Impact of Prophylactic Immediate Posttransplant Ganciclovir on Development of Transplant Atherosclerosis
A Post Hoc Analysis of a Randomized, Placebo-Controlled Study

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Background—Coronary artery disease occurs in an accelerated fashion in the donor heart after heart transplantation (TxCAD), but the cause is poorly understood. The risk of developing TxCAD is increased by cytomegalovirus (CMV) infection and decreased by use of calcium blockers. Our group observed that prophylactic administration of ganciclovir early after heart transplantation inhibited CMV illness, and we now propose to determine whether this therapy also prevents TxCAD.

Methods and Results—One hundred forty-nine consecutive patients (131 men and 18 women aged 48±13 years) were randomized to receive either ganciclovir or placebo during the initial 28 days after heart transplantation. Immunosuppression consisted of muromonab-CD3 (OKT-3) prophylaxis and maintenance with cyclosporine, prednisone, and azathioprine. Mean follow-up time was 4.7±1.3 years. In a post hoc analysis of this trial designed to assess efficacy of ganciclovir for prevention of CMV disease, we compared the actuarial incidence of TxCAD, defined by annual angiography as the presence of any stenosis. Because calcium blockers have been shown to prevent TxCAD, we analyzed the results by stratifying patients according to use of calcium blockers. TxCAD could not be evaluated in 28 patients because of early death or limited follow-up. Among the evaluable patients, actuarial incidence of TxCAD at follow-up (mean, 4.7 years) in ganciclovir-treated patients (n=62) compared with placebo (n=59) was 43±8% versus 60±10% (P<0.1). By Cox multivariate analysis, independent predictors of TxCAD were donor age (relative risk, 2.7; CI, 1.3 to 5.5; P<0.01) and no ganciclovir (relative risk, 2.1; CI, 1.1 to 5.3; P=0.04). Stratification on the basis of calcium blocker use revealed differences in TxCAD incidence when ganciclovir and placebo were compared: no calcium blockers (n=53), 32±11% (n=28) for ganciclovir versus 62±16% (n=25) for placebo (P<0.03); calcium blockers (n=68), 50±14% (n=33) for ganciclovir versus 45±12% (n=35) for placebo (P=NS).

Conclusions—TxCAD incidence appears to be lower in patients treated with ganciclovir who are not treated with calcium blockers. Given the limitations imposed by post hoc analysis, a randomized clinical trial is required to address this issue.

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illness in patients who were seropositive for CMV before the transplant, but it had no effect in seronegative recipients of hearts from seropositive donors. In the same study, ganciclovir was also shown to decrease the incidence of fungal infections,\(^1\) which suggests that the drug might have effects beyond its antiviral properties. The original trial was not designed to answer the question of whether ganciclovir could prevent TxCAD but simply to determine its efficacy for preventing acute CMV disease. In the present analysis, we examined the hypothesis that inhibition of CMV disease by ganciclovir prevents the development of TxCAD. Because some of us also have reported that the calcium channel blocker diltiazem prevented TxCAD when initiated early after transplantation,\(^3\) we proposed that any protective effect of ganciclovir would be greatest in patients at highest risk for TxCAD, ie, those not treated with a calcium blocker. To test these hypotheses, we performed a post hoc analysis of the incidence of TxCAD and death or retransplantation in patients enrolled in our previously reported trial of ganciclovir for prevention of CMV disease. We also analyzed the data to determine whether any protective effect of ganciclovir was related to pretransplant donor and recipient CMV serology or to posttransplant development of CMV illness.

### Methods

#### Patients

The methods for patient selection and randomization have been described previously.\(^10\) The use of calcium channel blockers for hypertension control or cyclosporine sparing after heart transplantation was not standardized but rather was based on individual physician choice. However, approximately equal numbers of patients received calcium blockers (diltiazem 60 to 200 mg/d \(n\) = 60) or nifedipine 30 to 60 mg/d \(n\) = 8) in both treatment groups, allowing for a subset analysis of the impact of ganciclovir in patients who received calcium blockers and those who did not. Acute rejection surveillance was standardized for all patients and involved routine surveillance endomyocardial biopsies performed according to previously described protocols.\(^12\)

#### Drug Schedule

Ganciclovir or placebo was administered during the initial 28 days after transplantation. The study drug was commenced on the first postoperative day but was delayed by up to 6 days due to acute-care problems in some patients. Therapy was delayed (starting on days 2 to 7) in 22% of patients, and this was similarly distributed in both groups. Patients were given intravenous infusions of ganciclovir or placebo at a dose of 5 mg/kg of body weight every 12 hours for 14 days, followed by 6 mg/kg once daily 5 days per week for 2 weeks. Therapy was modified according to each patient's creatinine clearance if this value was abnormal. This modification occurred in 38% of all patients (43% of the ganciclovir group and 32% of the placebo group). Dose modifications were rarely needed for thrombocytopenia (2%) or neutropenia (1%).

#### Coronary Angiography

Coronary angiography was performed annually after heart transplantation by the percutaneous femoral approach by use of standard angiographic techniques. For the purpose of this study, TxCAD was defined as the presence of any angiographic disease irrespective of severity because of the recognized underestimation of TxCAD by angiography\(^13\) and the previously reported prognostic impact of angiographically “minor” TxCAD lesions.\(^4\) The actuarial incidence of TxCAD was determined from these annual angiograms and from autopsy data.

### Outcome Measures

The outcome measures analyzed were development of TxCAD on annual angiograms, death, or retransplantation. Causes of death and reasons for retransplantation were recorded. Pathological findings at autopsy and examination of explanted hearts were reviewed where available. The secondary outcome measure examined was development of CMV illness, defined as a clinical syndrome consistent with CMV disease, with confirmatory evidence from histology, culture, or serology.

#### Statistical Analysis

##### Demographic Variables

The degree of comparability between the treatment and placebo groups was analyzed for the study population as a whole and for subsets stratified according to use of calcium blockers and recipient and donor CMV serological status before transplantation. Differences in continuous variables such as donor and recipient age were analyzed by the Student \(t\) test, and dichotomous variables such as sex, indications for transplantation, use of calcium blockers, and CMV serological status were analyzed by Pearson's \(\chi^2\) test.

##### Efficacy of Treatment

Only patients who survived beyond 1 year and thus had at least 1 coronary angiogram were included in this analysis. The efficacy of ganciclovir for prevention of TxCAD and death or retransplantation was examined. The actuarial incidence of TxCAD during follow-up was determined for ganciclovir and placebo groups for the study population as a whole. Multivariate regression analysis was performed to determine the independent effect of ganciclovir and confounding covariates obtained from a univariate analysis. The covariates included in this analysis model were lack of ganciclovir prophylaxis (placebo), CMV illness after transplantation, recipient seropositive before transplant, recipient seronegative/donor positive before transplant, calcium blocker treatment, \(\geq 3\) moderate acute rejection episodes, donor age \(\geq 40\) years, transplant site (institution), and average daily doses of cyclosporine and prednisone. Covariates found to have a probability value <0.1 were entered into a Cox proportional hazard model to determine their relative risk and 95% CI. Because of the known effect of calcium blockers to prevent TxCAD, a subset analysis was performed with patients stratified according to calcium-blocker use. Differences between survival curves were examined by log rank (Mantel-Haenszel) tests for equality of survival curves, and frequencies were examined by \(\chi^2\) tests.

Because ganciclovir was shown to be ineffective for preventing CMV illness in seronegative recipients of hearts from seropositive donors in this same cohort of patients, TxCAD frequency was determined in subsets of patients defined by the recipient's CMV serological status before transplantation. The actuarial incidence of death or retransplantation was compared in ganciclovir and placebo groups by use of similar analysis and stratification methods.

#### Methods of Analysis

All statistical tests presented are 2-sided. For all comparisons, significance was declared by a probability level <0.05. All statistical analyses were performed with SAS software, versions 5 and 6.

### Results

Of the 149 patients enrolled in the study (76 in the ganciclovir group and 73 in the placebo group), 121 survived beyond 1 year and were thus entered in this analysis. Table 1 shows the characteristics of the 2 groups before treatment, the proportion of patients receiving calcium channel blocking drugs in both treatment groups, and the reasons for exclusion from the current analysis. The treatment and placebo groups were comparable with regard to age, sex, ethnic origin, incidence of pretransplant CMV seropositivity in recipients and donors, and use of calcium channel–blocking drugs. Of the 149
patients initially enrolled in the trial, 28 were excluded from this analysis for the reasons summarized in Table 1. The first-year mortality rate of 13% was equally distributed in both treatment arms and was the major reason for exclusion from this analysis. The mean duration of follow-up was 4.7 ± 1.3 years, and no patient was lost to follow-up. Patients who developed a CMV illness requiring treatment with ganciclovir were not excluded from this analysis.

Table 2 shows the distribution of patients in the treatment and placebo groups and their stratification according to the use of calcium-blocking drugs, recipient and donor pretransplant CMV serological status, and rates of TxCAD at follow-up. Patients receiving calcium blockers were unequally distributed between ganciclovir (68) and placebo (53) treatment groups; however, the difference was not statistically significant. Distribution of seronegative recipients of seropositive donor hearts to ganciclovir (14) and placebo (13) treatment groups were similar and did not differ with respect to use of calcium channel blockers. Of the total 27 seronegative recipients of grafts from seropositive donors, 12 (44%) were in the calcium-blocker group compared with 15 (56%) in the group not receiving calcium blockers. The proportions of seronegative recipients randomized to ganciclovir or placebo differed in the calcium-blocker compared with no-calcium-blocker groups: among the 68 patients receiving calcium blockers, 4 patients (6%) were randomized to the ganciclovir group compared with 8 (12%) in the placebo group. For the 53 patients not receiving calcium blockers, 10 patients (19%) were randomized to the ganciclovir group compared with only 5 (9%) in the placebo group. In both the calcium-blocker and no-calcium-blocker groups, the rate of CMV illness was significantly lower in patients randomized to ganciclovir (no calcium blocker: 7 [25%] of 28 patients; placebo: 13 [52%] of 25 patients; P ≤ 0.05; calcium blocker: 3 [9%] of 34; placebo, 13 [38%] of 34; P = 0.01).

The 28 excluded patients did not differ from the patients included in this analysis with respect to donor age, recipient age, CMV serological status of donor and recipient, and the use of calcium channel blockers.

**Spectrum of Transplant CAD by Angiography**

In the ganciclovir group, of 29 patients with angiographic evidence of transplant CAD, 8 had 10% to 30% stenosis, 8 had 31% to 50% stenosis, 8 had 51% to 75% stenosis, and 5 had >75% stenosis. Similar findings were seen in the 30 patients in the placebo group, although the number of patients with mild and moderate severity of disease was somewhat higher. In the placebo group, 3 patients had 10% to 30% stenosis, 10 had 31% to 50% stenosis, 10 had 51% to 75% stenosis, and 7 had >75% stenosis.

**Actuarial Incidence of Transplant CAD**

For the study population as a whole, the actuarial incidence of TxCAD at follow-up was 43 ± 8% in patients treated with ganciclovir compared with 60 ± 11% in the placebo group (P < 0.1). In the no-calcium-blocker subset, actuarial incidence of TxCAD was lower in the ganciclovir treatment group than in the placebo group (32 ± 11% versus 62 ± 15%; P = 0.03) (Figure). In the calcium-blocker subset, the actuarial incidence of TxCAD in ganciclovir and placebo treatment groups was 50 ± 14% versus 45 ± 12% (P = NS).

**Frequency of Transplant CAD in Subset Analyses**

TxCAD occurred at rates of 50% and 44% in ganciclovir-treated and -untreated patients who received calcium blockers. The rate of TxCAD in the no-calcium-blocker/no-ganciclovir subset was 60% compared with 51% in the entire calcium-blocker–treated subset. For the study population as a whole, of the 14 seronegative recipients randomized to prophylactic ganciclovir, 4 (28%) developed TxCAD compared with 9 (69%) of 13 seronegative patients randomized to placebo. Among seropositive recipients, TxCAD developed in 22 (47%) of 48 patients randomized to ganciclovir compared with 21 (47%) of 46 patients in the placebo group.

**CMV-Seronegative Recipients**

In the no-calcium-blocker subset, the TxCAD rate in CMV-seronegative recipients of grafts from seropositive donors randomized to ganciclovir was 20% compared with 80% in
TABLE 2. Prevalence of TxCAD at Follow-Up (Mean 4 to 7 Years) in Patients Stratified According to Calcium-Blocker Use and Recipient and Donor CMV Serologic Status

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Ganciclovir (n=62)</th>
<th>Placebo (n=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients, n</td>
<td>TxCAD, n (%)</td>
</tr>
<tr>
<td>No Ca blocker (n=53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV R−/D+ (n=15)</td>
<td>10</td>
<td>2 (20)</td>
</tr>
<tr>
<td>CMV R+/D+ or D− (n=38)</td>
<td>18</td>
<td>7 (39)</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>9 (32)</td>
</tr>
<tr>
<td>Ca blocker (n=68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV R−/D+ (n=12)</td>
<td>4</td>
<td>2 (50)</td>
</tr>
<tr>
<td>CMV R+/D+ or D− (n=56)</td>
<td>30</td>
<td>15 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>17 (50)</td>
</tr>
</tbody>
</table>

TxCAD indicates number and percentage of patients with TxCAD at follow-up.

Multivariate Analysis of Predictors of TxCAD

Cox stepwise multivariate analysis was used to determine the contribution of multiple factors to the development of TxCAD in this study (see Table 3). Donor age >40 years and lack of ganciclovir prophylaxis were associated with significantly increased relative risks for TxCAD: 2.7 (P<0.01) and 2.9 (P<0.01), respectively.

Death or Retransplantation

Rates of death or retransplantation at follow-up in the study population as a whole were similar in patients randomized to ganciclovir compared with control (22±3% versus 17±5%; P=NS). These results did not differ when patients were stratified for calcium-blocker use. In the no-calcium-blocker group, actuarial incidence of death or retransplantation at follow-up in ganciclovir compared with placebo treatment groups was 14±3% versus 25±4% (P=NS); in patients receiving calcium blockers, the incidence of death or retransplantation was 28±18% versus 12±10% (P=NS).

Causes of Death

There was no difference in causes of death between ganciclovir and placebo treatment groups, regardless of use of calcium blockers. TxCAD accounted for 5 of 16 deaths in the ganciclovir group and 4 of 15 deaths in the placebo group. Among patients not treated with calcium blockers, 13% of deaths occurred in the ganciclovir group compared with 27% in the placebo group. This contrasts with the results in patients treated with calcium blockers, in whom 40% of deaths occurred in the ganciclovir group compared with 20% in the placebo group. None of these differences were statistically significant.

Discussion

The present study provides evidence suggesting that prophylactic treatment with ganciclovir initiated immediately after heart transplantation reduces the incidence of TxCAD and that this effect is most marked in patients not receiving calcium channel–blocking drugs. However, this analysis is potentially flawed and must be supported by a randomized, clinical trial to appropriately address the question of whether ganciclovir prevents TxCAD. Because this study was not...
Initially designed to assess this question, there are considerable limitations regarding the statistical power of such a post hoc analysis. Most importantly, the study was not powered to address the question of TxCAD in the study as a whole or in the multiple subsets analyzed nor to calculate the relative risk of multiple variables. Notwithstanding these limitations, for the study population as a whole there was a trend toward a decreased incidence of TxCAD in the ganciclovir-treated group. When multiple risk factors were considered together, lack of ganciclovir prophylaxis remained a significant predictor of TxCAD, together with donor age. Ganciclovir treatment was associated with a reduced incidence of TxCAD compared with placebo in patients not treated with calcium channel blockers. These results are consistent with several observational studies reporting a correlation of TxCAD with CMV infection and duration of viremia. Similar protective effect of early administration of calcium channel blockers in this analysis, in contrast to the results of published clinical trials. However, in prior trials, calcium blocker treatment was initiated early after transplantation, at the time of greatest vascular injury from ischemia/reperfusion, alloimmune responses, and hyperlipidemia. In the present study, the use of calcium-blocking drugs was not pre-specified and was likely begun later in the patient’s course as treatment for hypertension or as a cyclosporine-sparing strategy. Thus, the discrepancy of the observations regarding calcium-blocker effects on TxCAD compared with published data is likely explained in part by late initiation of the drug. An alternative explanation is that these findings are most likely due to the limitations of the sample size in the present study, which was insufficiently powered to evaluate the effect of ganciclovir in multiple subsets of patients.

In the original report of this randomized trial, we observed that ganciclovir was effective in preventing CMV illness in seropositive recipients but was ineffective in seronegative recipients of seropositive grafts. Therefore, we expected to find that CMV illness would predict development of TxCAD. In the multivariate analysis, however, neither CMV illness nor recipient serostatus proved to be an independent predictor of TxCAD. Rather, the results suggested that other factors, such as donor age and lack of ganciclovir prophylaxis, were stronger predictors of TxCAD. Aside from possible random effects as a consequence of the limitations of post hoc analysis, the lack of a correlation of TxCAD with CMV illness suggests that ganciclovir might prevent TxCAD by mechanisms independent of its ability to inhibit viral replication and CMV illness. This would be consistent with experimental studies showing a dose-dependent inhibitory effect of ganciclovir on smooth muscle cell replication of uninfected cells. Thus, the inhibitory effect of ganciclovir on CMV-enhanced graft atherosclerosis may not be linked only to viral inhibition but suggests that additional pathways may be involved.

The discordance between CMV illness and TxCAD is emphasized by the higher rates of TxCAD compared with the incidence of CMV illness. The results indicate that despite effective prevention of CMV illness with ganciclovir, TxCAD developed in some patients, raising the possibility of other contributing factors, such as donor age. Another possible explanation is that despite its efficacy in blocking viral replication and preventing CMV illness, ganciclovir was only partially effective in inhibiting activation of the gene products that are the cellular and molecular mediators of TxCAD. In the aortic allograft model of transplant atherosclerosis, ganciclovir prophylaxis did not alter the elevated expression of mitogens, and it reduced but did not completely block intimal thickening. Recent in vitro studies demonstrated that induction of cell surface expression of adhesion molecules (ICAM-1 and LFA-3) occurs as a direct consequence of CMV infection and is not blocked by antiviral treatment with ganciclovir or foscarnet. This suggests that despite effective antiviral therapy in transplant recipients, CMV-infected cells may continue to provide a focus for proinflammatory activity, which could contribute to the pathophysiology of TxCAD.

Another potentially important finding from the present analysis is that for the study population as a whole, a lower proportion (28%) of seronegative recipients randomized to
prophylactic ganciclovir developed TxCAD than did seronegative patients randomized to placebo (69%). This finding in seronegative patients contrasts with seropositive recipients, in whom TxCAD rates did not differ between patients randomized to ganciclovir versus placebo. Because the original trial showed that ganciclovir was ineffective in preventing CMV illness in these seronegative recipients, the observations from the present analysis suggest an effect of ganciclovir that is independent of its inhibitory actions on viral replication and CMV illness.

The limitations of the present study include the fact that it is retrospective; 19% of the original group of patients were excluded; subgroup analyses have been performed; treatment with calcium channel blockers was not randomized; and the numbers of patients in subgroups showing benefits was very small. Another important limitation is that TxCAD was assessed by angiography, which is known to underestimate the severity of the disease. The currently accepted gold standard for monitoring TxCAD, intravascular ultrasound, was not used in the present study. This is because the study predated the introduction of this technology, and no patient had intravascular ultrasound studies during the 5-year follow-up period.

The major limitation of the present study is that it was not powered to address the outcome of TxCAD, and the patients included in the analysis constitute a group “selected” because they survived beyond 1 year. This selection could have biased our results, because the patients with the worst CMV illness complicated by other infections and rejection may have died during the first year of follow-up. Furthermore, TxCAD is likely multifactorial and would require a larger sample size to adequately ensure unbiased distribution of high-risk patients among treatment and placebo groups. Similar considerations apply to the differential efficacy of ganciclovir for prevention of CMV illness in various subsets of patients. Notwithstanding these limitations, the results of the present study suggest that prophylactic immediate posttransplant ganciclovir administration decreases the incidence of TxCAD. This observation, if confirmed by a prospective randomized trial, suggests that prevention of CMV infection with ganciclovir, combined with other therapies to fully block the disease, could provide significant improvement in the outcome of patients after heart transplantation. These findings might also have important implications for prevention of coronary artery restenosis in native hearts.

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