A Novel Endothelin Antagonist, A-127722, Attenuates Ischemic Lesion Size in Rats With Temporary Middle Cerebral Artery Occlusion

A Diffusion and Perfusion MRI Study

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Background and Purpose—Endothelins (ETs) are potent vasoconstrictors. Plasma ET levels increase during acute brain ischemia and may worsen the ischemic damage. Diffusion-weighted MRI (DWI) and perfusion imaging (PI) are powerful tools for evaluation of acute cerebral ischemia. We studied the effects of A-127722, a novel ET_A-selective ET antagonist, on cerebral ischemic lesion size using 2,3,5-triphenyltetrazolium chloride (TTC) staining postmortem, on acute ischemic lesion development with DWI, and on the cerebral circulation using PI.

Methods—Twenty male Sprague-Dawley rats received either 5 mg/kg of A-127722 or vehicle (n=10 per group) intravenously 30 minutes and subcutaneously 4 hours after middle cerebral artery occlusion (MCAO). Whole-brain DWI and single-slice PI were done before initiation of treatment and repeated frequently thereafter up to 4 hours after MCAO. The animals were reperfused in the MRI scanner 90 minutes after the onset of MCAO. At 24 hours the animals were killed, and the brains were cut into six 2-mm-thick slices and stained with 2% TTC. Percent hemispheric lesion volume (%HLV) was calculated for each animal.

Results—Physiological parameters, body weight, neurological scores, and premature mortality (2 versus 2) did not differ between the two groups. No hypotension, abnormal behavior, or other adverse effects were seen. TTC-derived %HLV was 25.3±5.6% for controls and 16.2±9.6% for treated animals (36% reduction, P<.02). Six animals in each group had successful reperfusion as shown by PI. Among these animals, %HLV was 23.2±3.1% for controls and 9.3±4.4% for treated animals (60% reduction, P=.0001). The beneficial effect of A-127722 was limited to animals in which successful reperfusion was demonstrated. No difference in PI-detected perfusion deficit size was observed between the groups. DWI did not demonstrate significant in vivo lesion size differences.

Conclusions—A-127722 significantly reduced ischemic lesion size in rats without observable adverse effects. It is not clear whether the effect was due to vasodilatation of collateral arterioles not detectable by PI or whether A-127722 has neuroprotective properties that are independent of vascular effects. (Stroke. 1998;29:850-858.)

Key Words: cerebral ischemia • endothelins • magnetic resonance imaging • middle cerebral artery • rats

Endothelins are potent vasoconstrictors present in several species, including humans. The ET family consists of three isoforms: endothelin-1, endothelin-2, and endothelin-3 (ET-1, ET-2, and ET-3, respectively). ETs act through specific receptors with at least two distinct types. The ET_A receptor binds ET-1 preferentially, whereas the ET_B receptor does not have a preference among the three ET subtypes. ET_A receptors mediate vasoconstriction, and ET_B receptors usually mediate vasodilatation. ETs participate in the pathophysiology of a number of diseases, mainly as a result of potent vasoconstrictor effects. ET levels are elevated in plasma and cerebrospinal fluid of acute ischemic stroke patients. Increases in ET levels occur in various animal models of global and focal ischemia. Several ET antagonists are beneficial in a number of animal models of disease, including myocardial infarction, cerebral vasospasm induced by subarachnoid bleeding, and renal failure.

DWI and PI are novel imaging technologies that are sensitive for the early detection of focal brain ischemia. DWI is based on the random translational movement of water...
molecules in biological media. Ischemia causes a rapid decrease in water diffusion, and ischemic regions appear hyperintense on DWI only minutes after the induction of focal cerebral ischemia, whereas conventional MRI methods do not disclose any changes during the initial several hours after ischemia. The brain’s microcirculation (cerebral perfusion) can be evaluated by PI. PI is useful in evaluating acute stroke patients. With PI, it is possible to estimate the CBF index.

A-127722 is a novel, nonpeptide, ET\textsubscript{A} receptor–selective, competitive, and orally bioavailable ET antagonist. The characteristics of A-127722 were previously described in detail. Vasoconstrictor ET\textsubscript{A} and vasodilator ET\textsubscript{B} receptors are detectable in cerebral arteries and arterioles. In cerebrovascular pathophysiology, the targeting of constrictor ET\textsubscript{A} receptors with ET\textsubscript{A} receptor–selective antagonists may be beneficial rather than using combined ET\textsubscript{A}/ET\textsubscript{B} receptor antagonists. Since there is considerable evidence that ETs may play a role in the pathogenesis of cerebral ischemia, this study was designed to evaluate the effect of delayed application of A-127722 on focal cerebral ischemia in vivo with the use of DWI and PI and postmortem with the use of TTC staining in rats undergoing temporary MCAO.

### Materials and Methods

#### Animal Preparation

This study was approved by the Animal Research Committee of the University of Massachusetts Medical School (protocol No. A-643). Twenty male Sprague-Dawley rats weighing 300 to 330 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 intraperitoneal injection of chloral hydrate (400 mg/kg body wt). PE-50 polyethylene tubing was inserted into the left femoral artery for continuous monitoring of arterial blood pressure (78205D, Hewlett-Packard Inc) throughout the experiment and for measuring arterial pH, PaO\textsubscript{2}, and PaCO\textsubscript{2} (Corning).

**Experimental Focal Brain Ischemia**

Focal brain ischemia was induced by the intraluminal monofilament model of MCAO. 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 (Ethilon Nylon Suture, ETHICON Inc), with its tip rounded by heating near a flame and then coated with silicon (Bayer Inc), was inserted through an arteriotomy 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, and the posterior communicating artery. The animals were then placed in a 1-H home-built birdcage coil and were quickly placed into the bore of the magnet. In the MRI device, anesthesia was maintained with 1.0% of isoflurane delivered in air at 1.0 L/min. During the MRI measurements, body temperature was continuously monitored with a rectal probe with 0.1°C resolution (T type thermocouple, OMEGA Engineering Inc) and was maintained at 37.0°C with a thermostatically regulated heated air-flow system. Mean arterial blood pressure was continuously monitored and recorded every 30 minutes during the MRI protocol, and arterial blood gas samples were obtained through the left femoral arterial catheter at 60 and 150 minutes after MCAO while the animals were in the scanner. The animals were reperfused in the magnet mechanically by pulling the monofilament occluder approximately 10 mm caudally 90 minutes after MCAO.

#### MRI Measurements

The MRI studies were performed with a General Electric CSI-II 2.0-T/45-cm imaging spectrometer (General Electric Medical System) operating at 85.56 MHz for \textsuperscript{1}H equipped with ±20 G cm\textsuperscript{-1} self-shielding gradients (15-cm bore). \textsuperscript{1}H-weighted echo-planar imaging was used to perform dynamic contrast-enhanced PI. A coronal slice at the optic chiasm was acquired with a thickness of 2 mm (field of view, 25.6×25.6 mm; 64×64 pixel resolution). A total of 40 images (repetition time, 900 milliseconds; echo time, 38 milliseconds; data acquisition time, 65 milliseconds; number of excitations, 1) were obtained. A bolus injection of 0.15 mL of gadopentetate dimeglumine (Magnevist, Berlex Laboratory) was injected after acquisition of the seventh image. PI was done at 20, 70, 100, and 210 minutes after MCAO. The PI was used to determine whether A-127722 caused an increase in CBF in the ischemic territory. The perfusion data were processed to obtain an estimate of the CBF, as previously described. The CBF index was determined on a pixel-by-pixel basis from relative cerebral blood volume and mean transit time as CBF Index = Relative Cerebral Blood Volume/ Mean Transit Time. CBF index was chosen because it incorporates the information found in both the relative cerebral blood volume and the mean transit time. Abnormal perfusion was defined as CBF that fell 2 SDs below the mean of the contralateral hemisphere. The number of pixels with abnormal perfusion in the ischemic territory was calculated for each imaging time point and divided by the total number of pixels of the same slice of the right hemisphere, giving a percent hemispheric lesion area (%HLA= Number of Abnormal Pixels/Number of All Pixels in the Right Hemisphere in the Single Slice at Optic Chiasm).

The rate of diffusion of water was measured in vivo for each pixel, with the use of pulsed field gradient nuclear MR. The ADC is defined as:

$$ADC = -ln[M(k,\tau)/M_0](\gamma k^2 \tau^{-1})$$

where $k$ is the wave vector given by the time integral of the diffusion sensitizing gradient, $\tau$ is the observation time, and $M_0$ is the equilibrium magnetization at $k=0$. The ADC maps were obtained from an eight-slice diffusion-weighted echo-planar imaging pulse sequence. Half-sine shaped diffusion gradients were applied along the anterior-posterior (z) axis of the brain, with $k = yg(2\pi/\gamma)$ and $\gamma=\Delta/\delta$ (where $g$ is the gyromagnetic ratio and $g, \Delta$, and $\delta$ are the strength, separation, and duration, respectively, of the applied diffusion gradients). All data were acquired with $\delta$ of 10 milliseconds, $\Delta$ of 40 milliseconds, repetition time of 4 seconds, acquisition time of 65 milliseconds, and echo time of 92 milliseconds. The image size was 64×64 pixels with a pixel resolution of 400 $\mu$m.
(in-plane) and a slice thickness of 2.0 mm (axial plane). Eight contiguous slices encompassing the whole brain were acquired. The ADC maps were generated with the use of 10 b-values (k²) ranging from 63 to 1898 s/mm². ADC maps were obtained at 20, 30, 60, 83, 120, 150, 180, 210, and 240 minutes after MCAO. The ADC value for each pixel was calculated by performing linear regression to obtain the parameters of the ADC definition equation. The threshold value to define abnormal ADC values on ADC maps was evaluated as follows: to define abnormal diffusion values of water in the brain, we compared each pixel in the ischemic hemisphere with its homologous pixel in the normal hemisphere. As previously described, the side-by-side difference of ADC values (ΔADC) from homologous pixels, i.e., the ischemic and normal hemispheres that best define the ischemic lesion volume in vivo 2 hours or longer after MCAO is 29%, highly correlating with postmortem infarct volume. It is over 1000-fold selective for the ET A receptor over the ET B receptor (ET B

**Drug Characteristics**

A-127722 is the most potent ET antagonist yet described. It competitively inhibits ET-1 binding to human ET A receptors with a

\[ K_i \text{ of } 69 \text{ pmol/L}. \]

It is over 1000-fold selective for the ET A receptor over the ET B receptor (ET B

\[ K_i \text{ of } 139 \text{ nmol/L}. \]

In vitro, A-127722 exhibits a pA₂ of 10.5 for inhibition of ET-1–induced arachidonic acid release from human pericardium smooth muscle cells and a pA₂ of 9.2 for inhibition of ET-1–induced constriction of rat aortic rings. In vivo, at 10 mg/kg peroral, A-127722 maximally inhibits the pressor response to an intravenous bolus of ET-1 (0.3/kg), and this effect is still pronounced 24 hours after dosing. The plasma elimination half-life in the rat is 3.5 hours.

**Calculation of Ischemic Lesion Volume**

After the MRI protocol, the animals were removed from the magnet bore, the reperfusion was confirmed by the inspection of the position of the suture occluder, both catheters were removed, operation wounds were sutured, and animals were allowed free recovery from anesthesia in separate cages. Twenty-four hours after MCAO, the animals were scored neurologically according to a 6-point scale (0 = no deficit, 1 = failure to extend left forepaw fully, 2 = circling to the left, 3 = falling to the left, 4 = no spontaneous walking with a depressed level of consciousness, 5 = dead) modified from the original proposal by Zea Longa et al. The animals were then anesthetized 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 TTC at 37°C and fixed by immersion in a 10% buffered formalin solution. The unstained area was defined as ischemic lesion. 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 %HLV was calculated by the following equation, giving a correction for edema: %HLV = [(Total Lesion Volume) / (Right Hemisphere Volume – Left Hemisphere Volume)] × 100.

**Statistical Analyses**

Data are expressed as mean ± SD. Statistical analyses were performed with the unpaired t test or two-factor, repeated-measures ANOVA for continuous variables and the Mann-Whitney U test for nonparametric variables. A two-tailed value of P < .05 was considered significant.

**Results**

There were no significant differences in body weight, rectal (core) temperature, mean arterial blood pressure, blood pH, PaCO₂, or PaO₂, between the two groups (Table 1). We did not observe hypotension, abnormal behavior, or any other adverse effects. The neurological score at 24 hours after MCAO was 2.7 ± 1.3 in the controls and 3.1 ± 1.4 in the treated animals (P = .53). No animal had subarachnoid hemorrhage at postmortem. Two animals from each group died prematurely (8, 18, 21, and 24 hours after MCAO) and were graded 5 on the neurological scale. These animals underwent immediate craniectomy and TTC staining. All 4 animals that died prematurely had well-demarcated lesions compatible in size and shape with those seen 24 hours after focal ischemia. Reliability of TTC staining at 6 hours after focal ischemia has been demonstrated previously. The TTC-derived %HLV was 25.3 ± 5.6% (mean ± SD) in the control group and 16.2 ± 9.6% in the treated group (P < .02, 36% reduction). When the 4 animals that died prematurely were excluded from the statistical analyses, the %HLV was 23.2 ± 3.5% in the control group and 14.0 ± 9.6% in the treated group (n = 8 in each group, P = .025, 40% reduction).
TABLE 2. Results of MR Perfusion Imaging

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=10)</th>
<th>A-127722 (n=10)</th>
</tr>
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<tbody>
<tr>
<td><strong>All animals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-min PI</td>
<td>36.6±26.2</td>
<td>36.5±13.3</td>
</tr>
<tr>
<td>70-min PI</td>
<td>39.1±16.6</td>
<td>33.3±12.0</td>
</tr>
<tr>
<td>100-min PI</td>
<td>14.4±19.9</td>
<td>20.1±28.33</td>
</tr>
<tr>
<td>210-min PI</td>
<td>18.4±14.4</td>
<td>25.3±24.6</td>
</tr>
<tr>
<td><strong>Successfully reprefused animals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-min PI</td>
<td>27.5±14.0</td>
<td>31.6±8.5</td>
</tr>
<tr>
<td>70-min PI</td>
<td>27.8±9.3</td>
<td>30.5±11.6</td>
</tr>
<tr>
<td>100-min PI</td>
<td>6.7±11.9</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>210-min PI</td>
<td>7.3±2.8</td>
<td>6.7±3.6</td>
</tr>
<tr>
<td><strong>Nonreperfused animals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-min PI</td>
<td>50.1±21.1</td>
<td>43.8±16.9</td>
</tr>
<tr>
<td>70-min PI</td>
<td>48.8±11.1</td>
<td>45.5±6.0</td>
</tr>
<tr>
<td>100-min PI</td>
<td>33.0±29.5</td>
<td>49.2±22.8</td>
</tr>
<tr>
<td>210-min PI</td>
<td>43.7±12.6</td>
<td>53.1±8.5</td>
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CBF index is expressed in %HLA. P=.44 by two-factor, repeated-measures ANOVA for all animals.

PI showed perfusion deficits of comparable size in both groups 20 minutes after MCAO and did not change significantly after the commencement of A-127722 in treated animals. After reperfusion at 90 minutes, the region with perfusion deficit was reduced in both groups. At 210 minutes after induction of ischemia (120 minutes after mechanical reperfusion), the region with a perfusion deficit was not significantly different in the two groups (Table 2). Appropriate mechanical withdrawal of the monofilament occluder occurred in all 20 animals as proven later by examining the position of the occluder visually after the MRI protocol. The disappearance or near disappearance of the perfusion deficit on PI at the last PI time point was considered “successful reperfusion,” and the persistence of a perfusion deficit was considered “unsuccessful reperfusion” (Fig 1). Further analysis demonstrated that 6 animals in each group showed successful reperfusion on PI with little or no perfusion deficit on the 210-minute PI. Depending on the number of abnormal pixels in the left (intact) hemisphere and the clustering of perfusion deficits among all animals, we used a cutoff value of 10%HLA for defining reperefused (<10%HLA at 210-minute PI time point) and nonreperfused (>10% HLA at 210-minute PI time point). Some animals had complete or near complete reperfusion at 100-minute PI (10 minutes after mechanical reperfusion) but a substantially larger perfusion deficit at 210-minute PI (probably due to reocclusion at macrovascular or microvascular levels), and some animals showed no decrease in the size of the perfusion deficit at 100-minute PI but little perfusion deficit at the 210-minute PI time point (probably due to a lag time between the mechanical reperfusion and the reestablishment of the microcirculation). We used the 210-minute imaging time point for classification because this was the last PI. In A-127722–treated animals, successfully reperefused animals (n=6) had significantly smaller infarcts at postmortem (P<.0002) than nonreperfused A-127722–treated animals (n=4). In the control group, successful reperfusion did not make a difference in the final infarct volume when the %HLV of successfully reperefused (n=6) animals was compared with that of nonreperfused animals (n=4, P=.16). These results are summarized in Table 3. When the two groups of successfully reperefused animals are compared, the A-127722–treated group had significantly smaller (60% reduction) infarcts (9.3±4.4%, n=6) than controls (23.2±3.1%, n=6, P<.0001), whereas the two groups of nonreperfused animals (n=4 in each group, %HLV 26.6±2.7% for A-127722–treated animals and 28.4±7.5% for controls) demonstrated no difference in the final infarct size (P=.67). The neurological scores were calculated for subgroups (1.8±0.4 for controls and 2.2±1.0 for treated animals in the successfully reperefused

TABLE 3. TTC-Derived %HLV of Successfully Reperefused and Nonreperfused Animals

<table>
<thead>
<tr>
<th></th>
<th>Successfully Reperefused</th>
<th>Nonreperfused</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>All animals (n=20)</td>
<td>16.3±8.1 (n=12)</td>
<td>27.5±5.3 (n=8)</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>A-127722–treated animals</td>
<td>9.3±4.4 (n=6)</td>
<td>26.6±2.7 (n=4)</td>
<td>&lt;.0002</td>
</tr>
<tr>
<td>Controls</td>
<td>23.2±3.1 (n=6)</td>
<td>28.4±7.5 (n=4)</td>
<td>&lt;.2</td>
</tr>
</tbody>
</table>

Infarct volumes are mean±SD %HLV; n is number of animals.
group and $4.0\pm1.2$ for controls and $4.5\pm0.6$ for treated animals in the nonreperfused group; $P = .46$ and $P = .47$, respectively), and no difference was found between groups.

The in vivo ischemic lesion volumes expressed as %HLV for all animals were calculated with the use of the ADC maps derived from the DWI data (Fig 2A). The ischemic %HLV values before the initiation of drug infusion were not significantly different, and lesion evolution over 4 hours after MCAO did not show significant difference between the controls and the treated animals ($P = .99$). The control and treatment groups were again divided into the subgroups based on reperfusion. For the successfully reperfused animals (n=6 in each group), we observed a trend in ischemic lesion evolution over time in favor of the treated animals ($P = .13$, Fig 2B). In the nonreperfused subgroups (n=4 in each group), the pre-treatment lesion volumes were not significantly different, but at 4 hours after MCAO, the treated group tended to have larger ischemic lesions ($P = .15$, Fig 2C).

**Discussion**

Our results demonstrate that the ET$_A$-selective ET receptor antagonist, A-127722, significantly reduced the ischemic lesion size (36% reduction) in rats with 90 minutes of temporary MCAO without causing observable adverse effects when the therapy was started 30 minutes after MCAO. PI did not show significant differences in cerebral circulation over time between the two groups, suggesting that the effect is likely not due to vasodilatation leading to recirculation around the suture occluder. Furthermore, the beneficial effect was limited to those animals in which successful reperfusion was demonstrated. DWI data did not show a difference in lesion volumes in vivo overall, although a suggestive trend was seen in A-127722–treated animals that successfully reperfused.

**ETs Cause Neuronal Injury That Could Be Reversed by ET Antagonists**

Cerebral arteries are among the vessels most sensitive to ET-induced constriction. Topical administration of ET-1 onto cerebral arteries or arterioles in vivo resulted in a marked constriction of the vessels ($\approx 50\%$ reduction in vessel diameter). This effect is primarily mediated by ET$_A$ receptors since it is effectively antagonized by several ET$_A$ receptor antagonists. In contrast, the topical application of ET$_B$ receptor agonists onto rat basilar artery and cat cerebral resistance arterioles in vivo caused a dose-dependent dilatation ($\approx 20\%$ to $30\%$ increase in vessel diameter). ET-1 also produced strong contractions of human cerebral, meningeal, and temporal arteries that was mediated by ET$_A$ receptors. Abluminal administration of ET-1 to the MCA in anesthetized rats produces dose-dependent decreases in CBF and ischemic brain damage. Intrastriatal microinjections of ET-1 onto the rat MCA resulted in focal cerebral infarction similar to that produced by permanent occlusion of the artery. Intrastriatal injection of ET-1 induced a 60% reduction of striatal blood flow that led to focal ischemic damage. In a model of focal cerebral ischemia, intracisternal injection of ET-1 significantly increased the infarcted surface area. Blockage of endogenous ET with the ET antagonist BQ-123 significantly reduced neuronal death after global cerebral ische-
mia in a gerbil model of stroke. Patel et al recently reported a 45% decrease in cerebral infarct volumes in a cat focal ischemia model when therapy with PD156707, an ET(A) receptor antagonist, was initiated intravenously 30 minutes after permanent MCAO.

How Might A-127722 Reduce Ischemic Lesion Size?
The expected mechanism of attenuation of ischemic lesion size by A-127722 is increased blood flow to the ischemic site since the main biological effect of ETs is vasoconstriction. In this study PI before and after the drug injection in treated animals suggests that the anti-ischemic effect of this drug is not due solely to vasodilatation and subsequent increase in the collateral blood circulation into the ischemic region. Another study examining the effects of an ET(A) antagonist, PD156707, on CBF as measured by the laser-Doppler flowmetry method and on focal brain ischemia by postmortem examination in a permanent MCAO model in the cat found that a progressive improvement of CBF to preischemic levels occurred over 5 hours in drug-treated animals. The opposing findings on cerebral perfusion in their study and our study may be due to different methods of measurement in these two investigations. The pial collateral supply supports some degree of perfusion of the superficial cortical laminae, so that local CBF is generally higher in the upper cortical layers. Laser-Doppler flowmetry delivers information only from the cortex, whereas PI shows the whole ischemic region. Our study was a reperfusion study, and theirs was a permanent occlusion study; we used rats and they used cats. In their study the CBF increase was gradual over several hours, while in our study reperfusion increased the CBF into the ischemic territories dramatically at 90 minutes after MCAO; thus, perhaps a slow increase in CBF over time could not be determined because of the reperfusion effect. However, the sensitivity of PI to minor CBF changes is not yet known and requires further study. In the monofilament occlusion model, a potential problem could be the effect of a vasodilating agent inducing recirculation around the occluder by a maximum vasodilatation of internal carotid and middle cerebral arteries. This seems unlikely because such recirculation would cause a dramatic change in blood flow in the ischemic region, and PI should be able to detect it. Both ET-1 and ET-3 stimulate the production of several prostaglandins, thereby contributing to the inhibition of platelet aggregation. This effect is mediated by the ET(A) receptors; hence, A-127722 should not have an effect on platelet aggregation. A direct neurotoxic effect of ETs could not be shown. However, ETs may contribute to ischemic neuronal injury indirectly by stimulating the release of excitatory amino acids. Both ET-1 and ET-3 increased free intracellular Ca\(^2+\) levels in various cell cultures. ET antagonists might inhibit or decrease the release of excitatory amino acids and calcium accumulation into neural cells, which is thought to be instrumental in neuronal death. A-127722 does not inhibit MK801 binding, and therefore its positive effects on focal ischemia are probably not related to N-methyl-D-aspartate antagonism. The ET antagonists bosentan and FR139317 did not show an effect on induced spreading depressions in the cat brain cortex, suggesting that the anti-ischemic effects of ET antagonists are not due to inhibition of spreading depressions either. Hypothermia leads to neuroprotection. DWI is sensitive to brain temperature changes, and small changes in brain temperature can be demonstrated on ADC maps in rats. We did not observe any significant change in ADC values calculated from the intact brain hemispheres of treated animals before and during drug infusion (data not shown). This finding implies that A-127722 did not show its anti-ischemic effects as a result of brain hypothermia.

The beneficial effect of A-127722 on ischemic lesion size was observed at 24 hours after MCAO in this study. This model of focal cerebral ischemia provides large infarcts with good reproducibility and is suitable for MRI experiments. Studies with longer observation periods are required to determine whether this beneficial effect on ischemic lesion size persists.

Residual Perfusion Deficit in PI After Mechanical Reperfusion
Even though the mechanical reperfusion was appropriately done in all 20 animals, successful reperfusion, as shown by postreperfusion PI, was accomplished in only 12 animals (6 in each group). The persistence of a hypoperfused region resulted in large lesions (Table 3). Reperfusion proven by PI resulted in significantly smaller lesions in the treated group, possibly because of improved blood circulation and better delivery and penetration of A-127722 into the ischemic region. The persistence of hypoperfusion on PI (residual hypoperfusion) may be due to a clot in the right MCA trunk or due to the "no-reflow phenomenon." The no-reflow phenomenon is the inability to perfuse previously ischemic organs in a homogeneous manner after reopening of an occluded artery and may be caused by squeezing effects of perivascular astrocytic swelling on microvessels, parenchymal bleeding, intraluminal formation of microthrombi, detachment of endothelial cell fragments, platelet aggregation, endothelial cell swelling, polymorphonuclear leukocyte adherence to endothelial cells, vasoconstriction, and microvascular plugging by polymorphonuclear leukocytes, erythrocytes, and fibrin. Since we did not perform microscopic examination of the brain specimens, we cannot conclude the exact mechanisms of the residual hypoperfusion finding in PI after mechanical reperfusion.

Why Did DWI Not Demonstrate an In Vivo Reduction in Lesion Sizes in Favor of A-127722–Treated Animals?
The evolution of the in vivo lesion volume as measured by DWI did not demonstrate an effect in favor of the A-127722–treated group. The postmortem data, however, showed a significant treatment effect, especially in the successfully reperfused subgroup. In our previous experience with thrombolytic agents, we observed that successful reperfusion at an earlier time point is accompanied by a
rapid decrease in DWI-derived lesion volumes.\textsuperscript{42,43} In contrast, therapeutic effects of neuroprotective agents might occur more slowly and require longer MRI measurement protocols to confirm drug effects in vivo. We previously could not demonstrate a significant in vivo decrease in ischemic lesion volumes with basic fibroblast growth factor with a similar study protocol,\textsuperscript{44} whereas we could demonstrate a significant effect of the agent at postmortem as several studies had demonstrated before.\textsuperscript{45–48} Using a glycine site antagonist, we obtained a significant postmortem reduction in ischemic lesion size, while in the same study DWI did not show an in vivo effect during a 3.5-hour MRI protocol.\textsuperscript{49} With the same drug and with a similar study protocol, others have shown an in vivo lesion reduction by DWI at 6 hours after MCAO,\textsuperscript{50} suggesting a late effect. Lo et al\textsuperscript{51} demonstrated a significant reduction in ischemic lesion size in rats by DWI at 1 hour after commencement of MK-801, an N-methyl-D-aspartate receptor antagonist. Similar results were obtained at 3 hours after MCAO in rats treated with an \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) antagonist.\textsuperscript{52} These data suggest that in vivo lesion shrinkage seen by DWI when neuroprotective agents are used is likely to happen slowly and likely to be observed with rather long MRI measurement protocols. A delayed therapeutic effect is possible in the present study for several reasons. The second dose of A-127722 was injected after the MRI protocol was completed, ET activity continues in ischemic tissues for several days, and A-127722 has a long plasma half-life, suggesting a long-standing beneficial effect. The DWI-derived \%HLV at 4 hours after MCAO for the control group is almost identical to the TTC-derived \%HLV at 24 hours after MCAO, whereas for the treated group, postmortem \%HLV is only half the size of the DWI-derived \%HLV at a 4-hour time point after MCAO. Because ET antagonism is a novel therapeutic approach in brain ischemia and no previous studies exist, we chose a 4-hour MRI protocol to avoid hazards of prolonged anesthesia, such as increased mortality. For future studies, longer MRI protocols may be advantageous in demonstrating in vivo lesion shrinkage.

The vasoconstrictor effects of a single intravenous ET bolus last more than 60 minutes in animals\textsuperscript{53} and approximately 2 hours in humans.\textsuperscript{54} Since plasma levels of ET are high even several days after stroke,\textsuperscript{3} the overproduction of ET may continue for a long time after the initial cerebrovascular event. Therefore, treatment with ET receptor antagonists should be continued long enough to achieve complete suppression of ET effects. In this study we gave A-127722 as an intravenous bolus at 30 minutes and subcutaneously at 4 hours after induction of focal ischemia. A-127722 has a sufficiently long plasma half-time that, with the treatment regimen used, significant inhibition of ET\textsubscript{A}-mediated effects of ET-1 should have been achieved for the duration of the study. This study demonstrated that delayed treatment with A-127722 in rats with focal ischemia with reperfusion significantly attenuates infarct size without detectable changes in CBF. Thus, ET antagonism should be considered a therapeutic approach with reperfusion in the treatment of focal brain ischemia.

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References


Magnetic resonance images are able to provide both anatomic and functional information noninvasively. Specifically, DWI has been shown to be very sensitive in depicting regions of acute ischemic insult, while PI is able to reveal regions of perfusion abnormalities. These images are particularly advantageous in monitoring lesion evolution as well as the effectiveness of different therapeutic interventions.

In the accompanying article by Tatlisumak et al, both DWI and PI sequences were used up to 4 hours after ischemia to assess the lesion volume as well as the extent of reperfusion. The authors were able to utilize the PI data to divide rats into reperfusion and nonreperfusion groups. Based on this criterion, the lesion volume as well as the extent of reperfusion could be assessed in rats. J Cereb Blood Flow Metab. 1996;15:467–469.


clinical utilities of MRI in therapeutic interventions in acute cerebral ischemia.

Recently, several investigators have demonstrated that by combining information obtained from DWI and PI, it is possible to predict clinical outcomes in stroke patients. In the study by Tatlisumak et al, no significant differences in PI or DWI lesion volumes were noted between the treatment and control groups in the acute phase. The final histological outcomes based on TTC stain, however, showed a significant reduction in infarct volumes with ET antagonist treatment. The authors suggest that the lack of favorable MRI findings in the treatment group during the acute phase is consistent with a delayed effect of A-127722. The discrepancy may not be readily resolved with the notion that both the clinical studies and results shown in the accompanying article were based on a relatively small number of subjects. Further studies using a larger number of animals with MRI extended until the time of histological assessment of ischemic brain injury are needed to address questions that can be raised after the publication of the accompanying article.

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A Novel Endothelin Antagonist, A-127722, Attenuates Ischemic Lesion Size in Rats With Temporary Middle Cerebral Artery Occlusion: A Diffusion and Perfusion MRI Study
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