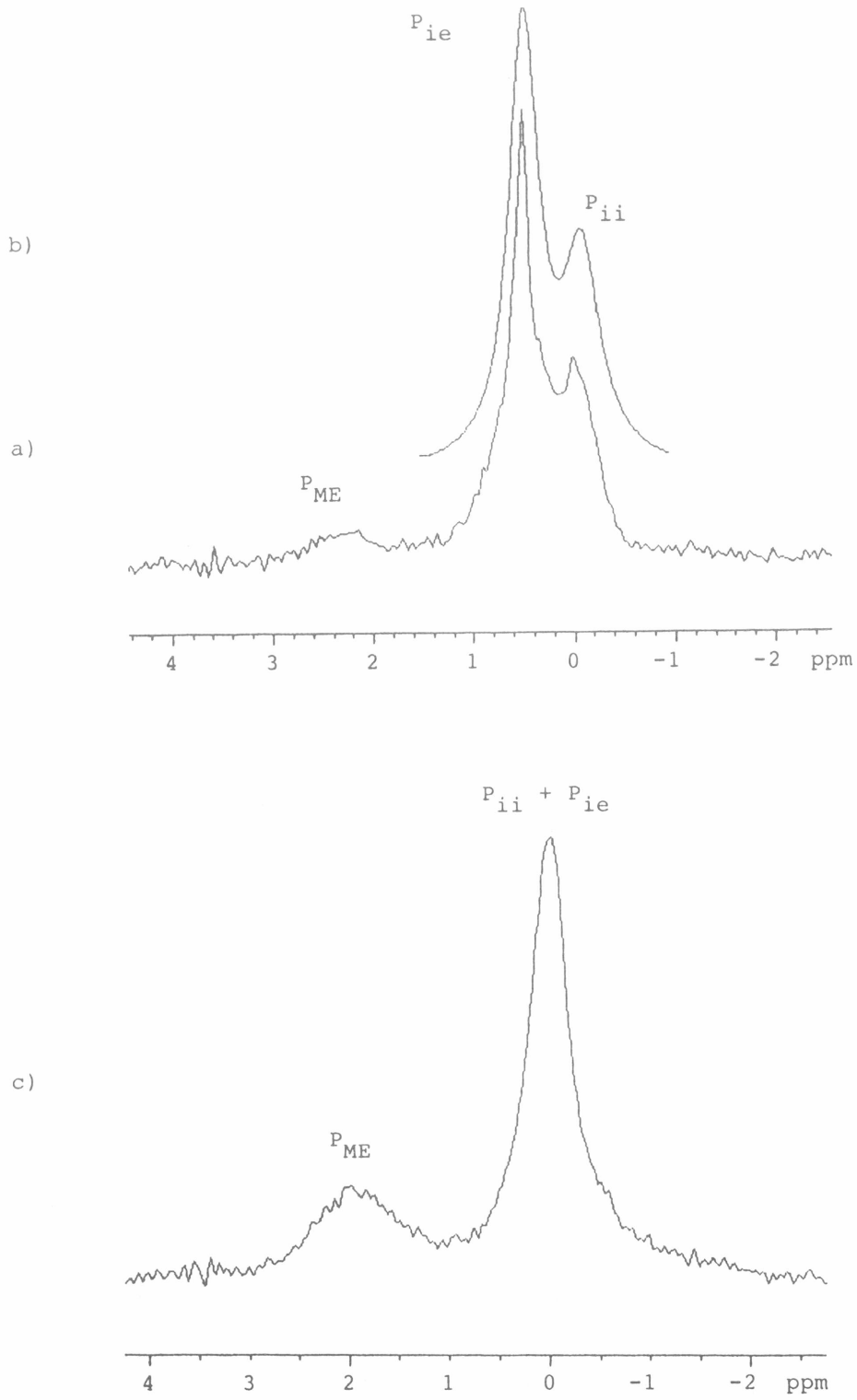

Fig. 1
The ^{31}P-MR spectra of the cadaverous kidney in Euro-Collins solution. a) spectrum of the first group, signals of P_{ii} and P_{ie} being separated, b) calculated spectra, c) spectra of the second group

Results

Sixty ^{31}P MR spectra from 18 different human cadaveric kidneys were obtained. The signal of P_{ME} was clearly separated from the inorganic phosphate signal in all the spectra (see Fig. 1). The spectra were divided according to the shape of the inorganic phosphate signal into two groups. The first group was characterized by splitting of the inorganic phosphate signal into P_{ii} and P_{ie} signals (Fig. 1a). This type of spectra was obtained from 13 renal grafts (72 %). The PME/P_{ii} ratios were determined from the intensities of signals after the deconvolution (Table 1). The second group of spectra was observed in five renal grafts, where only one signal of inorganic phosphate was observed (Fig. 1c). The deconvolution did not provide reproducible results in the determining intra- and extracellular inorganic phosphate signals. The intensity of the whole signal of inorganic phosphate was taken to represent P_{ii} and used for the calculation of P_{ME}/P_{ii}.

To decrease the errors of ^{31}P MR spectra measurement due to the saturation effects caused by using the surface coil, the limiting value of $P_{ME}/P_{ii}=0.5$ proposed by Bretan was corrected by using the saturation coefficient and $P_{ME}/P_{ii}=0.4$ was used as the lowest limit for good graft function.

The results of spectral analysis were compared with the function of the kidneys after transplantation. The clinical stage of patients with transplanted kidneys was evaluated on the first and the seventh days after transplantation according to the point scale (mentioned in Methods).

Discussion

P_{ME}/P_{ii} ratio ≥ 0.4 was found in 15 kidneys studied. Three kidneys had the P_{ME}/P_{ii} ratio ≤ 0.3. The P_{ME}/P_{ii} was correlated with the clinical stage of the patient according to the scale characterizing the function of the kidney graft on the first and the seventh day after the transplantation. The clinical data were available for 9 patients. Seven kidneys showed good function after the transplantation, while dialysis was necessary in two cases. There is a positive trend between good kidney function and increasing value of P_{ME}/P_{ii} (Table 1). The grafts with lower P_{ME}/P_{ii} ratios than 0.4 had lower function in the first days after transplantation. The correlation between P_{ME}/P_{ii} and kidney function in this small group of patients was relatively very high – the agreement between the clinical data and MRS results was 78 %.

Some questions about the application of this method in clinical practice are still open. One of them is the arrangement of the MRS examination of the grafts which should be performed at a certain time for all kidneys. This was not possible in this preliminary study. The second question concerns the evaluation and interpretation of the spectra. The meaningful calculation of the P_{ME}/P_{ii} ratio depends on the splitting of the inorganic phosphate signal into P_{ii} and P_{ie}. This splitting is probably caused by differences in pH values of the perfusion solution surrounding the kidney (P_{ie}) and of the kidney itself (P_{ii}). The relative chemical shifts of the P_{ii} and P_{ie} signals $\Delta\delta = \delta P_{ii} - \delta P_{ie}$ obtained using deconvolution rank from 0.2 to 0.7 ppm (see Table 1). The pH of Euro-Collins solution is around pH = 7 (Dhasmana *et al.* 1983) and the obtained range of $\Delta\delta$ corresponds to a difference of pH in the range 0.2–0.7. The buffering properties of Euro-Collins solution can cause that the pH gradient between the intracellular and extracellular solution is so small that only a single broad signal is observed in the spectrum. This can be the reason why only one signal in the range of inorganic phosphate chemical shifts was observed in 28 % of renal grafts. In these cases deconvolution leads to irreproducible results.

We also checked the shimming during the experiments because bad magnetic field homogeneity could be the cause of the broadening of the signal. Neither the long period of shimming, nor the complete change of starting shimming values did not change the shape of broad signals. For these cases we approximated P_{ii} intensity by the intensity of the whole signal of inorganic phosphate for the P_{ME}/P_{ii} evaluation. It is probably an overestimation of the P_{ii} and thus the P_{ME}/P_{ii} ratio is much lower than the real value. An example of this can be demonstrated on kidney No. 18. In contrast to the above mentioned assumption ($P_{ME}/P_{ii}=0.3$) the clinical data show a good function of kidney.

We have also observed quite opposite situation – very good signal splitting resulting in a high P_{ME}/P_{ii} ratio and unsatisfactory kidney function (kidney No. 1). Such cases can be explained by a long waiting period between the MRS examination and the transplantation. During this period, the ischaemic changes in the kidney could alter the concentration of the observed metabolites and the result of the spectroscopic examination would then not agree with the clinical status. The results show that the method of ^{31}P MR spectroscopy correlates with the clinical evaluation of kidney function. The limitation of the method concerns the cases where only one broad signal of inorganic phosphate is observed in the spectrum and a detailed study of this effect should be done in the future. The method should also be supplemented with the measurement of T2 maps (Kurková *et al.* 1994, Herynek *et al.* 1995). The combination of both methods can provide more data needed in clinical practice for the assessment of the viability of organs.

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