The Beta-hCG+Erythropoietin in Acute Stroke (BETAS) Study
A 3-Center, Single-Dose, Open-Label, Noncontrolled, Phase IIa Safety Trial

Steven C. Cramer, MD; Camille Fitzpatrick, NP; Michael Warren; Michael D. Hill, MD; David Brown, MD; Laura Whitaker, RN; Karla J. Ryckborst, RN; Lawrence Plon, PharmD

Background and Purpose—Animal data suggest the use of β-human chorionic gonadotropin followed by erythropoietin to promote brain repair after stroke. The current study directly translated these results by evaluating safety of this sequential growth factor therapy through a 3-center, single-dose, open-label, noncontrolled, Phase IIa trial.

Methods—Patients with ischemic stroke 24 to 48 hours old and National Institutes of Health Stroke Scale score of 6 to 24 started a 9-day course of β-human chorionic gonadotropin (once daily on Days 1, 3, and 5 of study participation) followed by erythropoietin (once daily on Days 7, 8, and 9 of study participation). This study also evaluated performance of serially measured domain-specific end points.

Results—A total of 15 patients were enrolled. Two deaths occurred, neither related to study medications. No safety concerns were noted among clinical or laboratory measures, including screening for deep vein thrombosis and serial measures of serum hemoglobin. In several instances, domain-specific end points provided greater insight into impairments as compared with global outcome measures.

Conclusions—Results support the safety of this sequential, 2-growth factor therapy initiated 24 to 48 hours after stroke onset. (Stroke. 2010;41:927-931.)

Key Words: growth factor ■ recovery ■ stroke treatment

Stroke remains a major source of disability. Most patients do not receive approved acute therapies, often because the time window for intervention is 4.5 to 8 hours after stroke onset. Furthermore, among those thrombolyzed, many nonetheless have significant long-term disability. Therapies are needed that both have a wider treatment time window and reduce disability in a larger fraction of affected subjects.1

Growth factor levels spontaneously increase in many brain regions after a stroke and are considered an important contributor to the process of spontaneous recovery.2,3 Increased growth factor levels due to exogenous administration, beginning 1 to 7 days after stroke, can further improve long-term outcome in preclinical experiments.4,5 Kolb et al6 used a sequential 2-growth factor approach, using epidermal growth factor and erythropoietin (EPO) to boost levels of new neural stem cells. As an extension of this approach, in a prior study in animals, we used β-human chorionic gonadotropin (hCG) in place of epidermal growth factor given the more extensive human experience with hCG. That study7 evaluated the effects of sequentially administered hCG, which promotes proliferation of endogenous neural stem cells,8 followed by EPO, which promotes differentiation of these cells into neural stem cells.8 Treatment initiated 24 hours after experimental stroke onset in rats was found to be safe and to improve long-term behavioral outcome.7 The hormone hCG is in the same growth factor family as nerve growth factor,9 crosses the blood–brain barrier,10 and enters the cerebrospinal fluid,11 and its receptor is normally present in adult rat brains.12 EPO is a growth hormone13 that also crosses the blood–brain barrier,14 and its receptor mRNA is readily detectable in central nervous system neurons and glia.15

The primary aim of the current study was to examine the safety of this sequential hCG plus EPO regimen translated to human subjects with stroke 24 to 48 hours old. The goal of the intervention was to act as a restorative rather than neuroprotective agent, promoting repair without reducing injury.1 This was a 3-center, single-dose, open-label, noncontrolled, Phase IIa safety trial.

A secondary aim evaluated domain-specific end points. Different neurological domains recover with different rates and extents after stroke, and furthermore restorative therapies might differentially influence recovery of these individual
Subjects and Overall Study Design

Subjects were consented and enrolled from September 2006 to February 2008 in accordance with local Institutional Review Boards at 3 North American sites: University of California Irvine Medical Center (n=11), Hoag Memorial Hospital Presbyterian (n=1), and the Foothills Hospital at University of Calgary (n=3). Entry and exclusion criteria are listed in Table 1. This study was registered at clinicaltrials.gov as #NCT00362414.

Administration of hCG followed by EPO (“B-E therapy”) is a 9-day course, initiated 24 to 48 hours after stroke onset, consisting of 3 once-daily intramuscular doses of hCG at 10 000 IU/dose, 1 day apart, on Days 1, 3, and 5 of study participation followed by a 1-day washout period followed by 3 once-daily intravenous doses of EPO at 30 000 IU/dose on Days 7, 8, and 9 of study participation (Figure 1). Patients with weight ≤45 kg (n=1) received half-dose EPO.

The primary outcome measure was safety through Day 90 assessed through adverse event reporting, serial examinations, blood testing, and a leg vein Doppler at Day 42. Secondary outcomes included the National Institutes of Health Stroke Scale (NIHSS) score, Barthel Index, and a battery of domain-specific end points (see subsequently) at 90 days.

Methods

Baseline Patient Characteristics

A total of 15 patients with moderate stroke were enrolled with demographic and baseline features as in Tables 2 and 3. An exception allowed enrollment of an 87 year old. Of the 15 enrollees, acute therapy included intravenous tissue plasminogen activator (n=1), intra-arterial tissue plasminogen activator (n=1), and MERCI thrombus retrieval (n=1). The baseline (ie, pretherapy) serum hCG level was normal in all cases. Baseline serum iron levels were low in 8 and normal in 7 subjects. Baseline serum ferritin was normal in 11 and

domains. This suggests the potential value of adding measures of separate behavioral domains to recovery assessments.16 As a secondary goal, the current study therefore also aimed to examine performance of domain-specific end points for measuring safety and detecting behavioral gains.

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Study Procedures

Study tests are outlined in Figure 1. Before therapy, subjects were screened, consented, and underwent baseline examination, scoring on NIHSS, blood testing, and (when possible) scoring on a battery of domain-specific end points. This battery of domain-specific end points consisted of the Action Research Arm Test,17 Fugl-Meyer Arm and Leg motor scores,18 Boston Naming Test,19 Line Cancellation Test,20 and the Trails A & B Tests.21 B-E therapy then started with the first dose starting 24 to 48 hours after stroke onset. Examination, urinalysis, blood chemistry testing, reticulocyte count, and the battery of domain-specific end points were each performed 4 more times through Day 90; measurement of serum blood counts and hemoglobin, 5 more times; and assessment of adverse events, 6 more times. On Day 42, a venous Doppler study of the legs was administered to screen for the presence of any deep vein thrombosis and the Barthel Index was scored. The NIHSS was repeated on Day 90, and the Geriatric Depression Scale22 was scored. When possible, MRI data were collected, consisting of diffusion-weighted images at baseline and fluid-attenuated inversion recovery images at Day 90.

Data Analysis

All analyses were 2-tailed with α=0.05.

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Table 2. Baseline Clinical Features

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>15</td>
</tr>
<tr>
<td>Age (range)</td>
<td>72 (23–87) years</td>
</tr>
<tr>
<td>Gender</td>
<td>9 male/6 female</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>9 white, 4 Hispanic, 2 Asian</td>
</tr>
</tbody>
</table>

Table 3. Clinical Data From Baseline to Day 90

<table>
<thead>
<tr>
<th>Scale</th>
<th>Baseline Score</th>
<th>Day 90 Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS, all patients</td>
<td>10 (6–19)</td>
<td>2 (0–6)</td>
</tr>
<tr>
<td>(n=15 at baseline, n=12 at Day 90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barthel Index</td>
<td>25 (0–100)</td>
<td>95 (70–100)</td>
</tr>
<tr>
<td>(n=11 at baseline, n=12 at Day 90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm Fugl-Meyer Motor Scale</td>
<td>32 (2–65)</td>
<td>62 (4–66)</td>
</tr>
<tr>
<td>(n=12 at baseline, n=12 at Day 90; normal score=66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg Fugl-Meyer Motor Scale</td>
<td>20 (3–34)</td>
<td>32 (20–34)</td>
</tr>
<tr>
<td>(n=10 at baseline, n=12 at Day 90; normal score=34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boston Naming Test</td>
<td>2 (0–10)</td>
<td>8 (2–10)</td>
</tr>
<tr>
<td>(n=13 at baseline, n=12 at Day 90; normal=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line Cancellation Test</td>
<td>96 (20–100)%</td>
<td>100 (87–100)%</td>
</tr>
<tr>
<td>(n=8 at baseline, n=12 at Day 90; normal=100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Action Research Arm Test</td>
<td>23 (0–57)</td>
<td>57 (0–57)</td>
</tr>
<tr>
<td>(n=9 at baseline, n=11 at Day 90; normal=57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trailmaking A, no. connected</td>
<td>8 (0–25)</td>
<td>25 (12–25)</td>
</tr>
<tr>
<td>(n=7 at baseline, n=12 at Day 90; normal=25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trailmaking B, no. connected</td>
<td>3 (0–11)</td>
<td>22 (6–25)</td>
</tr>
<tr>
<td>(n=5 at baseline, n=11 at Day 90; normal=25)</td>
<td></td>
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</tbody>
</table>

The value for age is the median (with range in parentheses).

Note that 8 of 12 subjects had Day 90 Barthel Index scores ≥95.

Safety of B-E Therapy

Clinical and laboratory data indicated no safety concerns and no serious adverse event related to study drug. Of the 12 patients assessed through Day 90, the median Day 90 score for NIHSS was 2; for the Barthel Index, 95, with 8 of 12 subjects having a score ≥95 (Table 3).

There were 2 deaths. One patient had a retroperitoneal hemorrhage associated with anticoagulant therapy given for a myocardial infarction that was concomitant with the index stroke, which ultimately led to multiorgan failure and death after a decision was made to change to palliative care. This patient received a total of 3 doses of hCG and 1 dose of EPO. A second patient had cardiac arrest 3 weeks after enrollment.

Two instances of deep vein thrombosis were identified. One patient with pre-existing pulmonary disease developed an upper extremity deep vein thrombosis. This patient received 3 doses hCG but because of the deep vein thrombosis, he received no EPO. A deep vein thrombosis (asymptomatic, in the calf) was diagnosed at the planned (Figure 1) Day 42 leg venous Doppler in 1 of the 13 patients for whom this result was available.

One enrollee with atrial fibrillation had 2 additional cerebral emboli before EPO, received the full B-E therapy course, and did well (Day 90 NIHSS score of 3). One enrollee lacked Day 90 follow-up evaluation due to recurrent mediastinal sepsis arising from cardiac surgery.

The serum hemoglobin and the reticulocyte count were each stable over time (repeated-measures analysis of variance, P>0.1 in each case for main effect of time; see Figure 2).

Infarct volume (baseline=diffusion-weighted images, Day 90=fluid-attenuated inversion recovery, data available at both time points in 7 patients) declined over time in 5 of 7 subjects. The mean baseline infarct volume was 55±38 mL. The mean within-subject change in infarct volume was a drop of 22%±34%.

elevated in 4. The median time from stroke onset to the start of B-E therapy was 40 hours (range, 19 to 48 hours). Most of the B-E therapy doses were given as an inpatient; however, in a few instances, patients received final B-E doses in an outpatient setting.

The mean within-subject change in infarct volume was a drop of 22%±34%.

In parallel with B-E therapy, serum hemoglobin and reticulocyte count (mean±SEM) showed no change over time, because the main effect of time was not significant in repeated-measures analysis of variance. Note that the initial increase in reticulocyte count from baseline to Day 6 preceded EPO administration and that the normal upper limit for reticulocyte count is 2.5%.
Serial Measurement of Domain-Specific End Points

This study also provided an opportunity to examine the performance of domain-specific end points, values for which are presented in Table 3. Note that measurement could not be obtained at baseline for some of these scales, typically due to reduced patient sensorium or the patient declining to complete the test with the Boston Naming Test performing best in this regard (data available in 13 of 15 patients) and Trailmaking B the worst (data available in 5 of 15 patients).

In some cases, the domain-specific end points provided insight into focal areas of dysfunction among patients with low global disability. For example, of 2 patients with Day 90 Barthel Index scores of 95 (little or no disability), 1 had an arm motor Fugl-Meyer score of 37 (moderate to severe arm weakness) and 1 could name only 4 of 10 items on the Boston Naming Test (moderate aphasias).

The domain-specific end points also provided finer resolution of outcomes in some cases. For example, among the 9 subjects with an NIHSS arm motor subscore of 0 at Day 90, the range of arm motor Fugl-Meyer scores was 53 to 66 (out of 66), indicating substantial arm motor deficits among some subjects with a perfect NIHSS arm motor subscore. Domain-specific measures also provided finer resolution of change over time. For example, 1 patient had modest improvement in NIHSS score, going from a baseline score of 10 to a Day 90 score of 5. In parallel, tremendous gains were measured through the arm motor Fugl-Meyer score, improving by 40 of 66 possible points.

Discussion

The BETAS study was a 3-center, single-dose, open-label, noncontrolled, Phase IIa trial that directly translated findings from prior preclinical investigations. The use of sequential growth factor administration was intended to increase endogenous neural stem cell proliferation. The current study enrolled a total of 15 patients with acute ischemic stroke that was overall moderate in severity. B-E therapy lasted 9 days and was initiated 24 to 48 hours after stroke onset.

The primary focus of this study was safety, and B-E therapy was found to be safe. Although no placebo group was included in this safety study, clinical outcomes such as 8 of 12 subjects having a Barthel Index score ≥95 at Day 90 compared favorably with placebo groups from prior stroke clinical trials. Neither of the 2 deaths was related to B-E therapy. Infarct volumes overall showed a trend toward reduction over time that were similar to those described elsewhere.

Hematologic effects of 3 EPO doses were negligible (Figure 2). Clinical applications of erythropoietic-stimulating agents such as EPO have recently come under review. Most of this attention has been focused on applications in oncology and renal failure, in which dozens or hundreds of doses are administered, which is much larger than the EPO exposure with B-E therapy. Also, the increased mortality rate described in association with EPO in a recent clinical stroke trial by Ehrenreich et al was not seen in the current study. There are several possible explanations for these differences; Ehrenreich et al used higher EPO doses as compared with B-E therapy (40 000 versus 30 000 IU), introduced the EPO at the time when the ischemic insult was actively evolving (EPO started within 6 hours poststoke versus EPO started 8 to 9 days poststroke in the current study), and gave EPO in parallel with tissue plasminogen activator (versus tissue plasminogen activator effects completely resolved by the time of EPO dosing in the current study).

Domain-specific end points have been useful in acute stroke research, but have only rarely been used in prior acute stroke trials. These measures have been suggested to have value for detecting differential effects that therapies might have on various functional aspects of stroke recovery. Domain-specific end points showed strengths in the current study, for example, in several instances provided greater insight than global outcome measures did into final level of impairment. Also, some of the domain-specific end points provided finer resolution of specific deficits and their change over time. Domain-specific end points also showed weaknesses, because a number of patients could not be satisfactorily evaluated at baseline, a topic that requires further study. In addition, the current battery of domain-specific end points did not directly examine quality of life, a priority to address in future studies.

The time window of B-E therapy suggests that it acts as a restorative rather than neuroprotective treatment. The time window of such therapies suggests the potential to reach a high fraction of patients with stroke. The current study provides data supporting the safety of sequential 2-growth factor therapy initiated 24 to 48 hours after stroke onset. A placebo-controlled, double-blind, Phase IIb trial of B-E therapy has been initiated (clinicaltrials.gov ID #NCT00938314).

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Disclosures

S.C.C. has received consulting fees from Stem Cell Therapeutics.

References


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