Delayed Treatment With an Adenosine Kinase Inhibitor, GP683, Attenuates Infarct Size in Rats With Temporary Middle Cerebral Artery Occlusion

Turgut Tatlisumak, MD; Kentaro Takano, MD, PhD; Richard A.D. Carano, PhD; Leonard P. Miller, PhD; Alan C. Foster, PhD; Marc Fisher, MD

Background and Purpose—Brain ischemia is associated with a marked increase in extracellular adenosine levels. This results in activation of cell surface adenosine receptors and some degree of neuroprotection. Adenosine kinase is a key enzyme controlling adenosine metabolism. Inhibition of this enzyme enhances the levels of endogenous brain adenosine already elevated as a result of the ischemic episode. We studied a novel adenosine kinase inhibitor (AKI), GP683, in a rat focal ischemia model.

Methods—Four groups of 10 adult Sprague-Dawley rats were exposed to 90 minutes of temporary middle cerebral artery (MCA) occlusion. Animals were injected intraperitoneally with vehicle, 0.5 mg/kg, 1.0 mg/kg, or 2.0 mg/kg of GP683 30, 150, and 270 minutes after the induction of ischemia by a researcher blinded to treatment group. The animals were euthanatized 24 hours after MCA occlusion, and brains were stained with 2,3,5-triphenyltetrazolium chloride. We measured brain temperatures in a separate group of 6 rats before and after administration of 1.0 mg/kg GP683.

Results—All treated groups showed a reduction in infarct volumes, but a significant effect was observed only in the 1.0 mg/kg–dose group (44% reduction, \( P = 0.0077 \)). Body weight, physiological parameters, neurological scores, and mortality did not differ among the 4 groups. No apparent behavioral side effects were observed. Brain temperatures did not change after drug injection.

Conclusions—Our results indicate that the use of AKIs offers therapeutic potential and may represent a novel approach to the treatment of acute brain ischemia. The therapeutic effect observed was not caused by a decrease in brain temperature. (Stroke. 1998;29:1952-1958.)

Key Words: adenosine ■ cerebral infarction ■ cerebral ischemia, focal ■ neuroprotection ■ temperature ■ rats

Adenosine is a ubiquitous autacoid with diverse physiological effects that include neuroprotection.1–3 Adenosine is formed from the hydrolysis of AMP by the enzyme 5’-nucleotidase and the hydrolysis of S-adenosylhomocysteine (SAH) by the enzyme SAH-hydrolase. In addition, adenosine is metabolized by adenosine kinase through phosphorylation to form 5’-AMP and by adenosine deaminase to form inosine and is released into the extracellular space by an equilibratory transport carrier.4 Enhanced energy requirements and increased ATP consumption in cells lead to a rapid increase in extracellular adenosine levels.5 Adenosine thus accumulates extracellularly to achieve high enough concentrations to activate its specific cell surface receptors and to initiate an array of responses within cells and the vasculature, leading to neuroprotection. The actions of adenosine are mediated through guanine-nucleotide binding protein–coupled cell surface receptors of 4 distinct types (A1, A2a, A2b, and A3).6 In the brain, A1 receptors are predominantly localized in the molecular layer of the cerebellum and in the hippocampus.7 Interestingly, N-methyl-D-aspartate receptors have a similar distribution.8 Moderate A1 receptor levels are found in the thalamus, caudoputamen, septum, and cerebral cortex.7 The A1 receptor distribution in the human brain parallels that seen in small animals.9 A2 receptors are present on smooth muscle and endothelial cells of cerebral vessels.3 A2a sites in humans and rodents are concentrated in the striatum, highly correlating with that of dopaminergic receptors.10 A1 receptors were described recently and shown to exist in several species including humans.6

Ischemia induces a dramatic, yet transient, increase in extracellular adenosine levels in both global and focal models,11–14 mainly caused by the efflux of intracellularly formed adenosine.15 In rats, the concentration of adenosine in the cerebrospinal fluid increased more than fourfold during transient ischemia.16 Results from in vivo and in vitro See Editorial Comment, page 1958.
experiments suggest a beneficial effect of this ischemia-mediated elevation in adenosine.\textsuperscript{17-19} In fact, excessive adenosine release appears to be one of the mechanisms by which the brain attempts to protect itself from cell injury.\textsuperscript{20} Although these observations may suggest a therapeutic potential for adenosine application in ischemic brain injury, its usefulness as a neuroprotective agent is limited by its rapid metabolism (human plasma half-life <5 seconds), cardiovascular side effects, and lack of apparent blood-brain barrier penetration.\textsuperscript{21} Although activation of adenosine receptors can be achieved by using adenosine analogues, another approach is to use adenosine regulating agents (ARAs) to influence the rate of adenosine metabolism or to inhibit adenosine reuptake into cells, thus increasing and prolonging extracellular endogenous adenosine levels. In several experimental studies of brain ischemia, the application of either adenosine analogues or ARAs has already been shown to be beneficial.\textsuperscript{1,3,22,23} Drugs that inhibit adenosine kinase are considered ARAs because of their ability to enhance the effects of endogenous adenosine in an event- and site-specific manner.\textsuperscript{24-26} The present study investigates the merits of adenosine kinase inhibition in brain ischemia with the novel, potent, and selective adenosine kinase inhibitor (AKI), (4-(N-Phenylamino)-5-phenyl-7-(5'-deoxy β-D-ribofuranosyl) pyrrolo[2,3-d]pyrimidine), GP683. Previously, GP683 has been demonstrated to be a potent anticonvulsant acting centrally through a theophylline-reversible mechanism (J.B. Wiesner et al, unpublished data). Presently, we have examined the effects of this AKI not only on cerebral infarct volume but also on brain temperature in a rat focal ischemia model.

Materials and Methods

Animal Preparation
All experiments and surgical procedures were approved by the Animal Research Committee of the University of Massachusetts Medical School (Protocol No. A-643). Forty male Sprague-Dawley rats weighing 290 to 360 g were used. Animals were housed under diurnal lighting conditions and allowed free access to food and water before and after the experiment. Anesthesia was induced by the injection of chloral hydrate (400 mg/kg body wt, IP) and repeated in 100 mg/kg doses as required throughout the surgery. PE-50 polyethylene tubing was inserted into the left femoral artery for continuous monitoring of blood pressure (model 78304A, Hewlett-Packard Inc) throughout the study and for measuring arterial pH, PaO\textsubscript{2} and PaCO\textsubscript{2} (Corning model 170-pH Blood Gas Analyzer, Corning Inc) at baseline and 90 minutes after the induction of focal ischemia. Rectal (core) temperature was continuously monitored with a rectal probe inserted to a 4-cm depth from the anal ring, and the core temperature was maintained at 37°C with a thermostatically controlled heating lamp (model 73ATD, YSI Inc) during the surgery.

Focal Cerebral Ischemia
Focal cerebral ischemia was induced by the suture occlusion model. Briefly, the right common carotid artery and the right external carotid artery were exposed through a ventral midline neck incision and were ligated proximally and permanently. A 4-0 nylon monofilament suture (Ethilon Nylon Suture, ETHICON Inc) with its tip rounded by heating near a flame and then coated with silicone (Bayer Inc) was inserted through an arteriectomy of the common carotid artery approximately 3 mm below the carotid bifurcation and advanced into the internal carotid artery to a point approximately 17 mm distal to the carotid bifurcation. Mild resistance indicated that the suture entered to the anterior cerebral artery, thus occluding the origins of the anterior cerebral artery, the middle cerebral artery (MCA), and the posterior communicating artery. Reperfusion was accomplished by withdrawing the occluder after 90 minutes of ischemia. Blood pressure and body temperature were continuously monitored and recorded at 30-minute intervals.

Drug Characteristics and Application
GP683 was synthesized by a procedure analogous to the one described by Erion et al\textsuperscript{27} for the synthesis of GP3269 except that the 4-fluoroanilino is replaced with an unsubstituted aniline. From pharmacokinetics experiments, a \( t_{1/2} \) of 1.4 hours was determined. In the present study, animals were given 1 of 4 different therapies (\( n=10 \) per group) in a blinded manner: vehicle, 0.5, 1.0, or 2.0 mg/kg GP683, IP, at 30, 150, and 270 minutes after the induction of focal ischemia. Pure (99.9%) dimethyl sulfoxide (DMSO, Sigma Chemical Co and Aldrich Chemical Co) was used as the vehicle to dissolve the drug. The rationale behind the range of GP683 doses selected for this study was based on an established ED\textsubscript{50} of 1.1 mg/kg, IP, in a rat maximal electroshock seizure model.\textsuperscript{28}

Calculation of Infarct Volume
After removal of the femoral catheter and closure of the wounds, the animals were allowed to recover from the anesthesia in separate cages. Twenty-four hours after MCA occlusion, the animals were examined neurologically using a 6-point scale (0=no deficit, 1=tail, 2=circling to the left, 3=falling to the left, 4=no spontaneous walking with a depressed level of consciousness, 5=dead) modified from that previously described by Zea Longa et al.\textsuperscript{30} The animals were then reanesthetized with chloral hydrate and killed. The brains were quickly removed and coronally sectioned into six 2-mm-thick slices. The brain slices were incubated for 30 minutes in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) at 37°C and fixed by immersion in a 10% buffered formalin solution. The unstained area was defined as infarcted tissue. Brain sections were photographed with a charge-coupled device camera (EDC-1000HR Computer Camera, ELECTRIM Corp), and images were stored on a microcomputer. Later, by use of an image analysis program (Bio Scan OPTIMAS), the areas of the infarcted tissue and the areas of both hemispheres were calculated for each brain slice. The uncorrected infarct volume was calculated by measuring the unstained area in each slice, multiplying it by slice thickness, and then summing all 6 slices. The infarcted areas in the caudoputamen and subcortex were traced manually on the images and calculated. Cortical infarct volume was calculated by subtracting the subcortical infarct volume from the uncorrected infarct volume. The corrected infarct volume was calculated to compensate for the effect of cerebral edema. The difference between the areas of the right and the left hemisphere in a slice was considered to be edema and subtracted from the infarct area of that slice (corrected infarct volume equals uncorrected infarct volume minus right hemisphere’s volume minus left hemisphere’s volume). The result was multiplied by slice thickness and all 6 slices were summed to find the total corrected infarct volume.

Measurement of Brain Temperature
In a separate experiment, regional brain temperature of 6 additional animals weighing 310 to 370 g was measured before and after treatment with GP683. These animals were housed, fed, and anesthetized similarly to those in the therapy experiment. Rectal (core) temperature was maintained at 37°C using the same heating lamp system. In these rats, the frontoparietal cranium was exposed by a midsagittal incision and 2 burr holes of 1.3 mm in diameter each were drilled on the right parietal skull at 0.5-mm caudal and 3.5-mm and 5.0-mm lateral to the bregma. With a 23-gauge needle as a guide, small copper constantan thermocouples, 0.03 inch (0.76 mm) in diameter (type IT-23, Physitemp Instruments Inc) were inserted into the right caudoputamen and lateral portion of the frontoparietal...
cortex, 5.0-mm ventral to the bregma, and fixed to the frontoparietal bone using dental cement (Durelon, Espe GmbH and Co. KG). Rectal (core), cortical, and caudoputaminal temperatures were continuously monitored and recorded every 15 minutes. After 1 hour of recording baseline values, 1.0 mg/kg GP683 was injected intraperitoneally. The temperatures of the 2 brain regions were recorded for 2 hours after drug injection, and animals were killed immediately thereafter. The precise locations of the thermocouples in the caudoputamen and the frontoparietal cortex of each animal were confirmed by visual inspection of coronally cut slices. The thermocouples used in this study were calibrated against a mercury thermometer in a water bath before the experiment.

Statistical Analysis

Values are presented as mean±SD. A 1-factor ANOVA and post hoc Scheffé’s test were used for statistical analyses. Neurological scores were evaluated using Kruskal-Wallis H test (corrected). Brain temperature measurements and the physiological parameters were evaluated using repeated-factor ANOVA. A 2-tailed P<0.05 was considered significant.

Results

There were no significant differences in body weight, rectal temperature, mean arterial blood pressure, arterial blood pH, PaCO2, and PaO2 among the 4 groups (Table 1). In the 2.0 mg/kg–dose group, blood pressure in 1 animal dropped 30 mm Hg within 1 minute after the first injection of GP683, but recovered to previous levels in approximately 3 minutes. After recovery from anesthesia, no animal showed any apparent abnormal behavior. Moreover, the neurological score at 24 hours did not differ among the 4 groups (Table 2). Three animals treated with GP683 at 2.0 mg/kg and 1 animal from each of the other 3 groups died prematurely. The overrepresentation of premature deaths in the 2.0 mg/kg–dose group was not statistically significant compared with the other groups. All 6 deaths occurred between 20 and 24 hours after induction of focal ischemia. These animals were assigned a score of 5 on the neurological assessment scale and underwent immediate craniectomy (within 1 hour after death) and TTC staining for determination of infarct volume.

The total (corrected and uncorrected) and regional (cortical, subcortical, and caudoputaminal) infarct volumes for control and GP683-treated groups are presented in Table 2. Both corrected and uncorrected infarct volumes in the 3 treated groups were smaller than those of the control group, and a significant reduction (44.1% and 34.5%, respectively) was observed only in the 1.0 mg/kg GP683–treated group (P<0.0077 and P=0.02, respectively). In a regional analysis of the data, cortical infarct volume was significantly smaller in the animals treated with 1.0 mg/kg GP683 than those in the control group (38.5% reduction, P=0.018, by post hoc Scheffé’s test). The subcortical and caudate-putaminal infarct volumes were not significantly different from control animals at any of the 3 doses of GP683 examined. When we reanalyzed the corrected infarct volumes after excluding the animals that died prematurely, infarct volumes were 185.6±40.6 mm3 for the control group (n=9), 144.4±34.8 mm3 for the 2.0 mg/kg–dose group (n=7), 98.4±44.4 mm3 for the 1.0 mg/kg–dose group (n=9), and 153.6±50.8 mm3 for the 0.5 mg/kg–dose group (n=9). The overall result of the study remained unchanged (P=0.0022 by single-factor ANOVA; P=0.0025 for the 1.0 mg/kg–dose group and not significant for other groups by post hoc Scheffé’s test).

The temperature study revealed no difference in the rectal, cortical, or caudate-putaminal temperatures over time in rats treated with 1.0 mg/kg GP683, IP (n=6, P>0.05 for all measurements, Table 3). This dose was chosen to assess...
TABLE 2. TTC-Derived Infarct Volumes and Neurological Scores

<table>
<thead>
<tr>
<th>Group</th>
<th>Corrected infarct volume</th>
<th>Uncorrected infarct volume</th>
<th>Cortex</th>
<th>Subcortex</th>
<th>Neurological scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group (n=10)</td>
<td>198.9±5.70</td>
<td>277.7±59.8</td>
<td>223.9±52.7</td>
<td>53.8±11.6</td>
<td>36.6±3.2</td>
</tr>
<tr>
<td>2.0 mg/kg-Dose Group (n=10)</td>
<td>164.9±49.7</td>
<td>248.9±69.9</td>
<td>200.5±62.8</td>
<td>48.5±17.6</td>
<td>32.3±7.2</td>
</tr>
<tr>
<td>1.0 mg/kg-Dose Group (n=10)</td>
<td>111.1±57.9</td>
<td>181.9±79.6</td>
<td>137.8±70.7</td>
<td>44.1±11.3</td>
<td>32.1±7.3</td>
</tr>
<tr>
<td>0.5 mg/kg-Dose Group (n=10)</td>
<td>152.9±48.0</td>
<td>246.1±56.4</td>
<td>194.4±46.7</td>
<td>51.7±13.2</td>
<td>34.5±6.7</td>
</tr>
</tbody>
</table>

Values are mean±SD. TTC indicates 2,3,5-triphenyltetrazolium chloride. Values are % reduction vs. control.

TABLE 3. Core (Rectal) and Regional Brain Temperatures Before and After Injection of GP683 (n=6)

<table>
<thead>
<tr>
<th>Time</th>
<th>Core, °C</th>
<th>Cortex, °C</th>
<th>Caudate Putamen, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>−60 min</td>
<td>36.9±0.2</td>
<td>35.9±0.7</td>
<td>36.3±1.2</td>
</tr>
<tr>
<td>−30 min</td>
<td>37.0±0.2</td>
<td>35.4±1.8</td>
<td>36.6±0.5</td>
</tr>
<tr>
<td>Drug injection</td>
<td>37.1±0.3</td>
<td>35.8±1.5</td>
<td>37.0±0.2</td>
</tr>
<tr>
<td>30 min after</td>
<td>36.9±0.1</td>
<td>36.3±1.2</td>
<td>37.0±0.3</td>
</tr>
<tr>
<td>60 min after</td>
<td>36.9±0.1</td>
<td>36.3±0.8</td>
<td>37.1±0.5</td>
</tr>
<tr>
<td>90 min after</td>
<td>36.9±0.1</td>
<td>36.3±0.8</td>
<td>37.1±0.6</td>
</tr>
<tr>
<td>120 min after</td>
<td>37.0±0.2</td>
<td>36.4±0.8</td>
<td>36.8±0.5</td>
</tr>
</tbody>
</table>

Values are mean±SD. The drug was injected intraperitoneally at 1.0 mg/kg dose to rats (n=6) without focal ischemia.

Discussion

This study demonstrates that the AKI, GP683, was effective in attenuating infarct volume when started 30 minutes after temporary MCA occlusion in rats. We obtained significant efficacy only with the 1.0 mg/kg dose. At this dose, there was no alteration in the physiological observations and core or regional brain temperature. The infarct volumes in the 2.0 mg/kg– and 0.5 mg/kg–dose groups were not different from the control group. However, in all treated groups, we observed a tendency toward smaller infarct volumes. We do not have a precise explanation for this “U-type” biphasic response. The lowest dose we used (0.5 mg/kg) probably was insufficient to evoke a maximum anti-ischemic response. Regarding the highest dose we used (2.0 mg/kg), it is possible that the treatment effect could decrease as the AKI concentration is increased from 1.0 to 2.0 mg/kg. This could be the result of a number of different but convergent events: higher levels of adenosine now acting at A2 receptors to offset the protection afforded by selective A1 receptor activation at lower adenosine levels, a “steal” effect resulting from vasodilatation in nonischemic regions, or a nonspecific but as yet unidentified effect of the drug itself at the higher dosing level. It should be noted that in the high dose (2.0 mg/kg) group, mortality was higher than in other groups (3 versus 1 in each group). Even though this increase in mortality was statistically insignificant, it requires further evaluation for possible toxic effects that may counterbalance the anti-ischemic effects of the drug. Reliability of TTC staining at 6 hours after focal ischemia has been demonstrated previously. Furthermore, Li et al have shown recently that TTC staining is reliable when the brain is harvested even 8 hours after death at room temperature. We analyzed our results including the animals that died prematurely because these animals usually have larger infarct volumes, and excluding these animals would have introduced a bias to the results of the study. Furthermore, a possible toxic effect of the drug, including an increase in the mortality rate, would have remained unreported.

There are a number of responses following adenosine receptor activation that underlie the neuroprotection seen in different animal models. Activation of the adenosine A1 receptors presynaptically attenuates excitatory amino acid (EAA) release and postsynaptically enhances the potassium and chlorine conductance in neurons, leading to membrane hyperpolarization and postsynaptic reduction of neuronal calcium influx, hyperpolarizes astrocyte cell membranes and improves the uptake of excessive extracellular potassium and glutamate by the astrocytes, and attenuates the basal and the N-methyl-D-aspartate–induced production of nitric oxide. Antagonism of adenosine’s actions resulted in an augmentation of EAAs intermittently in the brain. Cyclopenyladenosine, a selective A1 receptor agonist, attenuated traumatic cell death in rat hippocampal cell cultures free of glutamate, suggesting that adenosine can reduce neuronal injury via mechanisms other than the inhibition of EAA-induced toxicity. A2 receptor activation inhibits platelet aggregation, thus reducing the potential for vessel obstruction, inhibits neutrophil-mediated injury to endothelial cells.
by preventing the adherence of stimulated neutrophils to endothelial cells, attenuates the release of free radicals from neutrophils, and increases cerebral blood flow (CBF) by inducing smooth muscle relaxation on the microvasculature, leading to vasodilatation and thus improving the delivery of oxygen and nutrients to the brain regions at risk. In this regard, administration of acadesine, an ARA, significantly inhibited platelet aggregation in healthy men, reduced the frequency of recurrent platelet plugging in dogs with induced unstable angina pectoris, and reduced platelet deposition in ischemic regions in rats in a photodynamic stroke model. Muhonen et al. showed that in dogs with permanent occlusion of a branch of the MCA, topical application of adenosine had little effect on blood flow to collateral-dependent tissue, whereas topical application of 2-chloroadenosine increased blood flow to outer layers of collateral-dependent and normal cerebrum. Their findings suggest that the responses of the cerebral vasculature to adenosine and adenosine analogues may be different under various conditions such as during ischemia. Neuroprotective effects of adenosine agonists were also observed in vitro, suggesting that adenosine can influence brain cell metabolism independently of changes in CBF. Recently, A1 receptor stimulation without A2 or A3 receptor involvement was found to be beneficial in brain ischemia, but the mechanisms underlying this effect need further study. Adenosine agonists can induce hypothermia and might achieve neuroprotection in part by lowering brain temperature sufficient enough to attenuate EAA release. Currently, no data are available regarding an effect of AKIs on spreading depressions. Recently, adenosine was shown to reduce L-aspartate transport across the blood-brain barrier. It is not yet known whether this effect has a role in the neuroprotective properties of adenosine. Any effect of GP683 on spreading depressions has not been studied. Most adenosine agonists cause vasodilatation and increase CBF, a potential mechanism for neuroprotection. A vasodilator effect and an improvement of CBF as the mechanism of therapeutic effect after injection of GP683 is possible and needs to be studied further.

Presently, there are only a few reports on the application of AKIs in vivo. The present results compare favorably with a recent study investigating the application of another adenosine kinase inhibitor, 5'-deoxyiodotubercidin (5'dITU). In that study, the administration of 5'dITU (0.33 mg/kg, IV) at 30 minutes after the induction of ischemia also in a reversible rat stroke model resulted in a significant (32%) reduction in infarct volume. The potent AKI 5'-iodotubercidin failed to protect against cerebral ischemic injury in gerbils in a temporary bilateral carotid artery occlusion model, even though 5'-iodotubercidin has been shown by other investigators to lead to a fourfold increase of adenosine release rates in various experimental conditions. Inhibition of adenosine kinase was found to have a profound effect on adenosine release from rat hippocampal slices under several circumstances such as basal conditions, electric field stimulation, or energy depletion. Inhibition of adenosine kinase increased endogenous adenosine and depressed neuronal activity in hippocampal slices. Iodotubercidin increased CBF more than the adenosine deaminase inhibitor, erythro-9-[2-hydroxy-3-nonyl]adenine (EHNA), whereas their combination was more effective. A novel adenosine kinase inhibitor GP515 showed not only antiinflammatory effects that were mediated by adenosine but also inhibition of neutrophil adhesion to endothelial cells in rats.

The neuroprotective effect of AKIs is because of their ability to further elevate the already elevated brain adenosine levels during ischemia. EHNA increased extracellular adenosine levels in young rats’ striatum only, whereas 5'-iodotubercidin increased the extracellular adenosine levels in both young and old rats’ striatum, suggesting that the inhibition of adenosine kinase may be more effective than the inhibition of adenosine deaminase at potentiating endogenous adenosine levels. Fredholm and Lloyd originally found that inhibition of adenosine kinase elicited a large increase in adenosine release in rat hippocampal slices. Studies with rat cortical slices showed that AK inhibition with 5'-iodotubercidin resulted in an increase in basal adenosine release. However, a different profile was observed when adenosine release was induced by treatment with EAA receptor agonists. Under these conditions, adenosine release in the presence of AK inhibition was increased 3- to 7-fold over basal levels compared with only a 2- to 2.5-fold increase observed in the presence of adenosine deaminase inhibition. In 1 study, microdialysis probes were implanted in rat caudate nucleus along with concurrent measurements of CBF under basal conditions. AK inhibition with iodotubercidin increased regional brain adenosine levels by 170% and CBF by 140%, whereas adenosine deaminase inhibition by EHNA increased adenosine levels by 58% and CBF 27% only. Inhibition of adenosine kinase and adenosine deaminase increased brain adenosine levels. Even though we do not have data on the effects of GP683 on brain adenosine levels under different experimental conditions (including focal brain ischemia), data from experiments with other AKIs suggest that inhibition of the enzyme adenosine kinase is associated with a robust increase in extracellular brain adenosine concentrations in vivo.

Brain temperature falls gradually by 5°C to 6°C during ischemia, and low brain temperatures lead to protection from ischemic injury. The release of EAAs into the brain’s extracellular space was almost totally suppressed when intra-ischemic brain temperature was lowered from 36°C to 33°C. Because the degree of brain ischemic injury is dependent on intra-ischemic brain temperatures, failure to control or monitor brain temperature will introduce a bias in experimental brain ischemia studies. Previously, cyclohexyladenosine, an A1 receptor agonist, was shown to be neuroprotective, at least in part, by its hypothermic effect in gerbils in a global ischemia model. Our temperature study demonstrated that at its most effective dose GP683 does not induce a decrease of brain temperature when the core temperature is kept constant at 37°C, suggesting that the anti-ischemic effects of GP683 are mediated by mechanisms other than brain hypothermia (Table 3).

In the present study, the neuroprotective effect of the AKI GP683 was evident, with a 44.1% reduction of the corrected cerebral infarct volume at the 1.0 mg/kg dose. Furthermore, we did not observe behavioral abnormalities or changes in...
physiological parameters or brain temperature. Adenosine kinase inhibition represents a novel approach to the treatment of focal brain ischemia.

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References

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Editorial Comment

Adenosine is a potent endogenous modulator of neural transmission. Adenosine suppresses the release of excitatory neurotransmitters including glutamate, blocks selected calcium channels, and inhibits neutrophil activation. These receptor-mediated actions of adenosine are all considered salutary in the setting of cerebral ischemia-reperfusion. An increase in interstitial adenosine level in response to cerebral ischemia is probably a defense mechanism of the brain. Therapeutic strategies aiming to enhance the adenosine receptor mechanism have been explored to protect the brain from ischemic insult. Selected adenosine receptor agonists are among these putative therapeutic agents. However, the therapeutic effects of adenosine agonists are compounded by their potent systemic effects, especially their cardio depressant actions. Adenosine agonists may reduce cardiac output and cause hypotension. These adenosine effects are not desirable in the setting of acute cerebral ischemia. A more plausible approach is pharmacological modulation of adenosine metabolic pathways to reduce the rapid conversion to other purine metabolites. Adenosine kinase catalyzes adenosine phosphorylation to form 5′-AMP. An adenosine kinase inhibitor is thus expected to increase adenosine levels by preventing its catabolism and may be effective in enhancing the adenosine receptor mechanism for neuroprotection. Since the effect of enzyme inhibition on adenosine levels tends to be more substantial in the ischemic region with excessive formation of adenosine, adenosine kinase inhibitors may be site selective and less likely to cause systemic side effects.

In the preceding article by Tatlisumak et al, GP683, an adenosine kinase inhibitor, was noted to be effective in reducing the infarction volumes in a suture model of focal cerebral ischemia in rats. An important observation is that GP683 was effective given 30 minutes after induction of ischemia. The very narrow effective dose range in a bell-shaped dose-response curve is suggestive of probable adverse side effects of the largest dose (2 mg/kg) used in this study. There was a trend toward lower mean arterial pressure in this dose group. Excessive adenosine accumulation may also lead to diffuse cerebral vasodilatation, which may not be beneficial to the ischemic region. It would be interesting if, in future studies, the authors can systematically determine regional cerebral blood flow and measure interstitial adenosine levels in the ischemic and nonischemic regions to further assess the pharmacological actions of various doses of GP683. These additional studies may be helpful in maximizing the neuroprotective action of adenosine, which can be conferred through a selective inhibition of adenosine kinase.

Chung Y. Hsu, MD, PhD
Weili Lin, PhD
Guest Editors
Department of Neurology and Mallinckrodt Institute of Radiology
Washington University School of Medicine
St Louis, Missouri
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