Cerebroprotective Effect of a Non-N-Methyl-D-Aspartate Antagonist, GYKI 52466, After Focal Ischemia in the Rat

Stuart E. Smith, MSc, and Brian S. Meldrum, MBChir, PhD

Background and Purpose: Cerebroprotection after the administration of N-methyl-D-aspartate antagonists has been well documented. The present study sought to determine whether a cerebroprotective effect could be achieved with the administration of a non-N-methyl-D-aspartate antagonist, GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride; molecular weight, 330) after middle cerebral artery occlusion in the rat.

Methods: Neurological deficit and infarct volume 24 hours after permanent left middle cerebral artery occlusion in Fischer rats (n=7-13 per group per dose) were studied. Cerebral infarcts was visualized by the lack of reduction of 2,3,5-triphenyltetrazolium chloride.

Results: GYKI 52466 (10 mg • kg\(^{-1}\) i.p. at 0, 2, 4 hours) after middle cerebral artery occlusion had no effect on infarct volume. GYKI 52466 (10 mg • kg\(^{-1}\) i.v. for 5 minutes followed by 15 mg • kg\(^{-1}\) • hr\(^{-1}\) i.v. for 2 hours immediately after middle cerebral artery occlusion reduced cortical infarct volume by 68% (from 69 mm\(^3\) in vehicle-treated to 22 mm\(^3\) in GYKI 52466-treated animals; p<0.05). A 1-hour delay before initiation of drug infusion resulted in a 48% reduction in cortical infarct volume (from 60 mm\(^3\) vehicle-treated rats to 31 mm\(^3\) in GYKI 52466-treated rats; p<0.05). A 2-hour delay before initiation of drug infusion had no effect on cortical infarct volume. Neurological deficits (with blinded assessment after 24 hours) were improved after immediate treatment and after delayed treatment (1 or 2 hours).

Conclusions: The cerebroprotective effect of GYKI 52466 in the rat suggests a possible therapeutic role for non-N-methyl-D-aspartate antagonists given shortly after the onset of stroke.

KEY WORDS • cerebral ischemia • neuroprotection • non-N-methyl-D-aspartate antagonists • rats
Materials and Methods

Adult male Fischer F344 rats weighing 210–310 g were housed in groups of four to six in polyvinylchloride cages (350 mm wide×530 mm long×180 mm high) in an environment maintained at 19–22°C and a relative humidity of 55±3% with a 14-hour/10-hour light/dark cycle (light on from 6 AM to 8 PM). Food and water were available ad libitum. Halothane was used to induce (4% in a mixture of 70% N2O and 30% O2) and maintain (2%) anesthesia. Each rat was allowed to breathe spontaneously, and rectal temperature was maintained at 37°C (36.5–38°C) with a heating blanket. In some experiments, the left femoral vein was cannulated for drug administration and the caudal (tail) artery was cannulated for the continuous monitoring of arterial blood pressure, repeated blood sampling for serial measurements of blood glucose concentration, PaO2, PaCO2, and pH levels. Blood gases were analyzed using an ILL3404 analyzer (Instrumentation Laboratories, Cheshire, UK), and blood glucose was measured with Glucostix strips (Bayer Diagnostics, Ames Division, Slough, UK). Inner ear temperature and temporalis muscle temperature were monitored using thermoprobe.

All animals underwent subtemporal subperiosteal craniectomy (with intact zygoma) and exposure of the main trunk of the left MCA under 25-fold magnification of an operating stereomicroscope.21-22 The exposed artery was electrocauterized from its origin (including the lenticulostriate branch(es)) to its junction with the inferolateral frontoparietal cortex, the caudoputamen, and the globus pallidus. The boundaries between the areas of infarction and the normal adjacent areas were sharply demarcated.

GYKI 52466 (10 mg • kg−1 i.p. at 0, 2, and 4 hours) had no effect on infarct volume compared with that in vehicle-treated control rats (see Table 1). In these groups rectal temperature was maintained between 36.5°C and 38°C. Neurological deficits were not studied.

Infusion (0–2-hour) of GYKI 52466 (10 mg • kg−1 i.v. for 5 minutes followed by 15 mg • kg−1 • hr−1 for 2 hours) immediately after occlusion reduced the total infarct volume by 53% (from 152±51 mm3 in vehicle-treated rats to 71±43 mm3 in GYKI 52466-treated animals; p<0.05). Cortical infarct volume was reduced by 68% (from 69±17 mm3 in vehicle-treated rats to 22±11 mm3 in GYKI 52466-treated animals; p<0.05) (see Figure 1). Neurological deficit was also reduced (see Figure 2; vehicle score, 2.1±0.4; GYKI 52466 score, 1.5±0.7; p<0.05). A significant reduction of 10 mm Hg in mean arterial blood pressure was observed after the loading infusion of GYKI 52466 (10 mg • kg−1 i.v.) 60–65 minutes after the MCA occlusion (p<0.05, Student’s paired t test). A return to baseline mean arterial blood pressure occurred within

<table>
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<th>Results</th>
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<td>There was a consistent pattern of ischemic brain damage after permanent MCA occlusion. Lesions were seen in the dorsolateral frontoparietal cortex, the caudoputamen, and the globus pallidus. The boundaries between the areas of infarction and the normal adjacent areas were sharply demarcated.</td>
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| TABLE 1. Infarct Volumes 24 Hours After Middle Cerebral Artery Occlusion |
|---|---|---|
| | Total | Cortical | Noncortical |
| Vehicle, i.p. | 13 | 126±55 | 67±24 | 74±33 |
| GYKI 52466, i.p. | 7 | 124±29 | 61±19 | 61±18 |
| (10 mg • kg−1 for 0, 2, and 4 hours) |
| Values are mean infarct volumes in cubic millimeters (±SD). |

sections (1 mm thick) were cut from the frontal pole and then incubated for 30 minutes in microwells, each of which contained 2 ml 2% (wt/vol) triphenyltetrazolium chloride in saline at 37°C before storage in 0.1 M phosphate-buffered (pH 7.4) 5% (vol/vol) formaldehyde in saline for 1–5 days. The sections were photographed to scale, and slides were prepared and used to estimate the left and right hemispheric and the total, cortical, and noncortical infarct areas using an IBAS image analyzer (Kontron Elektronik, GmbH, Eching, FRG). These areas were used to calculate the hemispheric and infarct volumes in terms of cubic millimeters by use of a BASIC integration program that is based on the ends of spheres and truncated cones. Results are expressed as mean±SD and were analyzed using Student’s unpaired t test.

Delayed (1-hour) postocclusion infarction (1–3 hours) of GYKI 52466 (10 mg • kg−1 i.v. for 5 minutes followed by 15 mg • kg−1 • hr−1 for 2 hours) reduced cortical infarct volume by 48% (from 60±27 mm3 in vehicle-treated rats to 31±16 mm3 in GYKI 52466–treated animals; p<0.05) (see Figure 1). Neurological deficit was also reduced (see Figure 2; vehicle score, 2.0±0.6; GYKI 52466 score, 1.1±0.6; p<0.05). There were no significant changes in mean arterial blood pressure or rectal temperature (n=8). All other values were within normal ranges.
Delayed (2-hour) postocclusion infusion (2–4 hours) of GYKI 52466 (10 mg • kg\(^{-1}\) i.v. for 5 minutes followed by 15 mg • kg\(^{-1}\) • hr\(^{-1}\) for 2 hours) had no effect on infarct volume compared with vehicle-treated control rats (see Figure 1). However, neurological deficit was reduced (see Figure 2; vehicle score, 2.3±0.5; GYKI 52466 score, 1.5±0.5; \(p<0.05\)). A significant reduction of 10 mm Hg in mean arterial blood pressure was observed after the rapid loading infusion of GYKI 52466 (10 mg • kg\(^{-1}\) i.v.) 120–125 minutes after the MCA occlusion (\(p<0.05\), Student's paired \(t\) test). A return to baseline mean arterial blood pressure occurred within 10 minutes of this effect. All other values were within normal ranges. On recovery from anesthesia the animals displayed no behavioral side effects after the 2-hour infusion of GYKI 52466.

Hemispheric volumes for the infusion studies are included in Table 2 for purpose of review. For all groups rectal and inner ear temperatures were similar, ranging between 37.1°C and 37.7°C and 36.7°C and 36.9°C, respectively. The temporalis temperature was lower, ranging between 29.4°C and 33.2°C.

**Table 2. Hemispheric Volumes 24 Hours After Middle Cerebral Artery Occlusion**

<table>
<thead>
<tr>
<th>Time of infusion</th>
<th>Hemispheric volume (mm(^3))</th>
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<tr>
<td>Immediate i.v.</td>
<td>Left: 435±42, 397±48</td>
</tr>
<tr>
<td>GYKI 52466</td>
<td>Left: 429±32, 412±39</td>
</tr>
<tr>
<td>Delayed (1-hour) i.v.</td>
<td>GYKI 52466</td>
</tr>
<tr>
<td>Delayed (2-hour) i.v.</td>
<td>GYKI 52466</td>
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Values are mean hemispheric volumes in cubic millimeters (±SD). *Significantly smaller than the left hemisphere of vehicle-treated animals (\(p<0.05\)). †Significantly smaller than the left hemisphere of GYKI 52466–treated animals (\(p<0.05\)).

**Discussion**

GYKI 52466 reduces cortical infarct volume in rats when administered within 1 hour and improves neurological deficit when administered 0–2 hours after the ischemic insult. This therapeutic window for the cerebroprotective effect coincides with the peak increase of neurotoxic levels of glutamate and aspartate, which occurs 1 hour after occlusion during focal cerebral ischemia. A similar regimen of GYKI 52466 infusion (30 mg • kg\(^{-1}\) over 3 hours) selectively protects against the excitotoxic effects of kainate (but not NMDA) when focally injected into the hippocampus and reduces cell degeneration in the cortex.
and striatum after four-vessel occlusion in the rat. \textsuperscript{27} GYKI 52466 is a hydrophobic compound and has a fast onset of action and short-lived anticonvulsant effects in mice, rats, and baboons\textsuperscript{28,29}; therefore, these factors necessitate a continuous intravenous infusion of GYKI 52466 after the ischemic insult to observe a cerebroprotective effect. Two very recent reports describe the cortical cerebroprotective effects of another non-NMDA antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), and its administration (30 mg.kg\textsuperscript{-1}, i.v. at 0 and 60 minutes) or 30 mg.kg\textsuperscript{-1}, i.p. at 90, 120, and 180 minutes\textsuperscript{27} after permanent MCA occlusion in the rat. NBQX has a greater binding affinity for the AMPA than for the KA receptor and little or no affinity for the NMDA or the NMDA-glycine site. \textsuperscript{28} Results from binding studies for GYKI 52466 are not yet available. It has, however, been clearly demonstrated electrophysiologically that GYKI 52466 has selective non-NMDA versus NMDA antagonist properties at the doses used in this study. Thus, activation of non-NMDA receptors by glutamate appears to contribute to the infarction process after permanent MCA occlusion in the area of the ischemic penumbra. Blockade of glutamatergic neurotransmission at sites distant from the ischemic focus may also contribute to cerebroprotection. \textsuperscript{2} The GYKI 52466-induced reduction of mean arterial blood pressure by 10 mm Hg (in the group of animals that received delayed administration) is unlikely to have contributed to the cerebroprotective effect. Comparable degrees of cerebroprotection have been observed with competitive NMDA, noncompetitive NMDA, and non-NMDA antagonists after focal cerebral ischemia in rats, \textsuperscript{28,29,22,23,24} Of these classes of excitatory amino acid antagonists, the noncompetitive NMDA antagonists have the lowest therapeutic indexes in experimental animals. There were no adverse behavioral side effects observed after GYKI 52466 administration to rats in the present study. In mice GYKI 52466 and NBQX have therapeutic indexes of 2 and 6.6, respectively (where the therapeutic index is the median effective dose [ED\textsubscript{50}] for ataxia divided by the ED\textsubscript{90} for an anticonvulsant effect). \textsuperscript{19} From the physican's and patient's standpoint, a drug for the therapy of stroke needs to have a therapeutic index as high as possible, thus favoring competitive NMDA over non-NMDA antagonists as potential candidates for clinical trials. The case for a clinical trial of NMDA antagonists in stroke has been clearly stated. \textsuperscript{13} Further studies are required with non-NMDA antagonists before their clinical potential and relative merits compared with NMDA antagonists can be defined.

**Acknowledgment**

We thank Dr. Istvan Tarnawa from the Institute of Drug Research, Budapest, Hungary, for the supply of GYKI 52466.

**References**

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S E Smith and B S Meldrum

Stroke. 1992;23:861-864
doi: 10.1161/01.STR.23.6.861

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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