Soluble Human Complement Receptor 1 Limits Ischemic Damage in Cardiac Surgery Patients at High Risk Requiring Cardiopulmonary Bypass

Harold L. Lazar, MD; Paula M. Bokesch, MD; Frederick van Lenta, PhD; Carmel Fitzgerald, RN, MS; Constance Emmett, MS; Henry C. Marsh, Jr, PhD; Una Ryan, PhD; OBE and the TP10 Cardiac Surgery Study Group

Background—This study was undertaken to determine whether soluble human complement receptor type 1 (TP10), a potent inhibitor of complement activation, would reduce morbidity and mortality in high-risk patients undergoing cardiac surgery on cardiopulmonary bypass (CPB).

Methods—This was a randomized multicenter, prospective, placebo-controlled, double-blind study in which 564 high-risk patients undergoing cardiac surgery on CPB received an intravenous bolus of TP10 (1, 3, 5, 10 mg/kg) or placebo immediately before CPB. The primary endpoint was the composite events of death, myocardial infarction (MI), prolonged (≥24 hours) intra-aortic balloon pump support (IABP), and prolonged intubation.

Results—TP10 significantly inhibited complement activity after 10 to 15 minutes of CPB and this inhibition persisted for 3 days postoperatively. However, there was no difference in the primary endpoint between the 2 groups (33.7% placebo versus 31.4% TP10; P=0.31). The primary composite endpoint was, however, reduced in all male TP10 patients by 30% (P=0.025). TP10 reduced the incidence of death or MI in males by 36% (P=0.026), the incidence of death or MI in CABG males by 43% (P=0.043) and the need for prolonged IABP support in male CABG and valve patients by 100% (P=0.019). There was, however, no improvement seen in female TP10 patients. There were no significant differences in adverse events between the groups.

Conclusion—TP10 effectively inhibits complement activation during CPB; however, this was not associated with an improvement in the primary endpoint of the study. Nevertheless, TP10 did significantly decrease the incidence of mortality and MI in male patients. (Circulation. 2004;110[suppl II]:II-274–II-279.)

Key Words: complement ■ cardiopulmonary bypass ■ myocardial ischemia

T
he inflammatory response that results from complement activation during cardiopulmonary bypass (CPB) may result in myocardial and multiorgan dysfunction and contribute to postoperative morbidity and mortality.1–3 Complement activation results in increased levels of C3a and C5a, which mediate leukocyte activation, increasing the influx and activation of these inflammatory cells and resulting in endothelial disruption and tissue edema.4 Levels of C5b-9, termed membrane attack complexes, are increased and deposited in areas of infarcted and ischemic myocardium. Complement activation results in cell injury by promoting neutrophil adhesion to the endothelium and by directly lysing cellular membranes. The magnitude of this inflammatory response on CPB, particularly the level of C3a, has been shown to correlate with postoperative organ dysfunction and adverse clinical outcomes.2

The detrimental effects of complement activation during CPB have prompted the development of interventions such as leukocyte filters and heparin-bonded circuits in an attempt to inhibit complement activation and improve patient outcomes.5–7 Another approach is to directly inhibit complement activation with pharmacologic agents. Soluble complement receptor type 1, designated TP10, is a potent inhibitor of the classical and alternative complement pathways.8 TP10 was generated using recombinant DNA methods to truncate the naturally occurring, human, cell-surface complement regulatory protein CR1 yielding a soluble derivative. TP10 is produced in Chinese hamster ovary cell culture and purified to yield a 240 000-kDa glycoprotein that retains multiple complement inhibitory activities. TP10 exerts its biological actions by accelerating the decay of the convertases that activate C3 and C5 and by acting as a required cofactor in the

From Department of Cardiothoracic Surgery (H.L.L., C.F.), Boston University School of Medicine and Boston Medical Center, Boston, Mass; Department of Anesthesiology (P.M.B.), Emory University, Atlanta, Ga; Department of Laboratory Medicine (F.v.L.), Boston Medical Center, Boston, Mass; AVANT Immunotherapeutics, Inc (C.E., H.C.M., U.R.), Needham, Mass.

Correspondence to Harold L. Lazar, MD, Department of Cardiothoracic Surgery, Boston Medical Center, 88 East Newton Street, Boston, MA 02118. E-mail harold.lazar@bmc.org

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proteolytic degradation of C3b and C4b by factor I. As a result, TP10 inhibits the production by the complement cascade of the inflammatory anaphylatoxins, C3a and C5a, and of the membrane attack complex C5b-9.

In a porcine model of coronary artery bypass graft (CABG) surgery on CPB, TP10 infused at 10 mg/kg completely inhibited complement activation, decreased lung edema, and resulted in better preservation of regional wall motion and significantly less myocardial necrosis.9,10 This study was, therefore, undertaken to determine whether TP10 might also be effective in inhibiting complement activation in patients undergoing cardiac surgery on CPB and whether this would contribute to better clinical outcomes in patients at high risk.

Methods

Study Design

This was a prospectively randomized, placebo-controlled, parallel group, phase II trial involving 33 centers in the United States. Approval was obtained from the Institutional Review Boards of all centers and written informed consent was obtained from all patients. Inclusion criteria included patients at high risk undergoing cardiac surgery on CPB, such as repeat sternotomy, ejection fraction ≤30%, combined CABG and valve surgery, or urgent and emergent CABG or valve surgery using Society of Thoracic Surgery criteria. Patients were excluded if they required cardiopulmonary resuscitation within 24 hours of randomization, they were not expected to survive 7 days after surgery, had renal or hepatic failure, had a known complement deficiency or immune deficiency syndrome, or had documented HIV infections.

The primary endpoint was the composite incidence of death, myocardial infarction (MI), prolonged ventilatory (≥24 hours), and prolonged (≥24 hours) intra-aortic balloon pump support. Secondary endpoints included the time from treatment to death or an MI, postoperative length of stay, and the degree of complement suppression. In addition, the effect of TP10 on the length of ventilatory support and intensive care unit length of stay, the incidence of arrhythmias, changes in cognitive function using mini-mental state examination (MMSE) scores, chest tube drainage, blood product utilization, and the incidence of adverse events were also recorded.

Study Protocol

After anesthetic induction, patients were assigned to 1 of 5 treatment groups using a blocked randomization. All patients received a single intravenous infusion of TP10 (1 mg/kg, 3 mg/kg, 5 mg/kg, or 10 mg/kg), or placebo over 30 minutes before mediasternotomy. The infusion was completed before CPB. An upper dose of 10 mg/kg was selected based on previous studies in adult lung transplantation patients and other indications, which showed that this dose was associated with the most complete inhibition of complement for up to 3 days. After discussion with the FDA, smaller doses were also used to generate a dose–response curve. CPB was conducted using moderate hypothermia (32°C to 36°C), hemodilution, and cardioplegia as per practice standard at each institution. No attempt was made to alter or standardize anesthetic or surgical techniques. All patients were followed-up for a 28-day period and were monitored by a data safety and monitoring board.

Myocardial Infarction

All electrocardiogram (ECG) and creatine kinase (CK)-myocardial band (MB) data were forwarded to a core laboratory (Washington University, St. Louis, Mo) for adjudication and diagnosis of MI. In patients undergoing valvular surgery, the presence of a Q or non-Q MI was based on the Minnesota Code Classification using an adaptation of the Nova Code for serial comparison. A 2-grade worsening of the code qualified as a Q MI. For patients undergoing CABG, MI was diagnosed according to the same ECG criteria or by the magnitude of the CK-MB elevation. The upper limits of normal for the local laboratories at each center were used as the peak value observed in the analysis. A diagnosis of a non-Q MI was made when the peak CK-MB was ≥10 upper limits of normal in the absence of ECG criteria for Q MI. The overall diagnosis of MI was made by the presence of either Q MI or non-Q MI.

Biological Assays

Serum and plasma samples were immediately centrifuged and stored at −80°C until assayed. Hemolytic (CH50) assays were performed at a core laboratory as previously described.11 TP10 concentrations were measured in plasma samples at a core laboratory using a specific biotinylated monoclonal TP10 antibody enzyme-linked immunosorbent assay. Plasma levels of C3a and SC5b-9 were analyzed at a core laboratory using a specific biotinylated monoclonal antibody enzyme-linked immunosorbent assay (Quidel, San Diego, Calif).11 The presence of anti-TP10 antibodies were screened using the enzyme-linked immunosorbent assay method at a core laboratory. The specificity of positive samples was confirmed by competitive enzyme-linked immunosorbent assay using antisera specific for the Knops/McCoy minor blood group antigens, which are found on CR1.12

Schedule of Assessments

Complete blood counts, biochemistry profiles, and urinalyses were obtained before surgery, 24 hours after the end of the drug infusion, and 24 hours before discharge from the hospital. Samples for total CK and CK-MB were obtained every 8 hours for the first 24 postoperative hours. MMSE were administered the day before surgery, 24 hours after surgery, and 28 days after surgery. A 12-lead ECG was recorded before and every 24 hours after surgery until postoperative day 5 and again on postoperative day 28.

Statistical Analyses

The primary analysis was a comparison of the response rate in the placebo group, with the average of the rates in the 5- and 10-mg/kg groups, because previous experimental and clinical studies showed the greatest suppression of complement activation was achieved at these doses. The study was powered assuming a reduction in the primary endpoint event rate from 25% in the placebo group to 15% in the combined 5- and 10-mg/kg groups. All data represent the mean±SD. Hypothesis tests involving dose–response was 1-tailed, with statistical significance defined as P≤0.05. All other hypothesis tests were 2-tailed, with statistical significance defined as P≤0.05. The Kruskall–Wallis test was used to compare values among the different patient cohorts with adjustment for multiple comparisons. Spearman correlations were used to assess correlation between TP10 concentration and clinical outcome parameters. All data analyses were conducted using SAS version 6.12 software by an independent monitor.

Results

The results are summarized in Tables 1 to 3 and Figures 1 to 4. Of the 564 patients enrolled, 549 received the study drug. There were 23 deaths, and 13 subjects were randomized but did not receive the study drug.

Patient profiles are reviewed in Table 1. The groups did not differ in age, gender, the type or urgency of the surgery performed, and the time on CPB. The majority of patients (61.2%) underwent CABG, 31% had combined CABG and valve surgery, and 7.8% had only valve surgery. Only 58 patients (10.3%) received aprotinin and separate analyses of these patients showed no effect of aprotinin on outcome.

The pharmacokinetics of TP10 were analyzed using a 2-compartment model with a weighting scheme of 1 per concentration (Figure 1). Cmax was dose-dependent and ranged from 21.1±6.7 mg/mL at 1 mg/kg dose to 219.3±66.5 mg/mL at 10 mg/kg. TP10 plasma concentra-
tions declined biphasically after dosing. $T_{\text{max}}$ was independent of dose and ranged from 0.78±0.5 hours at 1 mg/kg dose to 0.84±0.7 hours at 10 mg/kg. Area under the time–plasma concentration curve was dose-dependent and ranged from 167.5±49.8 μg·h/mL at 1 mg/kg to 1668.9±601 μg·h/mL at 10 mg/kg. The elimination half-life of TP10 was 56.6 and 55.1 hours for the 5- and 10-mg/kg dose groups, respectively.

All TP10 groups showed marked suppression of available serum complement activity (CH50) compared with the placebo group (Figure 2). This effect was seen after only 5 to 10 minutes on CPB and persisted for 3 days postoperatively. The greatest suppression was seen in the 5- and 10-mg/kg doses. Complement activation, as evidenced by C3a and SC5b-9 generation, was greatest during and up to 2 hours after CPB and was profoundly inhibited in all patients receiving TP10 (Figures 3 and 4). CH50 values from males and females in each dose group were comparable (data not shown).

The event rate in the primary endpoint was lower in the TP10 group (31.4% versus 33.7% in placebo) but failed to achieve statistical significance ($P=0.32$) in the intent-to-treat population. In the subgroup of patients undergoing combined CABG and valve surgery, TP10 treatment yielded a 74% relative reduction in QMI (from 9.3% in placebo to 2.4%; $P=0.038$) and completely eliminated the incidence of prolonged intra-aortic balloon pump support (7.0% in placebo versus 0.0%; $P=0.005$).

Prospectively defined analyses of treatment interaction revealed that gender had a significant effect on the primary endpoint ($P=0.011$). Significant treatment interactions were not observed for age, weight, ethnicity, study center, or type of surgery. Subsequent analysis of the primary endpoint in the male subpopulation revealed a significant 30% relative reduction in event rate (35.4% in placebo to 24.6% for TP10; $P=0.026$). There was, however, no effect on the primary endpoint seen in female patients ($P=0.962$). Further subgroup analysis of the male subpopulation revealed additional significant improvements in components of the primary endpoint, lending support to the positive finding in the composite endpoint. TP10 reduced the incidence of death or MI in all male patients by 36% (26.8% for placebo versus 17.1% for TP10; $P=0.025$) and the incidence of MI in all male TP10 patients by 40% (23.0% for placebo versus 13.9% for TP10; $P=0.003$). Male CABG patients treated with TP10 experienced a 43% reduction in the incidence of death or MI (22.6% placebo versus 12.8% TP10; $P=0.043$). Male TP10 patients undergoing combined CABG and valve procedures experienced a 49% reduction in MI (35.7% for placebo versus 18.3% for TP10) and a 100% reduction in the need for prolonged intra-aortic balloon pump support (7.1% for placebo versus 0% for TP10; $P=0.019$). Female patients receiving TP10 showed no significant differences from the placebo group (Table 2).

TP10 achieved these beneficial effects without any increase in the incidence of adverse effects (mean number of adverse effects=8.9 for placebo versus 9.2 for TP10). Because of the importance of complement in host defense against infectious pathogens, special attention to adverse events related to infection is appropriate. As seen in Table 3, there were no apparent effects on the incidence of reported adverse events of infection in any treatment group, including postoperative wound infections. Twenty-three deaths occurred while patients were undergoing study: 11 in the placebo group and 12 in TP10-treated patients. Specific adverse events resulting in death are summarized in Table 4. TP10 had no effect on the incidence of cardiac arrhythmias, hospital, or intensive care unit length of stay, cognitive function, blood loss or blood product utilization, weight gain, or infections.

**Discussion**

Our study has shown that TP10 inhibits complement activation on CPB and as a result improves outcomes in male patients undergoing high-risk cardiac surgery on CPB. This improvement appears mainly because of decreased myocardial necrosis. These results confirm earlier experimental
studies, which show that TP10 given before periods of ischemic injury will limit infarct size during reperfusion.\textsuperscript{9,10} The most likely mechanism involves the ability of TP10 to suppress all complement activation, which decreases the harmful effects of neutrophil activation. Weissman et al have demonstrated in a rat model that pretreatment with TP10 before ischemia significantly decreases leukocytes in the peri-infarct zone and completely abolishes membrane attack complex deposition in infarcted and ischemic tissue.\textsuperscript{13} The suppression of C3a and SC5b-9 in our human studies supports these earlier experimental studies.

We found that TP10 had no effect on neurocognitive outcome in this study. Furthermore, we found no significant change in the MMSE from baseline at 7 or 28 days after CPB in any of the treatment groups, including placebo. In a recent study of cardiac surgery patients on CPB using the anti-C5 monoclonal antibody pexelizumab, Shernan et al also found no improvement in postoperative MMSE scores with anti-complement therapy.\textsuperscript{14} Although the MMSE is useful for screening gross neurological impairment, other more sensitive cognitive function tests may be required to document subtle changes in memory and learning.

### TABLE 2. Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Placebo</th>
<th>1 mg/kg</th>
<th>3 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>Difference From Placebo (90% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Endpoint</td>
<td>209</td>
<td>51</td>
<td>55</td>
<td>45</td>
<td>48</td>
<td>10.8%</td>
<td>0.0264</td>
</tr>
<tr>
<td>Males: base</td>
<td>74 (35.4%)</td>
<td>17 (33.3%)</td>
<td>23 (41.8%)</td>
<td>10 (22.2%)</td>
<td>13 (27.1%)</td>
<td>(1.6%, 19.9%)</td>
<td>0.9616</td>
</tr>
<tr>
<td>N of Events (event rate)</td>
<td>73</td>
<td>21</td>
<td>15</td>
<td>23</td>
<td>24</td>
<td>-15.9%</td>
<td>0.0227</td>
</tr>
<tr>
<td>Females: base</td>
<td>21 (28.8%)</td>
<td>8 (38.1%)</td>
<td>9 (60.0%)</td>
<td>10 (43.5%)</td>
<td>11 (45.8%)</td>
<td>(-30.7%, -1.1%)</td>
<td>0.9723</td>
</tr>
<tr>
<td>N of Events (event rate)</td>
<td>209</td>
<td>51</td>
<td>55</td>
<td>45</td>
<td>48</td>
<td>9.1%</td>
<td>0.9754</td>
</tr>
<tr>
<td>MI</td>
<td>209</td>
<td>51</td>
<td>55</td>
<td>45</td>
<td>48</td>
<td>-15.4%</td>
<td>0.5960</td>
</tr>
<tr>
<td>Males: base</td>
<td>48 (23.0%)</td>
<td>7 (13.7%)</td>
<td>19 (34.5%)</td>
<td>4 (8.9%)</td>
<td>9 (18.8%)</td>
<td>(1.6%, 16.7%)</td>
<td>0.9706</td>
</tr>
<tr>
<td>N of Events (event rate)</td>
<td>73</td>
<td>21</td>
<td>15</td>
<td>23</td>
<td>24</td>
<td>-16.2%</td>
<td>0.9706</td>
</tr>
<tr>
<td>Females: base</td>
<td>12 (16.4%)</td>
<td>5 (23.8%)</td>
<td>4 (26.7%)</td>
<td>7 (30.4%)</td>
<td>8 (33.3%)</td>
<td>(-28.7%, -2.2%)</td>
<td>0.3877</td>
</tr>
</tbody>
</table>

Difference from placebo is the placebo event rate minus the average event rate of the 5- and 10-mg/kg groups. \( P \) based on a z-score statistic. MI indicates myocardial infarct; IABP, intra-aortic balloon pump.

### TABLE 3. Perioperative Infections

<table>
<thead>
<tr>
<th>Event</th>
<th>Placebo (n=272)</th>
<th>1 mg/kg (n=72)</th>
<th>3 mg/kg (n=70)</th>
<th>5 mg/kg (n=68)</th>
<th>10 mg/kg (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulitis (%)</td>
<td>5 (1.8%)</td>
<td>2 (2.8%)</td>
<td>1 (1.4%)</td>
<td>2 (2.9%)</td>
<td>2 (3.0%)</td>
</tr>
<tr>
<td>Pneumonia (%)</td>
<td>8 (2.9%)</td>
<td>1 (1.4%)</td>
<td>2 (2.9%)</td>
<td>1 (1.5%)</td>
<td>2 (3.0%)</td>
</tr>
<tr>
<td>Urinary tract infection (%)</td>
<td>11 (4.0%)</td>
<td>2 (2.8%)</td>
<td>1 (1.4%)</td>
<td>1 (1.5%)</td>
<td>4 (6.0%)</td>
</tr>
<tr>
<td>Postoperative wound infection</td>
<td>14 (5.1%)</td>
<td>7 (9.7%)</td>
<td>5 (7.1%)</td>
<td>4 (5.9%)</td>
<td>2 (3.0%)</td>
</tr>
</tbody>
</table>
In our study, female patients did not benefit from TP10 therapy. Differing outcomes in women are not an uncommon finding, because women have failed to show clinical benefits from earlier trials using beta blockers and ACE inhibitors. Women have been shown to have poorer outcomes after CABG surgery. In the Society of Thoracic Surgery database consisting of 334,913 patients undergoing CABG surgery, women, who represented 28% of the database, had an operative mortality of 4.5% compared with 2.6% for men (P < 0.0001). This persisted even after the use of the internal mammary artery. Female gender has also been shown to be an independent risk factor for increased mortality after combined CABG and valve procedures. Female CABG patients may be more susceptible to poorer outcomes because they have a higher incidence of diabetes, heart failure, left ventricular hypertrophy, and diastolic dysfunction, all of which contribute to higher postoperative mortality. An other reason for the lack of improvement for women in clinical trials is their small sample size. Only 28% of the population of this study was women, and this small sample size limits the detection of statistical benefit because the study was only powered to show a benefit in the overall population. TP10 resulted in equal suppression of complement in both male and female patients in our study. Hence, the mechanism for the lack of benefit for females by TP10 may involve factors other than complement suppression. Further studies are being planned to address these issues and to recruit a larger number of female cardiac surgery patients at high risk.

TP10 showed no increase in adverse events compared with placebo. A single intravenous dose of TP10 before CPB inhibited available complement hemolytic activity (CH50) for 3 days and the production of C3a and SC5b-9 during the 2 hours after CPB. Only a single bolus before the onset of CPB is required to protect the myocardium. There were no side effects or adverse events associated with administering
this drug relative to placebo, as might be expected for a soluble version of a natural human receptor.

In conclusion, although the study failed to meet its prospective primary endpoint, subsequent analysis indicated the complement suppression with TP10 was effective in decreasing mortality and MI in male patients undergoing cardiac surgery on CPB. TP10 was well-tolerated and readily administered as an intravenous single bolus infusion over 30 minutes. Although the beneficial effects of TP10 were not extended to females in this study, further studies with larger numbers of women are ongoing to see whether this group of patients will also benefit from TP10 therapy.

References

TABLE 4. Summary of Patient Deaths

<table>
<thead>
<tr>
<th>Gender (M/F)</th>
<th>Dose (mg/kg)</th>
<th>Placebo</th>
<th>Gender (M/F)</th>
<th>TP10</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Pulmonary embolus</td>
<td>1</td>
<td>M Respiratory arrest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Cardiogenic shock</td>
<td>1</td>
<td>M Cardiac arrest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Myocardial infarction</td>
<td>3</td>
<td>M Cardiopulmonary arrest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F Pulmonary hypertension</td>
<td>3</td>
<td>M Septic shock</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Septic shock</td>
<td>3</td>
<td>F Brain stem insult</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Metabolic acidosis</td>
<td>3</td>
<td>M Myocardial infarction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Respiratory failure</td>
<td>5</td>
<td>F Acute respiratory failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Exsanguination</td>
<td>5</td>
<td>M Multiple arterial emboli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Perioperative myocardial infarction</td>
<td>5</td>
<td>M Refractory hypoxemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Cardiac arrest</td>
<td>10</td>
<td>F Pulmonary emboli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Pneumonia</td>
<td>10</td>
<td>M Multisystem organ failure, acute renal failure, respiratory failure, and sepsis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F Ischemic colitis requiring surgical intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Soluble Human Complement Receptor 1 Limits Ischemic Damage in Cardiac Surgery Patients at High Risk Requiring Cardiopulmonary Bypass

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