31P-Magnetic resonance spectroscopy (31P-MRS) of human allografts after renal transplantation

Andree Klemm1, Reinhard Rzanny3, Reinhard Fünfstück1, Wolfram Werner2, Jörg Schubert2, Werner A. Kaiser3 and Günter Stein1

Departments of 1Internal Medicine IV and 2Urology, 3Institute of Diagnostic and Interventional Radiology, Friedrich-Schiller-University, Jena, Germany

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Abstract

Background. 31P-Magnetic resonance spectroscopy (31P-MRS) can be used as a non-invasive tool for measuring the relative intracellular concentrations of several phosphorus metabolites in different organs. Various pathological conditions are characterized by different metabolic patterns. We studied the value of 31P-MRS after renal transplantation with both an uneventful and a clinically complicated course.

Methods. We determined the relative concentrations of phosphate-containing metabolites in renal allografts of humans with 31P-MRS (1.5 Tesla) in the first few weeks after transplantation; 18 patients with an uneventful clinical course and 10 patients who required dialysis after transplantation were examined. Six patients with a stable allograft function 2–3 months after transplantation served as controls.

Results. In patients with primary allograft function, we found a significant correlation between the phosphomonoester/phosphodiester-ratio (PME/PDE) (r = 0.66, r < 0.01) and the time after transplantation, but no correlation between the nucleoside triphosphate (β-NTP)-concentration (r = −0.11) and the time course. In the patients with primary or early allograft dysfunction caused by histologically proven rejection (n = 5), we found a low β-NTP compared to patients with an uncomplicated clinical course (0.09 ± 0.01 vs 0.15 ± 0.03), but no differences in the PME/PDE ratio (0.73 ± 0.21 vs 0.80 ± 0.21). In contrast, the PME/PDE ratio was lowered in three patients with delayed graft function caused by acute tubular necrosis (0.45 ± 0.07 vs 0.80 ± 0.21), but the β-NTP concentration was not reduced (0.15 ± 0.003 vs 0.15 ± 0.03). The 31P-MR spectrum of two patients with cyclosporin A damage was not altered compared to the controls.

Conclusions. 31P-MRS can be used in patients in the early period after renal transplantation. A significant correlation between the PME/PDE ratio and the time course but no change in the β-NTP concentration was found in patients with primary allograft function in the first 4 weeks after renal transplantation. Different patterns of 31P-MR spectra were observed depending on the different causes of primary and early transplant dysfunction.

Introduction

Primary and early allograft dysfunction remains a challenge for the nephrologist. A rapid diagnosis is necessary, because a delay in treating early rejection deteriorates the prognosis of the allograft function. On the other hand, the immediate anti-rejection treatment based on a mere clinical suspicion of rejection alone endangers those patients who have a delayed graft function caused by an acute tubular necrosis (ATN) or a cyclosporin A (CSA) damage through possible side effects of anti-rejection treatment. The only reliable method for recognizing the cause of primary transplant dysfunction is the invasive transplant biopsy. The diagnostic value of non-invasive procedures–sonography and scintigraphy–is limited to providing the accurate diagnosis in such cases [1]. Colour Doppler sonography is an improvement in diagnostic management after renal transplantation, especially for vascular complications. However, the measurable alterations of the vessel indices as evidence for renal parenchymal damage have a high sensitivity to recognize a rejection, but there is a lack of specificity [2]. The first hope in MRI failed because the loss of corticomedullary differentiation is a non-specific sign and was found both in ATN and rejection [1,3]. 31P-Magnetic resonance spectroscopy (31P-MRS) enables the non-invasive evaluation of relative concentrations of different intracellular phosphorus metabolites. Substrates of energy metabolism like nucleoside tri-
phosphates (NTP) or inorganic phosphate (Pi) as well as metabolites of the cell membrane metabolism like phosphomonoesters (PME) or phosphorylesters (PDE) can be estimated. These metabolic parameters were investigated in various organs of animals and humans in the past. Various pathological conditions were characterized by different metabolic patterns. Chan and Shapiro had evaluated the renal allograft metabolism by $^{31}$P-MRS in animals [4]. Alterations in phosphate metabolism could be shown for allograft rejection and ischaemia, whereas allografts with CSA damage did not show any difference to controls. Well resolved and spatially localized $^{31}$P-MRS spectra from orthotopic and transplanted kidneys were described by Boska et al. using an image selected in vivo spectroscopy (ISIS) sequence. These recorded spectra were comparable to the spectra received from animal experiments [4,5]. Grist et al. demonstrated that the $^{31}$P-MR spectra alter during episodes of allograft dysfunction due to rejection, but these examinations were not performed in patients immediately after transplantation; the mean examination time was 31 months after transplantation (range 1–72 months). The authors found that elevated Pi/ATP and PDE/PME-ratios are associated with allograft rejection [6]. Recently, Heindel et al. found an increase of Pi/ATP and a reduced pH in human allografts during rejection, whereas a decreased PME/PDE ratio but no change in pH was seen in case of ATN. These examinations were made in an earlier time after renal transplantation (from day 12 to day 69 after transplantation, mean: day 20 ± 10) [7].

Another field for use of $^{31}$P-MRS in nephrology is the assessment of allograft viability before transplantation [8–11]. The aim of this work was to measure the relative concentrations of phosphate-containing metabolites in renal allografts of humans after the cold and warm ischaemia period during the first few weeks after renal allograft transplantation using $^{31}$P-MRS. The second aim was to clarify and extend the knowledge, whether the $^{31}$P-MRS is suitable as a non-invasive tool for diagnosis in primary and early allograft dysfunction in humans.

Patients and Methods

Patients

Thirty-four transplanted patients who received a renal allograft were examined with $^{31}$P-MRS. An informed consent was obtained from all subjects, and examinations were approved by the local review board.

The patients were divided into three groups:

(i) Six patients (five males, one female; age 44 ± 8 years) with an uneventful course and a stable allograft function (serum creatinine 113 ± 12 μmol/l) examined with $^{31}$P-MRS in the time period of 2–3 months after renal transplantation served as controls.

(ii) Eighteen patients (14 males, four females; age 47 ± 12 years) without dialysis requirement and an uncomplicated clinical course after transplantation were measured between day 4 and 28 after transplantation to evaluate the time-dependent alterations of phosphate-containing metabolites after transplantation in case of a primary allograft function. Each patient was examined once in this time. The decline in serum creatinine values after transplantation is shown in Figure 4.

(iii) Ten patients (seven males, three females; age 48 ± 11 years) with a primary or early allograft dysfunction were examined to determine the potential use of $^{31}$P-MRS in the noninvasive differential diagnosis of primary and early transplant dysfunction. In all these cases, one or more renal biopsies were taken for diagnosing.

Acute tubular necrosis (ATN)

An ATN was the cause of primary allograft dysfunction in three cases. These subjects were anuric and required dialysis treatment. The $^{31}$P-MRS measurements were performed on days 10, 14 or 27 after transplantation. In the following, renal function improved and serum creatinine levels fell to normal values in two patients. In the third case, the allograft had to be ectomized because of an irreversibly tubulointerstitial damage.

Cyclosporin A (CSA) damage

Two patients had a CSA damage, which was supposed histologically and confirmed by a blood level of CSA repeatedly above 250 ng/ml. One patient had to be dialyzed, the other patient had a serum creatinine of 440 μmol/l. $^{31}$P-MRS measurements were performed on days 10 or 14 after transplantation, respectively. The daily diuresis was 2 or 2.3 l respectively in these cases. The allograft function improved immediately after reducing the daily CSA-dosage in these two cases.

Allograft rejection

Three patients needed dialysis treatment because of a primary allograft dysfunction caused by an early rejection. One patient with an interstitial rejection was examined with $^{31}$P-MRS on day 12. Allograft function improved after rejection treatment with high-dose-steroids and dialysis treatment could be finished in this case. In contrast, the allografts of two patients with vascular rejection had to be explanted because of persistent transplant dysfunction despite antirejection treatment and a very poor prognosis as seen from subsequent biopsies. These two patients were examined with $^{31}$P-MRS on days 8 or 13, respectively.

Furthermore, two patients with primary allograft function but vascular rejection 4 or 9 weeks respectively after transplantation were examined during the rejection episodes. These persons had to return to dialysis treatment after unsuccessful anti-rejection treatment.

Method

The $^{31}$P-MRS spectra were obtained with a Philips Gyroscan II System (1.5 Tesla) equipped with a spectroscopy accessory (Philips Medical Systems). In order to obtain spectra of well-defined volume in renal allografts, we used a gradient-controlled localization method with a surface coil (10 cm) and an ISIS sequence with three adiabatic 180° pulses [12].
The repetition time (TR) was 2.5 s, the free induction decay (FID) sampling rate was 2000 Hz, the number of sampling points and averages was 1024. The examination was performed in a supine position. The surface coil was centered over the renal allograft on the patient’s skin and then fixed. After the scout image in three levels (T₁TSE with TR/TE = 450/12,90) the volume of interest (VOI; 100–150 ml) was placed and adapted to the size of renal allograft (Figure 1a). Prior to 31P-MRS measurements shimming was done resulting in line widths of 8–12 Hz. The average FID was corrected for DC, zero-filled to 2048 data points and filtered by exponential multiplication (8 Hz). Phase correction was applied after Fourier transformation. Spectral data were processed using Gyroscan standard software package (Philips) (Figure 2). The values for the metabolites were calculated as relative concentrations related to the total peak area of the 31P-MR spectrum. The statistical analysis of the relative metabolite concentration and metabolite ratios was made by linear regression and correlation in patients with primary allograft function. The various metabolite concentrations were compared with the unpaired t-test or by means of descriptive statistics with calculation of confidence intervals (95%).

Results

A representative 31P-MR spectrum of renal allograft with normal function obtained from a patient 16 days after transplantation is shown in Figure 1b. The six major peaks of this spectrum include PME, Pi, PDE and the γ-, α-, and β-phosphorous resonances of NTP. Only the β-peak of NTP was used for calculation of NTP, because the γ- and α- peaks contain phosphorous resonances of other nucleotides like nucleoside diphosphates (NDP) and NAD/NADP as well [13,14].

Neither the calculated relative mean concentrations of the metabolites PME, PDE, Pi and β-NTP nor the PME/PDE ratio differed between the group of 18 patients with primary allograft function measured on days 4–28 after transplantation and the six control subjects with stable renal function 2–3 months after transplantation (Figure 3). However, when the metabolite concentrations from patients with primary allograft function (day 4–28 after transplantation) were correlated with the time elapsed after transplantation, a significant correlation between PME ($r=0.50; \ P<0.05$), PME/PDE ratio ($r=0.66; \ P<0.01$) and the time course was observed (Figure 4). The correlation between PDE and time course was not significant ($r=-0.40, \ P=0.1$). The β-NTP ($r=-0.11$) and Pi ($r=-0.33$) concentrations remained unchanged during the first 4 weeks after transplantation. The Pi peak (Pi < 1% of total peak area) was hardly detectable on days 24 and 25 after transplantation in two patients, who had a considerable polyuria with low serum level of anorganic phosphate after transplantation. A cor-
Fig. 3. Relative metabolite concentrations in renal allografts of patients with uneventful clinical course immediately or 2–3 months after transplantation.

<table>
<thead>
<tr>
<th>Function</th>
<th>PME* ± SD</th>
<th>PDE* ± SD</th>
<th>PME/PDE</th>
<th>Pi* ± SD</th>
<th>β-NTP* ± SD</th>
<th>α-NTP* ± SD</th>
<th>γ-NTP* ± SD</th>
</tr>
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<tbody>
<tr>
<td>Primary allograft function; day 4-28 after transplantation (n=18)</td>
<td>0.19±0.03</td>
<td>0.25±0.05</td>
<td>0.80±0.21</td>
<td>0.07±0.03</td>
<td>0.15±0.03</td>
<td>0.18±0.04</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>Stable allograft function; 2-3 months after transplantation (n=6)</td>
<td>0.20±0.02</td>
<td>0.27±0.04</td>
<td>0.77±0.06</td>
<td>0.07±0.02</td>
<td>0.14±0.01</td>
<td>0.17±0.03</td>
<td>0.15±0.03</td>
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*Estimated as percentage peak area (± SD) related to the total peak area (without Pcr) of the $^{31}$P-MR spectrum.

Fig. 4. Course of serum creatinine and PME/PDE ratio in 18 patients with primary allograft function after transplantation.

Relation between allograft Pi-concentration and serum anorganic phosphate did not exist. A small phospho-creatine (Pcr) peak (4.9 ± 1.9%) could be identified in 10 of the 34 recorded spectra indicating the absence of significant contributions from surrounding tissues.

The relative metabolite concentrations in the allografts of patients with primary or early allograft dysfunction due to rejection, ATN or CSA damage, and examples of the $^{31}$P-MR spectra are shown in Figures 5 and 6. A decreased intracellular β-NTP-level (0.09 ± 0.01) was found in patients with vascular (n=4) or interstitial (n=1) rejection (Figure 6a). The calculated confidence interval (95%) is clearly different from that of patients with a well-functioning allograft. In contrast, the calculated PME/PDE ratio in rejected allografts (0.73 ± 0.21) did not differ from the patients with primary allograft function (0.80 ± 0.21) (Figure 6b). No difference in intracellular β-NTP-concentration was seen in patients with ATN (n=3) and CSA damage (n=2) compared to patients with primary

Fig. 5. $^{31}$P-MR spectra recorded from allografts of patients with primary allograft dysfunction due to different causes.
Fig. 6. Comparison of intracellular β-NTP concentration and PME/PDE ratio in renal allografts of patients (i) with stable transplant function 2–3 months after transplantation, (ii) with primary allograft function 4–28 days after transplantation, and (iii) with primary and early allograft dysfunction due to rejection, ATN and CSA damage. Mean values and confidence intervals (95%) are expressed.

allograft function (Figure 6a). A decreased PME/PDE ratio was observed in the three patients with ATN (0.45 ± 0.07), whereas the patients with CSA damage did not differ in the PME/PDE ratio from patients with primary allograft function (Figure 6b).

Discussion

The consistent intracellular β-NTP levels in patients with primary allograft function measured from day 4 to day 28 after transplantation indicate an early restitution of the intracellular metabolism of high-energy nucleotides after reperfusion in human allografts. It is known from earlier animal studies that the recovery of NTP needs a few hours only [8]. However, it was found that complete recovery of nucleotide metabolites can occur even when the functional recovery after a prolonged warm ischaemia is still incomplete [15]. The normal β-NTP-level in the three patients with primary allograft dysfunction due to ATN is consistent with these findings. In contrast, we observed a decreased β-NTP-level in the three patients with primary allograft dysfunction caused by vascular or interstitial rejection and in the two patients with vascular rejection 4 or 9 weeks respectively after transplantation. The low β-NTP is in line with the results of Chan and Shapiro obtained from rejected allografts in rats [4,14]. The absence of spectroscopic changes in CSA nephrotoxicity has already been described in animal and human allografts and was confirmed in our two patients with CSA damage [4,6].

The phospholipid metabolism is strictly associated with cell membrane synthesis and degradation. The phospholipids are represented in the 31P-MR spectrum by two peaks, the PME peak and the PDE peak, with every peak containing several compounds. The PME metabolites phosphocholine and phosphoethanolamine are precursors of phosphatidylcholine and phosphatidylethanolamine which are major components of the phospholipids of the cell membrane. In contrast, the PDE glycerophosphorycholine and glycerophosphorylethanolamine are formed as intermediate metabolites during cell membrane degradation. Earlier findings regarding tumour tissue and the developing brain indicate that an elevated PME level is considered to be a reflection of an increased membrane phospholipid metabolism with a higher degree of cell growth and proliferation [16–18]. Murphy et al. observed an increase in PME and a decrease in PDE in the regenerating rat liver after partial hepatectomy. They supposed the undergoing cell proliferation to be the cause for these spectral changes [19]. An elevation in the PME/PDE ratio was also observed in rat kidneys 21 days after an ischaemia insult [20]. For these reasons, we suggest that the correlation between the PME peak area, PME/PDE ratio and the time elapsed after transplantation in patients with primary allograft function reflects the cell membrane regeneration process still occurring during the first few weeks after ischaemia even with an uneventful clinical course.

The decreased PME/PDE ratio in patients with delayed graft function due to ATN was also found by Heindel et al. [7]. Contrary to the report of Grist et al. we could not observe a general decreased PME/PDE ratio (or an increased PDE/PME, respectively) in our patients with allograft rejection, the PME/PDE ratio varied between 0.54 and 1.14 [6]. In rejected rat allografts an elevated PME/PDE ratio was even found as compared to controls [14].

A PCr peak could only be identified in nine of the 34 recorded 31P-MR spectra. According to some authors, the PCr peak is caused completely by the surrounding muscle tissue (6,21). However, a small PCr peak was also detected in the spectra of isolated porcine and rat kidneys [8,15,22,23]. The absence of PCr peak in human kidneys during cold storage is caused by a rapid loss of PCr after explantation [9,10]. Nevertheless, we suggest that the main part of the PCr peak in some of our 31P-MR spectra derives from the
surrounding tissue. The influence of muscle tissue on other peaks of the spectra is negligible, because the PCr level in skeletal muscle is high compared to the other phosphorus metabolites, and the PCr peak is only small.

The considerably reduced Pi peak (<1% of total peak area) in two patients with well functioning allograft 24 and 25 days after transplantation indicates a loss of intracellular inorganic phosphate. This loss is probably due to the known temporary hyperphosphatemia after renal transplantation caused by the damage of tubular cells and secondary hyperparathyroidism. A decreased Pi has also already been described in muscle tissue of patients after transplantation [24]. These findings indicate that the intracellular Pi detected by $^{31}$P-MRS may not only depend from the energy state of cells: because of the variety of the Pi/NTP ratio observed in our cases which was also reported by Grist et al. the calculation of the Pi/NTP-ratio seems not to be reliable [6]. The energetic state should rather be assessed by the relative signal intensity of the $\beta$-NTP peak. The pH value was not calculated because of the wide variation range in humans [5]. In rat allografts no change in intracellular pH-value was found during rejection [4,14].

In conclusion, $^{31}$P-MRS can be used in patients in the first time after renal allograft transplantation and has the potential of a non-invasive tool in the differential diagnosis of primary renal allograft dysfunction because of different spectroscopic patterns in different forms of graft dysfunction. However, a confirmation of the hitherto existing findings in a larger number of patients is necessary before $^{31}$P-MRS could be recommended in clinical use after transplantation. The use of higher field MRS with better peak resolution and new MRS-techniques with a smaller voxel size may improve the in vivo MRS in the next years.

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References

23. Meier D, Pons M, Binswanger U, Böiger P. Posttransplant hypophosphatemia related to muscle phosphate content as detected by nuclear magnetic resonance spectroscopy, EDTA/ERA XXIXth Annual Congress 1992, Paris; (Abstr.): 214

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