Nimodipine in Animal Model Experiments of Focal Cerebral Ischemia
A Systematic Review

J. Horn, MD; R.J. de Haan, PhD; M. Vermeulen, MD; P.G.M. Luiten, PhD; M. Limburg, MD

Background and Purpose—Based on the results of animal experiments, clinical trials were performed with nimodipine, which did not demonstrate a beneficial effect on outcome after stroke. The aim of this study was to determine whether the evidence from animal experiments with nimodipine supported the use of nimodipine in clinical trials.

Methods—We performed a systematic review of animal experiments with nimodipine in focal cerebral ischemia. Studies were identified by searching Medline and Embase. We assessed whether these studies showed a beneficial effect of active treatment. In-depth analyses were performed on infarct size and amount of edema, and subgroup analyses were performed on the length of the time window to the initiation of treatment and the methodological quality of the studies.

Results—Of 225 identified articles, 20 studies were included. The methodological quality of the studies was poor. Of the included studies, 50% were in favor of nimodipine. In-depth analyses showed statistically significant effects in favor of treatment (10 studies). No influence of the length of time to the initiation of treatment or of the methodological quality on the results was found.

Conclusions—We conclude that the results of this review did not show convincing evidence to substantiate the decision to perform trials with nimodipine in large numbers of patients. There were no differences between the results of the animal experiments and clinical studies. Surprisingly, we found that animal experiments and clinical studies ran simultaneously. (Stroke. 2001;32:2433-2438.)

Key Words: animal models ■ calcium channel blockers ■ cerebrovascular disorders ■ nimodipine ■ meta-analysis

The recognition of the existence of a penumbral zone in ischemic stroke, as described by Astrup and Siesjo,1 was a major trigger in the search for an effective neuroprotective agent. Massive calcium influx into cells was found to be a final common pathway leading to cell death. Therefore, calcium channel blockers might protect the penumbral zone from becoming necrotic. Early steps on the road toward neuroprotective treatments in stroke were, as usual, in the form of animal experiments. Positive results of treatment with nimodipine, an L-type calcium channel blocker, in animal models of acute focal ischemia were reported, and investigations of this agent were started in patients in the early 1980s. In 1988, a randomized controlled trial in stroke patients showed a beneficial effect.2 On the basis of these promising results, calcium antagonists in stroke were studied in more clinical trials,3-6 in which, finally, 7665 patients were randomized. These trials failed to confirm the beneficial effect of active treatment,7 but in a meta-analysis of 9 trials with nimodipine in acute ischemic stroke, a statistically significant effect in favor of nimodipine was found when treatment was started within 12 hours of stroke onset.8 This again raised hope for neuroprotective treatment in stroke patients. However, in a recent systematic review performed for the Cochrane collaboration7 on the effects of calcium antagonists in stroke, this positive effect of early treatment could not be confirmed.

Similar discrepancies between initial successful experimental animal data and lack of effect in clinical application have been reported for a variety of potentially neuroprotective drugs. These agents either evoked unacceptable adverse reactions or did not have any beneficial effect.9-12 These disappointing results have raised doubts about the interpretation and validity of the results of animal stroke models with respect to subsequent clinical research.

The aim of the present investigation was to determine whether the evidence from animal experiments was in favor of nimodipine, which would mean that these models were inadequate in the prediction of treatment response in patients or that the data were inconclusive, which would mean that the clinical trials were based on insufficient evidence.

Materials and Methods

Literature Search and Inclusion Criteria

Our literature search for the present review was restricted to published results of animal studies, which were identified by...
searching Medline (1966 to 1999) and Embase (1980 to 1999) with the use of the search terms “nimodipine” (limited to “animal”) and “cerebral ischemia.” Reference lists of identified articles were also searched.

Studies were included if they fulfilled the following criteria: (1) the study assessed the effect of nimodipine on focal cerebral ischemia; (2) a group of control animals was described; (3) nimodipine was administered after the induction of ischemia; (4) the effect of nimodipine was assessed in animals or in whole brains, not in slices or samples of brain tissue; (5) nimodipine was not tested in combination with other neuroprotective agents; (6) there was a restriction to the original study results (reviews and duplicate publications were excluded); and (7) the studies were published in English, French, or German. The selection of trials (unblinded) was performed (by J.H.) on the basis of title and abstract. In case of doubt, the whole publication was evaluated.

Data Extraction
From the included studies, the following data were extracted: animal species; number of animals in treated and control groups; method of allocation to treatment group; method to induce ischemia; method, dosage, and time of drug administration; method to assess efficacy (blinded assessment); and results of treatment.

Methodological Quality of Studies
On the basis of recommendations published in 1999,13 we designed an 8-point rating system to assess the methodological quality of the included animal experimental studies. One point was attributed for each of the following characteristics: (1) the dose/response relationship that was investigated, (2) randomization of the experiment, (3) optimal time window of the treatment investigated, (4) monitoring of physiological parameters, (5) blinded outcome assessment, (6) assessment of at least 2 outcomes (infarct size and 1 functional outcome), (7) outcome assessment in the acute phase (1 to 3 days), and (8) outcome assessment in the chronic phase (7 to 30 days). The points were granted when in the study report these items were mentioned. Studies scoring 4 points were graded as “poor methodological quality,” and studies with ≥4 points were scored as “good methodological quality.”

Outcome Assessment and Statistical Analyses
For each study, we defined whether a positive (nimodipine beneficial) or negative (no difference between active and placebo treatment or deleterious effect of nimodipine) result was reported.

Analysis in depth was possible only for a limited selection of trials, which reported data on the impact of nimodipine on infarct size or amount of edema. Per study, we calculated the effect size (the mean of the treatment group minus the mean of the control group divided by the pooled standard deviation of the 2 groups) and pooled the individual effect sizes accordingly. Because our data was shown to be heterogeneous, we used a random-effects model.14 Statistical uncertainty was expressed in 95% CIs.

Subgroup Analyses
In view of the common opinion that treatment has to be started as soon as possible after the induction of ischemia,15 we performed a subgroup analysis on data from studies in which nimodipine was started within or later than 1 hour after ischemia. Difference in the frequency of treatment results was analyzed with the Fisher exact test. Finally, we investigated whether study methodology influenced the results of the experiments.

Results

Description of Studies
We identified 225 articles. On the basis of predefined criteria, 205 articles were excluded (a list of these studies is available from the author). Table 1 lists the various reasons for exclusion. Many studies were excluded because they described the effect of nimodipine in global ischemia models or because nimodipine was administered before the induction of ischemia. We also identified several duplicate publications.16–24 Twenty studies fulfilled the inclusion criteria; the characteristics of these studies are listed in Table 2, in which the studies are ordered by increasing length of the time interval between the induction of ischemia and the initiation of treatment. In total, 234 animals were treated with nimodipine (216 animals served as controls) after the induction of focal cerebral ischemia. In all studies, the animals were anesthetized during the surgical procedures, only 1 study explicitly reported that the animals were allowed to recover before the induction of ischemia.25 Parameters such as body temperature, blood glucose levels, and blood pressure were maintained within strict limits in most animal experiments.

In 12 of 19 studies, treatment was started within 1 hour after the induction of ischemia.16,21,25–34 One study did not state the length of this interval.35 The methodological quality of the included studies was poor (median 2.85 points, range 1 to 5). Only 2 studies mentioned randomization of the animals,27,33 and in 6 studies, the outcome was assessed by a blinded assessor.21,25,26,28,32,36 One study reported “double-blind” assessment of effectiveness.37 Although 9 studies assessed 2 outcome measurements (functional and histopathologic),18,25,27,32,35–39 only 3 studies assessed outcome in the chronic phase.18,32,36

Outcome
Of the 20 included studies, 10 reported a positive effect of nimodipine, and 10 did not. In 7 studies, exact data about infarct size were presented21,28–31,36,37 (Figure 1). The pooled effect size in favor of nimodipine was −1.2 (95% CI −1.7 to −0.7).

Extracting data regarding the amount of edema was possible in 3 studies18,27,34 (Figure 2), revealing a pooled effect size of −0.6 (95% CI −1.2 to −0.1) in favor of nimodipine.

Subgroup Analysis
Additional analysis of the studies in which treatment was started within 1 hour after the induction of ischemia resulted in 4 positive and 8 negative studies. Treatment that was
## Table 2. Characteristics of 20 Included Studies (Ordered by Length of Time Interval Between Ischemia and Start of Treatment)

<table>
<thead>
<tr>
<th>First Author, Year of Publication</th>
<th>Animal (Treated/Control), n</th>
<th>Method of Ischemia</th>
<th>Method of Administration</th>
<th>Assessment</th>
<th>Result (Methodological Score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartkowski, 1988</td>
<td>Rats (31/23)</td>
<td>MCA occlusion, unknown which method and whether permanent or not</td>
<td>20 μg/kg per h IV for 24 h, started after occlusion (time window unknown)</td>
<td>Neurological outcome and infarct size 24 h after occlusion</td>
<td>Positive (3)</td>
</tr>
<tr>
<td>Meyer, 1986</td>
<td>New Zealand White rabbits (10/10)</td>
<td>Surgical occlusion of MCA, permanent</td>
<td>0.5 μg/kg per min IV for 4 h immediately after occlusion</td>
<td>CBF, intracellular brain pH, and EEG amplitude measurement until 4 h after occlusion</td>
<td>Positive (1)</td>
</tr>
<tr>
<td>Snape, 1993</td>
<td>Lister hooded rats (8/4)</td>
<td>Photochemically induced cortical infarction</td>
<td>0.5 mg/kg IP 5 min after irradiation</td>
<td>Size of infarcted area 24 h after surgery</td>
<td>Negative (3)</td>
</tr>
<tr>
<td>Greenberg, 1990</td>
<td>Cats (8/6)</td>
<td>Microsurgical occlusion of left MCA for 1 h (3-h reperfusion)</td>
<td>5 μg/kg per min for 3 min and 1 μg/kg per min until end of reperfusion, IV started 5 min after occlusion</td>
<td>Infarct size, cytosolic free calcium, amplitude depression on EEG, 4 h after occlusion</td>
<td>Positive (3)</td>
</tr>
<tr>
<td>Lyden, 1988</td>
<td>New Zealand White rabbits (34/18)</td>
<td>Anesthesia, injection of microspheres into CCA (ECA ligated)</td>
<td>5 or 50 μg/kg for 2 min, IV started 5 min after injection of microspheres</td>
<td>Neurological outcome (normal, abnormal, or dead) after 18 h (blinded assessment)</td>
<td>Negative (4)</td>
</tr>
<tr>
<td>Deng, 1997</td>
<td>Wistar rats (10/14)</td>
<td>Occlusion of right MCA (Tamura method), permanent</td>
<td>5 mg/kg SC, started 5 min after occlusion</td>
<td>Neurological deficit, amount of brain edema, 24 h after occlusion</td>
<td>Positive (3)</td>
</tr>
<tr>
<td>Gotoh, 1986</td>
<td>Sprague-Dawley rats (5/5)</td>
<td>Occlusion of MCA (Tamura method), permanent</td>
<td>1 μg/kg per min IV until death, started 5 min after occlusion</td>
<td>Volume ischemic damage, 4 h after occlusion and local CBF (35 min after occlusion)</td>
<td>Negative (3)</td>
</tr>
<tr>
<td>Berger, 1989</td>
<td>Sprague-Dawley rats (7/7)</td>
<td>Surgical occlusion of left MCA, permanent</td>
<td>0.5 μg/kg per min IV for 4 h, started 15 min after occlusion</td>
<td>Infarct volume, local cerebral pH and local CBF, 4 h after occlusion</td>
<td>Negative (2)</td>
</tr>
<tr>
<td>Berger, 1988</td>
<td>Sprague-Dawley rats (5/6)</td>
<td>Surgical occlusion of left MCA, permanent</td>
<td>0.5 μg/kg per min IV for 75 min, started 15 min after occlusion</td>
<td>Infarct volume, local cerebral pH and local CBF, 75 min after occlusion</td>
<td>Positive (2)</td>
</tr>
<tr>
<td>Hakim, 1986</td>
<td>Sprague-Dawley rats (4/8)</td>
<td>Occlusion of left MCA (Tamura method), permanent</td>
<td>0.5 μg/kg per min IV, started 15 min after occlusion</td>
<td>Infarct size, local cerebral pH and local CBF, 3 h after occlusion</td>
<td>Negative (2)</td>
</tr>
<tr>
<td>Sauter, 1986</td>
<td>SHR/KYO rats (6–8/8)</td>
<td>Surgical coagulation of left MCA, permanent</td>
<td>Bolus 0.6 mg/kg per d SC, 15, 105, and 195 min after occlusion</td>
<td>Neurological score (blinded observer), MRI to assess infarct size, biochemical analysis, 3 d after occlusion</td>
<td>Negative (4)</td>
</tr>
<tr>
<td>Dinnaj, 1990</td>
<td>Wistar rats (8/8)</td>
<td>Surgical occlusion of right CCA and MCA, permanent</td>
<td>2 μg/kg per min IV for 60 min, started 30 min after occlusion</td>
<td>Changes in cortical microcirculation</td>
<td>Negative (2)</td>
</tr>
<tr>
<td>Nishikibe, 1988</td>
<td>Mongolian gerbils (17/24)</td>
<td>Surgical occlusion of left CCA, permanent</td>
<td>0.01 or 0.1 mg/kg IP, bolus injection, administered 30 min after occlusion</td>
<td>Regional CBF and MAP in 3 types of ischemic severity</td>
<td>Negative (2)</td>
</tr>
<tr>
<td>Selcuklu, 1993</td>
<td>New Zealand White rabbits (6/6)</td>
<td>Surgical occlusion of ICA, permanent</td>
<td>1 μg/kg per d IV (3 animals treated for 6 h, 3 treated for 24 h), started 1 h after occlusion for both groups</td>
<td>Blinded neurological examination, infarct volume, volume of edema, 7 d after occlusion</td>
<td>Positive (5)</td>
</tr>
<tr>
<td>de la Torre, 1991</td>
<td>Cats (4/4)</td>
<td>Surgical ligation of MCA, permanent</td>
<td>1 μg/kg per min for 60 min IV started 1 h after occlusion</td>
<td>Rate of cytoprotection and CBF, 4 h after occlusion</td>
<td>Negative (2)</td>
</tr>
<tr>
<td>Germano, 1986</td>
<td>Sprague-Dawley rats (5/9)</td>
<td>Microsurgical coagulation of MCA, permanent</td>
<td>20 μg/kg for 10 min, IV started 1 h after occlusion</td>
<td>Neurological evaluation, MRI, and histopathologic assessment of infarct size, 12 h after occlusion</td>
<td>Positive (3)</td>
</tr>
<tr>
<td>Germano, 1987</td>
<td>Sprague-Dawley rats (24/24)</td>
<td>Surgical transection of left MCA, permanent</td>
<td>2 μg/kg per min IV for 10 min, started 1, 2, or 3 h after occlusion (n=8 in each group)</td>
<td>Neurological evaluation (double blind) and infarct size, 24 h after occlusion</td>
<td>Positive (5)</td>
</tr>
<tr>
<td>Kobayashi, 1988</td>
<td>Rats (26/26)</td>
<td>MCA occlusion, unknown which method and whether permanent or not</td>
<td>20 μg/kg IV, 1 or 4 h after occlusion</td>
<td>Neurological outcome and infarct size, 24 h after occlusion</td>
<td>Negative (3)</td>
</tr>
<tr>
<td>Roda, 1995</td>
<td>Long-Evans rats (5/5)</td>
<td>Permanent right MCA occlusion and 90-min occlusion of both CCAs, 24-h reperfusion</td>
<td>40 μg/kg IA as bolus given twice, 90 min and 110 min after occlusion</td>
<td>Infarct size 24 h after occlusion</td>
<td>Positive (2)</td>
</tr>
<tr>
<td>Hara, 1990</td>
<td>Wistar rats (11/15)</td>
<td>Occlusion of left CCA for 3 h (3-h reperfusion)</td>
<td>30 μg/kg and 6 mLAg IV, started 3 h after occlusion, at pump rate 0.2 mL/min for 30 min</td>
<td>Mortality (after 7 d), neuronal damage, and brain edema (6 h after occlusion)</td>
<td>Positive (3)</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; KYO, Kyoto; MCA, middle cerebral artery; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; CBF, cerebral blood flow; and MAP, mean arterial pressure.
started ≥1 hour after the induction of ischemia resulted in 5 positive and 2 negative studies (P = 0.17).

In the 4 studies with a good methodological quality,25,32,36,37 2 showed an effect in favor of nimodipine, and 2 did not.

Discussion
In the present investigation, we focused on whether the evidence from animal experiments was in favor of nimodipine, which would mean that these models were inadequate to predict the treatment response in patients or that the data from animal experiments were inconclusive, which would mean that the clinical trials were based on insufficient evidence. The selected studies did not provide an answer regarding the efficacy of treatment with nimodipine (50% of the studies were in favor of nimodipine). Nor did subgroup analyses on the length of the time window to treatment and the methodological quality of the studies demonstrate beneficial treatment results. Because we had no information that time window to treatment would differ between different animal models, we decided not to speculate and performed the rough time analysis as presented. More detailed subgroup analyses on the effects of different doses of nimodipine or infusion periods could not be performed because of the small number of included studies. In-depth analyses on the effect of nimodipine on infarct size and the amount of edema showed statistically significant beneficial results. These data could be extracted only from a small subsample of publications, which turned out mainly to be reports with positive treatment results. However, this supports the fact that both infarct size and functional outcome should be assessed in focal ischemia.

We found that the methodological quality of the included studies was poor. Issues such as randomization, masked treatment allocation, blinded outcome assessment, and intention to treat analyses, which are very important issues and are now generally required in clinical studies, were especially neglected in these animal experiments.40 Surprisingly, 1 study reported a double-blind assessment of effectiveness.

We realize that systematic reviews carry hazards such as publication bias and a bias for good quality studies.41,42 Evaluating “old” studies from the early 1980s increases these hazards. Because most experiments were performed >10 years ago, we did not approach the authors for detailed information about their studies. We considered it to be unlikely that the authors either possessed or would remember the data required. By limiting our search strategy to the electronic databases and the reference lists of articles, we were unable to estimate the number of unpublished manuscripts. For clinical trials with calcium antagonists in ischemic stroke, we found that at least 18% of all studies remained unpublished.7 In these unpublished trials, results were significantly worse for patients in the treatment group. One might assume that in experimental animal research, this percentage would not be different. If such a publication bias for negative animal experiments exists, this would result in an even more “negative” conclusion of the present review. Other possible sources for bias are selection of studies and data extraction.
The fact that this was done by 1 author might have increased this risk; however, criteria for study inclusion and data extraction were strict. The number of manuscripts written in a “foreign language” (ie, Chinese, Japanese, Russian, or Polish) was limited (2%). We assumed that translating these manuscripts would not result in more useful data.

Was the animal model used suitable to predict the effectiveness of nimodipine in stroke patients? Several authors have discussed the discrepancies between the results of animal models in stroke and the results of clinical studies. A possible explanation for these discrepancies was the difference in the age of the patients. Older stroke patients often have comorbidity, such as diabetes and hypertension. Furthermore, in animal experiments, physiological parameters (blood pressure, [brain] temperature, and blood glucose levels) can be kept within strict limits, and neuroprotective treatment can be started immediately or shortly after the induction of ischemia. Finally, major differences exist in the assessment of the effectiveness of animal experiments measuring histopathologic parameters compared with trials measuring functional ability in stroke patients.

Results of animal experiments with nimodipine in focal cerebral ischemia were reported until 1997, which was 15 years after the first clinical study. It is surprising that these animal experiments ran a course parallel to several clinical studies, because it is reasonable to assume that the clinical studies are preceded by animal studies. However, as early as 1982, the first results of clinical studies on the effect of nimodipine on cerebral blood flow in stroke patients were reported. On the basis of the hypothesis of the calcium-dependent final common pathway in cell death and his previous experiments, an author started a “single-blind” pilot study, which showed that treatment with nimodipine was promising. In 1988, the same author reported the results of a placebo-controlled, double-blind, randomized clinical trial. In the introduction to his article, results of animal experiments with nimodipine were quoted. Only 1 experiment dealt with focal cerebral ischemia; others concerned global ischemia or the use of nimodipine before the induction of ischemia. The authors concluded that nimodipine was statistically significantly better than placebo regarding mortality and changes in neurological deficit (modified Mathew scale). However, repeat analysis of data on the functional outcome of patients in this trial, by dichotomizing the score on the disability item in the modified Mathew scale, shows a difference that was not statistically significant. The supposedly positive results of this first randomized controlled trial were followed by a small trial, which showed similar results. In the introduction to that article, the authors refer to an animal experiment by Germano et al. Subsequent reports on larger clinical trials refer in the introduction to a single positive animal experiment, to the proven effectiveness of nimodipine in the treatment of subarachnoid hemorrhage, and primarily to the positive results of the first randomized controlled trial with nimodipine in ischemic stroke.

We conclude that the results of the animal experiments reviewed in the present investigation did not show convincing empirical evidence to substantiate the decision for trials with nimodipine in stroke patients. In fact, there were no differences between the results of animals experiments and clinical studies. Surprisingly, we found that animal experiments and clinical studies ran simultaneously.

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References


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