Ebselen in Acute Ischemic Stroke
A Placebo-Controlled, Double-blind Clinical Trial

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Background and Purpose—The effect of ebselen, a seleno-organic compound with antioxidant activity through a glutathione peroxidase–like action, on the outcome of acute ischemic stroke was evaluated in a multicenter, placebo-controlled, double-blind clinical trial.

Methods—Patients diagnosed as having acute ischemic stroke who could receive drug treatment within 48 hours of stroke onset were enrolled. Oral administration of ebselen granules suspended in water (150 mg BID) or placebo was started immediately after admission and was continued for 2 weeks. The major end points were the Glasgow Outcome Scale scores at 1 month and 3 months after the start of treatment. The modified Mathew Scale and modified Barthel Index scores at 1 month and 3 months were also studied as secondary outcome measures.

Results—Three hundred two patients were enrolled in the trial. Intent-to-treat analysis of 300 patients (151 given ebselen and 149 given placebo) revealed that ebselen treatment achieved a significantly better outcome than placebo at 1 month (P=.023, Wilcoxon rank sum test) but not at 3 months (P=.056, Wilcoxon rank sum test). The improvement was significant in patients who started ebselen within 24 hours of stroke onset but not in those who started treatment after 24 hours. There was a corresponding improvement in the modified Mathew Scale and modified Barthel Index scores.

Conclusions—Early treatment with ebselen improved the outcome of acute ischemic stroke. Ebselen may be a promising neuroprotective agent. (Stroke. 1998;29:12-17.)

Key Words: clinical trials  neuroprotection  stroke outcome

Ebselen, 2-phenyl-1,2-benzisoselenazol-3[2H]-one, is a lipid-soluble seleno-organic compound that potently inhibits lipid peroxidation through a glutathione peroxidase–like action. Because it is active against membrane hydroperoxides such as phospholipid hydroperoxides and glutathione peroxidase but not glutathione peroxidase, this agent effectively inhibits both nonenzymatic and enzymatic (the lipoxygenase pathway of the arachidonate cascade) lipid peroxidation in vitro. Since the involvement of reactive oxygen intermediates in permanent brain damage due to ischemia (ie, infarction) has been supported by several studies, ebselen has been suggested to have the potential to protect the brain against ischemic insults. It was previously reported that recirculation-induced edema as well as postischemic hypoperfusion was markedly improved in a cat model of prolonged middle cerebral artery occlusion and that infarct size was reduced in rats with transient middle cerebral artery occlusion by ebselen pretreatment. Ebselen also significantly decreased cerebral edema and reduced infarct size in rats with permanent middle cerebral artery occlusion by postoperative treatment. These promising results prompted us to investigate the clinical value of ebselen in patients with acute ischemic stroke.

Subjects and Methods

Selection of Patients
Sixty-eight Japanese neurological and neurosurgical units (see “Appendix”) joined the trial. Eligible patients were those who were diagnosed as having acute ischemic stroke including thrombosis and embolism by the assessment of symptoms and by CT and who could receive drug treatment within 48 hours of onset. Patients with the following conditions were excluded from the trial: transient ischemic attacks; pregnancy; surgery interfering with assessment of neurological function; previous stroke with residual neurological impairment; major cardiopulmonary, hepatic, renal, or metabolic disease; or hemorrhagic stroke. This clinical protocol was approved by the institutional review board of each study site.

Drug Administration
Informed consent was obtained from the patient or the nearest relative. Patients were randomized to ebselen or placebo with the use of separate randomization lists balanced for each participating center. Treatment with ebselen or placebo (fine granules dispersed in water; 150 mg BID) was started within 48 hours after acute ischemic stroke and was continued for 14 days. The oral route (a gastric tube was used in patients with disturbance of consciousness) was selected for administration because ebselen is insoluble in water but is rapidly absorbed from the gastrointestinal tract and maintains a stable plasma concentration. In previous clinical trials, this method of administration was...
well tolerated and did not cause vomiting or diarrhea. The dose of 300 mg/d was found to achieve a better outcome in the preceding phase IIb trial, a double-blind dose-finding trial involving daily doses of 100, 200, 300, and 400 mg for 2 weeks (T.Y., unpublished data, 1993).

Clinical Management

Patients were managed according to the protocol of the attending investigator with only minor restrictions. Hypervolemia (administration of albumin or dextran combined with intravenous fluid supplements) and 10% glycerol were used as routine prophylactic measures. Concomitant treatment with calcium channel blockers such as nifedipine and nicardipine, warfarin, heparin, and aspirin was left to the discretion of the attending investigator. Administration of ticlopidine, urokiniase, tissue plasminogen activator, and sodium ozagrel was prohibited for the first 2 weeks.

Assessments and End Points

On admission a medical history was obtained, and general physical and neurological examinations were performed. Patients were monitored clinically throughout their hospital stay by assessment of blood pressure, pulse rate, and neurological status, including the Glasgow Coma Scale and the Japan Coma Scale, and by hematology and biochemical blood tests.

Angiography on admission was not mandatory in this trial, and the decision was left to the attending investigator. All patients were required to undergo repeated CT scanning on completion of treatment (at approximately day 14) and at approximately day 30, in addition to routine scans on admission, at approximately day 7, and on exacerbation of neurological deficits. The LDAs on each CT were classified as follows: none, small (a lacunar or small infarct <2 cm), multiple (multiple small infarcts), medium (between small and large, involving one cerebral lobe), and large (an infarct covering the whole territory of the anterior, middle, or posterior cerebral artery). The major end points were the GOS scores at 1 and 3 months after the start of treatment. The outcome at each period was categorized as follows: good recovery, moderate disability, severe disability, survival but in a vegetative state, and death. Neurological status was assessed by the modified Barthel Index, and functional status was assessed by the modified Barthel Index. The modified Mathew Scale evaluates 13 neurological items with a maximal value of 200, 300, and 400 mg for 2 weeks (T.Y., unpublished data, 1993).

Effect of Ebselen on Outcome

A previous phase IIb study, a double-blind dose-finding trial conducted between March 1992 and July 1993, showed that a good outcome of GOS score (good recovery or moderate disability) was achieved in approximately 47% of patients at 3 months by 300 mg/d of ebselen. Since the estimated percentage of the placebo group achieving such a score was approximately 35%, it was calculated that a minimum of 260 patients was required for the trial to have an 80% chance of detecting a 12% increase in the GOS. Comparison of demographic data, clinical treatment, and clinical parameters between the groups was done by the \( \chi^2 \) test without continuity correction, the Wilcoxon rank sum test, and the Student’s \( t \) test. The \( \alpha \)-level used in the comparison of study end points was .05 (two-tailed). The dependence of drug efficacy on prognostic factors such as age, sex, and the site of LDAs, as well as the relationships between the delay of starting treatment and the GOS score, were assessed by appropriate stratified analyses. The data obtained by these comparisons are presented with nominal two-tailed probability values unadjusted for multiplicity. Complete analysis was done on an ITT basis, and a PC analysis was also performed for the major end points.

Results

Enrollment of Patients

Between June 1994 and December 1996, 302 patients were enrolled in the trial (152 received ebselen and 150 received placebo). Selection of patients for the ITT and PC analyses was performed by the review committee before code opening, and 300 patients were subjected to ITT analysis. The other 2 patients were excluded from ITT analysis because of a diagnosis of subarachnoid hemorrhage (ebselen group) and transient ischemic attacks (placebo group). Fifty-eight patients were excluded from PC analysis because of the delay of starting treatment beyond 48 hours (n=16); concomitant administration of ticlopidine (n=8), urokiniase or tissue plasminogen activator (n=20), barbiturate (n=1), or sodium ozagrel (n=10); and incomplete test drug administration (n=3). Thus, 242 patients (118 in the ebselen and 124 in the placebo group) were subjected to PC analysis.

Equivalent of the Two Groups

A clinical profile of the patients subjected to ITT analysis is shown in Table 1. Demographic variables showed no statistically significant differences between the ebselen and placebo groups. The mean age was 65 years (range, 33 to 85 years) in the ebselen group and 65 years (range, 22 to 85 years) in the placebo group, and mean time from the onset of stroke to the start of treatment was 29.7 hours (range, 3 to 91 hours) in the ebselen group and 26.9 hours (range, 4 to 96 hours) in the placebo group (\( P=\text{.106, Student’s } t \) test). Distribution of the baseline modified Mathew Scale score in three categories, <35 (severe impairment), \( \geq 35 \) and <75 (moderate impairment), and \( \geq 75 \) (mild impairment), was not statistically significant (\( P=\text{.160, Wilcoxon test} \). The peak systolic and diastolic blood pressures were similar in both groups at all times. Administration of 10% glycerol was done in a similar percentage of both groups (88 patients in the ebselen group and 90 in the placebo group). Mild hypervolemia was used in 44% and 49% of the ebselen and placebo groups, respectively. The use of other drugs such as calcium channel blockers, heparin, warfarin, and aspirin was also similar in both groups, employed in 15 and 14, 10 and 10, 2 and 7, and 2 and 1 patients of the ebselen and placebo groups, respectively. Routine physical therapy was done in 68% and 72% of the ebselen and placebo groups, respectively.

Effect of Ebselen on Outcome

Ten patients died in the ebselen group (6.6%), and 15 died in the placebo group (10.0%), and therefore the overall mortality rate was not significantly reduced (\( P=\text{.288, } \chi^2 \) test).
The GOS scores obtained after 1 month and 3 months in the ITT and PC analyses are shown in Fig 1. One patient in the placebo group missed the 1-month evaluation because of early discharge from the hospital but returned for the 3-month evaluation at the outpatient clinic. Four patients (3 and 1 in the ebselen and placebo groups, respectively), who were alive but not scored on outcome scale because of incomplete drug administration or discharge from the hospital before the assessment, were excluded from analysis. The difference between the ebselen and placebo groups was statistically significant at 1 month in both ITT analysis and PC analysis (P=.038 at 1 month and P=.049 at 3 months, Wilcoxon test) and PC analysis (P=.016 at 1 month and P=.027 at 3 months, Wilcoxon test; data not shown). However, there were no significant differences when ebselen treatment was started after 24 hours in both ITT analysis (P=.385 at 1 month and P=.644 at 3 months, Wilcoxon test) and PC analysis (P=.390 at 1 month and P=.715 at 3 months, Wilcoxon test; data not shown). In the stratified analysis of the GOS scores in relation to the site of LDAs, there were no statistically significant differences between the ebselen and placebo groups for patients with perforator infaracts in both ITT analysis (P=.537 at 1 month and P=.979 at 3 months, Wilcoxon test) and PC analysis (P=.693 at 1 month and P=.688 at 3 months, Wilcoxon test). The overall outcome was nearly the same in both groups. However, there were significant differences for patients with cortical infaracts in both ITT analysis (P=.020 at 1 month and P=.039 at 3 months, Wilcoxon test) and PC analysis (P=.016 at 1 month and P=.033 at 3 months, Wilcoxon test). A good recovery was significantly more common in the ebselen group than in the placebo group. There were no significant differences in the baseline characteristics of the patients, including the type of rehabilitation, between the two groups in these stratified analyses.

**Effect of Ebselen on Secondary Outcome Measures**

Changes of the modified Mathew Scale and modified Barthel Index scores in ITT analysis are shown in Table 2. Three patients in the ebselen group, who were alive but not scored on both outcome scores because of incomplete drug administration or discharge from hospital before the assessment, were excluded from analyses. Two patients in the placebo group, who were alive but not scored on both outcome scores because of discontinuation of drug administration due to aggravation of neurological status, were excluded from the analysis of modified Barthel Index. For the modified Mathew Scale, scores at 2-week evaluation were also regarded as the scores of 1 and 3 months for these patients. In the analysis of both scores, the ebselen group was found to have a higher proportion of patients with no or mild impairment (range of scale, 75 to 100) and with no or mild disability (range of scale, 75 to 100), respectively. These differences reached statistical significance at 1 and 3 months. There was not a significant difference between the ebselen and placebo groups in baseline neurological impairment. Changes of neurological status, functional status, and GOS scores of ITT patients with severe or moderate impairment (<75, modified Mathew Scale) are shown in Table 3. Ebselen treatment achieved a significant improvement in each outcome measure. Thus, there was a corresponding improvement in both secondary outcome measures, as was the case with the GOS score.

**Clinical and Laboratory Events**

The following complications and clinical events were respectively observed in the ebselen and placebo groups: new cerebral infarction (5 [3%] and 6 [4%], P=.742), new hemorrhagic infarction.

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**TABLE 1. Clinical Profile of the Ebselen and Placebo Groups**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Ebselen (%)</th>
<th>Placebo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤65</td>
<td>68 (45)</td>
<td>70 (47)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>83 (55)</td>
<td>79 (53)</td>
</tr>
<tr>
<td>Stroke type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombosis/embolism</td>
<td>71 (47)/61 (40)</td>
<td>74 (50)/62 (42)</td>
</tr>
<tr>
<td>Unclear</td>
<td>19 (13)</td>
<td>13 (9)</td>
</tr>
<tr>
<td>Site of LDAs (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left/right</td>
<td>77 (51)/70 (46)</td>
<td>66 (45)/79 (54)</td>
</tr>
<tr>
<td>Left and right</td>
<td>4 (3)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Site of LDAs (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perforating/cortical</td>
<td>53 (35)/61 (40)</td>
<td>50 (34)/54 (36)</td>
</tr>
<tr>
<td>Both regions</td>
<td>32 (21)</td>
<td>37 (25)</td>
</tr>
<tr>
<td>Size of LDAs (initial/final)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>56 (37)/7 (5)</td>
<td>59 (40)/6 (4)</td>
</tr>
<tr>
<td>Small</td>
<td>22 (15)/34 (23)</td>
<td>23 (15)/30 (20)</td>
</tr>
<tr>
<td>Multiple</td>
<td>23 (15)/31 (21)</td>
<td>24 (16)/35 (24)</td>
</tr>
<tr>
<td>Medium</td>
<td>36 (24)/50 (34)</td>
<td>25 (17)/38 (26)</td>
</tr>
<tr>
<td>Large</td>
<td>14 (9)/27 (18)</td>
<td>18 (12)/39 (26)</td>
</tr>
<tr>
<td>Start of treatment after onset, d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>61 (40)</td>
<td>67 (45)</td>
</tr>
<tr>
<td>≥2</td>
<td>80 (53)</td>
<td>76 (51)</td>
</tr>
<tr>
<td>Mean time from onset to treatment, h</td>
<td>29.7</td>
<td>26.9</td>
</tr>
<tr>
<td>Modified Mathew Scale on admission (mean±SD)</td>
<td>70±20</td>
<td>66±20</td>
</tr>
<tr>
<td>&lt;35 (severe)</td>
<td>15 (10)</td>
<td>18 (12)</td>
</tr>
<tr>
<td>≥75 (mild)</td>
<td>70 (47)</td>
<td>58 (39)</td>
</tr>
</tbody>
</table>

Values in parentheses do not total 100% because of rounding.
(35 [23%] and 26 [17%], *P*=.218), gastrointestinal bleeding (5 [3%] and 6 [4%], *P*=.742), nausea/vomiting (2 [1%] and 5 [3%], *P*=.244), and respiratory infection (11 [7%] and 26 [17%], *P*=.007). The overall incidence of adverse reaction was slightly higher in the ebselen group than in the placebo group (7.3% versus 3.3%), but there was no significant difference between the two groups (*P*=.127, χ² test). No significant changes in laboratory data were noted. The causes of death (presumed or confirmed) in the ebselen and placebo groups were as follows: recurrence/deterioration of cerebral infarction (6 and 9), sepsis (1 and 0), pneumonia (2 and 3), brain hemorrhage (0 and 1), acute myocardial infarction (0 and 1), gastrointestinal bleeding (1 and 0), and pulmonary embolism (0 and 1). Ebselen therapy was not suspected to have contributed to the death of any patient.

**Discussion**

A number of studies have shown that oxidative stress (generation of active oxygen and lipid peroxidation) occurs within ischemic brain tissue.6,7,18 Peroxidation of cell membrane phospholipids leads to an increase of intracellular free radicals when the intrinsic antioxidant systems is jeopardized by an energy crisis. Such oxidative stress has been suggested to aggravate tissue damage primarily through impairment of the cerebral microcirculation.6 Peroxidation of membrane phospholipids...
triggered by oxidative stress appears to underlie ischemic brain damage. Ebselen is reported to reach inside cells as a result of its reactive binding to the intracellular thiol groups such as glutathione, inhibits lipoxygenase in the arachidonate cascade, blocks the production of superoxide anions by activated leukocytes, inhibits inducible nitric oxide synthase, and exhibits a sustained defense line effect against peroxynitrite. Accordingly, ebselen has the potential to influence the key reactions involved in ischemic brain damage. Among the multiple intrinsic antioxidant systems, glutathione peroxidase plays a major role in intracellular redox regulation. The neuroprotective effect of ebselen demonstrated in the present trial may be explained by these mechanisms.

In the present study both groups were well balanced, and there were no significant differences in baseline characteristics. Patients in three impairment categories of modified Mathew Scale were evenly distributed on admission, and the number of patients with mild impairment was slightly more in the ebselen group, but the difference was not statistically significant. Both ITT and PC analyses revealed a significant difference in the overall outcome scores between the ebselen and placebo groups, and the percentage of patients with a good outcome was always approximately 10% higher in the ebselen group. Despite the obvious trend for spontaneous improvement from 1 month to 3 months, a difference between the groups was maintained. The efficacy of ebselen on outcome was also observed in patients with severe or moderate impairment, as shown in Table 3. Stratified analysis provided the following information. The outcome of patients who received early ebselen treatment was significantly superior to that of patients who received later treatment. In addition, the effect of ebselen on outcome was more prominent in patients with lesions involving the brain cortex than in those with deep-seated lesions. These findings may suggest that ebselen protected the brain from ischemic insults. The possible influence of baseline characteristics on the evaluation of drug efficacy was examined for each end point by Cochran-Mantel-Haenszel analysis, and no significant influence was detected.

It is generally believed that a large number of centers enrolling a small number of patients per center will increase the risk of skew in randomization and ancillary care. To minimize this problem, the diagnosis was done by CT, the grading of patients by guest on August 26, 2017 http://stroke.ahajournals.org/ Downloaded from
was conducted by modified Mathew Scale, and the evaluation of efficacy was based on the GOS score. The study committee reviewed the uniformity and appropriateness of the final judgment of each investigator. In addition, the racial and socioeconomic status of patients in Japan is quite homogeneous. Therefore, valid results could be obtained with this study design. The overall incidence of abnormal laboratory findings was similar in the ebselen and placebo groups. The incidence of respiratory infections was significantly lower in the ebselen group, and this drug was not reported to cause infection in an animal study.21 Thus, we found no evidence of the potential problems of antioxidant therapy. There was no evidence that ebselen contributed to any of the causes of death. The present clinical trial may support the safety of this agent at a dose of 300 mg/d.

In conclusion, the outcome of patients with ischemic stroke was significantly improved by early treatment with ebselen. Hence, ebselen may be a useful neuroprotective agent for the treatment of acute ischemic stroke.

Acknowledgment
This study was supported by Daiichi Pharmaceutical Co, Ltd, Tokyo, Japan.

Appendix
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Participating Centers
This trial was performed with the cooperation of the doctors and staff of the following neurological and neurosurgical institutions and hospitals in Japan: Asahikawa Red Cross Hospital, Kitami Central Hospital, Bibai Rosai Hospital, Kusharo Rosai Hospital, Kusharo General Hospital, Azabu Neurosurgical Hospital, Nakamura Memorial Hospital, Shinjapporo Neurosurgical Hospital, Hokkaido University, Hokkaido Neurosurgical Hospital, Otaru Neurosurgical Hospital, Research Institute for Brain and Blood Vessels—Akita, Iwate Medical College, Iwate Prefectural Central Hospital, Sendai National Hospital, Kohnan Hospital, Fukushima Medical College, Aizu Central Hospital, Ashikaga Red Cross Hospital, Usunomia Saiseikai Hospital, Jichi Medical College, Nagoaka Red Cross Hospital, Chuo University Hospital, Kameda General Hospital, Keai Hospital, Chiba Emergency Medical Center, Keio University, Tokyo Women’s Medical College, Showa University, Kitou Rosai Hospital, Nipon Medical School, Nipon Medical School First Hospital, Tokai University, Kitasato University, Yokohama General Hospital, Juntendo University Izu-nagakyo Hospital, Shizuoka Prefectural General Hospital, Hamamatsu Rosai Hospital, Fuji Health University, Nagoya Eikaiakai Hospital, Nagoya National Hospital, Nagoya City University, East Nagoya National Hospital, Aichi Saiseikai Hospital, Toyama Medical and Pharmaceutical University, Fukui Red Cross Hospital, Mazuru City Hospital, National Cardiovascular Center, Kitano Hospital, Iseikai Hospital, Hanwa Memorial Hospital, Oono Memo-

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Stroke. 1998;29:12-17
doi: 10.1161/01.STR.29.1.12

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