Preliminary Report of the Effects of Complement Suppression With Pexelizumab on Neurocognitive Decline After Coronary Artery Bypass Graft Surgery

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Background and Purpose—Pharmacological modulation of complement activation recently has been postulated as a therapeutic target in the treatment of neurological injury. We hypothesized that pexelizumab, a humanized scFv monoclonal antibody directed against the C5 complement component, would limit deficits in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass.

Methods—The Phase II Pexelizumab study was a 914-patient, double-blind, placebo-controlled, 65-center study of patients undergoing coronary artery bypass graft surgery. Patients were randomized to pexelizumab bolus, bolus plus infusion, or placebo. Neurological and neurocognitive functions were assessed as secondary endpoints at baseline and on postoperative days (POD) 4 and 30. Cognitive deficits were assessed with multivariable tests accounting for baseline cognition, age, diabetes, years of education, sex, elevated creatinine, history of myocardial infarction, neurological disorder or congestive heart failure, and cardiopulmonary bypass time.

Results—Pexelizumab had no statistically significant effect on the primary composite endpoint or on overall cognition. When domain specific effects were examined, a decline of at least 10% in the visuo-spatial domain was observed on POD 4 in 56% of patients receiving placebo compared with 40% receiving pexelizumab by bolus and infusion ($P=0.003$). Similarly, on POD 30, a 10% decline was present in 21% of patients in the placebo group versus only 12% of the drug bolus plus infusion group ($P=0.016$). No differences were seen between treatment groups in any of the other domains.

Conclusions—Pexelizumab administration for 24 hours perioperatively had no effect on global measures of cognition but may reduce dysfunction in the visuo-spatial domain. (Stroke. 2004;35:2335-2339.)

Key Words: antibodies ■ cognitive disorders ■ inflammation ■ ischemia ■ reperfusion injury

Although mortality for patients undergoing cardiac surgery continues to decline, unacceptable rates of postoperative cognitive decline remain, occurring in 53% of patients immediately after surgery and in 30% after 6 months. Quality of life may be diminished for these patients who anticipate that postoperative improvements in physical status will generally improve their lives. Additionally, deterioration in cognitive functioning strains the availability of critical healthcare resources.

Coronary artery bypass grafting (CABG) requiring cardiopulmonary bypass (CPB) is associated with an ischemia–reperfusion (IR) injury, which triggers a complex inflammatory response in the heart as well as in the lungs, kidneys, gut, and brain. Although cerebral embolization is among the inflammatory mediators that alter vascular permeability and activate leukocytes, endothelial cells, and the coagulation cascade, pexelizumab, a 25-kDa recombinant, humanized, single-chain antibody, blocks C5 cleavage in the classical, alternate, and lectin complement pathways. In a preliminary study of 35 patients undergoing CABG surgery, administration of pexelizumab reduced total complement...
activity, soluble C5b-9 formation, and leukocyte activation. Patients receiving the highest dose also demonstrated an 80% reduction in new cognitive deficits. Therefore, to assess this potential benefit of pexelizumab in a larger cohort of patients, we evaluated cognitive function in the 914 patients enrolled in the Phase II Pexelizumab study.

Materials and Methods

Study Design
The Phase II Pexelizumab study was a prospective, multicenter, randomized, double-blinded, placebo-controlled trial approved by each site’s institutional review board. Patients were eligible for enrollment if they were aged 40 years or older and had at least 1 of the following risk factors for ischemia: age 60 or older, previous CABG surgery, history of neurologic event, history of New York Heart Association class III or IV congestive heart failure, history of ≥2 myocardial infarctions (MI) or an MI that occurred >48 hours but <4 weeks before surgery, or creatinine ≥1.3 mg/dL. Patients were excluded if the weight was >120 kg, had a preoperative mechanical assist device such as an intra-aortic balloon pump, were in cardiacogenic shock within 72 hours of surgery, had planned carotid or aortic arch surgery, experienced an MI within 48 hours, or had a ventricular pacemaker, left bundle branch block, renal insufficiency (creatinine ≥2 mg/dL), uncontrolled diabetes (glucose >400 mg/dL), history of malignancy, presence or suspicion of active infection, history of dementia, or history of alcohol or drug abuse within 2 years of screening. After informed consent was obtained, 914 patients undergoing elective CABG with or without valve surgery were enrolled at 65 sites throughout the United States. Patients were prospectively stratified into CABG (n=800) and CABG plus valve (n=114) surgery, and randomized to 1 of 3 treatment arms initiated immediately before CPB: placebo, pexelizumab 2.0 mg/kg bolus (bolus), or pexelizumab 2.0 mg/kg bolus followed by 0.05 mg/kg per hour for 24 hours (bolus + infusion). The primary endpoint was the combined incidence of all-cause mortality (from any cause), MI (defined as new Q-wave by electrocardiogram criteria or non-Q-wave by myocardial band isoenzyme of creatine kinase >60 ng/mL), left ventricular dysfunction (use of 4 or more inotropes, left ventricular assist device, or intra-aortic balloon pump), and new central neurologic deficit (an increase of 1 point on the National Institutes of Health Stroke Scale [NIHSS]) on postoperative day (POD) 4. Secondary endpoints included neurocognitive dysfunction, bleeding, transfusion rates, and renal function. Patients undergoing CABG plus valve surgery were excluded from the current analysis to achieve a more homogenous sample.

Measurement of Neurologic Function
The NIHSS, quantifying neurologic deficits in 11 categories, was administered preoperatively and again on POD 4 and 30. In addition, a clinical diagnosis of postoperative stroke was recorded.

Measurement of Neurocognitive Function
Trained study personnel examined patients preoperatively and again on POD 4 and 30—time points that coincided with the assessment of the primary outcome variable in this trial; the same person administered all 3 tests. Instruments used to evaluate neurocognitive function were chosen to adequately assess key cognitive domains within the limited testing periods typically available for cardiac surgical patients and included: (1) Symbol–Digit Modalities Test (SDMT), which is a timed test of psychomotor speed, working memory, and sustained visual attention; (2) Mini-Mental State examination, which assesses orientation, attention, immediate, and short-term recall, language, and ability to follow simple verbal and written commands; (3) Animal Fluency Test, which requires patients to name as many different animals as possible in 1 minute; (4) Clock Drawing Test, which asks patients to draw the face of a clock showing the numbers and the hands set to a specific time; and (5) Rey Auditory–Verbal Learning Test, which assesses a patient’s ability to recall a 15-word list after each of 5 presentations and a 30-minute delayed recall.

Statistical Analysis of Overall Cognition
Factor analysis was completed as previously described, producing 4 standardized factor scores representing independent and equally weighted domains of cognitive function. Two summary measures were calculated to represent cognitive function. Cognitive deficit (the binary outcome) was defined as a decline of 1 SD or more in performance on at least 1 of the 4 domains. To quantify overall cognitive function and the degree of learning (ie, practice effect from repeated exposure to the testing procedures), a composite cognitive index was first calculated as the sum of the 4 domain scores. A continuous change score (the continuous outcome) was then calculated by subtracting the baseline from the follow-up cognitive index. Cognitive deficit and the change score were examined with multivariable logistic and linear models, accounting for baseline cognitive index, age, sex, education level, CPB time, elevated creatinine (≥1.3 mg/dL), and histories of neurologic disorder, MI, and congestive heart failure.

Cognitive Domains
Because pexelizumab therapy has been shown to have domain specific effects, each of the cognitive tests (Animal Fluency, Clock-Face, Rey Auditory–Verbal Learning Test, and SDMT) was compared post hoc among treatment groups using the Kruskal–Wallis (3-group), the Wilcoxon (mean change) and Kolmogorov–Smirnov (percent change) tests. To assess the relevance of percent change in the tests, we modeled the score at baseline on age and educational level in all 728 patients with data. Based on the effect of age observed (Figure 1), we chose a 10% decrease to represent a significant decline, and defined deficit as a decline of 10% or more. Covariables tested in the multivariable models included baseline score, age, diabetes, years of education, sex, and history of congestive heart failure.

Mini-Mental State Examination and NIHSS
The Kruskal–Wallis nonparametric test was used to compare groups. Data were analyzed using SAS software (version 8.2, SAS Institute Inc.). P<0.05 was considered significant.

Results
Of the 800 patients undergoing CABG alone, 267 (33.4%) were randomized to receive pexelizumab as a bolus and 263 (32.9%) received a bolus followed by an infusion for 24
hours. There were no statistically significant differences between the 3 treatment groups in baseline demographic characteristics. (Table 1)

Mean serum pexelizumab concentration time plots for the 2 dosing regimens revealed similar pharmacokinetic profiles. Complete suppression of hemolytic activity was seen within 1 hour of treatment with pexelizumab but was sustained for 24 hours in only the bolus + infusion group. There were no statistically significant differences in the primary study endpoint between treated and placebo patients on POD 4 or 308 (Figure 2).

The incidence of clinically defined stroke was 2.25% in the bolus group, 2.28% in the bolus + infusion group, and 1.11% in the placebo group (P=0.52). Cognitive deficit (binary outcome) was present on POD 4 in 57% of patients in the bolus group, 48% in the bolus + infusion group, and 46% receiving placebo (P=0.07). On POD 30, this deficit had declined to 33%, 35%, and 34%, respectively (P=1.0). The cognitive index (continuous outcome) at POD 4 and 30 was \(-0.27\pm0.44\) and \(0.04\pm0.38\), \(-0.21\pm0.5\) and \(0.05\pm0.36\), and \(-0.2\pm0.5\) and \(0.09\pm0.41\) in the bolus, bolus + infusion, and placebo groups (P=0.26 for POD 4 and 0.42 for POD 30). There were also no statistically significant differences in the MMSE or NIHSS scores between any of the treatment groups. Multivariable modeling failed to demonstrate any differences between the treatment groups.

On POD 4, a decline of at least 10% was seen in the SDMT (visuo-spatial domain) in 56% of patients receiving placebo compared with 40% receiving pexelizumab by bolus plus infusion (P=0.003) and 49% in those receiving a bolus only (P=0.01; Figure 3). Similarly, on POD 30, a 10% decline was present in 21% of patients in the placebo group, but in only 12% of the bolus plus infusion group (P=0.016) and 16% of the bolus group (P=0.05). Comparison of the change in raw scores also revealed that patients receiving pexelizumab by infusion showed significantly less decline at POD 4 on the SDMT, and significantly more improvement at POD 30, compared with placebo group, with both the univariate (POD 4, P=0.03; POD 30, P=0.007) and multivariable tests (POD 4, P=0.02; POD 30, P=0.003). No differences were seen between treatment groups when evaluating the Animal Fluency, Clock Drawing, and Rey Auditory–Verbal Learning Test tests.
Adverse events were similar in all treatment groups (Tables 2 and 3). The study agent was discontinued because of an adverse event in 4 placebo-treated patients (1%), 3 bolus-treated patients (1%), and 3). The study agent was discontinued because of an adverse event in 4 placebo-treated patients (1%), 3 bolus-treated patients (1%), and 3). The study agent was discontinued because of an adverse event in 4 placebo-treated patients (1%), 3 bolus-treated patients (1%), and 3).

**Discussion**

Cerebral IR injury produces a profound inflammatory response characterized by neutrophil, macrophage, and platelet accumulation, upregulation of adhesion molecules, blood–brain barrier destruction, and cytokine production. During central nervous system inflammation, complement activation plays a direct role in neuronal cell death and has been implicated in many disease processes, including subarachnoid hemorrhage, Alzheimer disease, trauma, and stroke.

In a study of patients who died after ischemic stroke, Lindsberg et al demonstrated complement deposition within areas of necrosis and concluded that activation of the terminal complement pathway with membrane attack complex assembly occurs within cerebral infarct zones. Others have shown that complement depletion before cerebral IR injury may have neuroprotective effects in animal models. Huang et al used a mouse model of middle cerebral artery occlusion and reperfusion to demonstrate that administering a potent inhibitor of complement activation before IR injury significantly reduced neurologic deficit and the accumulation of neutrophils and platelets. There were also trends toward smaller infarct volumes and improved cerebral blood flow.

Production of proinflammatory complement byproducts during cardiac surgery is facilitated by exposure of the circulating blood to the bio-incompatible surfaces of the extracorporeal circuit and to endotoxin. Substantial evidence supports a particularly critical role during CABG and CPB for the late complement components C5α and C5b-9 in inflammation-mediated tissue injury. C5α is a potent phosphatidylinositol, vasoconstrictor, and promoter of cytokine production. Both C5α and C5b-9 promote leukocyte activation and chemotaxis, whereas C5b-9 also promotes leukocyte–endothelial cell interactions and cell lysis. Thus, complement inhibition with pexelizumab therapy represents a novel neuroprotective strategy for cerebral IR injury.

In this study, pexelizumab infusion had no statistically significant effect on the primary endpoint or on overall measures of cognition. Consistent with the domain specific effect seen in the previous cardiac surgery trial, pexelizumab ameliorated only the decline in the visuo-spatial domain (SDMT) at POD 4 and 30. Changes in the SDMT are a sensitive indicator of the decline in cognitive function observed in normal aging; typically, a 7% to 10% decline in cognitive function can occur over 6 to 10 years. In monozygotic or dizygotic twins, an 8% decline occurred over 5 years, starting at age 56. In a study of 4870 patients aged 58 to 70, an average 7% decline over 6 years was observed. Normotensive patients followed-up for 10 years declined by 3.94 points in their SDMT scores. To assess the relevance of percent change in the SDMT, we modeled expected baseline score on age, adjusting for education, in all patients with valid baseline scores (Figure 1). At the mean age of 68, the predicted score is 30, with a 10% decline corresponding to a decrease of 3 points. At the rate of 0.38 points per year observed in our sample at baseline, this rate represents the decline expected over a period of 8 years, similar to the effect of aging from 68 to 76 years.

Our detection of an improvement only in the visuo-spatial domain is a concern but is consistent with previous reports on pexelizumab and with potential mechanisms of injury during cardiac surgery. In a study of 35 CABG patients, the incidence of visuo-spatial deficits was 55% in the placebo group compared with 11% in those receiving pexelizumab. Furthermore, a preponderance of strokes in the posterior cerebral circulation distribution has been reported in patients undergoing cardiac surgery, and most pure posterior cerebral artery infarcts in nonsurgical patients are caused by cardiac or intra-arterial embolism. Grosset et al showed that 30% of cardiogenic emboli enter the posterior circulation, which is more than the 20% expected from the relative proportion of blood flow to this region. Thus, the selective vulnerability of the parietooccipital region to the embolic injury commonly seen during cardiac surgery may be evidenced by greater
visuo-spatial abnormalities. Given the variability of the anatomy and supply zones of the major cerebral arteries,\textsuperscript{25} and the nonuniform expression of complement within brain tissue,\textsuperscript{26} our exploratory analysis is justified. However, a larger trial is necessary to further assess the significance of this improvement in only the visuo-spatial domain.

The primary limitation of our study is the number of statistical comparisons. Because this was an exploratory investigation, multiple comparisons were made so that any potential benefit or risk could be examined. These values are reported as observed, without statistical adjustment for the multiple comparisons. In consideration of these multiple comparisons, the study sample size at alpha=0.010 would provide 94\% power to detect a 10\% difference with deficit rates of 50\%, 40\%, and 30\% in the 3 treatment groups. Second, only the SDMT showed a difference between treatment groups. Testing protocols for assessing cognitive function typically include a variety of test measures that target specific cognitive domains but which may not be appropriate for detecting changes over time. Tests such as the SDMT are considered speed tests (not power tests) capable of detecting relatively small changes in function.\textsuperscript{27} Furthermore, the SDMT is more likely to be sensitive to changes over time because successful performance on this test requires a broad range of cognitive processes, including attention, memory, visual scanning, motor integration, planning, and visuo-spatial judgment, and the test shows a broader distribution of scores and is not generally constrained by either floor (too difficult) or ceiling (too easy) effects. Also, there is an expected “learning effect” that occurs with repeat administration of the SDMT, as well as with other standardized cognitive tests. Empirical observations show that re-testing alone, without any intervention, should actually be associated with an increase in SDMT score of \textapprox 6\% to 7\% within a 30-day period. Hence, any decline in scores with subsequent re-testing likely underestimates the true decline in function caused by the attenuating effect of concomitant learning. A final limitation to our study is the limited follow-up of the study subjects. Although previous studies have shown that short-term decline predicts dysfunction at 5 years after surgery,\textsuperscript{2} any assessment of the long-term therapeutic effect of perioperative complement suppression requires more extensive follow-up.

In conclusion, pexelizumab had no statistically significant effect on the primary composite endpoint or on overall cognition. When domain specific effects were examined, complement suppression for 24 hours perioperatively with pexelizumab did ameliorate the decline in visuo-spatial function.

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References
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