EARLY CHANGE OF MYOCARDIAL WATER DURING ACUTE CARDIAC ALLOGRAFT REJECTION

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To determine the changes in myocardial water during acute cardiac rejection and the effects of Ciclosporin (CYA) on the myocardial water, 90 heterotopic cardiac transplants were performed in rats which were divided into 3 groups, namely those receiving 1) Lewis x Lewis isografts, 2) Lewis x Brown Norway allografts and 3) CYA treated allografts (15 mg/kg/day). The water content was measured in both recipient and donor hearts at 2, 4, 6 and 8 days after transplant. Pathological specimens were examined by light and electron microscopy, and scored on a 0 to 4+ scale of increasing evidence of rejection. The water content of the isografts showed no significant change throughout the post operative period. In contrast, the allografts had significant increase of water content as early as 2 days after transplant, compared to the isografts and recipients hearts. A significant difference in cellular infiltration was noted between isograft and allograft 4 days after transplant. CYA suppressed significantly the increase of myocardial water and cellular infiltration in the allografts.

These data suggest that myocardial edema may precede cellular infiltration during the rejection process and it may be suppressed with CYA treatment. The measurement of myocardial water may be useful in early detection of acute cardiac allograft rejection and for examining the therapeutic effects of CYA.

The success of human cardiac transplantation has steadily increased over the past several years making it an appropriate life-saving procedure in patients with end stage heart disease. Despite significant improvement in transplantation techniques, rejection remains the major obstacle to successful long-term cardiac graft function and consequently, patient survival. Improved survival from cardiac transplantation depends in part, on early detection of rejection and early institution of appropriate therapy. At the present time, the endomyocardial biopsy technique is one of the most reliable methods of monitoring the progress of rejection postoperatively.

The outstanding histologic features of rejection are interstitial edema, vascular engorgement, capillary and venular fibrinous thrombi, focal exudate of red blood cells and fibrin, and infiltration of large monocytes. Among these findings, interstitial edema is a constant factor at all stages of acute rejection and the earliest change during the rejection process. However, this interstitial edema has not been evaluated precisely by endomyocardial biopsy technique, because edema is often confused as a traumatic artifact produced during sample processing.

The purpose of this study was to determine 1) myocardial water changes during rejection

Key Words:
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Ciclosporin

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process; 2) the relationship between myocardial water and cellular infiltration; and 3) the effects of Ciclosporin as an immunosuppressive agent on myocardial water in allogeneic cardiac transplantation.

MATERIALS AND METHODS

1) Animals
Male Lewis (LEW) rats weighing 250-300g served as recipients of heterotopic abdominal cardiac grafts from LEW (syngeneic) or male Brown Norway (BN) rats weighing 250-300g (allogeenic).

<table>
<thead>
<tr>
<th>Grades</th>
<th>Histological appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no rejection</td>
</tr>
<tr>
<td>1</td>
<td>focal interstitial lymphocytic infiltration</td>
</tr>
<tr>
<td>2</td>
<td>focal interstitial lymphocytic infiltration with myocytic necrosis</td>
</tr>
<tr>
<td>3</td>
<td>moderate, diffuse lymphocytic infiltration with myocytic necrosis</td>
</tr>
<tr>
<td>4</td>
<td>massive, diffuse lymphocytic infiltration with myocytic necrosis</td>
</tr>
</tbody>
</table>

2) Transplantation
Heterotopic cardiac grafts were transferred to the abdominal great vessels of the recipients by a technique modified from Ono and Lindsey. Rats were anesthetized with atropine sulfate (0.04 mg/kg) i.m. and ketamine hydrochloride (50 mg/kg) i.m. and maintained with Halothane. After injecting 2 ml of heparinized saline (250 units/ml) into the vena cava, the vena cavae and pulmonary veins were ligated. The heart was removed and immediately placed in 4°C saline. The ascending aorta was anastomosed in an end-to-side fashion to the infrarenal aorta of the recipient with 8-0 monofilament nylon. The pulmonary artery was joined to the inferior vena in a similar fashion. Total ischemic time of the donor hearts was between 20 to 30 min. Donor and recipient hearts were removed at 2, 4, 6 and 8 days after transplantation. The right ventricle was fixed in a 10% formalin solution for the histological scoring. A piece of left ventricle was fixed in 0.1 mol/L phosphate-buffered 3% glutaraldehyde for electron microscopic examination. The rest of the left ventricle of each heart was weighed and dried to a constant weight. The percentage of myocardial water was calculated using following formula.

\[
\text{percent myocardial water} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100
\]

TABLE II SUMMARY OF WATER CONTENTS AND HISTOLOGICAL SCORES IN 3 GROUPS

<table>
<thead>
<tr>
<th>Group</th>
<th>POD</th>
<th>N</th>
<th>%H₂O recipient</th>
<th>%H₂O donor</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>7</td>
<td>76.1 ± 0.5</td>
<td>76.4 ± 0.5</td>
<td>1.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7</td>
<td>76.2 ± 0.5</td>
<td>76.8 ± 1.2</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6</td>
<td>75.6 ± 0.7</td>
<td>76.4 ± 0.6</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7</td>
<td>75.9 ± 0.3</td>
<td>75.4 ± 2.2</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>8</td>
<td>75.8 ± 0.8</td>
<td>77.6 ± 1.1*</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>75.5 ± 0.8</td>
<td>76.9 ± 1.0*</td>
<td>2.2 ± 0.8*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>8</td>
<td>75.6 ± 0.4</td>
<td>80.1 ± 0.9**</td>
<td>2.1 ± 1.0*</td>
</tr>
<tr>
<td>8(NB)</td>
<td>5</td>
<td>75.6 ± 0.9</td>
<td>80.4 ± 0.8**</td>
<td>3.0 ± 1.4*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>6</td>
<td>75.2 ± 0.3</td>
<td>76.5 ± 1.5</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7</td>
<td>75.8 ± 1.0</td>
<td>76.3 ± 1.0</td>
<td>0.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6</td>
<td>75.9 ± 0.6</td>
<td>76.1 ± 2.0</td>
<td>0.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9</td>
<td>75.7 ± 1.2</td>
<td>76.1 ± 1.0</td>
<td>1.4 ± 0.7**</td>
</tr>
</tbody>
</table>

POD = post operative days; N = number of rats; %H₂O = water content; HS = histological scores
B = beating hearts; NB = non-beating hearts; *p < 0.01 vs recipient hearts of each group; **p < 0.01 vs donor hearts of group 1; ***p < 0.05 vs donor hearts of group 1; **p < 0.01 vs beating hearts at day 8 in group 2

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3) Histology
   The pathological specimens were embedded in paraffin and stained with hematoxylin-eosin. There were submitted in a blind fashion for histopathological evaluation. The samples were scored on a 0 to 4+ scale, using the criteria in Table I.

4) Experimental groups
   Group 1 (n = 26): LEW recipients received LEW syngeneic cardiac grafts. All of the donor hearts in this group were beating at the time of sacrifice.
   Group 2 (n = 36): LEW recipients received BN allogeneic cardiac grafts. Five donor hearts had stopped beating prior to sacrifice at Day 8. These data are displayed separately from the beating hearts.
   Group 3 (n = 28): LEW recipients of allogeneic BN cardiac graft were treated with Ciclosporin (CYA: Sandoz Ltd, Basel, Switzerland). CYA in ethanol at 100 mg/ml was administered orally both just prior to transplant and thereafter at 15 mg/kg/day.

5) Statistics
   After completion of the entire experimental series, the data were analyzed using the least square regression analysis and the analysis of variance? The data are expressed as the mean ± SD for the tables and mean ± SEM for the graphs. In all cases, a p value of less than 0.05 was considered to be statistically significant.

RESULTS
   The data on myocardial water and histological scores are summarized in Table II.

1) Change of myocardial water during rejection process
   Figure 1 shows the change of myocardial water in the donor hearts in the 3 groups. In group 1, there was no significant change of myocardial water throughout the post operative period, even compared with the recipient hearts. In group 2, a significant change in myocardial water was noted as early as the second day and was higher than that of the recipient hearts as well as the donor hearts of group 1. The myocardial water increased remarkably at Days 6 and 8. In group 3, CYA completely suppressed the increase of myocardial water in the allografts.

2) Change in histological scores
   Figure 2 demonstrates the changes in histological scores of the donor hearts in the 3 groups.
   In group 1, no significant change was observed in histological scores throughout the post operative period. The significant difference in histo-
Fig. 2. The change in histological scores of donor hearts in 3 groups during post operative days.

Fig. 3. Ultrastructural changes in a capillary of the rejecting heart 2 days after transplant in group 2. Endothelial injury such as pinocytotic vesicles, narrowing of the lumen, and perivascular edema was noted. (C: capillary, E: endothelial cell, M: mitochondria, arrow: pinocytotic vesicle).

Fig. 4. Ultrastructural changes in a capillary of the heart 2 days after transplant in group 3. A small amount of pinocytotic vesicles were found in the CYA-treated heart. (C: capillary, E: endothelial cell, M: mitochondria, arrow: pinocytotic vesicle).

3) Ultrastructural change in the capillaries in 3 groups at the early stage of post transplant period.

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Figure 3 demonstrates the ultrastructural change in the capillaries of the heart two days after transplant in group 2 when the heart showed the significant increase in myocardial water. The capillaries showed signs of endothelial injury, such as swelling of the endothelial cells, a large number of pinocytic vesicles, perivascular edema and narrowing of the capillary lumen. Figure 4 demonstrates the ultrastructure of the capillaries in the CYA-treated hearts two days after transplant. In contrast to the nontreated hearts, the structure of the capillaries was well preserved.

4) Relationship between histological scores and myocardial water

Figure 5 shows the relationship between histological scores and myocardial water of the donor hearts in group 2. The correlation coefficient (r) was 0.34 (p > 0.05). There was no significant correlation between them.

DISCUSSION

In this study, we demonstrated the presence of early change of myocardial water preceding cellular infiltration and the progression of the myocardial edema in the rejection process. In addition, CYA treatment completely suppressed the accumulation of myocardial water in the allogeneic cardiac grafts.

Early detection of acute cardiac rejection is critically important in heart transplantation. Present criteria for detecting acute cardiac rejection includes decreased QRS summation, right axis deviation, arrhythmias, and gallop rhythm. These changes are non-specific and lag behind the onset of rejection by 24–48 hours. At the present time, endomyocardial biopsy is the most useful technique in diagnosing acute rejection and examining the efficacy of immunosuppressive therapy. Although this technique provides the evidence of the histological changes in rejecting hearts, it cannot quantitatively evaluate myocardial edema, which is a constant histologic finding at all stages of rejection.

Myocardial edema, as we demonstrated, appears at the early stage of rejection and increases with progression of rejection. However, the mechanism underlying the change of myocardial water during the rejection process remains unclear. The early change of myocardial water may be subsequent to the change of endothelial permeability. As we have demonstrated ultrastructurally, the microvascular endothelium is the primary target of immunologic injury; this in turn induces the loss of integrity of the allograft microcirculation. Our results demonstrated a significant increase in water content in the allografts, but not in the isografts nor in CYA-treated allografts. This suggests that this endothelial injury may be caused by the immunologic reaction and not by the ischemic injury during the transplant procedures. It is therefore important to note that this early change of myocardial water subsequent to endothelial injury may be used as an indicator in the detection of an acute rejection episode. However, in the clinical situation it might be difficult to differentiate between the myocardial edema caused by heart preservation or surgical procedures and that caused by rejection at the early post operative period.

We demonstrated that the myocardial water in the allografts increased rapidly with time in the post transplant period. With progression of rejection, more capillaries are destroyed and exudation of fibrin and blood occurs. These changes would be partly responsible for the rapid increase in myocardial edema. However, in this study, there was no significant correlation between myocardial water and histological grades of rejection, which was contrary to Scott's results. The myocardial water may move in a
complex manner during the rejection process. Bieber demonstrated that interstitial edema can occur without cellular infiltration at the early stage of rejection. Barnard's group also mentioned that the degree of interstitial edema does not always match the severity of cellular infiltration. In addition, lymphatic drainage may be one of the factors that effect on the myocardial water. In our study, five of the non-beating hearts at Day 8 of group 2, revealed necrosis upon histological examination and showed significant decreases of myocardial water, compared with the beating hearts at Day 8. Aystole, which is the end stage of rejection, may result from the lack of the coronary perfusion secondary to capillary or venular thrombosis. In this setting, myocardial water may not transudate into the interstitial space. Although the process is unclear, changes in myocardial water may reflect these phenomena and may not be parallel with cellular infiltration.

CYA, which is a potent immunosuppressive agent suppressed the myocardial edema as well as the cellular infiltration. It is noteworthy that CYA suppressed endothelial injury, which is thought to be caused by humoral factors other than the cellular reaction. This suggests that CYA may suppress both cellular and humoral immunity.

In summary, myocardial edema occurs at the earliest stage of rejection and CYA suppresses this phenomenon. These results suggest that myocardial water may be a good indicator for detecting cardiac rejection and examining the efficacy of immunosuppressive therapy. In addition, changes in myocardial water may be the basis of electrocardiographic decrease of QRS voltage summation and the echocardiographic increase of left ventricular mass in the rejecting hearts. Quantitative non-invasive techniques, such as magnetic resonance imaging that measure the myocardial water, may prove to be applicable for early detection of rejection in future.

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