AMPA Antagonist ZK200775 in Patients With Acute Ischemic Stroke
Possible Glial Cell Toxicity Detected by Monitoring of S-100B Serum Levels

Jan-Willem Elting, MD; Geert A. Sulter, MD; Markku Kaste, MD; Kennedy R. Lees, MD; Hans C. Diener, MD; Marc Hommel, MD; Mark Versavel, MD; Albert W. Teelken, PhD; Jacques De Keyser, MD

Background and Purpose—S-100B and neuron-specific enolase (NSE) serum concentrations can be used as peripheral markers of glial cell and neuronal damage, respectively. We investigated these markers in a clinical trial with the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) antagonist ZK200775 in acute ischemic stroke patients.

Methods—In a multicenter, double-blind, randomized, placebo-controlled phase 2 trial, 61 ischemic stroke patients were treated with either placebo or active drug in a dose-finding design. Twenty-five patients received placebo, 12 patients received a total dose of 262.5 mg in 48 hours (dose group 1), and 13 patients received a total dose of 525 mg in 48 hours (dose group 2). Eleven patients received a total dose of 105 mg over a period of 6 hours (dose group 3; reduction of total dose and infusion time because of adverse events in group 2). Serum concentrations of S-100B and NSE were analyzed with the use of a monoclonal sandwich immunoluminometric assay. Neurological outcome was assessed with the National Institutes of Health Stroke Scale (NIHSS).

Results—In group 2 there was a significant transient worsening in the mean NIHSS score 48 hours after the start of treatment. The mean increase was 11 points. This was due to reduction of consciousness (stupor and coma) in 8 of 13 patients. Neurological deterioration in group 2 was associated with a higher increase of S-100B concentrations, but not of NSE concentrations, than in the placebo group. The trial was stopped prematurely for safety reasons.

Conclusions—The AMPA antagonist ZK200775 transiently worsened the neurological condition in patients with acute ischemic stroke. Our results suggest that in addition to neuronal dysfunction, glial cell toxicity may have occurred. It may be useful to introduce monitoring of serum markers of brain damage in phase 2 trials with glutamate receptor antagonists. (Stroke. 2002;33:2813-2818.)

Key Words: excitatory amino acid antagonists • nerve tissue protein S-100 • neuron-specific enolase • neurotoxins • receptors, AMPA • stroke, acute • stroke, ischemic

Neuroprotective drugs are effective in animal stroke models but have failed to show clinical efficacy in stroke patients.1–3 In the ischemic penumbra there is a buildup of extracellular glutamate, which leads to an overstimulation of \(N\)-methyl-\(D\)-aspartate (NMDA)– and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)–type glutamate receptors. This is associated with excitotoxicity because of an accumulation of calcium in the cells. Among the glutamate receptor blockers, NMDA antagonists have been the most frequently studied, but the occurrence of side effects is one of the factors limiting their use in humans.4 AMPA antagonists also hold promise as neuroprotective compounds in acute ischemic stroke, but thus far they have not reached phase 3 clinical trials.5 ZK200775 is a quinoxalinedienedione-like drug with high affinity at the AMPA/kainate receptor site with little activity at the NMDA site. It behaves as a neuroprotective drug in rodent models of ischemia and head trauma.6 Three phase 1 studies in healthy subjects showed acceptable side effects at dosages required to obtain serum levels that were neuroprotective in the animal models. Side effects included mild and reversible sedation, visual disturbances,
and memory impairments (unpublished Schering research reports AG99, AO12, and AO13, 1999).

S-100B and neuron-specific enolase (NSE) can serve as peripheral markers of brain damage in various neurological diseases.7,8 S-100B exists predominantly in glial cells, while NSE is mainly found in neurons. In patients with acute ischemic stroke, serum levels of these proteins correlate with infarct size, as well as with neurological and functional outcome.9–11 Temporal patterns of both proteins are usually of the biphasic type, with peak levels around the second to fourth day after stroke onset.12,13 It has been suggested that these biochemical serum markers could serve as surrogate tools to evaluate neuroprotective therapies.1,9,10,14

The AMPA antagonist ZK200775 was studied in a phase 2 multicenter, double-blind, randomized, placebo-controlled dose-escalating trial to evaluate safety in patients with an acute ischemic stroke. The details of the clinical and pharmacokinetic data will be reported elsewhere but will be briefly mentioned here. In this article we assessed whether there were any treatment effects on the serum concentrations of S-100B and NSE. Efficacy was not a primary goal of this study, but the National Institutes of Health Stroke Scale (NIHSS) was used as a secondary outcome parameter.

Subjects and Methods

Subjects

Patients were included from August 1997 to August 1998 in 10 hospitals across Europe (see Appendix). Ethics committee approval was obtained at each study center. Inclusion criteria were stroke severity on the NIHSS between 2 and 21. For patients who developed symptoms overnight, stroke onset was defined as the last time the patient was known to be symptom free. Female patients were included only if they were postmenopausal or surgically sterilized. Exclusion criteria included cerebral hemorrhage, renal insufficiency, ventricular arrhythmia (atrial fibrillation was not an exclusion criterion), use of thrombolitics, severe hypertention, and severe concomitant disease likely to influence clinical assessment during the study (such as dementia or metastasized cancer). Other drug treatments such as aspirin and heparin were allowed.

All patients gave their written informed consent to participate in this study. Written consent was also accepted from the next of kin or close family member if the patient was unable to write. Intact ability to understand information and to communicate (best NIHSS language score 0 or 1, best NIHSS dysarthria score 0 or 1) was an additional requirement for inclusion.

Treatment was started within 24 hours of stroke onset in all patients. Patients were randomly assigned to either placebo or trial drug. The study was directed by a steering committee, and safety was monitored by an independent safety board (see Appendix). An interim safety analysis was performed after each dose group, after which decisions were made to proceed with a higher dose or adjusted dosing. The first dose group received a loading dose of 25 mg in 30 minutes followed by a maintenance dose of 237.5 mg over 47 hours and 30 minutes. The second group received a loading dose of 50 mg in 30 minutes followed by a maintenance dose of 475 mg over 47 hours and 30 minutes. The third group received a loading dose of 50 mg in 30 minutes followed by a maintenance dose of 55 mg over 5 hours and 30 minutes. This reduction of total dose and infusion time was decided after the end of dose step 2 because of adverse events in group 2. This study was part of a first phase 2 study that focused on safety and tolerability. Sample size was therefore determined on a pragmatic basis.

Methods

S-100B and NSE

Venous blood samples were taken on admission (baseline), after 24, 48, 72, and 96 hours, and after 7 days. Within 1 hour of collection, all samples were centrifuged and stored at −20°C until analysis. After clotting and centrifugation at 4000 rpm for 10 minutes, NSE and S-100B were analyzed with the use of monoclonal sandwich immunoluminometric assays (San gốc) and a fully automated LIA-mat system.

Hemolysis has no influence on serum S-100B levels but can increase serum NSE levels.15,16 Therefore, hemolytic samples were identified by visual inspection of the serum, and the amount of NSE attributable to hemolysis was determined by using the following formula: NSE = 0.665 × hemoglobin − 0.533. This equation was obtained from earlier experiments in which it was shown that increasing levels of free serum hemoglobin correlated highly significantly (r = 0.998) with serum NSE levels (A. Klaren, BS, unpublished data, 1998). This amount was subtracted from the total amount of serum NSE. Samples in which the estimated amount of NSE as a result of hemolysis was >50% were excluded from the analysis.

Neurological Assessment

Stroke severity was assessed with the NIHSS on admission, after 2 days, after 7 days, and after 4 weeks. Efficacy analysis was not a primary goal of this study because of safety aspects and a wide admission window of 24 hours, but the difference in NIHSS score between week 4 and admission was used as a secondary “outcome parameter.” Patients were considered to have a neurological improvement when the difference between these scores was ≥4 points, no major change when the difference ranged from 3 to −3, and neurological deterioration when the difference was <−4 points.17

Statistical Analysis

To analyze S-100B and NSE data, the area under the curve (AUC) and the individual peak levels were used as summary measures.18 Peak values were not determined if there was a missing value before or after the peak level unless the missing value was on day 7, because earlier studies showed that this is an unlikely time point for peak levels of both serum markers. AUC values were not calculated if there was >1 missing value or if the first or last value was missing. Interpolation was used if missing values for other time points were present. Log transformation was used when these variables followed a nonnormal distribution. One-way ANOVA was performed to compare the S-100B and NSE data between treatment groups. Correction for multiple comparisons was performed with the Dunnett T3 method for unequal variances or the Bonferroni method in case of equal variances.19 To compare proportions between groups, χ² tests and Fisher exact tests were used. Multiple regression analysis with a backward stepwise strategy (probability of F for removal ≥0.1, probability of F for entry ≥0.05) was used to assess the influence of several baseline variables on maximum S-100B values.

Results

Stroke Type and Severity

Demographic and baseline characteristics of the placebo group and the 3 active treatment groups (termed group 1, group 2, and group 3) are shown in Table 1. With regard to baseline characteristics, group 1 had a higher percentage of stroke due to small-vessel disease (50%) than the other groups. In group 1 hypertension and diabetes were more often present, and in group 2 there were more previous nondisabling strokes or transient ischemic attacks than in the placebo group. Figure 1 shows the NIHSS scores in the placebo group and the 3 active treatment groups. Baseline NIHSS scores were similar for the 58 patients used in the S-100B and NSE analysis (F = 1.8; P = 0.16), with comparable baseline values between the placebo group (6.3 ± 4.4), group
3 (7.0±4.1), and group 2 (7.5±4.4) but lower baseline values in group 1 (4.0±2.2).

After 48 hours, a highly significant increase was seen in NIHSS scores in group 2 (P=0.008 compared with placebo, P=0.018 compared with group 3, and P=0.03 compared with group 1). Eight of 13 patients in group 2 had a reduction of consciousness, 1 had a confusional state, and 1 showed stroke progression. The maximum incidence and intensity of these adverse events usually occurred >24 hours after the start of infusion (range, 4 to 48 hours). These events led to a prompt discontinuation of study medication. In the placebo group and group 3, there were 1 and 2 patients, respectively, with events of comparable severity, but mild somnolence was more frequently observed. One patient died after 96 hours in group 2 because of cerebral edema with transtentorial herniation, and 2 patients died of severe pulmonary edema and progressive stroke after 4 weeks in the placebo group. Monitoring of vital parameters (blood pressure, pulse, O2 saturation, temperature) and blood glucose showed no major differences between the groups.

The NIHSS score at 4 weeks was highest in treatment group 2 but did not differ significantly from the other groups (F=2.423; P=0.07). When categorized into 3 outcome groups reflecting deterioration, no change, or improvement, there was no difference in outcome between treatment groups (group 3 versus placebo, P=0.81; group 1 versus placebo, P=0.08; group 2 versus placebo, P=0.70, Fisher exact test).

CT scanning was performed in all patients at baseline and in 77% of patients at day 7, depending on the judgment of the investigator. Baseline CT scan results were consistent with ischemic stroke in all patients. Two patients had hemorrhagic transformation of the infarct on day 7. The trial was stopped prematurely because of safety concerns, especially the reduction in consciousness.

S-100B and NSE
S-100B values were obtained for 96% of all intended samples; 4% were lost because of nonsampling or death of the patient. NSE values were obtained for 94% of all intended samples; 5% were lost because of nonsampling or death, and 1% were lost because of uncorrectable amounts of hemolysis. Nine percent of all NSE samples were hemolytic but could be corrected with the correction formula. The patient in group 2 who died after 96 hours was excluded from the analysis because neither peak levels nor AUC could be calculated reliably. The maximum serum levels of both markers were very high in this patient (S-100B, 11.25 ng/mL; NSE, 22.18

**TABLE 1. Demographic and Clinical Data**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo Group</th>
<th>Dose Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dose</td>
<td>265.5 mg/48 h</td>
<td>525 mg/48 h</td>
</tr>
<tr>
<td>Time between stroke onset and start trial drug, h</td>
<td>16.3±5.2</td>
<td>15.7±4.6</td>
</tr>
<tr>
<td>% SAE resulting in discontinuation of trial drug</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Time between start trial drug and discontinuation, h</td>
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<td>NA</td>
</tr>
<tr>
<td>No. of patients</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Sex (M/F)</td>
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<td>10/2</td>
</tr>
<tr>
<td>Age, mean±SD</td>
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<td>65.9±8.8</td>
</tr>
<tr>
<td>Weight, kg</td>
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<td>83±13</td>
</tr>
<tr>
<td>Large vessel occlusion vs lacunar infarction, %</td>
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<td>50 vs 50</td>
</tr>
<tr>
<td>History of†</td>
<td></td>
<td></td>
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<tr>
<td>Cardiac disease</td>
<td>48</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>Previous stroke or TIA</td>
<td>8</td>
<td>25</td>
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</table>

*P<0.05 vs placebo.
†History values are percentages.
Note the large percentage of lacunar strokes, hypertension, and diabetes in group 1. SAE indicates serious adverse event; NA, not applicable.

**Figure 1.** Mean±SEM values of NIHSS total scores at all time points for all treatment groups (n=58). *P<0.05 vs group 2 (ANOVA, Bonferroni adjustment). Deceased patients and patients with hemorrhagic transformation of the infarct were excluded (n=3). Group 2 received the highest dose of ZK200775, and group 3 received the lowest dose. The increase in mean NIHSS score in dose group 2 was due to reduction of consciousness (stupor and coma), which was transient in nature. After 4 weeks no differences in NIHSS scores were found between the groups.
ng/mL) but were obtained during the stage of transtentorial herniation and were therefore considered unreliable. Two patients with hemorrhagic transformation of the infarct were excluded from the analysis because cerebral hemorrhage causes different S-100B and NSE patterns.20 The remaining 58 patients entered the S-100B and NSE analysis.

Individual peak levels of S-100B could be determined in all but 1 patient and for NSE in all but 5 patients (all because of missing values). In 4 patients AUC values for S-100B and in 13 patients AUC values for NSE could not be determined because of missing values at baseline or at 7 days.

The evolution over time of the S-100B and NSE levels in all treatment groups between admission and day 7 is shown in Figures 2 and 3, respectively. NSE values followed a normal distribution after corrections for hemolysis were done. Individual maximum values and AUC did not differ significantly between the treatment groups. S-100B values followed a skewed distribution in all groups, which was normalized after log transformation. Compared with the other groups, S-100B levels in group 2 were higher between 48 and 96 hours after start of treatment. There was a significant difference in baseline characteristics on S-100B results, we performed a multiple regression analysis using the NIHSS baseline value, stroke type (lacunar versus large-vessel occlusion), time between stroke onset and start of treatment, and the treatment group variable (ordered according to increasing dose: 0-placebo, 3-highest dose) as independent variables.

Excluded variables (because of missing values). In 4 patients AUC values for S-100B and in 13 patients AUC values for NSE could not be determined because of missing values at baseline or at 7 days.

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In the final model, only NIH score at baseline and the treatment group variable (ordered according to increasing dose) contributed significantly.

### Discussion

Several AMPA antagonists have been developed as neuroprotective compounds and have entered clinical development. None of these compounds has reached phase 3 clinical trials, and unacceptable adverse events can be the main obstacle. The most prominent finding of this study was that the administration of the AMPA antagonist ZK200775 in ischemic stroke patients resulted in a transient neurological deterioration, which was associated with a higher than expected rise in serum S-100B levels. The level of sedation was more severe in stroke patients and occurred later than in normal subjects during the phase 1 studies. Besides a longer infusion time and higher doses in stroke patients, blood-brain barrier disruption, with increased tissue concentrations, may be responsible for these differences. Although baseline stroke characteristics were not equally distributed in all treatment groups, this is not a sufficient explanation for the difference in serum S-100B levels between the placebo group and dose group 2. In fact, the placebo group, group 2, and group 3 were quite similar, but group 1 consisted of less severe strokes.

There was a relation between S-100B levels and stroke severity, but the results of the multiple regression analysis indicate that ZK200775 treatment had an independent effect on S-100B levels. In the present study time to drug administration was relatively late for a putative neuroprotective compound, and we cannot rule out that the results may have been different when the drug had been given within 3 to 6 hours after stroke onset. However, the available data show no significant influence of time to treatment on the S-100B levels. The drug did not affect serum NSE levels.

It is tempting to hypothesize that the drug caused a reversible neuronal dysfunction while having a toxic effect on glial cells. This is different from neurotoxicity, which rather would lead to an increase in serum NSE levels, as we have previously shown in patients with hyperglycemic cortical ischemic stroke.21 Direct suppression of synaptic transmis-
sions via neuronal AMPA receptor blockade can explain reduced neuronal activity, as some in vitro experiments suggest.6 This has also been demonstrated in animal experiments with AMPA antagonists; local glucose metabolism decreases in rats treated with 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX), a selective AMPA antagonist with a biochemical and pharmacological profile similar to that of ZK200775.22,23 This reflects decreased neuronal metabolic demands during decreased synaptic transmission. Multiple cortical areas but also deeper brain structures such as the thalamus were involved. These phenomena were accompanied by side effects similar to those observed in this study. Moreover, brain stem structures may also have been involved since subcortical electroencephalographic signs were found in healthy subjects receiving ZK200775 (subcortical and cortical signs detected visually in pharmacological electroencephalograms of healthy male volunteers are induced by the competitive AMPA antagonist ZK200775; H. Ott, PhD, and W. Scheuler, MD, unpublished data, 2000).

Glial cell damage can also contribute to neuronal dysfunction since glial cells are important for neuronal homeostasis; there are several known mechanisms by which astroglial damage leads to neuronal dysfunction.24 One of these is interference with neuronal glucose metabolism, in which astrocytes play an important role.25 We can only speculate about the mechanism by which ZK200775 may have caused glial cell toxicity. While nothing is known about a possible toxic effect of AMPA antagonists on astrocytes, a direct effect cannot be excluded. Presynaptic AMPA receptors, which regulate the synaptic release of glutamate, exist.26,27 Blockade of these receptors by AMPA antagonists can lead to a decrease in extracellular glutamate levels.28 Since glutamate is an essential element for glutathione synthesis in astroglia, the consequence is a lowering of the astrocyte antioxidative defense system, leading to an increased vulnerability to free radical damage.29,30 Support for this theory is that inhibition of the glutamate transporter by α-aminoacidic acid also leads to a decreased intracellular availability of glutamate and subsequent selective damage to astrocytes.30–32

Our study is another example of how toxicity of glutamate antagonists may prohibit their clinical use. A trend toward increased mortality was observed in stroke patients treated with the NMDA antagonist Seltofel, and this was interpreted as a neurotoxic effect.33 The present study suggests that AMPA receptor antagonists may have toxic effects on glial cells, at least in patients with brain ischemia. We suggest that monitoring of serum markers of brain damage should be included in phase 2 trials with glutamate receptor antagonists.

Appendix

Steering Committee

The following are in alphabetical order: Dr H. C. Diener, Abteilung Neurologie, Universität Essen, Essen, Germany; Dr M. Hommel, Service Neurologie, CHU Hopital Michalon, Grenoble, France; Dr M. Kaste, Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland; Dr J. De Keyser, Academisch Ziekenhuis, Groningen, Netherlands; Dr K. R. Lees, University Department of Medicine and Therapeutics, Gardiner Institute, Western Infirmary, Glasgow, UK; H. Steiner, Schering AG, SBU Therapeutics CV/CNS, Berlin, Germany; Dr M. Versavel, University of Antwerp, at that time at Schering AG, SBU Therapeutics CV/CNS, Berlin, Germany.

Safety Board

The following are in alphabetical order: Dr W. Hacke, Department of Neurology, University of Heidelberg, Heidelberg, Germany; Dr J. Mau, Department of Statistics in Medicine, Heinrich-Heine Universität, Düsseldorf, Germany; Dr K. Pooek, Medizinische Fakultät der RWTH Aachen, Aachen, Germany.

Study Centers and Principal Investigators

The following are ordered according to decreasing numbers of stroke patients contributed to the study; see protocol dated February 5, 1999: Dr M. Kaste, Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland. Dr Busse, Neurologische Klinik, Klinikum Minden, Minden, Germany; Dr K. R. Lees, University Department of Medicine and Therapeutics, Gardiner Institute, Western Infirmary, Glasgow, UK; Dr J. De Keyser, Academisch Ziekenhuis, Pállajt–Hámé Central Hospital, Lahti, Finland; Dr H. C. Diener, Abteilung Neurologie, Universität Essen, Essen, Germany; Dr P. Marx, Freie Universität Berlin, Universitätsklinikum Benjamin Franklin, Berlin, Germany; Dr J. De Keyser, Academisch Ziekenhuis, Groningen, Netherlands; Dr J. Sivenius, Kuopio University Hospital, Kuopio, Finland; Dr J. Cäckebeke, Onze Lieve Vrouw Ziekenhuis, Aalst, Belgium; Dr M. Hommel, Service Neurologie, CHU Hopital Michalon, Grenoble, France; Dr R. L. Haberli, Abteilung für Neurologie und Klinische Neurophysiologie, Städt. Krankenhaus München-Harlaching, München, Germany.

Acknowledgment

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References

3. Lees KR. Neuroprotection with the NMDA antagonist Selfotel, and this was interpreted as a neurotoxic effect.33 The present study suggests that AMPA antagonists may prohibit their clinical use. A trend toward increased mortality was observed in stroke patients treated with the NMDA antagonist Seltofel, and this was interpreted as a neurotoxic effect.33 The present study suggests that AMPA receptor antagonists may have toxic effects on glial cells, at least in patients with brain ischemia. We suggest that monitoring of serum markers of brain damage should be included in phase 2 trials with glutamate receptor antagonists.


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