Non-invasive diagnostic of cardiac allograft vasculopathy by $^{31}$P magnetic resonance chemical shift imaging

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Background: Coronary angiography is still the gold standard for the diagnosis of cardiac allograft vasculopathy (CAV) for which alternative non-invasive diagnostic approaches are currently investigated. In this study, we assessed whether $^{31}$P magnetic resonance chemical shift imaging can diagnose CAV by studying variations in cardiac high-energy phosphates in a population of adult heart transplant recipients.

Methods and results: CAV was defined by coronary angiography as the presence of diffuse coronary irregularities with significant concentric narrowing on epicardial or distal coronary arteries. Eight patients with CAV (group A), and 18 patients without CAV (group B) were included in this study and compared to nine healthy volunteers (group C). Patients and volunteers underwent $^{31}$P three-dimensional chemical shift imaging to determine the ratio of phosphocreatine (PCr) and adenosine tri-phosphate (ATP). PCr/ATP was significantly lower in group A (1.51 ± 0.50) than in groups B and C (1.98 ± 0.53 ($p = 0.003$) and 2.14 ± 0.31 ($p = 0.001$)), respectively. Time from transplant, number of episodes of acute rejection, and left ventricular ejection fraction (LVEF) were not significantly different between patient groups. A PCr/ATP value of 1.59 was the optimal cut-off value to predict CAV (specificity and sensitivity of 100% and 72%, respectively).

Conclusion: Clinically, in vivo $^{31}$P chemical shift imaging is a promising, non-invasive method to detect the potential modifications of high-energy phosphates related to CAV and to better screen indications for coronary angiograms. This may be relevant for coronary angiography follow-up and adjustments of immunotherapy regimen. © 2005 Elsevier B.V. All rights reserved.

Keywords: MRS; Coronary circulation; Transplantation

1. Introduction

Despite general improvement in long-term outcome of heart transplantation (HTx), cardiac allograft vasculopathy (CAV) is still recognized to limit life expectancy of recipients, as it remains the most important predictor of mortality after 5 years following HTx. CAV appears and evolves silently in HTx recipients. Its diagnostic therefore requires careful follow-up with coronary-angiography or intravascular ultrasound [1]. Since the current diagnostic practice is costly and repeatedly exposes the patients to the risk of catheterization, a constant effort is made to establish a reliable non-invasive method for the diagnostic of CAV. Non-invasive diagnosis of CAV involves echocardiography and radionuclide imaging techniques [2]. Because the information given by both methods is based on comparison with normal myocardial areas, and because the diffuse nature of the coronary lesions (including small intramyocardial vessels) potentially threatens the entire myocardium, echocardiography and radionuclide studies performed in resting conditions have a low sensitivity for CAV diagnosis with a specificity below 75% [2,3]. More recently, multi-detector computed tomography has been shown to detect CAV with a sensitivity of 83% and a specificity of 95% [4]. Although these results are very promising to achieve a non-invasive diagnosis of CAV, multi-slice computed tomography exposes patients to a significant cumulated irradiation when repeated. CAV is characterized by diffuse microvascular injury that might cause alterations in myocardial energetics. In vivo $^{31}$P magnetic resonance spectroscopy (MRS) studies on animals and humans have shown modifications of high-energy phosphates (HEP) potentially related to CAV [5,6]. In the present study, we have evaluated the potential of an optimized three-dimensional $^{31}$P chemical shift imaging (CSI) protocol to non-invasively diagnose CAV in HTx recipients.
2. Materials and methods

2.1. Subjects

Informed consent was obtained from 26 selected HTx recipients receiving systematic treatment of post-transplantation hyperlipidemia and from nine healthy volunteers with no known heart disease. The institutional committee of ethics approved the study. Patients were included in the study according to the following criteria: time from HTx more than 2 years before inclusion, absence of contraindications to MR techniques, and no ongoing rejection at the time of the study. Three patients, for which $^{31}$P-MRS was technically not possible, were excluded. Group A ($n = 8$) included patients presenting signs of diffuse CAV as described on the report of the last coronary angiogram performed before $^{31}$P-MRS. Diffuse CAV was defined by the presence of diffuse coronary epicardial or distal disease with significant concentric lumen narrowing. Group B ($n = 18$) included 16 patients with normal angiograms and two patients with non-significant focal proximal lesions not related to diffuse CAV. Coronary angiograms were systematically reviewed by two experts (T.C. and J.Q.) before assignment of patients to a specific group. Previous history of acute rejection was defined as the presence of at least one episode of moderate to severe acute rejection documented by biopsy with transient modification of immunotherapy as a consequence (at least ISHLT grade 2 rejection [7]). For transplanted patients, left ventricular function was assessed by transthoracic echocardiography performed at the time of the MR study. Group C ($n = 9$) included the healthy volunteers as a control group. The average time between coronary angiography and MR was 3 months.

2.2. MR acquisition

All MRS measurements were performed using a Siemens Magnetom Vision Plus 1.5 T MR system equipped with a broadband channel. We used a commercially available $^{31}$P/$^{1}$H surface coil (Siemens, linear coil $\phi = 22$ cm for $^{1}$H TX/RX and $^{31}$P TX, quadrature coil $\phi = 12$ cm for $^{31}$P RX) for radiofrequency transmission and reception. Odam MAGLIFE (Schiller Medical, Wissembourg, France) equipment was used for ECG gating. A reference vial filled with 80 ml of 1M phenyl phosphonic acid (PPA) was placed on the rear of the radiofrequency coil for flip angle calibration purposes. All subjects were positioned supine in the scanner. The surface coil was placed on the chest of the subjects and fixed using velcro tapes. Automated MAP-shimming was performed followed by the acquisition of 21 short-axis images (multi-slice Snapshot-FLASH bright blood). These images were used in post-processing as a reference for the positioning of the slice Snapshot-FLASH bright blood). These images were used to optimize sensitivity and specificity of the diagnostic test. Unless stipulated differently in the text or legends, data are expressed as mean ± SD. For all tests, statistical significance was achieved for $p < 0.05$. Statistical analyses were carried out using SPSS software v12.0.1 (SPSS Inc., Chicago, IL, USA).

2.3. Post-processing

The set of phase-encoded FIDs was Fourier-transformed in spectral dimension. Local spectra were obtained using voxel-shifting technique and integration over a volume covering the anterior and septal part of the left ventricle at a level in between apex and base. These volumes were manually defined using the proton reference image data set. As no additional spatial filter was applied, the spatial resolution was conserved after post-processing. The spectra were filtered using a line broadening of 12 Hz and fitted in time domain using the AMARES algorithm [9] in order to obtain the signal amplitudes for the phosphocreatine (PCr), $\gamma$ATP and $\beta$ATP resonances. All signal amplitudes were corrected for partial saturation taking into account the local flip angle, the individual mean repetition time and literature values for the longitudinal relaxation time $T_1$ at 1.5 T [10]. The ATP contribution from blood was calculated using the 2,3-diphosphoglycerate (DPG) signal amplitude [11] and subtracted from the ATP integrals. The ATP concentration was assumed to be represented by the $\beta$ATP signal amplitude.

2.4. Statistics

Data for each continuous variable were tested for significant deviation from a normal distribution by using the Kolmogorov–Smirnov test. Data were compared with ANOVA analysis followed by PLSD Fisher test or non-parametric tests appropriately. Categorical data were compared with the chi-square test. For the transplanted hearts, we checked the relation between PCr/ATP and the following data: sex, donor age, time from transplant, past history of acute rejection, ventricular function, and CAV as diagnosed by the coronary angiogram. All variables were included in a multiple stepwise regression analysis where variables were entered if $p < 0.05$ and removed if $p > 0.1$. Optimal cut-off value for PCr/ATP to predict CAV was determined by receiver-operating characteristic (ROC) analysis to optimize sensitivity and specificity of the diagnostic test. Unless stipulated differently in the text or legends, data are expressed as mean ± SD. For all tests, statistical significance was achieved for $p \leq 0.05$. Statistical analyses were carried out using SPSS software v12.0.1 (SPSS Inc., Chicago, IL, USA).

3. Results

The demographic data of studied hearts are summarized in Table 1. Volunteers were younger than HTx recipients, but were calculated to obtain a nominal spatial resolution of $2.1 \times 2.1 \times 3.0 \text{ cm}^3$. This nominal spatial resolution is equivalent to the voxel size at half maximum of the point-spread function. Two thousand two hundred thirty-two phase-encoded FIDs were sampled with 256 points and a spectral width of 2 kHz. A hard pulse of 300 $\mu$s duration was used as excitation pulse. No decoupling or Nuclear Overhauser Effect pulses were used. The total duration of the entire protocol ranged from 40 to 55 min depending on the heart rate.
the organ age was not significantly different at the time of the study. Mean time between HTx and the study was 6.5 ± 3.6 years for all patients. The distribution of the usual risk factors for natural arteriosclerosis was not significantly different between groups A and B. Fig. 1 shows typical spectra obtained from patients with and without CAV along with the respective regions of interest. The spectra illustrate a significant decrease of the PCr/ATP ratio in group A. The regions of interest shown correspond to the contours of the point-spread function at levels of 64% (inner contour) and 33% (outer contour) of the maximum. The voxel profile with the limits of possible signal contamination from regions outside the voxel is hereby objectively visualized. As shown in Fig. 2, PCr/ATP was significantly lower in group A than in group B \((p = 0.003)\) or C \((p = 0.001)\). Reciprocally, a multiple stepwise regression analysis confirmed the presence of CAV.

Table 1
Demographic data of studied hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>A ((n = 8))</th>
<th>B ((n = 18))</th>
<th>C ((n = 9))</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender (%)</td>
<td>75</td>
<td>61.1</td>
<td>66.6</td>
<td>0.16</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>42 ± 15</td>
<td>35 ± 8</td>
<td>—</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean time since HTx (years)</td>
<td>7.7 ± 2.5</td>
<td>6.0 ± 3.9</td>
<td>—</td>
<td>0.25</td>
</tr>
<tr>
<td>Organ age (a) (years)</td>
<td>49.1 ± 15.8</td>
<td>39.4 ± 5.84</td>
<td>39.4 ± 5.8</td>
<td>0.11</td>
</tr>
<tr>
<td>LV EF</td>
<td>70 ± 4.6</td>
<td>70 ± 7.5</td>
<td>—</td>
<td>0.94</td>
</tr>
<tr>
<td>Episodes of acute rejection ((n))</td>
<td>2.62 [0.63–4.62]</td>
<td>1.77 [0.82–2.73]</td>
<td>—</td>
<td>0.43</td>
</tr>
<tr>
<td>Past infection with CMV (%)</td>
<td>62.5</td>
<td>44.4</td>
<td>—</td>
<td>0.67</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>87.5</td>
<td>88.9</td>
<td>—</td>
<td>0.57</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>87.5</td>
<td>83.3</td>
<td>—</td>
<td>0.75</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>0</td>
<td>5.5</td>
<td>—</td>
<td>0.67</td>
</tr>
<tr>
<td>Arteriosclerosis (%)</td>
<td>25</td>
<td>11.1</td>
<td>—</td>
<td>0.75</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>12.5</td>
<td>11.1</td>
<td>—</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Variables are expressed as mean ± SD or mean [95% confidence interval]. LV EF: left ventricular ejection fraction; CMV: cytomegalovirus

\(a\) Calculated as "age of donor + time from HTx".

Fig. 1. Typical spectra obtained from transplanted patients with (b) or without (a) CAV along with the respective regions of interest.

Fig. 2. Box and whiskers representation of PCr/ATP for transplanted patients with or without CAV (groups A and B) and for healthy volunteers (group C).
4. Discussion

This study shows that transplant patients with diffuse CAV as shown on the coronary angiogram exhibit a significantly altered PCr/ATP ratio when compared with transplanted patients with normal coronary angiograms and with healthy volunteers. On the other hand, transplant recipients with normal coronary angiograms exhibit a PCr/ATP ratio comparable to healthy volunteers. Reciprocally, the presence of CAV was the only predictor variable of the PCr/ATP ratio. These results are in line with the hypothesis that microvascular lesions due to diffuse cardiac allograft vasculopathy might induce cellular ischemia within poorly perfused myocardial areas like the interventricular septum.

Although the sensitivity of the diagnostic test was higher than that of echocardiography and radionuclide imaging techniques for the diagnosis of CAV [1–3], its specificity was comparable. This yielded a high negative predictive value (93%), which could allow reducing the frequency of coronary angiograms in transplanted patients with a resting PCr/ATP ratio above 1.59. These results have to be interpreted taking into account the conditions of the study, i.e., (i) the employed MR protocol and (ii) the prevalence of CAV in the patient population. (i) 3D CSI benefits from a good localization quality, especially in the presence of breathing motion. This is important in order to avoid signal contamination from surrounding chest muscle tissue and ventricular blood. The average spectral quality was sufficient for quantification using AMARES. The nominal spatial resolution of the measurement technique was 2.1 cm × 2.1 cm × 3 cm, resulting in voxel dimensions of 13 mL. In short-axis view, this voxel dimension is optimized to study the myocardial energetics potentially altered by the disease of distal coronary arteries rather than to visualize vessels and lesions directly. (ii) The best cut-off value of a diagnostic test determined by ROC curve analysis depends on the prevalence of the disease in the studied population [12]. The prevalence of CAV in the group of transplanted patients (30.8%) was lower than that expected from literature data with respect to the mean time from transplant (42% as diagnosed by coronary angiography at 5 years post-transplant [13]). Because the patients included in the present study were submitted to the classical inclusion criteria for MR exams (excluding, for example, patients with permanent pacing), the characteristics of the patient population studied here might slightly deviate from a standard population of HTx recipients. The lower observed prevalence in our series may also be a result of the beneficial effect of the treatment of post-transplantation hyperlipidemia which every patient was systematically receiving [14].

Modulations of cellular energetic metabolism have been found in atherosclerotic coronaropathy and in heart transplant recipients [15–19] in particular using 31P-MRS. Few studies have suggested that in heart transplant recipients variations in high-energy phosphates (HEP) might be related to CAV [5,6]. Variations in HEP with significant decrease in PCr/Pi and ATP/Pi have been found using 31P-MRS in a rat heart transplant model of CAV [5]. Also using 31P-MRS, Evanochko et al. [6] have shown differences in PCr/ATP decrease under stress in patients early after transplantation. The authors suggested that abnormal decrease of PCr/ATP in some patients revealed a transient ischemic event possibly related to CAV. All these patients had normal coronary angiograms. In the present study, patients were selected later after transplantation and had normal or abnormal coronary angiograms. We could therefore show the potential of the non-invasive PCr/ATP measurement to detect CAV. Alteration of the PCr/ATP ratio in patients with CAV was observed at rest and might be due to lower perfusion of the myocardium related to a global alteration of the cardiac microcirculation in the heart, which is characteristic for CAV. Consistent with this hypothesis, Muehling et al. [20] have shown using perfusion MRI that the endomyocardium/epicardium perfusion ratio was lower at rest in a similar group of patients with CAV compared to transplanted patients without CAV.

The longitudinal analysis of intra-individual variations of PCr/ATP is one interesting perspective of our study. In particular, close follow-up of patients with PCr/ATP below 1.59 and no diagnosed CAV on the coronary angiogram (false positives) at the time of the study has to be performed in order to clarify whether 31P-MRS can detect CAV at a very early stage before the apparition of coronary lesions. If the apparition of CAV can be detected by intra-individual time-related variations of PCr/ATP, one potential benefit for heart transplant recipients would be a reduction of the number of coronary angiographies performed systematically during
post-transplant follow-up in selected patients. Moreover, if CAV can be detected before the apparition of lesions on the coronary angiogram, targeted adjustments of immunosuppres- sive regimen might improve late survival after HTx [21].

In conclusion, in vivo $^{31}$P chemical shift imaging is a promising, non-invasive method to detect the potential modifications of high-energy phosphates related to CAV and to better screen indications for coronary angiograms. This may be relevant for coronary angiography follow-up and adjustments of immunotherapy regimen.

5. Study limitations

The definition of CAV is difficult in general, and in the present study, diffuse CAV was assumed to be present only in case of coronary epicardial or distal disease. Therefore, not all arteries were equally involved among patients with diffuse CAV. This may have induced variations in the results and at least partly may have caused the observed overlap in PCR/ATP between groups A and B.

Acknowledgements

Grant support: Programme Hospitalier de Recherche Clinique 2001 (Assistance Publique-Hôpitaux de Marseille). Centre National de la Recherche Scientifique (UMR 6612).

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References


Appendix A. Conference discussion

Dr M. Kamler (Essen, Germany): Did you look at the coronary flow reserve with intravascular ultrasound?

Dr Caus: No. We don’t have this technique available in Marseille, so I cannot answer your question.