DP-b99, a Membrane-Activated Metal Ion Chelator, as Neuroprotective Therapy in Ischemic Stroke

Hans-Christoph Diener, MD; Dietmar Schneider, MD; Yair Lampl, MD; Natan M. Bornstein, MD; Alexander Kozak, PhD; Gilad Rosenberg, MD; on Behalf of the Study Group

Background and Purpose—DP-b99 is a chelator of zinc and calcium ions that acts selectively within cell membranes and has neuroprotective properties in animal models of stroke. We present the results of a multicenter, double-blind, placebo-controlled, randomized trial to assess the safety and potential protective effects of DP-b99 in acute ischemic stroke.

Methods—One hundred and fifty stroke patients with signs of cortical involvement and a National Institutes of Health Stroke Scale (NIHSS) score of 7 to 20 received a 4-day course of intravenous 1 mg/kg per day DP-b99 or placebo within 1 to 9 hours of stroke onset. Treatment with recombinant tissue plasminogen activator was not allowed.

Results—No major differences in mortality rate, causes of death, adverse events, safety laboratory tests, and ECG parameters were found between the 2 groups. The baseline NIHSS score of the 72 DP-b99– and 75 placebo-treated patients in the intent-to-treat cohort was (mean±SD) 12.2±4.0 and 12.6±3.3, respectively; the time to needle (mean±SD) was 6:36±1:47 and 6:28±1:33 hours, respectively; and the age (mean±SD) was 73.3±9.9 and 72.0±9.6 years, respectively. The 90-day median change from baseline (the primary end point) was −6.0 and −5.0 NIHSS points in the DP-b99 and placebo groups, respectively (nonsignificant). At 90 days, there was a significantly better outcome in the DP-b99 group compared with the placebo group (modified Rankin scale score of 0, 1, or same as prestroke): 30.6% and 16.0%, respectively (P=0.05). The recovery rate was unaffected by the time to needle. Further analyses indicated that the 90-day median change from baseline in patients with an entry NIHSS score of 10 to 16 was 8.0 and 5.0 points in the DP-b99 and placebo groups, respectively (P=0.03).

Conclusions—In this small-scale study, the primary end point of change in NIHSS score from baseline to day 90 was not met. However, secondary end points demonstrated a significantly improved 90-day recovery rate with treatment with DP-b99 when compared with placebo. In addition, in patients with baseline NIHSS scores of 10 to 16, a significant post hoc change in NIHSS score from baseline to day 90 was observed. No major safety problems were identified. These findings need to be confirmed with a larger prospective study of strokes involving the cortex. (Stroke. 2008;39:1774-1778.)

Key Words: ischemic stroke ■ neuroprotective therapy ■ membrane-activated metal ion chelator ■ randomized trials ■ placebo-controlled studies

Thrombolysis with alteplase (recombinant tissue-type plasminogen activator) is the only approved treatment for acute stroke in a 3-hour time window.1 Less than 5% of patients are currently being treated with recombinant tissue-type plasminogen activator. Thus, there is an urgent need for neuroprotective therapies that have a lower bleeding risk than thrombolysis and can be offered to a higher percentage of patients over a longer time period after the event. Cerebral tissue can be protected in animal models by a variety of medications that attenuate neuronal injury after ischemia.2 No neuroprotectant has been approved for use in patients recently, even NXY-059, which seemed to be a promising agent for neuroprotective therapy after ischemic stroke, failed in the largest clinical trial program to date.3–5

Excessive concentrations of divalent metal ions such as zinc and calcium are known mediators of damage in acute ischemic stroke.6 DP-b99 is a membrane-activated lipophilic chelator of divalent metal ions that is designed to chelate these ions only in the vicinity of membranes and when their concentrations exceed physiologic levels, thus affecting the function of proteins that require these ions for their activation.7 In cellular systems, DP-b99 has been shown to atten-
uate detrimental zinc-dependent membrane-associated processes, such as calpain activation and tumor necrosis factor-α–induced activation of matrix metalloproteinase-9. In rodent models of cerebral ischemia, DP-b99 therapy reduced infarct volume and neuron-specific enolase release and increased the survival rate. Two of the problems hindering the extension of findings from animal stroke models to the clinic are the immense differences between laboratory animals and patients in terms of absolute infarct size and the much longer treatment window commonly called for in the clinical setting. When moving from bench to bedside, it is therefore reasonable to try and shorten the time needed to achieve adequate tissue drug levels—and overcome the longer diffusion distances in the poorly perfused human penumbra—by increasing the neuroprotectant’s serum levels beyond those used in the laboratory setting (safety permitting), so as to create a gradient that will promptly and effectively drive the drug into the target tissue. In the present study, which included only patients with cortical involvement and thus, those most likely to experience detrimental zinc-dependent membrane-associated processes, such as calpain activation and tumor necrosis factor-α–induced activation of matrix metalloproteinase-9, DP-b99 therapy reduced infarct volume and neuron-specific enolase release and increased the survival rate.

Two of the problems hindering the extension of findings from animal stroke models to the clinic are the immense differences between laboratory animals and patients in terms of absolute infarct size and the much longer treatment window commonly called for in the clinical setting. When moving from bench to bedside, it is therefore reasonable to try and shorten the time needed to achieve adequate tissue drug levels—and overcome the longer diffusion distances in the poorly perfused human penumbra—by increasing the neuroprotectant’s serum levels beyond those used in the laboratory setting (safety permitting), so as to create a gradient that will promptly and effectively drive the drug into the target tissue. In the present study, which included only patients with cortical involvement and thus, those most likely with large hemispheric infarcts, the dose used (1 mg/kg per day) was 2 to 3 orders of magnitude higher than that used in preclinical in vivo studies. Previous exposure of healthy volunteers and stroke patients to repeated DP-b99 administrations of 1 mg/kg per day have shown this dose to be safe.

In an earlier small-scale study of this dose administered to acute stroke patients for 2 consecutive days (with a treatment window of 12 hours), a larger decrease from baseline on the National Institutes of Health Stroke Scale (NIHSS) was observed with DP-b99 compared with placebo after a 30-day follow-up. The present study was the first prospective evaluation of the effects of DP-b99, a member of a novel class of divalent metal ion chelators, on neurologic function and recovery after acute ischemic stroke.

Patients and Methods
This was a double-blind, placebo-controlled, randomized, multicenter trial that compared DP-b99 with placebo in the treatment of acute ischemic stroke. The study protocol and informed consent procedures were approved by the ethics committee of each participating center.

Trial Subjects
Male and female acute stroke patients who consented to participation, who were 18 to 85 years old, had clinical signs of hemorrhagic gray-matter involvement (language dysfunction, neglect, and/or visual field defect), and had a baseline NIHSS score of 7 to 20 were enrolled. Computed tomography or magnetic resonance imaging was used to exclude patients with cerebral hemorrhage. Patients were also excluded if they were eligible for thrombolytic therapy, had a prestroke modified Rankin scale (mRS) score ≥3, had rapid neurologic improvement before randomization, had had a stroke within the preceding 3 months in the same assumed cerebral territory, or had comorbidities that could confound interpretation of the trial results or limit the subject’s life expectancy to <3 months.

Study Drug Administration
Study drug DP-b99 1 mg/kg per day or matching placebo (sucrose 1 mg/kg per day and mannitol 1.25 mg/kg per day) was administered as a 2-hour intravenous infusion. The first infusion started within 9 hours of symptoms onset and was followed by 3 additional infusions at 24±3-hour intervals.

Study Design
A central computerized, randomization system allocated the subjects to DP-b99 or placebo (1:1) and stratified them to achieve 50% treatment initiations within 1 to 6 hours of symptoms onset (ie, once 1 temporal stratum enrolled 50% of the planned enrollment for the entire study, enrolment continued only into the other stratum). NIHSS score was recorded at baseline, on each of the 4 treatment days, and 30 and 90 days after randomization. The mRS was used to assess outcome on study days 30 and 90. Subjects were evaluated for safety throughout the study by clinical observations, repeated ECG and vital signs recordings, serum chemistry, blood counts, and coagulation parameters testing. All investigators were required to either hold a valid certification for administration of the NIHSS or to become certified in the administration of this scale through the National Stroke Association NIHSS Certification Services. An independent drug safety monitoring board evaluated the safety data at regular intervals.

End Points and Statistical Considerations
The primary end point was the change in NIHSS score from baseline to day 90. A sample size of 75 subjects per treatment group provided >90% power to detect a 4-point between-group difference in NIHSS score change from baseline at a significance level of ≤0.05. The 2 main secondary end points were recovery as assessed by mRS (90-day score ≤1 or return to the prestroke score) and by NIHSS (90-day score ≤1). Other prespecified end points included recovery as assessed by either mRS or NIHSS, recovery (90-day NIHSS score ≤1) by baseline NIHSS (categorized as 7 to 10, 11 to 15, and 16 to 20), and mRS score distribution at day 90. Missing NIHSS data of subjects who died from causes directly attributable to the index stroke were imputed with the highest possible score (n = 42), whereas other missing data were imputed by using the last observation carried forward. Adjudication of deaths directly attributable to the stroke was performed blindly by the data safety monitoring board.

Results
From February 2005 to July 2006, 150 patients (75 per treatment group, safety cohort) were randomized in 25 centers in Germany, Israel, and South Africa. Table 1 presents the patients’ baseline characteristics by treatment group.

Mortality
There were 12 deaths in the DP-b99 group (16%) and 15 in the placebo group (20%). No difference in the pattern of causes of death was observed between the groups. Brain edema was the most common cause of death, with 4 cases in the DP-b99 and 6 in the placebo group. Kaplan-Meier analysis suggested no significant between-group difference in 90-day survival probability (P = 0.56).

Safety
All study subjects except 1 in the DP-b99 group experienced at least 1 adverse event (AE). AE severity distribution was similar in the 2 groups: 37% and 33% of the patients in the DP-b99 and placebo groups, respectively, reported severe AEs. Thirty-five (47%) and 28 (37%) patients reported serious AEs (SAEs) in the DP-b99 and placebo groups, respectively (P = 0.32, Fisher’s exact test). None of the SAEs was considered related or probably related to the study drug. SAEs occurring in ≥2 patients per group in the DP-b99 and placebo groups, respectively, were as follows: brain edema/herniation, 11% and 7%; cerebral hemorrhage, 5% and 5%; pneumonia, 4% and 5%; anemia, 3% and 0%; cardiac arrest, 3% and 0%; multiple organ failure, 3% and 1%; recurrent stroke, 3% and 4%; convulsions, 1% and 3%; and septic...
The mean SD time to needle in the intent-to-treat cohort was yielded results similar to those of the intent-to-treat patients. All efficacy results presented herein relate to the intent-to-treat cohort; analyses of the per-

Efficacy

The intent-to-treat cohort included 72 DP-b99– and 75 placebo-treated patients. All efficacy results presented herein relate to the intent-to-treat cohort; analyses of the per- protocol cohort (68 DP-b99– and 71 placebo-treated patients) yielded results similar to those of the intent-to-treat patients. The mean±SD time to needle in the intent-to-treat cohort was 6:33±1:47 and 6:28±1:33 hours in the DP-b99 and placebo groups, respectively.

The main efficacy results are presented in Table 2. The distribution of mRS scores at 90 days by treatment group is displayed in Figure 1. The recovery rate was unaffected by the time to needle: in the 1- to 6-hour window, the recovery rate as assessed by mRS was 25.0% and 16.2% in the DP-b99 (n=36) and placebo (n=37) groups, respectively, and in the 6- to 9-hour window, it was 36.1% (n=36) and 15.8% (n=38), respectively (P=0.492, logistic regression with time to needle as the covariate).

Prespecified analysis of recovery by baseline NIHSS score revealed that the proportional response to DP-b99 in patients with a baseline score of 11 to 15 was greater than in patients having a baseline score of 7 to 10, as shown in Table 2. Post hoc analyses confirmed that the largest difference between treatment groups in the 90-day recovery rate occurred in patients in the middle range of baseline NIHSS scores (Figure 2). Distinctly, in the DP-b99– and placebo-treated patients with a baseline score of 10 to 16 (n=32 and 49, respectively), the median NIHSS change from baseline to 90 days was −8.0 and −5.0 points, respectively (P=0.038, Wilcoxon rank-sum test), and the proportion of patients who had recovered by day 90 (as assessed by either mRS or NIHSS) was 34.4% and 12.2% (P=0.025, Fisher’s exact test), respectively.

Discussion

The results of this small-scale study are encouraging, very interesting, and may be explained by the potential mechanisms of action of DP-b99. In contrast to most neuroprotective agents previously tried in acute stroke, DP-b99 is unique, as it allows the simultaneous interference with a variety of pathophysiologic processes, from apoptosis to inflammation, rather than just a single damaging process. The results of this small, proof-of-concept phase II study indicate that DP-b99 is safe and might be effective as neuroprotective therapy for stroke. The study failed to meet its primary end point but showed positive results for a number of predefined secondary end points. The result, however, has to be replicated in an adequately powered larger study.

At first glance, the placebo group in this study seems to have had a much worse outcome than would have been expected on the basis of the clinical course of placebo groups in other recent trials with similar baseline severity. For example, the mean baseline NIHSS scores of the placebo groups in the SAINT I and SAINT II studies were 12.5 and 13.3, respectively, compared with 12.6 in the present study, yet the 90-day recovery rates as assessed by the mRS in the former 2 studies were 31% and 27%, respectively, whereas the corresponding rate in our study was only 16%. However, it is important to realize that these apparently similar baseline NIHSS scores resulted most likely from different stroke syndromes that had dissimilar prognoses. In the SAINT

### Table 1. Baseline Characteristics of the Study Patients

<table>
<thead>
<tr>
<th></th>
<th>DP-b99 (n=75)</th>
<th>Placebo (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y (SD)</td>
<td>73.3 (9.9)</td>
<td>72.0 (9.6)</td>
</tr>
<tr>
<td>Female, %</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>Mean body mass index, kg/m² (SD)</td>
<td>27.8 (4.8)</td>
<td>27.5 (5.0)</td>
</tr>
<tr>
<td>Baseline NIHSS score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>12.2 (4.0)</td>
<td>12.6 (3.3)</td>
</tr>
<tr>
<td>Median (minimum, maximum)</td>
<td>11.5 (7.0, 19.0)</td>
<td>12.0 (7.0, 19.0)</td>
</tr>
<tr>
<td>Historical mRS score distribution, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>67</td>
<td>76</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Percentage of prior strokes in past 10 years</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>Diabetes, % of group</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Any cardiovascular history, % of group</td>
<td>89</td>
<td>92</td>
</tr>
<tr>
<td>Hypertension, % of group</td>
<td>83</td>
<td>87</td>
</tr>
<tr>
<td>Atrial fibrillation, % of group</td>
<td>40</td>
<td>37</td>
</tr>
</tbody>
</table>

### Table 2. Efficacy Results (Intent-to-Treat Cohort)

<table>
<thead>
<tr>
<th>End Point</th>
<th>Placebo Group (n=75)</th>
<th>DP-b99 Group (n=72)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary end point: median change in NIHSS score from baseline to day 90</td>
<td>−5.0</td>
<td>−6.0</td>
<td>0.560†</td>
</tr>
<tr>
<td>Recovery by mRS (day 90 score&lt;1 or return to prestroke score), %</td>
<td>16.0</td>
<td>30.6</td>
<td>0.050†</td>
</tr>
<tr>
<td>Recovery by NIHSS (day 90 score&lt;1), %</td>
<td>12.0</td>
<td>23.6</td>
<td>0.084†</td>
</tr>
<tr>
<td>Recovery by either mRS or NIHSS, %</td>
<td>18.7</td>
<td>37.5</td>
<td>0.016†</td>
</tr>
<tr>
<td>Recovery (day 90 NIHSS score&lt;1) by baseline NIHSS score, %</td>
<td>See Figure 1</td>
<td>See Figure 1</td>
<td>0.044‡</td>
</tr>
</tbody>
</table>

*Wilcoxon rank-sum test.
†Fisher’s exact test.
‡Cochran-Mantel-Haenszel test controlling for prestroke mRS score.
studies, the neurologic eligibility criteria required patients to have only limb weakness, whereas in the current trial, the inclusion criteria required a language dysfunction, visual field defect, or neglect. In other words, the DP-b99 study focused on patients with cortical involvement, who most likely had rather large hemispheric infarcts and hardly ever lacunar strokes, whereas the SAINT trials enrolled an assortment of different stroke syndromes, including patients with lacunar infarcts, whose prognosis is far better than that of patients with larger infarcts. This becomes further evident when examining the incidence of AEs that are proportional to infarct size, such as brain edema: in the placebo groups of SAINT I and SAINT II, the rates of the SAE brain edema were 3% and 1.7%, respectively, compared with 7% in our study. In addition, the mean age of the placebo-treated patients in the present DP-b99 study (72 years) was greater than that of the SAINT I and SAINT II placebo groups (68.5 and 69 years, respectively), a factor known to adversely affect recovery after stroke.

In this study, the higher recovery rate with DP-b99 therapy was observed in both the pre-6-hour window and in the 6- to 9-hour window. (The somewhat higher recovery rate in the 6- to 9-hour window probably does not reflect a trend toward a greater treatment effect with delayed drug administration, because the difference in effect size between the earlier and later treatment windows was statistically insignificant). In the ischemic penumbra, detrimental inflammatory and proapoptotic processes continue to evolve hours to days after the onset of ischemia, and such processes may be important targets for neuroprotection. As mentioned earlier, DP-b99 can attenuate the activity of several neuroinflammatory elements, such as microglial activation, tumor necrosis factor-α activation, and the activity of matrix metalloproteinase-9, as can other members of this family of 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid-based compounds. Interestingly, though not studied with DP-b99 per se, other closely related compounds of the same chemical family have been shown in vivo to upregulate hypoxia-inducible factor 1, a factor that is part of the adaptive response of mammalian cells to hypoxia, which is induced up to 24 hours after cerebral ischemia and can be neuroprotective against cell death triggered by ischemia and oxidative stress. In vitro, DP-b99 protects neurons from death induced by H2O2 even if it is added 4 to 6 hours after the insult, suggesting that the events affected by DP-b99 take place relatively late after the addition of H2O2. Further experiments showed that DP-b99 inhibits H2O2-induced calpain activity to a similar extent as commercially available calpain inhibitors, which are known neuroprotectants in experimental brain ischemia. Calpain, a calcium-dependent cysteine protease, is activated under various neurotoxic conditions, including ischemia, and is a key player in the cascade that eventually leads to apoptosis; inhibition of calpain activity may be part of the mechanism by which DP-b99 mediates its neuroprotection. Taken together, DP-b99 may attenuate a variety of stroke-related pathophysiologic processes, including those that occur in the hours and days of the subacute phase, and can thus enjoy a wide temporal treatment window.
One strength of this study was the ability to safely use a dose of study medication that results in plasma concentrations that exceed those used in animal experiments. Considering the larger infarct size in humans compared with that in animals and the need to promptly drive the drug into the poorly perfused tissue, this may have contributed to the higher recovery rate in the DP-b99–treated group. Other strengths include the inclusion of patients with cortical involvement. Such patients may have a potentially better likelihood of benefit from neuroprotective therapies. The major shortcomings of this trial are the small sample size and the fact that the primary end point was missed. The positive outcomes in secondary end points still could be a play of chance. This phase II trial served the purpose of finding out whether there is a biologic signal of efficacy and will aid in the design of subsequent studies with DP-b99.

Appendix

Enrolling Investigators

Germany: H.-C. Diener, Essen (Principal Investigator); D. Schneider, Leipzig; B. Grewing, Bad Neustadt; M. Goertler, Magdeburg; R. Huber, Ulm; D.G. Nabavi, Münster; R.U. Kaminski, Wurzen; A. Hetzel, Freiburg; J. Marx, Mainz; J. Glahn, Minden; L. Harms, Berlin; T. Eils, Bergisch Gladbach; F. Stoegebauer, Osnabrueck; R. Haberl, Munich-Harlaching; M. Sitzer, Frankfurt; D. Sander, Munich; H. Buchner, Recklinghausen; J. Sobesky, Cologne; Israel: Y. Lamp, Holon; N.M. Bornstein, Tel Aviv; T. Ben-Hur, Jerusalem; B. Weller, Haifa; D. Tanne, Petach Tikva; D. Yamitsky, Haifa; South Africa: J.S. Roos, Cape Town.

Data Safety Monitoring Board

F. Fazekas, Universitätssklinik für Neurologie, Graz (neurologist, chairman); Y. Caraco, Division of Medicine and Clinical Pharmacology Unit, Hadassah University Hospital at Ein Karem, Jerusalem (internist, clinical pharmacologist); I. Steiner, Neurological Sciences Unit, Hadassah University Hospital at Mount Scopus, Jerusalem (neurologist); J. Tál, TechnoStat Ltd, Kfar Sava, (statistician); D. Tzivoni, Department of Cardiology, Shaare Zedek Medical Center, Jerusalem (cardiologist).

Source of Funding

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Disclosures

Drs Kozak and Rosenberg are employees of, and hold stock options in, D-Pharm Ltd. Dr. Diener received honoraria for participation in clinical trials, contribution to advisory boards or oral presentations from: Abbott, AstraZeneca, Bayer Vital, BMS, Böhringer Ingelheim, D-Pharm, Fresenius, GlaxoSmithKline, Janssen-Cilag, MSD, Novartis, Novo-Nordisk, Paion, Parke-Davis, Pfizer, Sanofi-Aventis, Sankyo, Servier, Solvay, Thrombogenics, Wyeth, Yamaguchi. Financial support for research projects was provided by AstraZeneca, GSK, Böhringer Ingelheim, Novartis, Janssen-Cilag, Sanofi-Aventis. The Department of Neurology at the University Duisburg-Essen received research grants from the German Research Council (DFG), The German Ministry of Education and Research (BMBF), the European Union, the Bertelsmann Foundation and the Heinz-Nixdorf Foundation.

References

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