Analysis of the distribution of histologic myocardial lesions during acute cardiac rejection. Experimental study in rodents

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Abstract

Background and method: Asymmetric distribution of histologic lesions have been reported in grafted hearts that could hamper the interpretation of right ventricular endomyocardial biopsy. Heterotopic heart transplantations were performed in rats (n = 59) and guinea pigs (n = 20). Grafted hearts were examined by a pathologist who established the degree of cardiac rejection in the four cardiac cavities.

Results: Forty cardiac rejections were diagnosed in rats and ten in guinea pigs. An asymmetric distribution of histologic lesions was observed in 34 (68%) rejected hearts with greater lesions in the auricular myocardium than in the ventricular myocardium (n=25, 50%). One (n=18) or two degrees (n=7) differentiated the severity of rejection between atria and ventricles. Cardiac rejection score was significantly greater in atria than in ventricles (3.12±0.18 vs. 2.6±0.2 (P<0.01) in rats and 2.35±0.37 vs. 1.6±0.47 (P<0.001) in guinea pigs. There were histologic lesions of rejection in the auricular myocardium in seven cases, although the ventricular myocardium was completely normal. In nine (18%) other grafted hearts the degree of rejection was equal in the auricular myocardium and ventricular septum but was greater than the degree of rejection noted in the right and left ventricular free walls. Conclusion: The distribution of histologic lesions of acute cardiac rejection in rodents was heterogeneous in grafted hearts which exhibited greater lesions in the atria than in ventricles. This should be taken into consideration in the evaluation of new methods of detection of cardiac rejection and in the diagnosis of acute cardiac rejection in humans.

Keywords: Heart transplantation; Cardiac rejection; Histopathologic examination; Asymmetric distribution; Atria

1. Introduction

Acute cardiac graft rejection is a frequent complication of cardiac transplantation [1]. Endomyocardial biopsy remains the gold standard for the diagnosis of acute rejection after cardiac transplantation in humans [2]. At this time, it is the reference method to evaluate the sensitivity and specificity of the new non-invasive methods to diagnose cardiac allograft rejection [3]. Endomyocardial biopsy is routinely performed in the right ventricle although some observers have reported that histologic myocardial lesions were more severe in the auricular than in the ventricular myocardium [4]. This could introduce a bias in the interpretation and validation of the new methods of detection of cardiac rejection and should be taken into consideration in the diagnosis of acute rejection in cardiac transplanted patients.

The aim of our study was to analyze the distribution of histological lesions of acute rejection in the auricular and ventricular myocardium of grafted hearts and to determine the differences in degree of rejection in cardiac cavities in heterotopic heart transplantation performed in rodents.

2. Materials and methods

2.1. Animals

Allografts were performed in rats and guinea pigs using allogeneic animals. The rats (Long Evans and Wistar), were divided into two groups: a first allogeneic group not treated with immunosuppressive drugs, a second allogeneic receiving cyclosporine (15 mg/kg s.c. once daily). To test the species dependence of distribution of cardiac rejection lesions, we performed heterotopic heart transplantation in a small group of guinea pigs. The number of guinea pigs was smaller than rats because the rate of mortality during
anesthesia and surgery was higher. The guinea pigs group (Three colored and Dunkin Hartley) comprised only one allogeneic not treated with immunosuppressive drugs. All animals were adult males. Weights ranged from 200 to 300 g. All animals received care in compliance with the ‘Principles of Laboratory Animals Research’ and the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication no. 85-23, revised 1985).

2.2. Surgical procedure

Rats were anesthetized with ketamine (100 mg/kg) and largactil (45 mg/kg) administered intraperitoneally. Guinea pigs were anesthetized with ketamine (100 mg/kg) administered intraperitoneally and a spontaneously inhaled mixture of ether±air which was stopped just after the incision. Heterotopic heart transplantation was performed in the abdomen according to the modified technique of Ono and Lindsey [5]. Cardiac arrest and preservation of the donor heart was made with a cold (4°C) heparinized cardioplegia solution.

2.3. Tissue analysis – histological examination

The interval to removal of grafted hearts for histological examination after cardiac transplantation ranged from 1 to 8 days in order to represent all grades of rejection. The transplanted hearts were excised, immediately placed in Bouin’s solution and then embedded in paraffin. Several longitudinal cross sections of grafted hearts were performed in order to include the four cardiac cavities on the same slide. The samples were evaluated blind by a pathologist who examined and compared the auricular and ventricular myocardium. Non-specific postoperative lymphocytic infiltration in the epimyocardium was excluded in the morphologic evaluation because these lesions are known to be related to the heterotopic model. The degree of rejection was graded according to the standardized grading system of the International Society for Heart Transplantation [6]. The histopathologic grade of heart rejection was scored according to the standardized grading system of the International Society for Heart Transplantation [6] (grade 0 = 0; grade IA = 1; grade IB = 1.5; grade II = 2; grade IIIA = 3; grade IIIB = 3.5; grade IV = 4) and the differences in the degree of rejection between auricular and ventricular myocardium were noted and compared.

2.4. Statistical method

Scores of rejection in the auricular and ventricular myocardium are expressed as mean ± standard error and compared using the Student test for paired samples. Statistical significance was determined assuming significance for $P \leq 0.05$.

3. Results

Fifty-nine heart transplantations were performed in rats: 42 using allogeneic rats not treated with cyclosporine, 17 using allogeneic rats receiving cyclosporine.

Twenty heart transplantations were performed in allogeneic guinea pigs.

3.1. Histological examination

Forty histologic heart rejections were diagnosed in rats: 30 in the allogeneic group not treated with cyclosporine and ten in the allogeneic group receiving cyclosporine. There were no histologic lesions of rejection in 19 heart grafts in rats: 12 in the allogeneic group not treated with cyclosporine and removed early after transplantation, seven in the allogeneic group receiving cyclosporine and removed early after the transplantation.

Histological examination of heart grafts in the guinea pigs showed acute cardiac rejection in ten cases and no rejection in ten cases.

Grafted hearts without histological lesions of rejection were excluded of the present analysis.

Fig. 1 shows the degree of rejection in the atrium and ventricle according to the Billingham classification and shows the asymmetric distribution of rejection between atria and ventricle.

3.2. Distribution of histologic myocardial lesions in atrial and ventricular myocardium (Table 1)

3.2.1. Rats

An asymmetric distribution of rejection was observed in 26 (65%) grafted hearts in 40 grafted hearts with acute rejection (allogeneic group not receiving cyclosporine $n = 21$, receiving cyclosporine $n = 5$). The histologic lesions were greater in the auricular myocardium than in the ventricular myocardium in 17 cases (42.5%) (Fig. 2). In nine cases, histologic lesions were equal in the auricular and ventricular septum and greater than the histologic lesions in the free walls of the right and left ventricles. In the other allogeneic grafts ($n = 14$; 35%), rejection was most often severe (grade 4, $n = 9$; grade 3B, $n = 2$; grade
3A, \( n = 1 \); grade 1B, \( n = 1 \); grade 1A, \( n = 1 \) in the atria and ventricles.

The difference in rejection in the auricular and ventricular myocardium was equal to one (\( n = 12 \)) or two degrees (\( n = 5 \)) according to the Billingham classification. It was important to note that in four cases there were evident histologic lesions in the auricular myocardium (grade 1A, \( n = 1 \); grade 1B, \( n = 2 \); grade 2, \( n = 1 \)) although the ventricular myocardium was completely normal. In four cases, the grade of rejection was superior to 3 in atria and inferior to 3 in the ventricle. We compared the severity score of grafted heart rejection between the auricular and ventricular myocardium according to the standardized grading system of the International Society for Transplantation. There was a statistically significant difference between the auricular and ventricular scores: \( 3.12 \pm 0.18 \) vs. \( 2.6 \pm 0.2 \) (\( P < 0.01 \)) in rats.

Histologic lesions in the ventricular myocardium were never more severe than in the auricular myocardium. The same degree of rejection was observed in the right and left atrial myocardium and the atrial septum.

The same degree of rejection in the right and left ventricles was observed in the majority of grafted hearts, except in five cases which exhibited greater histologic lesions in the right ventricle than in the left. In these five cases the difference in rejection was equal to one (\( n = 2 \)) or two degrees (\( n = 3 \)). However the higher degree of rejection was not

### Table 1

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Asymmetric (( n = 34 ))</th>
<th>Symmetric (( n = 16 ))</th>
</tr>
</thead>
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<tr>
<td>Allogeneic rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
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</tr>
<tr>
<td>A &gt; VS and V</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>A = VS &gt; V</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>A &gt; V</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

\( A, \) atrium; \( V, \) ventricle; \( VS, \) ventricular septum.

**Fig. 2.** Asymmetric distribution of histologic lesions in the myocardium during acute cardiac rejection. Example of an asymmetric distribution of histologic lesions in a longitudinal cross section including the four cavities in a rat not treated with an immunosuppressive agent. In the right atrium (A, Hematoxylin, eosin, safran, 250x) there was a diffuse lymphocytic and neutrophilic infiltrate with aggressivity for myocytes (grade 3B). In the left atrium (B, Hematoxylin, eosin, safran, 250x) there was a diffuse lymphocytic and neutrophilic infiltrate, prominent oedema and myocyte necrosis (grade 4). In the ventricular septum (C, Hematoxylin, eosin, safran, 250x) there was a focal perivascular lymphocytic infiltrate without damage to adjacent myocytes (grade 1A). In the left ventricle (D, Hematoxylin, eosin, safran, 250x) there was an interstitial lymphocytic infiltrate only (grade 1B).
greater than the degree of rejection in the auricular myocardium. The score of grafted heart rejection did not differ statistically between the right and left ventricles (2.6 ± 0.2 vs. 2.5 ± 0.3; NS).

3.2.2. Guinea pigs
The same results were noted in ten rejected grafted hearts in guinea pigs: eight (80%) had more histologic lesions of rejection in the auricular than in the ventricular myocardium (difference equal to one degree n = 6 or two degrees n = 2); and two had an equal degree of rejection (grade 3A, n = 1; grade 4, n = 1). There were histologic lesions of rejection in the atria in three cases without evident lesions in the ventricles (grade 1A, n = 2; grade 1B, n = 1). In two cases the grade of rejection was superior to 3 in atria and inferior to 3 in the ventricle. We compared the severity score of grafted heart rejection between the auricular and ventricular myocardium according to the standardized grading system of the International Society for Transplantation. There was a statistically significant difference between the auricular and ventricular scores: 2.35 ± 0.37 vs. 1.6 ± 0.47 (P < 0.001) in guinea pigs. Two grafted hearts showed an asymmetric distribution of histologic lesions between the right and left ventricles.

In summary, whatever the species of animal studied, analysis of the atrial myocardium showed more severe histologic lesions than in ventricles in 25/50 rejected grafted hearts.

4. Discussion
Right ventricular endomyocardial biopsy remains the gold standard for the diagnosis of acute cardiac rejection. Moreover endomyocardial biopsy is the reference method used in the evaluation of new non-invasive methods of detection of acute cardiac rejection [3]. However an asymmetric pattern of rejection has been reported in grafted heart rejections [4,7,8] which could delay the diagnosis of grafted heart rejection and introduce a bias in the interpretation and validation of new methods of detection of cardiac rejection. Two recent studies [7,8] showed early alteration of atrial electrical activity during acute cardiac rejection such as intraauricular conduction disturbance or prolongation of action potential and established a good correlation between the severity of modifications of the electrical activity of atria and the score of grafted heart rejection in atria.

We systematically studied the distribution of histologic lesions of cardiac rejection in four cardiac cavities in heterotopic heart transplantations performed in rodents and we observed an asymmetric distribution of rejection in 68% of rejected grafted hearts, with greater lesions in the atrial myocardium (50%) or ventricular septum (18%) than in the ventricular myocardium. No histologic lesions of rejection in ventricles were observed without equal or more severe lesions in the atria; and, surprisingly, and perhaps most important seven (14%) grafted hearts (four in rats, three in guinea pigs) showed histologic lesions in the atria although the ventricles were completely normal and in six cases (four in rats, two in guinea pigs) the grade of rejection was superior to 3 in atria and inferior to 3 in ventricles. Tahara et al. [8], using the same experimental model (n = 6), also observed a greater score of rejection in atria than in the ventricles (2.8 ± 0.4 vs. 1.3 ± 0.2; P < 0.05). A difference of blood flow between atria and ventricles in heterotopic model can’t explain these data because similar observations have been made in orthotopic model. In fact, in a previous study Avitall et al. [4] noted that histopathologic examination of the right and left atria revealed more severe rejection than in the ventricles in a small number (n = 9) of orthotopic cardiac transplantations performed in dogs. The difference of degree of rejection was not specified in this study. Bieber et al. [9] reported earlier histologic lesions in the conduction system in 12 dogs but did not specify the degree of rejection associated in the atria or ventricles. The impact of the surgical procedure has been evaluated in nine experiments. Rats were killed less than 24 h after surgery and the auricular and ventricular myocardium appeared as normal at the histological examination excluding major lesions due to the surgery. The data obtained from orthotopic heart transplantation and our suggest that the asymmetric pattern of rejection is not linked to the experimental model of transplantation (working heart or not) and the surgical procedure. Therefore, the asymmetric pattern of rejection should be taken into evaluation and validation of new non-invasive techniques for the diagnosis of acute cardiac rejection.

In 1984 Haverich et al. [10], who studied the distribution of lesions of cardiac rejection in the right and left ventricles, reported significantly higher rejection scores in the right ventricle than in the left ventricle in orthotopic cardiac transplantation performed in primates. Arai et al. [11] demonstrated that, in rats, greater intramyocardial lymphocytic accumulation in right ventricle than left. However these authors did not analyze the atrial myocardium. As with Rose et al. [12], we did not find the same results, changes of rejection were equally distributed between the left and right ventricular free walls. However, we often observed histologic lesions more severe in the ventricular septum than in the free walls of ventricles, as reported by Haverich et al. [10].

The mechanism of the asymmetric distribution of histologic lesions in the cardiac myocardium during acute rejection is not known, but it seems to be independent of the species, of immunosuppressive treatment and of experimental model of heart transplantation. Several hypothesis can be suggested: increased vascularization in the atria; differences in physical properties such as wall thickness, wall tension, oxygenation between the atria and ventricles and differences in cellular antigenic receptors between the atria and ventricles.

Our results suggest performing endomyocardial biopsy in the right atrium for early diagnosis of acute cardiac rejection. ISHT 2 rejection has been demonstrated a strongest predictor
of rejection classified as $\geq$ ISHT 3A and appears to be of clinical significance [13]. Endomyocardial biopsy in the right atrium showing a higher grade of rejection in atrium could be useful to distinguish a true acute rejection from Quilty lesions and to decide a treatment. Konno et al. [14] developed and evaluated right atrial biopsy in humans and Sekiguchi et al. [15] reported good results of right atrial endomyocardial biopsy in 100 patients suffered from cardiac arrhythmia. No major complications were encountered in their series of more than 100 right atrial cardiac biopsies. Nonetheless in the clinical setting the right atrial biopsy should be used carefully and not systematically because four or five biopsy pieces are required in the transplanted patients increasing the risk of perforation. The application of right atrial endomyocardial biopsy in transplanted patients could be useful to physicians following up patients and an alternative technique in certain circumstances such as in patients with right ventricular pacing lead, in the presence of fibrosis in repetitive ventricular biopsy.

Moreover a histological examination of the atrium myocardium should be performed in the study whom the aim is to evaluate and validate new methods of detection of acute cardiac rejection.

4.1. Limits of the study

The study of the evolution of histological lesions in different cardiac cavities would be necessary to define the value of acute cardiac rejection in atria. This require to realize serial multichamber biopsies in each grafted hearts in a larger experimental model such as the dog or pig.

The distribution of histologic lesions of acute cardiac rejection in heterotopic heart transplantation performed in rodents is asymmetric in the myocardium with more severe lesions in the atria than in the ventricles. This result should be taken into account for the evaluation of new non-invasive techniques for the diagnosis of acute cardiac rejection. If these results are confirmed in transplanted patients, right atrial endomyocardial biopsy should be valuable for physicians following up transplanted patients in certain circumstances.

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References