GM1 Ganglioside Therapy in Acute Ischemic Stroke

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For the Italian Acute Stroke Study—Hemodilution+Drug

Eleven of 31 clinical centers participating in the Italian Acute Stroke Study-Hemodilution carried out a preliminary study on the effectiveness of ganglioside GM1 in acute stroke; 502 patients were randomized to GM1 (GM1, n=121), GM1 plus hemodilution (GM1+H, n=128), placebo (P, n=130), or placebo plus hemodilution (P+H, n=123) groups ≤12 hours after onset of a hemispheric cerebral infarct. The patients were treated for 15 days and were evaluated on Days 21 and 120 after the onset of stroke. Intention-to-treat analysis failed to show any differences in neurologic deficit, mortality, or neurologic disability among the groups. Efficacy analysis showed a significantly higher degree of neurologic improvement in GM1 group patients compared with patients in the P group during the first 15 days. GM1-treated patients (GM1 and GM1+H groups) showed a significantly higher degree of neurologic improvement during the first 10 days compared with the placebo-treated patients (P and P+H groups). These differences were no longer statistically significant at Day 120. Our results provide a rationale for the planning of a larger, multicenter trial of GM1 ganglioside in acute stroke. (Stroke 1989;20:1143–1149)

Improved understanding of the pathophysiology of acute focal cerebral ischemia has led to experimental therapeutic strategies that attenuate ischemic neural damage. These strategies include efforts to increase microcirculatory blood flow and decrease the activities of endogenous neurotoxic substances produced in the ischemic penumbra to lengthen neuronal survival and improve tissue repair.1,2 Despite expectations, none of the several drugs or procedures hitherto tested has provided clear-cut positive results.3 Many factors (including inadequate sample size, brief follow-up, uncertainties about diagnosis, selection of nonhomogeneous or unrepresentative subgroups of patients and, finally, insensitivity of the criteria adopted to evaluate outcome) could have affected the results of those trials.4,5 Taking into account these crucial parameters, a multicenter trial was performed in which some centers, in the context of the Italian Acute Stroke Study—Hemodilution (IASS-H),6 tested the ganglioside GM1.7

Subjects and Methods

During 18 months, 11 of the 31 clinical centers participating in the IASS-H performed a randomized double-blind controlled trial to test the efficacy of GM1 in acute stroke. The general design of the trial was planned by a Steering Committee and described in a protocol manual. Included were patients with a first hemispheric cerebral infarction diagnosed clinically ≤12 hours after the onset of stroke. Excluded were patients with brainstem or cerebellar signs or symptoms, history or evidence of a previous stroke, resolution of the symptoms and signs during the recruitment period, other severe disabling diseases, cerebral hemorrhage, brain tumor, and other misdiagnosis. A registry was kept of excluded as well as included patients. Informed consent was obtained from the patients or their relatives. The stroke registry is summarized in Table 1.

The 502 patients were randomized to one of four treatment groups: ganglioside GM1 (n=121), GM1 plus hemodilution (GM1+H, n=128), placebo (P,
n = 130), and placebo plus hemodilution (P+H, n = 123). GM1 was administered intravenously in a dose of 100 mg diluted in 50 ml normal saline and infused over 10 minutes. An equal amount of normal saline was administered as placebo. The treatment was repeated daily during the first 15 days of hospitalization. Patients allocated to the GM1+H or P+H groups were submitted to normovolemic hemodilution according to the schedule of the IASS-H.

On admission, a detailed medical history and a clinical description of the ischemic event of each patient were obtained. Then a general clinical evaluation (including blood pressure, heart rate and rhythm, and electrocardiogram [ECG]) and a quantitative neurologic examination according to the Canadian Neurological Scale (CNS) were performed. Scores for the CNS range from 1.5 to 10 points; the higher the score the less the neurologic deficit (Table 2). The clinical diagnosis of first ischemic stroke had to be confirmed by computed tomography (CT) ≤48 hours after the onset of symptoms. Hospitalization lasted ≥15 days. During that period each patient underwent daily neurologic examination and an evaluation of the general clinical and cardiologic status, including, for the first 7 days, determinations of liver transaminases, creatine phosphokinase, blood urea nitrogen, lactic dehydrogenase, leukocyte and erythrocyte counts, creatinine, serum proteins and electrolytes, blood glucose level, and hematocrit. In addition, an ECG was carried out on Days 3, 7, 15, and 120 after stroke onset. All patients had a Doppler study of the neck vessels. A B-mode echocardiogram was performed in all patients without atherosclerotic lesions of the neck vessels documented by Doppler sonography.

All randomized patients received the prescribed treatment and were followed until death or Day 120, regardless of whether exclusion criteria were subsequently identified. The patients were evaluated daily for the first 15 days, and then again on Days 21 and 120. The outcome measures were mortality rate, neurologic disability measured by the modified Rankin’s Classification of Neurologic Disability Status (DS) (Table 3) in which 1 signifies normal function and 6 maximal impairment, and degree of neurologic improvement (DNI) defined as the difference between the CNS on Days 1–15, 21, and 120 and the CNS at admission.

The causes of death were classified as fatal stroke, cardiac cause, or other cause of death based on clinical and/or pathologic data. In every patient in whom autopsy was not performed, the cause of death was reviewed by the Executive Committee in consultation with the physician of...
the clinical center involved. Information on patients who died after hospital discharge was obtained from death certificates.

On Day 120, all patients still living underwent a cerebral CT scan and an ECG, and neurologic deficit (CNS score) and disability (DS score) were evaluated.

Data were collected onto a form of which one copy remained at the clinical center, a second copy went to the Fidia Clinical Research Department, and a third copy remained at the clinical center, a second copy at the Fidia Clinical Research Department. The study was approved by the Ethics Committee at each clinical center. Consequently, 427 patients (247 men and 180 women, mean ± SD age 68 ± 11 years; Table 5) had a first ischemic hemispheric stroke. Consequently, 427 patients (247 men and 180 women, mean ± SD age 68 ± 11 years; Table 5) had a first ischemic hemispheric stroke. Consequently, 427 patients (247 men and 180 women, mean ± SD age 68 ± 11 years; Table 5) had a first ischemic hemispheric stroke.

For the purposes of the efficacy analysis, we considered only those patients with a first ischemic hemispheric stroke. We excluded 32 additional patients since they did not want to continue in the study; these patients were homogeneous.

### Results

Between January 1985 and June 1986, 502 consecutive patients with acute stroke were randomized within an average of 6.3 hours after the onset of symptoms. A CT scan was performed in 439 patients within 48 (mean 14) hours and in 60 patients within 48–96 hours. Only three patients failed to undergo a CT scan, but each of these patients had an autopsy.

One hundred ninety-nine of the 502 randomized patients had a history of heart disease, 74 of diabetes, and 231 of hypertension; 140 patients smoked >10 cigarettes/day. On admission, 87 patients had atrial fibrillation. One hundred ninety-nine patients had a maximum arterial blood pressure of >170 mm Hg, and 175 had a minimum arterial blood pressure of >100 mm Hg; 147 patients had both maximum and minimum arterial blood pressure greater than those values. These risk factors for stroke were homogeneously distributed among the treatment groups.

Table 4 reports the number of patients randomized into each group, the group mean CNS and DS scores, and mortality on Days 15, 21, and 120. Intention-to-treat analysis showed no statistically significant differences among groups.

CT and/or pathology demonstrated a previous stroke in 23, a hemorrhage in 40, a brainstem and/or cerebellar lesion in three, and a neoplasm in nine patients. Consequently, 427 patients (247 men and 180 women, mean ± SD age 68 ± 11 years; Table 5) had a first ischemic hemispheric stroke.

For the purposes of the efficacy analysis, we considered only those patients with a first ischemic hemispheric stroke. We excluded 32 additional patients since they did not want to continue in the study; these patients were homogeneous.

### Search and Sort

The search identified 502 patients randomized to the Italian Acute Stroke Study Group. The patients were divided into four groups: GM1, GM1+H, P, and P+H. The CNS and DS scores were collected on Days 1, 15, 21, and 120. The Mann-Whitney nonparametric test, with Bonferroni’s correction for multiple comparisons, was used to compare DS scores of surviving GM1-treated (GM1 and GM1+H groups) and placebo-treated (P and P+H groups) patients. DNI was also tested on subgroups of patients defined by CNS scores at admission and by the time between the onset of symptoms and randomization.

### Table 3

**Rankin’s Classification of Neurologic Disability Status (Modified)**

<table>
<thead>
<tr>
<th>Description</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>No significant disability: able to carry out all previous usual activities</td>
<td>1</td>
</tr>
<tr>
<td>Slight disability: unable to carry out all previous activities but able to attend to bodily needs without assistance</td>
<td>2</td>
</tr>
<tr>
<td>Moderate disability: requiring some help for bodily needs and with walking</td>
<td>3</td>
</tr>
<tr>
<td>Moderately severe disability: requiring some help for bodily needs and unable to walk without assistance or physical device</td>
<td>4</td>
</tr>
<tr>
<td>Severe disability: unable to attend to bodily needs without assistance and/or to walk</td>
<td>5</td>
</tr>
<tr>
<td>Very severe disability: bedridden, incontinent, and requiring constant nursing care</td>
<td>6</td>
</tr>
</tbody>
</table>

### Table 4

**Clinical Evolution Among Patients Randomized to Italian Acute Stroke Study Group**

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>15</th>
<th>21</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CNS</td>
<td>n</td>
<td>CNS</td>
</tr>
<tr>
<td>GM1</td>
<td>121</td>
<td>5.5</td>
<td>98</td>
<td>7.3</td>
</tr>
<tr>
<td>GM1+H</td>
<td>128</td>
<td>5.8</td>
<td>110</td>
<td>7.5</td>
</tr>
<tr>
<td>P</td>
<td>130</td>
<td>6.2</td>
<td>108</td>
<td>7.4</td>
</tr>
<tr>
<td>P+H</td>
<td>123</td>
<td>5.7</td>
<td>102</td>
<td>7.2</td>
</tr>
<tr>
<td>Total</td>
<td>502</td>
<td>418</td>
<td>58</td>
<td>411</td>
</tr>
</tbody>
</table>

GM1, 100 mg/day for 15 days; H, normovolemic hemodilution; P, placebo (normal saline); CNS, Canadian Neurological Scale score as measure of neurologic deficit; DS, modified Rankin Scale class as measure of neurologic disability; M, mortality.
TABLE 5. Clinical Evolution Among Patients With First Ischemic Hemispheric Stroke, Italian Acute Stroke Study Group

| Group | Time from onset (hr) | Age (yr) (mean±SD) | 120 | 21 | 15 | Day | Time from onset (hr) | Age (yr) (mean±SD) | 120 | 21 | 15 | Day |
|-------|---------------------|--------------------|-----|----|----|-----|---------------------|--------------------|-----|----|----|-----|-----|-----|---------------------|--------------------|-----|----|----|-----|-----|-----|---------------------|--------------------|-----|----|----|-----|-----|-----|
| GM1   | 6.0                 | 67±12              | 93  | 5.6 | 74 | 7.5 | 3.1 | 15 | 74  | 7.6 | 3.0 | 15 | 63  | 8.4 | 2.1 | 22 |
| GM1+H | 6.3                 | 69±11              | 105 | 5.8 | 94 | 7.4 | 3.2 | 9  | 93  | 7.6 | 3.1 | 9  | 84  | 8.3 | 2.5 | 17 |
| P     | 7.0                 | 68±11              | 119 | 6.1 | 100| 7.3 | 3.2 | 14 | 96  | 7.6 | 3.0 | 17 | 82  | 8.4 | 2.3 | 29 |
| P+H   | 6.4                 | 67±10              | 106 | 5.7 | 91 | 7.1 | 3.4 | 12 | 90  | 7.3 | 3.3 | 13 | 79  | 8.1 | 2.6 | 19 |
| Total | 6.3                 | 68±11              | 427 | 5.8 | 359| 7.1 | 3.4 | 50 | 353 | 7.3 | 3.3 | 54 | 308 | 8.1 | 2.6 | 87 |

GM1, 100 mg/day for 15 days; H, normovolemic hemodilution; P, placebo (normal saline); CNS, Canadian Neurological Scale score as measure of neurologic deficit; DS, modified Rankin Scale class as measure of neurologic disability; M, mortality.

Sixteen percent (n=15) of the patients in the GM1 group and 12% (n=14) in the P group died within the first 15 days of hospitalization. By Day 21, three more patients (14%) in the P group had died. By Day 120, 24% of the patients in the GM1 group and 24% in the P group had died. No significant difference between groups in mortality was detected.

The survival time of all GM1-treated patients (GM1 and GM1+H groups) with first ischemic hemispheric stroke was not significantly better than that of the placebo-treated individuals (P and P+H groups) (Table 5). ANOVA of DNI for these 174 patients revealed significant effects in favor of GM1 up to Day 15 (p=0.056 on Day 15) (Figure 1). The greatest effect was found among patients with a CNS score of <4.0 points at admission (p=0.03 on Day 15) (Figure 2).

The survival time of all GM1-treated patients with first ischemic hemispheric stroke was not significantly better than that of the placebo-treated individuals (P and P+H groups) (Table 5). ANOVA was also performed on the subgroup of patients randomized <6 hours after onset. Neurologic outcome of these patients was not significantly better than that of patients randomized >6 hours after onset.

**Discussion**

Current evidence suggests that neuronal death secondary to primary brain damage may be related to either deficits in the availability/activity of neurotrophic factors or to increased activity of
neuronototoxic agents, such as excitatory amino acid neurotransmitters. Treatment with GM1 has been reported to ameliorate the deficit following various types of central nervous system injuries in animals,8,14-18 including different models of focal ischemia.19-22

The mechanism of action of GM1 is still under investigation; however, a fundamental prerequisite is that the agent interact with neuronal membranes with respect to their capacity to transfer, modulate, and respond to signals. In vitro studies have shown that GM1 enhances the cellular response to neuronotrophic factors27-33 and that GM1 decreases glutamate-induced neuronotoxicity.34,35 Thus, these two mechanisms could be involved in GM1-induced facilitation of central nervous system recovery in vivo and could provide the experimental rationale for the use of gangliosides in an acute stroke trial.

As to our protocol, we chose the time-to-randomization limit of ≤12 hours after stroke onset because experimental work suggests a better effect of GM1 when treatment is started as soon as possible after the insult. The CT scan, performed on almost all randomized patients, enabled us to confirm the clinical diagnosis of ischemic stroke and to delete from the efficacy analysis a significant number of patients (approximately 10%) whose nonischemic brain lesions clinically mimicked ischemic stroke. As criteria for monitoring GM1 effectiveness and clinical outcome, we considered neurologic deficit, neurologic disability, and mortality. The CNS, the internal consistency of which has been defined, was chosen because it takes into account the most important parameters for stroke patients.9 Furthermore, the CNS is brief and easy to use, thus minimizing interobserver variability. The DS was chosen for its simplicity and reproducibility,10 and turned out to be a fortunate choice since we found a close correlation between the DS scores and the CNS scores when measuring neurologic impairment. To evaluate both the acute and the long-term effects of GM1, a 120-day follow-up was used. The homogeneity of baseline characteristics of our patients, including medical history, suggests that our randomization procedure was successful and that comparable treatment groups were achieved.

In conclusion, our data stress that, during 15 days of treatment of GM1 started ≤12 hours after the onset of symptoms, ischemic stroke patients showed a significantly greater degree of neurologic improvement than placebo-treated patients. We also found that the lower the CNS scores at admission (<4.0 points), the greater the degree of neurologic improvement. The significance of this effect was clearly evident during the first 15 days of the study, which corresponds to the duration of treatment, and suggests that a more prolonged administration of GM1 in future trials would be opportune. Our results should encourage further testing of the clinical efficacy of GM1 during the acute phase of ischemic stroke.

Appendix 1. Complete List of Participants


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References


KEY WORDS • cerebrovascular disorders • clinical trials • gangliosides
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*Study--Hemodilution + Drug.*

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