HuEP5C7 as a Humanized Monoclonal Anti–E/P-Selectin Neurovascular Protective Strategy in a Blinded Placebo-Controlled Trial of Nonhuman Primate Stroke

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Abstract—Although inhibiting interaction of β2 integrins with cognate immunoglobulin class adhesion receptor ligands is an effective neuroprotective strategy in small mammal models of stroke, the strategy has failed in human trials. A completely different antiadhesion receptor strategy was therefore rigorously tested in a model that may more closely approximate human reperfused stroke. Early leukoadhesive events in postischemic cerebral microvessels are mediated by upregulated selectin-class adhesion receptors on endothelial cells. Therefore, a blocking antibody prepared against common P- and E-selectin epitopes was humanized to suppress complement activation and tested in a reperfused hemispheric stroke model in Papio anubis (baboon). Histological examination of postischemic cerebral microvessels revealed a strong upregulation of E- and P-selectin expression. Placebo-blinded administration of the humanized anti-human E- and P-selectin monoclonal antibody (HuEP5C7, 20 mg/kg IV, n=9; placebo, n=9) immediately after the onset of 1 hour of temporary ischemia resulted in trends showing reduced polymorphonuclear leukocyte (PMN) infiltration into ischemic cortex, reduced infarct volumes (by 41%), improved neurological score (by 35%), and improved ability to self-care (by 39%). Importantly, there was no evidence of systemic complement activation, immune suppression, or pathological coagulopathy associated with this therapy. These data suggest that a humanized anti-E/P-selectin antibody approach is safe and may be effective as a clinical treatment for human stroke. (Circ Res. 2002;91:907-914.)

Key Words: cell adhesion molecules ■ E-selectin ■ P-selectin ■ Papio ■ brain ischemia

Experimental data implicates leukocyte adhesion receptors in the pathogenesis of cerebral injury in small animal models of stroke.1–9 However, both anti–intracellular adhesion molecule-1 (ICAM-1) and anti-CD11/18 strategies have failed dramatically in human trials.10–12 Several explanations are possible. First, proinflammatory microvascular failure leading to “no-reflow” might be important in rodent stroke, yet of limited relevance to primate stroke, due to differences in cerebrovascular collateralization.13 Alternatively, no-reflow may be relevant to primate stroke, but its critical nature is obscured by the clinically tested antiadhesion strategies potential to fix serum complement,3,10,11,14 which may exacerbate stroke outcome.3,15–17 There is also evidence from both trials that the therapeutic antibodies interfered with endogenous immunoregulatory defenses to promote the development of clinically significant infections and fever, thereby negating any potential cerebroprotective effects. This deleterious immunomodulation is not entirely unexpected, because ICAM-1 and CD11/18 are critical to numerous host defenses such as leukocyte adhesion, diapedesis, oxidative burst, and selectin expression.4 Theoretically, interfering with selectin-mediated leukocyte adhesion may affect immune regulation differently because selectins are more proximal in the adhesion cascade yet are less highly adhesive,3,4 and this strategy may simultaneously block microvascular thrombosis, which is likely to be particularly important in stroke.17,17a,18 P-selectin, which modulates postischemic no-reflow in murine stroke,2,5,6 represents a particularly attractive target for blockade given that P-selectin is rapidly translocated to the activated platelet surface where it participates in microvascular thrombosis and can modulate platelet-leukocyte-endothelial interactions. E-selectin is also highly upregulated in ischemic cerebral microvessels6,9,19–21 and participates in postischemic hypoperfusion, and its blockade has been asso
associated with improved outcomes in mice. Blocking selectin-mediated events might therefore be expected to preserve microvascular patency without many of the severe immunomodulatory side effects seen with antiintegrin approaches.

Although stroke remains the leading cause of combined death and disability, treatment options remain severely constricted and attempts to develop new strategies have been foiled by the high-profile failures of multiple clinical trials (antioxidants, calcium antagonists, glycine receptor antagonists, NMDA receptor antagonists, and others). In all cases, preliminary trials in rodents or other small mammals had suggested efficacy. Therefore, the present study was performed in subhuman primates despite the inherent difficulties of such a trial, because the results are more likely to be relevant to humans than smaller or more distantly related species. Experiments were designed to establish therapeutic efficacy of a combined anti-\(E/P\)-selectin approach. Because of speculated reasons for failure of the two prior antiadhesion receptor clinical trials, the present studies were prospectively designed to determine whether a humanized anti-\(E/P\)-selectin antibody (HuEPC7) causes complement fixation in stroke or clinical evidence of immune suppression.

Materials and Methods

Study Design and Statistical Analysis

Investigators were blinded until all data were collected and analyzed. Statistical analysis was completed using unpaired two-tailed analysis with the Student’s \(t\) test for nonparametric data and similar two-tailed analysis with Fisher’s exact test for parametric data. Significant differences were defined by a value of \(P<0.05\). Sample-size calculations were performed based on historical data from our group using the same baboon stroke model. Further guidance was provided by prior work with a well-defined murine model and functionally similar anti-\(E\) and anti-\(P\)-selectin antibodies.\(^9,10\) Experiments were designed anticipating a 60% reduction in infarct volume and similar improvements in neurological outcome between control/treatment arms. Experimental and control cohorts were determined to require 9 animals each, based on 0.80 power for an \(\alpha\) of 0.05 and a \(\beta\) of 0.1.

Animals

Eighteen baboons (Papio anubis; SouthWest Biological Research Foundation, San Antonio, Tex) weighing 18.5 ± 0.7 kg (range 11.4 to 24.1 kg) were studied. Experimental cohorts were composed of HuEPC7-treated (\(n=9\)) and placebo-treated animals (\(n=9\)). All animals were disease-free for a period of at least 90 days before inclusion. A team of veterinarians was responsible for medical and health. Mean baseline values of hematocrit, serum glucose, peripheral white blood cells (WBCs), platelet, and polymorphonuclear leukocyte (PMN) counts were not different between cohorts. All procedures were approved by the Institutional Animal Care and Use Committee at Columbia University and performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Humanization of HuEPC7

The antibody HuEPC7 (in 0.01% Tween 80, 20 mmol/L sodium citrate, 120 mmol/L sodium chloride, \(pH 6.0\)) was provided by Protein Design Laboratories, Inc. HuEPC7 is a humanized IgG\(_2\) monoclonal antibody against human E- and P-selectin.\(^{27}\) Parent murine EP-SC7 monoclonal IgG\(_2\) antibody was developed by immu- 

nizing mice with transfected cell lines expressing human E-selectin and human P-selectin.\(^{28}\) HuEPC7 was generated by grafting the complementarity-determining regions of EP-SC7 into a human IgG\(_2\) deficient in Fc receptor binding due to a defined mutation in the heavy chain constant region (IgG\(_2\).M3). Binding of HL-60 cells and leukocytes to CHO cells that express human E- and P-selectin is blocked by HuEPC7. HuEPC7 inhibits neutrophil binding to cytokine-activated endothelial cells that express E- and P-selectin as well as P-selectin-mediated platelet binding. HuEPC7 does not mediate antibody-dependent complement- or cell-mediated cytotoxicity. HuEPC7 cross-reacts with E- and P-selectin in most nonhuman primate species, including baboons, with similar binding affinity. Safety and pharmacokinetic investigations of intravenous HuEPC7 administration to a maximum dose of 100 mg/kg in healthy macaques demonstrated no apparent adverse effects and a prolonged elimination half-life (12 days at 100 mg/kg).\(^{27}\)

Specificity of HuEPC7

ELISAs were performed to establish specificity of HuEPC7 and control antibodies for relevant adhesion molecules. Recombinant human P-selectin, E-selectin, VCAM-1, and ICAM-1 (R&D systems) were diluted to 2 \(\mu g/mL\). ELISA/RIA plates were coated with 50 \(\mu L\) of human antigen in 20 mmol/L Tris-Cl (\(pH 8.5\)) overnight. Each well was washed 3 times with 0.05% Tween 20 in PBS. Antibody (100 \(\mu L\); biotinylated HuEPC7 or biotinylated HuM291) was added at various dilutions (500 ng/mL, 250 ng/mL, 100 ng/mL, 0 ng/mL) and incubated for 2 hours at room temperature (RT). Wells were then washed with 0.05% Tween 20 in PBS 3 times. Working dilution of Streptavidin-HRP (100 \(\mu L\)) was added, and the mixture incubated for 30 minutes at RT. Wells were then washed 3 times with 0.05% Tween 20 in PBS. Substrate solution (100 \(\mu L\)) was added and incubated for 20 minutes at RT, after which 50 \(\mu L\) of stop solution was added. Optical density was determined by using a microplate reader.

For positive control antibodies, monoclonal anti-P-selectin, anti-E-selectin, and anti-ICAM-1 (DAKO) were diluted at 1:10000. An extra step of biotinylated anti-mouse IgG was added after incubation with the monoclonal antibody. Each assay was run 3 times with 9 repeats for each dilution.

Experimental Primate Model of Reperfused Hemispheric Cerebral Ischemia

Each animal underwent surgery to induce cerebral ischemia as outlined in a previously described clinically relevant model of nonhuman primate stroke.\(^{29}\) Briefly, via a transorbital craniectomy, each anterior cerebral artery and the left internal carotid artery were occluded with micro-Yasargil aneurysm clips. Motor-evoked potentials (MEPs) were utilized in conjunction with a 5 minutes temporary occlusion and a 10-minutes recovery period to assess for variations in susceptibility to stroke. The aneurysm clips were then reapplied and the test agent (HuEPC7 20 mg/kg, or dose-volume equivalent placebo) was administered intravenously. The infusion was completed 25 minutes after the onset of vessel occlusion. After 1 hour of vessel occlusion, the clips were removed, a layer of gelfoam was placed over the dural defect, and methyl methacrylate was used to fill the orbit. Marline stroke models have demonstrated that increasing ischemic duration correlates with increased severity of cerebral injury. One hour was chosen as the occlusion duration because it provided the optimal balance between incidence of major infarction (>20%) and degree of neurological insult that permits at least 3 days survival.\(^{28}\) In developing this nonhuman primate stroke model, 90 minutes of occlusion caused such severe infarcts that a significant proportion of animals were unable to be extubated, whereas 30 minutes of occlusion failed to cause major infarction in a significant proportion of the animals (data not shown). Postoperatively, the animal remained intubated for 18 hours. Physiological parameters were strictly controlled throughout the experiment. Animals were then extubated and monitored closely in the ICU.

A trained, blinded, veterinarian assessed animal viability just before the 72-hour MRI scan. This decision was based on the following guidelines: (1) ability to sit upright, (2) ability to control oral secretions in a manner that precludes aspiration, and (3) ability to effectively self-feed. Animals meeting these criteria (self-caring) were allowed to awaken from their 72-hour MRI and were observed.
to the 9th postoperative day, at which time they underwent a second MRI and were euthanized without awakening. Animals failing to self-care were euthanized immediately after the 72-hour MRI as previously described.25

**Serum Levels of HuEP5C7**

At 30 minutes and 120 minutes after occlusion, and at 24, 48, 72 hours, and 9 days if self-caring, blood was collected into Vacutainers (Becton Dickinson), allowed to clot at RT for 30 minutes, and serum was isolated by centrifugation and stored at −70°C. Serum levels of HuEP5C7 were determined by anti-idiotypic antibody binding to HuEP5C7. Microplates (Nunc A/S) precoated with anti-idiotypic antibody in PBS were used to capture HuEP5C7 from baboon serum and then blocked for 1 hour with a casein and surfactant containing solution (Super Block, Scy-Tek). Calibration controls and specimens were diluted in Super Block, and added to the wells in triplicate and incubated at RT for 1 hour. After several washes in PBS containing Tween 20, sheep anti-human IgG2 -HPR (The Binding Site, UK) was incubated at RT for 1 hour. After several washes in PBS containing Tween 20, sheep anti-human IgG2 -HPR (The Binding Site, UK) was added and incubated at RT for 1 hour. Microplates were washed with PBS/Tween followed by incubation with o-phenylene diamine (OPD) solution. Color development was stopped with sulfuric acid (HCl buffer as a negative control (DAKO Corp). Amplification was performed using biotinylated goat anti-rabbit IgG (H+L) (Vector Labs), and avidin-biotin-peroxidase-complex (Elite ABC kit, Vector Labs). Staining and counterstaining were developed with peroxidase substrate solution (VECTOR ABC peroxidase substrate kit) and methyl green, respectively.

**Quantitative Neurological Assessment**

All animals were neurologically intact (neurological function score of 100) before beginning the study. Two blinded independent observers conducted daily neurological examinations using the Spetzler primate neurological function scale, a previously validated assessment scale.25,29 In this scoring system, higher function is reflected in a higher score.

**MRI**

MRI (T2-weighted) was performed to measure infarct volume at 72 hours (and again at 9 days if self-caring). Images were obtained on 1.5 Tesla MRI scanners (3-mm slice thickness, no intervening tissue). Two independent, blinded observers conducted daily neurological examinations using the Spetzler primate neurological function scale, a previously validated assessment scale.25,29 In this scoring system, higher function is reflected in a higher score.

**Necropsy**

Tissue samples from all major organ systems were obtained and underwent gross inspection for signs of infection or other morbidities.

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**Figure 1.** ELISA analysis for antibody affinity. HuEP5C7 demonstrates dose-dependent affinity for E-selectin and P-selectin, but does not demonstrate affinity for ICAM-1 or VCAM-1. Control antibody (HuM291) does not demonstrate affinity for E-selectin, P-selectin, ICAM-1, or VCAM. Antibody concentration (ng/mL) reported on x-axis, optical density quantified on y-axis.
Renal Histological Analysis
Sections (1×1×0.5 cm) were taken from each animal’s kidneys bilaterally immediately postmortem. The sections (cortex and medulla) were paraffin embedded, sampled at 5-mm intervals, and stained with H&E. Sections were examined for evidence of antibody complex or complement deposition.

Results

Physiology
Animal size, perioperative central venous pressure, arterial PCO₂, temperature (core and brain), hematocrit, serum glucose, and mean arterial blood pressure analysis demonstrated no differences between cohorts. MEP dropout time and time to recovery was the same for both cohorts, and all animals examined showed vessel patency on 72 hours postoperative MRA. MRA identified no cerebrovascular abnormalities. Intracranial pressure (ICP) increased steadily postoperatively in both cohorts, but treated animals demonstrated significantly lower mean postoperative ICP (placebo, 13.4±3.2 cmH₂O, n=9; treated, 9.6±1.08 cmH₂O; n=9, P<0.01).

HuEP5C7 Specificity
ELISA confirmed a concentration-dependent affinity of HuEP5C7 for E- and P-selectin. No ICAM-1 or VCAM-1 cross-reactivity was observed. Control antibody (HuM291) did not demonstrate affinity for any of the adhesion molecule substrates and commercially available recombine anti-adhesion molecule antibodies had binding properties as expected (Figure 1).

P- and E-Selectin Upregulation, Neutrophil Accumulation, and Fibrin Deposition Early After Reperfused Ischemia in Control Animals
Immunohistochemical staining revealed upregulation of P-selectin and E-selectin in the endothelium of both ischemic and nonischemic cortex at 72 hours, but the strength of the staining was markedly increased in the ischemic versus the nonischemic cortex. At 9 days, neither selectin was expressed. At 72 hours, focal fibrin accumulation was demonstrated the ischemic microvasculature, but was absent in the nonischemic microvasculature (Figure 2). Myeloperoxidase staining demonstrated increased PMN accumulation in the ischemic parenchyma versus the contralateral nonischemic parenchyma (Figure 3). These data indicate that ischemia/reperfusion induces ipsilateral expression of selectin-class adhesion receptors, and that this expression is functionally associated with cerebral microvascular leukosequestration.

Effect of HuEP5C7 Administration on Soluble Selectin Levels, Cerebral Neutrophil Accumulation, and Circulating WBC Counts
Almost coincident with termination of the HuEP5C7 infusion, circulating drug levels reached 437±37 mg/mL (n=9), and then steadily declined to reach 98±6 mg/mL (n=5) by 9 days after operation (Figure 4). These levels are well above those associated with in vitro blockade of selectin-mediated leukocyte recruitment. Placebo-treated animals had significant elevations in both serum E- and P-selectin levels by 24 hours with a slow return to baseline by 9 days. In contrast, antibody-treated animals demonstrated a marked suppression of these elevations (Figure 5). There was a 60% reduction in the mean accumulation of PMNs in the ischemic cortex of the HuEP5C7-treated animals (121±40 cells/cm²) versus placebo animals (298±140 cells/cm²), although relatively large SEMs precluded assigning statistical significance (Figure 6). Before ischemia, there was no difference in circulating WBC levels between cohorts. After ischemia (30 minutes, 2 hours, and 24
hours), the treated cohort had significantly elevated WBC levels ($P=0.01$). This leukocytosis returned to baseline levels at 48 hours, 72 hours, and 9 days (Figure 7). Likewise circulating neutrophils increased 360% in treated animals versus 83% in untreated animals (Figure 8). The WBC differentials showed preferential increases in neutrophils versus other leukocytes (not shown).

**Infarct Volume, Neurological Function, and Mortality**

All cohorts demonstrated a strong correlation between neurological score and infarct volume ($r=0.80$, $P<0.0001$). For all clinical outcome analyses, treatment with HuEP5C7 trended toward improved outcome (41% smaller infarct volumes at euthanasia, a greater ability to self-care, and 35% improvement in neurological score; Figure 8). The placebo-treated cohort contained nearly 3 times as many neurologically devastated animals.

**Complement Activation**

Baseline C3a levels were 1530±123 ng/mL (mean±SD; n=6) in the placebo group and 1670±275 ng/mL (mean±SD; n=6) in the HuEP5C7 cohort ($P=NS$). There was no significant elevation in C3a in either cohort at any time after occlusion (not shown). There was no measurable C5b-9 activation at baseline or at any subsequent time point (not shown). Histopathological examination of renal tissue revealed no evidence complement deposition (not shown).

**Infectious and Hemorrhagic Complications**

There were no deaths before euthanasia in either cohort. Careful clinical monitoring of the animals receiving treatment with HuEP5C7 revealed no fever, pneumonia, or urinary tract infection. Postmortem analysis of intracranial, intrathoracic, and intraabdominal contents revealed no evidence of systemic or focal infection. There was no gross or radiographic evidence of intraparenchymal cerebral hemorrhage in any animals. There was one minor wound infection in a placebo animal and none in the treated animals.

**Discussion**

Inflammatory cascades are increasingly recognized as important pathological mediators in tissue ischemia and reperfusion. In the brain, ischemia triggers activation of common transcription factors, such as nuclear factor-$\kappa$B, hypoxia inducible factor 1, interferon regulatory factor 1, and STAT3, which triggers induction of multiple proinflammatory genes. The induced genes include both cytokines (such as platelet-activating factor, tumor necrosis factor-$\alpha$ [TNF$\alpha$], and interleukin-1$\beta$ [IL-1$\beta$]) as well as glycoprotein adhesion receptors. Ischemic microvascular endothelial cells are quite active in this regard, expressing multiple leukocyte adhesion receptors, including ICAM-1, P-selectin, and E-selectin, each of which appears to play a critical role in the pathogenesis of postischemic hyperperfusion and progressive cerebral tissue
injury in experimental stroke.1–9 When these adhesion receptors engage their respective cognate leukocyte ligands, leukocytes roll, adhere, and then diapedes through the vessel wall. The rapid accumulation of leukocytes in ischemic cerebral foci results in microvascular plugging, initiation of local coagulation cascades, and the release of a variety of toxins from activated leukocytes. In rodent models, adhesion receptor blockade (with specific antibodies) or genetic knockout has resulted in consistent improvements in functional outcome and reduced infarct volume. Despite this data, clinical trials in humans using both anti-ICAM and anti-CD11/18 antibodies have failed,10–12 calling into question both the relevance of these receptors to human stroke and the relevance of murine ischemia models. As treated patients may actually have been hurt by interference with the ICAM-CD11/18 interaction, the present series of experiments were undertaken in a nonhuman primate model for both scientific and ethical reasons.

The devastating failure of these clinical trials has yielded some important mechanistic insights into trial design and antiadhesion receptor agent composition. Albeit speculative, there are several hypotheses as to why these trials failed to realize the benefits seen with antiadhesion receptor strategies in rodents: (1) the ICAM-1 trial used a nonhumanized murine antibody that fixed complement,14 (2) CD11/18 may actually play a limited role in human stroke, especially in the absence of meaningful reperfusion,7,30 and (3) there is possibly clinically significant immunosuppression in neurologically compromised geriatric populations, raising the risk for infectious pulmonary complications. It is possible that these complications obscured any demonstrable benefit of these therapies.

The present experiments were designed using an anti-E/P-selectin–based therapeutic approach for three reasons. First, despite an absence of clinically evident infections in short-term survival murine stroke experiments using antintegrin strategies, interference with CD18- and ICAM-1–mediated leukocyte adhesion might result in increased vulnerability to infection11 in disabled, elderly hosts, the typical victims of stroke. Unlike CD18 blockade, HuEP5C7 does not impair host defense in primate model of Staphylococcus aureus subcutaneous infection.52 Another reason for choosing an anti-E/P-selectin therapy was to provide upstream interference with platelet-leukocyte-endothelial interactions, the

Figure 6. PMNs, per square cm of cross sectional area (20× magnification), in placebo (298±140, n=9) vs treated animals’ ischemic cortex (121±40, n=9).

Figure 7. A, Circulating WBC counts in treated and placebo cohorts. Treated animals experienced significant elevation in WBC counts 30 minutes, 120 minutes, and 24 hours after occlusion vs placebo animals. Placebo: baseline=8.7±0.8×10⁶/mL; n=9; preischemia=9.2±1.4×10⁶/mL; n=9; 0.5 hours ischemia=8.1±0.9×10⁶/mL; n=9; 2 hours=7.8±0.8×10⁶/mL; n=9; 24 hours=7.8±1.3×10⁶/mL; n=8; 48 hours=6.5±0.8×10⁶/mL; n=8; 72 hours=10.2±5.6×10⁶/mL; n=5; 9 days=8.3±1.4×10⁶/mL; n=5. HuEP5C7: baseline=10.1±7.0×10⁶/mL; n=8; preischemia=10.2±0.7×10⁶/mL; n=9; 0.5 hours ischemia=11.3±0.7×10⁶/mL; n=9; 2 hours=11.6±1.0×10⁶/mL; n=8; 24 hours=15.8±1.7×10⁶/mL; n=9; 48 hours=14.5±3.2×10⁶/mL; n=9; 72 hours=11.1±1.5×10⁶/mL; n=9; 9 days=7.9±2.5×10⁶/mL; n=5. B, Circulating PMN counts in treated and placebo cohorts. Treated animals demonstrate significantly elevated circulating PMN counts vs controls at 0.5, 2, 24, and 72 hours after occlusion. Placebo: baseline=3.1±0.8×10⁶/mL; n=9; preischemia=6.4±1.3×10⁶/mL; n=9; 0.5 hours ischemia=5.2±0.9×10⁶/mL; n=9; 2 hours=5.2±0.6×10⁶/mL; n=9; 24 hours=5.7±1.2×10⁶/mL; n=8; 48 hours=5.9±0.9×10⁶/mL; n=8; 72 hours=2.6±1.0×10⁶/mL; n=5; 9 days=4.4±1.0×10⁶/mL; n=5. HuEP5C7: baseline=3.7±0.6×10⁶/mL; n=8; preischemia=7.2±0.9×10⁶/mL; n=9; 0.5 hours ischemia=8.2±0.9×10⁶/mL; n=9; 2 hours=8.3±0.9×10⁶/mL; n=8; 24 hours=13.1±1.6×10⁶/mL; n=9; 48 hours=10.5±2.2×10⁶/mL; n=9; 72 hours=8.3±1.8×10⁶/mL; n=8; 9 days=5.4±2.1×10⁶/mL; n=5.
combination of which might have a profound effect on microvascular failure while still allowing for some degree of immunological competence through L-selectin- and ICAM-1-dependent pathways. The third reason for blocking both P- and E-selectin was the well-documented redundancy of these receptors in a variety of inflammatory settings and the availability of a humanized monoclonal antibody that could be easily and safely utilized in human trials.27,32

The hemispheric reperfused primate stroke model was selected because it reliably mimics the pathophysiology of large vessel embolic stroke in humans in terms of degree and location of tissue damage. Possible limitations of this study are that this model uses a trial occlusion before the 1 hour ischemic period and that the therapy was delivered immediately after the onset of ischemia. The recording of MEPs during the trial occlusion helps to confirm adequate clip placement and identify potential anatomic/physiological outliers.25 Due to the trial occlusion, this model may be of particular relevance to people who present with transient ischemic attacks (TIAs) preceding stroke. In any case, as experimental conditions were identical between groups, discerning differences between treatments should be possible. The early delivery of therapy was chosen to provide the greatest chance of demonstrating treatment efficacy, thereby establishing the relevance of an antiselectin strategy in primate stroke while minimizing the use of animals in light of ethical and economic considerations.

In this model, we show that the cortical microvasculature expresses both P- and E-selectin in reperfused ischemia, with a dramatic associated influx of neutrophils by 72 hours after reperfusion. In these animals, peripheral levels of these soluble selectins are also elevated. Blinded treatment with the humanized anti–E/P-selectin antibody resulted in marked suppression of peripheral selectin levels and attenuation of cerebral neutrophil influx. We are unable to, as yet, determine to what degree the reduction of detectable sE- and sP-selectin levels in the peripheral blood was due to interference by HuEP5C7 with the assay versus decreased positive feedback and a decreased amount of neurological insult.

Circulating WBCs were elevated during the time period corresponding with the highest agent levels. This increase was primarily due to neutrophil elevations at these times points. All other leukocytes showed little or no elevation. P-selectin and E-selectin–deficient mice can have increased peripheral circulation PMN counts.33 Rodent studies utilizing an anti-integrin antibody have repeatedly demonstrated increased circulating WBCs concomitant with decreased leukocyte migration.35-38 These findings in mice and rats parallel those observed in our primate model after HuEP5C7 administration. Our data support the hypothesis put forth in the aforementioned studies that the increase in circulating WBCs is due to the sequestering of leukocytes in the intravascular space due to the inability of the PMNs to undergo rolling. This hypothesis is further supported by the decreased presence of neutrophils in the brain parenchyma in HuEP5C7 animals. Despite these findings there was no evidence of elevated temperatures, pneumonia, urinary tract infections, or wound infections in any of the treated animals, suggesting that the antiselectin approach in stroke may be superior, at least in terms of safety, to an anticomplement or antimigrin approach.

Treated animals also demonstrated dramatic reductions in infarct volumes and improvements in neurological function and survival, and there was no evidence of activation of the peripheral complement cascade, all in direct contrast to what was seen with the failed clinical strategies. Because P-selectin is also expressed on platelets and has been shown to decrease fibrin deposition in murine cerebral ischemia/reperfusion injury, it is important to assess for hemorrhagic conversion of these large reperfused strokes. Gradient echo imaging and serial sectioning postmortem revealed no evidence of hemorrhagic conversion in any animals. These data are concordant with murine data, in which the addition of selectin-blocking carbohydrates to a complement-inhibitory protein also did not increase the degree of intracerebral hemorrhage after stroke.17

Together these data suggest that despite the small under-powered cohort size, P- and E-selectin are potentially relevant...
targets in primate stroke due in large part to the fact that cerebral neutrophil influx is abrogated without inducing clinically deleterious immunosuppression or complement activation. In light of the increasing evidence that many Phase III stroke studies have demonstrated worse outcomes in the experimental arms than in controls, these data also point to the important use of translational (subhuman primate) models in confirming preliminary findings in rodent systems before proceeding with human trials.

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