Novel Implantable Device to Detect Cardiac Allograft Rejection

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Background—Allograft rejection remains the nemesis of solid organ transplantation. Soul Mate is a novel implantable wireless data transmission system that analyzes 9 intramyocardial electrogram parameters recorded from 4 or 6 configurations of 2 or 3 epicardial leads to detect allograft rejection. This study determined the ability of the Soul Mate to detect early rejection of transplanted hearts.

Methods and Results—Five dogs underwent heterotopic cervical heart transplantation and simultaneous implantation of the Soul Mate’s Cardiac Rejection Monitoring Device. Dogs were initially immunosuppressed, but subsequent drug discontinuation allowed allograft rejection to appear. Allograft biopsies were performed at regular intervals to determine rejection grade, which was compared to a calculated rejection score determined as percent change from baseline of values for each intramyocardial electrogram. There was significant correlation between the biopsy results and the evolution of 5 parameters. The strongest correlation ($r=0.939; P<0.001$) was obtained using the “general median” parameter from 4 configurations, assessed 1 day before the biopsy, with a sensitivity of 85.7% and a specificity of 100% compared to the myocardial biopsy results.

Conclusions—The Soul Mate allograft rejection monitoring system accurately detected transplanted heart rejection in a canine model noninvasively with continuous sampling. This proof-of-concept study suggests that the Soul Mate could be used to more intensely and more frequently monitor cardiac allografts for rejection. (Circulation. 2009;120[suppl 1]:S185–S190.)

Key Words: biopsy ■ heart failure ■ rejection ■ transplantation

Each year ≈5000 patients worldwide undergo heart transplantation (HTx). Despite improved immunosuppressive therapy, acute and chronic allograft rejection remains the most significant factor limiting the success of HTx. Early, precise, and accurate detection of rejection, with subsequent effective management, is important to minimize allograft damage and prolong morbidity-free survival. Currently, the most reliable technique for the diagnosis of acute allograft rejection is endomyocardial biopsy (EMB). However, because of its cost, associated complications, invasiveness, and inability to completely survey the allograft, EMB falls quite short of optimal diagnosis of rejection and does not allow for compulsive daily monitoring. Also, EMB can be performed only in specialized centers, and results are not immediately available. Other simpler, less invasive, more sensitive methods for detecting rejection in real time are critically needed. Electrical activity of the heart is closely related to its functional state, and analysis of intramyocardial electrocardiography (IMEG) has been considered sensitive and specific for allograft rejection.1–4 The peak-to-peak amplitude (PPA) of the unipolar IMEG has been shown sensitive to a variety of alterations in myocardial physiology1–6 but has been limited by the inability to easily transmit large quantities of continuous monitoring data. Signal-averaged electrocardiography has helped in the management of HTx rejection in clinical applications,7–9 but it is difficult to perform and not frequently done.

TransWorld Heart Corporation recently developed the Soul Mate Heart Transplant Monitoring System to monitor electrophysiological changes with the aim of allowing earlier diagnosis of graft rejection and help with acute and long-term patient management. The Soul Mate uses wireless information transmission to a centralized data reduction center with Internet-accessible daily analysis of 9 IMEG parameters recorded from 6 vectors of the heart. The purpose of this study was to assess the efficacy of this novel device to detect cardiac allograft rejection noninvasively in a canine model.
Materials and Methods

Device Description
The Soul Mate Heart Transplant Monitoring System (Figure 1) records and transfers IMEG data to the TransWorld Central Monitoring Center. The Cardiac Rejection Monitoring Device records IMEG signals through 3 standard leads, at programmable times of up to 2 minutes in either bipolar or unipolar configurations. One lead is placed on the epicardial surface of the right ventricle (RV) and two on the left ventricle. The device records information of the following 9 IMEG parameters (Figure 2) from QRS complexes for each ventricular configuration at the frequency of 1000 Hz: area under dominant peak (AUDP; mV × ms), area under minor peaks (mV × ms), area under the curve (sum of AUDP and area under minor peaks; mV × ms), base-to-dominant peak amplitude (mV), PPA (mV), nadir ECG duration (ms), total ECG duration (ms), slew rate of dominant peak upslope (mV/ ms), and slew rate of dominant peak downslope (mV/ ms).

Data transfer is achieved by holding the OneLife Wand over the Cardiac Rejection Monitoring Device, and stored data are transferred to the Wand via telemetry. The data are then transferred from the Wand to the Home Call Box through a Bluetooth wireless connection. For data analysis, the Home Call Box automatically sends the data to the Central Monitoring Center.

Experimental Design
The study protocol was approved by the Cleveland Clinic’s Institutional Animal Care and Use Committee. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, DC, 1996).

Five mongrel dogs (Hodgins Kennel, Inc, Howell, Mich) weighing 26.0±2.1 kg underwent heterotopic HTx,10–12 receiving allografts from 5 dogs weighing 8.9±0.9 kg. After the HTx and Cardiac Rejection Monitoring Device implantation, data were collected and stored automatically by the Cardiac Rejection Monitoring Device every 4 to 6 hours and transferred to the TransWorld Central Monitoring Center twice per day.

Biopsy specimens were taken at regular intervals to determine biopsy rejection grade (BG), and the results were compared with analyzed data to evaluate the efficacy of the device.

Surgical Procedures
Donor dogs were anesthetized with 15 mg/kg of intravenous thiopental, ventilated through an endotracheal tube, and placed in the right lateral position. Through a left thoracotomy, the heart was harvested using potassium crystalloid cardioplegia and placed in cold saline. An atrial septal defect was created by removing the foramen ovale, and the mitral leaflets were removed to create mitral regurgitation. Cardioplegia solution was injected every 20 minutes.

The recipient dogs were anesthetized with 3 mg/kg of intravenous propofol, intubated, and placed on the left side. The right common carotid artery and external jugular vein were exposed. The brachiocephalic trunk of the donor dog’s explanted heart was anastomosed to the recipient’s carotid artery in an end-to-side fashion under systemic heparinization (100 U/kg). The donor’s main pulmonary artery was anastomosed to the recipient’s right jugular vein. After the HTx was completed, screw-in myocardial leads were placed on the lateral wall and posterior wall of the left ventricle and the RV free wall of the donor heart. The leads were tunneled subcutaneously and attached to the Cardiac Rejection Monitoring Device, which was placed in the subcutaneous pocket on the back of the animal.

Immunosuppressive Protocol
The animals were given methylprednisolone (500 mg) during the HTx procedure. On the day of the HTx, oral immunosuppressive therapy was started, consisting of cyclosporine (20 mg/kg per day) and prednisone (0.5 mg/kg per day). The dosage of cyclosporine was adjusted to therapeutic blood levels between 400 and 600 ng/mL. Cyclosporine was discontinued at either 22 or 24 days after the HTx, with the exception of 2 animals that were euthanized during this period of immunosuppressive therapy. Prednisone was continued at the same dose during the entire postoperative course to prevent rebound adrenal insufficiency.

Surveillance EMB and Pathological Evaluation
Follow-up biopsies were performed through an incision under local and general anesthesia. Three to 4 full-layer biopsy specimens were taken from the ventricular septum with a biopsy needle (Tru-Cut Biopsy Needle, 18-G; Cardinal Health, Dublin, Ohio) and fixed in 10% buffered formalin. During the period of cyclosporine administration, a biopsy study was performed 3 times per week. After cessation of cyclosporine, daily biopsy studies were performed until the allograft stopped beating.

All biopsy specimens were sectioned at 3-step levels, stained with hematoxylin and eosin, and evaluated for the presence or absence of rejection by a cardiac pathologist blinded to the status of immunosuppressive therapy, external findings of the allograft, and the data from the device. BG was determined according to the revised classification scheme of the International Society for Heart and Lung Transplantation.13 In brief, rejection is classified as grade 0R (none), 1R (mild), 2R (moderate), and 3R (severe).

Data Analysis
The median of the noninvasively transmitted IMEG values was obtained during each recording session for 1 day per lead configuration as representative of a single parameter for that day. Two possible input sources were evaluated for each parameter, including the median of the normalized values obtained from 6 configurations (3 ventricular leads in both unipolar and bipolar mode) and from 4 configurations, excluding the RV lead. In addition, a calculation was performed by taking the parameter “general median,” which was the median of the individual medians of the 5 parameters (area under the
curve, AUDP, base-to-dominant peak amplitude, PPA, and slew rate of dominant peak upslope) as input. A sliding baseline consisted of the average values of 1 parameter over 3 consecutive days before the actual considered date. Calculated rejection grade (CG) 2 was deemed to exist when the value of the parameter was between 50% and 70% of the baseline, and CG 3 when the value was <50%. When the value of a single parameter had an average slope >8% per day or the animal was in CG 2 or 3, the baseline remained frozen.

CG were obtained each day and compared with BG taken on the corresponding date. Analysis was also performed between BG and CG just 1 day before the acquisition of biopsy data to determine the capability of the Soul Mate as an early rejection detecting device. Correlation coefficients between BG and CG for each parameter were analyzed using SPSS software (version 11.5; SPSS, Inc, Chicago, Ill).

Statement of Responsibility
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
The experimental course of each of the 5 animals, including International Society for Heart and Lung Transplantation grades of rejection for biopsy specimens, is shown in Figure 3. After the third post-HTx day, 44 biopsies were performed. Figure 4 shows representative histological findings of biopsy specimens taken from experimental animal 2 on days 2, 12, 28, and 31 postoperatively. These findings demonstrate no, mild, moderate, and severe rejection, respectively.

During the experiments, 69 035 individual heartbeats were recorded and analyzed. The 6 parameters (area under the curve, AUDP, base-to-dominant peak amplitude, PPA, slew rate of dominant peak upslope, and general median) successfully detected rejection in experiments 2 and 3. In experiment 1, the 6 parameters reported grade 2 rejections not detected by biopsy on the day of the termination, although the allograft had stopped beating, probably because of ischemia caused by a thrombus in its aortic root. In experiment 2, detection was 1 day earlier in 3 parameters (AUD, AUDP, and general median) with 4 configurations, but there was 1 false-negative episode by the device on day 5. In experiment 3, rapid progression of allograft rejection was observed, leading to complete graft failure and cardiac arrest on day 10. Detection of rejection occurred 2 days earlier than by biopsy in 6 parameters with 4 configurations (not considering the RV lead). In experiments 4 and 5, progressive rejection was not observed even after cessation of immunosuppressive therapy. Figure 5 shows examples of the changes in 6 IMEG parameters with time after heart transplantation obtained in experiments 2, 3, and 5 from 4 configurations.

Table 1 details the comparisons between BG and CG obtained through the general median parameters from 6 configurations and 4 configurations. When rejection grades are classified as negative (grade 0 or 1) or positive (grade 2 or 3), the corresponding diagnostic indices had a sensitivity of 85.7% and a specificity of 97.3% based on data from 6
configurations, and a sensitivity of 85.7% and a specificity of 91.9% based on data from 4 configurations. When BG was compared with CG, obtained 1 day before biopsy, the indices had a sensitivity of 71.4% and a specificity of 100% based on data from 6 configurations, and a sensitivity of 85.7% and a specificity of 100% based on data from 4 configurations. In Table 2, correlation coefficients between CG and BG using each parameter are shown. Significant correlations \((r>0.75; \ P<0.001)\) were obtained between the biopsy results and the 6 parameters (area under the curve, AUDP, base-to-dominant peak amplitude, PPA, slew rate of dominant peak upslope, and general median). In the rest of the 4 parameters, there were no correlations \(>0.7\). The strongest correlation \((r=0.939)\) was obtained using the general median with 4 configurations 1 day before obtaining the biopsy data.

**Discussion**

A decline in R-wave amplitude from a surface 12-lead ECG has been considered indicative of organ rejection since the early years of HTx. This decline likely reflects a decrease in functional myocardial cell mass as a result of myocyte injury and necrosis, which occurs with moderate-to-severe inflammation caused by rejection. Several reports suggest that IMEG recordings are more sensitive and specific for diagnosing graft rejection.\(^1\)\(^-\)\(^6\),\(^11\) Recently, IMEG monitoring has been clinically applied for patient management after HTx;\(^14\)\(^-\)\(^16\) however, the Soul Mate system has several significant advantages over this method, because it can measure, analyze, and transmit 9 IMEG parameters from 6 vectors. Of 9 parameters, 5 were significantly \((r>0.75; \ P<0.001)\) correlated to biopsy results and demonstrated the Soul Mate’s ability to make an early diagnosis of allograft rejection. By applying the general median parameter, the strongest correlation coefficient was obtained when BG was compared with CG obtained 1 day before biopsy. The sensitivity of 85.7% and specificity of 100% to determine biopsy-proven cardiac allograft rejection demonstrated the capability of this device to more effectively and safely monitor heart transplant patients. We believe that the ability of this device to provide more frequent recording of parameters that characterize allograft rejection will allow earlier diagnosis of significant rejection episodes requiring ad hoc immunosuppressive therapies as well as critical fine-tuning of day-to-day maintenance immunosuppressive strategies.

Unfortunately, contemporary management of patients after HTx relies primarily on a “cookbook” approach, with standard multidrug protocols usually prescribed to prevent rejection and maintain graft integrity and optimal function. Immunosuppressive drugs, however, are toxic and cause renal and hepatic insufficiency, as well as the risk of infection and malignancy in these patients that is directly related to the degree of immunosuppression prescribed.\(^17\) Management of
immunosuppressive strategies by EMB does not enable daily fine-tuning of drug administration and doses that follow a minimalist strategy. The Soul Mate can fulfill this challenge. It reported rejection not detected by biopsy on the days of experiment termination. In the absence of significant cellular rejection, allograft failure in this animal could be explained by ischemia from the autopsy findings. However, it may also be explained by the occurrence of antibody-mediated rejection, which, without additional immunohistochemical stains, routine EMB cannot detect. In a previous animal study, the sensitivity of IMEG was much higher than that of EMB (100% versus 12.5%) to detect antibody-mediated rejection.

For early detection of rejection, 6 parameters with 4 configurations (not considering the RV lead) appear to have provided the best results. In contrast, Everett et al. concluded that the sensitivity of detecting rejection increased with the increase in the number of leads, using unipolar PPA as a parameter in their animal study. We believe that using the input source without considering the RV lead enables earlier detection of rejection.

One important limitation of this study is that we used a heterotopic rather than an orthotopic HTx model. The effects of preload and afterload on the course of allograft rejection can be debated. Because the primary purpose of our experiment was to prove the concept that IMEG can detect and quantitate cardiac allograft rejection, we preferred this approach because the graft would not be burdened by the need to maintain an adequate hemodynamic load to sustain animal survival. Another limitation may be the fact that the immunosuppressive therapy used in this study was not entirely consistent with that employed in humans and that the donor dogs were not cross-matched with recipient dogs. The allograft in experiment 3 suffered from severe rejection and stopped beating on day 10, even though therapeutic cyclosporine blood concentration levels were noted and would have been predicted to have prevented this event (Figure 3). In contrast, the allograft in experiment 4 demonstrated only mild rejection 9 days after the cessation of cyclosporine. Another limitation is that the optimum “cut-offs” to determine CG grades (>50 and 70% baseline) and the 5 IMEG parameters used to derive the general median parameter were determined after the study. Also, these optimized values are specific to this animal model and may be different for human transplant rejection. Further studies, especially in humans, will be necessary to validate the proper cut-offs and the IMEG parameters for the general median calculation.

Finally, the number of animals was small, and the duration of each experiment was short. Further studies are warranted to evaluate the effects of myocardial and electrode fibrosis changes, diastology changes, ischemia (transplant vasculopathy), or infection on the sensitivity and accuracy of chronic IMEG parameter measurements to detect rejection. In addition, the effects of therapeutic interventions (such as bolus steroids) on the IMEG parameters need to be evaluated. To further validate results, human clinical trials would be a next step, given the probable safety of the device as extrapolated from experiences with simple pacemakers and defibrillators in HTx patients and demonstration that the unit can detect and monitor acute rejection, as well as transmit data via telemetry.

Table 1. Comparison Between BG and CG

<table>
<thead>
<tr>
<th>Configuration/Parameter</th>
<th>CG 0 or 1</th>
<th>CG 2</th>
<th>CG 3</th>
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</thead>
<tbody>
<tr>
<td>6 Configurations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG 0</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BG 1</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BG 2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>BG 3</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4 Configurations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG 0</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BG 1</td>
<td>27</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>BG 2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BG 3</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>6 Configurations 1 day before biopsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG 0</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BG 1</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BG 2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BG 3</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4 Configurations 1 day before biopsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG 0</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BG 1</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BG 2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>BG 3</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Correlation Coefficient Results

<table>
<thead>
<tr>
<th>Configurations</th>
<th>AUC</th>
<th>AUDP</th>
<th>BDPA</th>
<th>PPA</th>
<th>SRDPD</th>
<th>General Median</th>
<th>AUMP</th>
<th>NED</th>
<th>TED</th>
<th>SRDPU</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Configurations*</td>
<td>0.834</td>
<td>0.833</td>
<td>0.885</td>
<td>0.870</td>
<td>0.832</td>
<td>0.885</td>
<td>0.122</td>
<td>0.354 H11021</td>
<td>0.550</td>
<td>0.551</td>
</tr>
<tr>
<td>4 Configurations†</td>
<td>0.787</td>
<td>0.834</td>
<td>0.896</td>
<td>0.817</td>
<td>0.837</td>
<td>0.820</td>
<td>0.228</td>
<td>0.009</td>
<td>0.550</td>
<td>0.571</td>
</tr>
<tr>
<td>6 Configurations 1 day before biopsy‡</td>
<td>0.869</td>
<td>0.931</td>
<td>0.881</td>
<td>0.881</td>
<td>0.763</td>
<td>0.881</td>
<td>0.175</td>
<td>0.387 H11002</td>
<td>0.397 H20648</td>
<td>0.628</td>
</tr>
<tr>
<td>4 Configurations 1 day before biopsy§</td>
<td>0.884</td>
<td>0.931</td>
<td>0.890</td>
<td>0.881</td>
<td>0.852</td>
<td>0.939</td>
<td>0.17</td>
<td>0.084 H20648</td>
<td>0.397 H20648</td>
<td>0.628</td>
</tr>
</tbody>
</table>

Correlation coefficient using the data obtained from *all 6 configurations, †4 configurations excluding the RV lead, ‡all 6 configurations 1 day before the biopsy data, and §4 configurations, excluding the RV lead, 1 day before the biopsy data.

AUC indicates area under the curve; BDPA, base-to-dominant peak amplitude; AUMP, area under minor peaks; NED, nadir electrocardiogram duration; SRDPU, slew rate of dominant peak downslope; SRDPU, slew rate of dominant peak upslope; TED, total electrocardiogram duration.

The data in bold indicate r>0.75.

/ / H11002

P = 0.05.

/ / H20648

P = 0.001.
Conclusions
We conclude that the Soul Mate cardiac allograft rejection monitoring system demonstrated the capability, in real time, to accurately and noninvasively detect early acute allograft rejection in a heterotopic canine model. This approach could be used as a noninvasive tool for guiding the frequency and timing of obtaining an EMB. This device would potentially reduce the number of biopsies needed and result in earlier detection and treatment of rejection. It is possible that the device could actually replace EMB and allow vastly more frequent allograft rejection assessment that would assist the clinician with day-to-day, evidence-based adjustments of complicated and toxic immunosuppression cocktails. Furthermore, using the transtelephonic measurements could be beneficial for patients who can be monitored at great distances from the transplant center. There is a potential that the Soul Mate system would offer a method for less invasive and more effective management of HTx patients.

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Disclosures
J.B.Y. is a consultant for TransWorld Heart Corporation.

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