Neuroprotection by the Selective Cyclooxygenase-2 Inhibitor SC-236 Results in Improvements in Behavioral Deficits Induced by Reversible Spinal Cord Ischemia

Paul A. Lapchak, PhD; Dalia M. Araujo, PhD; Donhuang Song, MD; Justin A. Zivin, MD, PhD

Background and Purpose—Cyclooxygenase-2 (COX-2), an enzyme that is induced in the central nervous system after various insults, has been localized to neurons and in cells associated with the cerebral vasculature, where it may be involved in the inflammatory component of the ischemic cascade. COX-2 is part of the initial reaction that involves the arachidonic acid cascade, which produces molecules that support an inflammatory response. The present study evaluated the pharmacological effects of a specific long-acting COX-2 inhibitor, SC-236, in a reversible rabbit spinal cord ischemia model using clinical rating scores (behavioral analysis) as the primary end point.

Methods—SC-236 was administered (10 to 100 mg/kg SC) 5 minutes after the start of occlusion to groups of rabbits exposed to ischemia induced by temporary (10 to 40 minutes) occlusion of the infrarenal aorta. Behavioral analysis, which allowed for the calculation of an ET50 value representing the duration of ischemia (minutes) associated with a 50% probability of resultant permanent paraplegia, was conducted 18 and 48 hours later. A drug was determined to be neuroprotective if it prolonged the ET50 significantly compared with the appropriate control group.

Results—Since SC-236 is not readily soluble in aqueous solutions, it was dissolved in 100% dimethyl sulfoxide (DMSO) for subcutaneous administration. Therefore, the vehicle-treated control group consisted of rabbits given an equal volume of DMSO without drug. In the DMSO-treated control group, the ET50 assessed 18 hours after initiation of aortal occlusion was 18.84 ± 3.19 minutes. In contrast, treatment with 100 mg/kg of SC-236 given 5 minutes after the start of occlusion prolonged the ET50 of the group significantly to 30.04 ± 3.55, an effect that was still evident 48 hours later. In addition, lower doses of the drug (10 and 50 mg/kg) also showed a trend for an increase in ET50. SC-236 (100 mg/kg) did not significantly alter body temperature after a subcutaneous injection.

Conclusions—The present study suggests that COX-2 plays an important role in the ischemic cascade of events that translate into ischemia-induced behavioral deficits and furthermore that selective COX-2 inhibitors may be useful in the treatment of ischemic stroke to improve behavioral functions. (Stroke. 2001;32:1220-1225.)

Key Words: behavior, animal • cyclooxygenase inhibitors • cytokines • inflammation • ischemia • neuroprotection • spinal cord • spinal cord blood flow

Ischemia activates a cascade that leads to the induction and expression of genes in a variety of cell types throughout the central nervous system (CNS).1–7 Because it is associated with the production of both cytokines and immediate early genes that may be detrimental to CNS cells,8 the inflammatory mediator pathway has been implicated as a potential contributor to ischemia-induced deficits. The product of one such immediate early gene, cyclooxygenase-2 (COX-2) (an inducible form of COX), has become the focus of attention because it is the rate-limiting enzyme involved in arachidonic acid metabolism, thereby generating prostaglandins and thromboxanes, molecules that play important roles in supporting and sustaining the inflammatory response.9 COX-2 can be induced in neurons and in cells associated with the cerebral vasculature after various CNS insults, including global ischemia.10–13 Since COX-2 immunoreactivity also has been detected in the human brain after cerebral ischemia, it has been proposed that COX-2 and its reaction products participate in ischemic injury in the human brain.14

In view of these findings, the possibility that COX-2 may be a crucial component in ischemia-induced neurodegeneration has been the subject of several recent studies that used rodent models of focal ischemia. The COX-2 inhibitors SC-58125 and NS-398 have been shown to prevent delayed death of hippocampal neurons15 and to reduce infarct size16 after global ischemia, respectively. In contrast, Hara et al17 reported that total infarct volume was unaffected by NS-398. Nevertheless, prostaglandin levels in the peri-ischemic region...
were decreased in response to NS-398 treatment, prompting the authors to conclude that COX-2 inhibition may be important in peri-ischemic pathophysiology. However, to our knowledge, administration of a COX-2 inhibitor has not been shown to improve behavioral function(s) after transient spinal cord ischemia.

In consideration of the potential for COX-2 as a target for pharmacological intervention, the main purpose of the present study was to determine whether administration of a COX-2 inhibitor attenuates the behavioral deficits consequent to an ischemic insult in an established, validated animal model (the reversible rabbit spinal cord ischemia model), using a well-defined clinical rating scale. The selective COX-2 inhibitor 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-benzene-sulfonamide (SC-236) was chosen on the basis of its selectivity (high COX-2/COX-1 ratio of 1780/1), high potency (in vitro inhibition in the nanomolar range), and long plasma half-life (117 hours with a dose of 20 mg/kg in rodents).

Materials and Methods

Spinal cord ischemia was produced in the rabbit spinal cord ischemia model by occluding the aorta for up to 40 minutes, as described previously. Briefly, the aorta of male New Zealand White rabbits weighing 3 to 5 kg was exposed at the level of the renal arteries through a midline abdominal incision, and a small-diameter Tygon tube was used to form a snare around the aorta just distal to the left (caudal) renal artery. The ends of this tube were threaded through a small plastic button followed by a larger-diameter tube, and the incision was closed around the tubing so that the free ends were accessible externally. Rabbits were allowed to recover from anesthesia for at least 2 hours, at which time it is possible to establish whether sensations and motor activity are normal. Pulling on and clamping the protruding small tubing occludes the aorta a predetermined period, and release restores blood flow, after which all tubing is removed and the small hole in the abdominal wall is closed with surgical clips. The duration of ischemia was selected to span all grades of damage, ranging from full recovery to permanent paraplegia. This group also included abnormal animals that did not hop normally, were less responsive than normal to pinching of the hind limbs, and exhibited variable bowel and bladder function. In this group, the graders were blinded at all times as to the treatments the animals received.

SC-236 was administered at a dose of 10 to 100 mg/kg SC, 5 minutes after initiation of aortic occlusion. Two groups of vehicle-injected (1 mL/kg) control animals, one given dimethyl sulfoxide (DMSO), the vehicle required to solubilize SC-236, and the other normal saline, also were included in the study. After 48 hours, animals were euthanatized with the use of Beuthanasia-D (Schering Plough Animal Health Care Corporation). All animal use procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of the San Diego Veterans Administration Medical Center.

The duration of occlusion for individual animals was predetermined to be from 10 to 40 minutes, providing a wide range of ischemia for each experimental (drug- or vehicle-treated) group. The group ET$_{50}$ representing the duration of ischemia (minutes) associated with a 50% probability of resultant permanent paraplegia, was statistically analyzed and graphically demonstrated by using computer construction of an ischemic duration quantal "dose-response" curve for each group that is similar to the LD$_{50}$ curves of pharmacological studies. Statistical significance was assessed with the group t test and adjusted for multiple comparisons with the Bonferroni correction ($P>0.05$). Neuroprotection was demonstrated if a drug significantly prolonged the ET$_{50}$ compared with the corresponding control (vehicle-treated) group.

### TABLE 1. Effects of SC-236 Treatment on Behavioral Outcome of Ischemic Rabbits Measured After Aortic Occlusion

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>18 h</th>
<th>48 h</th>
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<tbody>
<tr>
<td>Saline (n=9)</td>
<td>22.97±1.87</td>
<td>22.97±1.87</td>
</tr>
<tr>
<td>DMSO (n=14)</td>
<td>18.84±3.19</td>
<td>20.24±2.09</td>
</tr>
<tr>
<td>SC-236 (10 mg/kg; n=14)</td>
<td>28.23±5.85</td>
<td>23.18±4.39</td>
</tr>
<tr>
<td>SC-236 (50 mg/kg; n=12)</td>
<td>25.06±4.36</td>
<td>25.06±4.36</td>
</tr>
<tr>
<td>SC-236 (100 mg/kg; n=13)</td>
<td>30.04±3.55*</td>
<td>30.04±3.55*</td>
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</table>

Percentage of rabbits that are paraplegic as a function of the duration of ischemia (aortic occlusion) in minutes measured 18 hours after spinal cord ischemia. The curve labeled DMSO-treated (solid line) shows the development of paraplegia in DMSO-treated rabbits. The curve labeled COX-2 inhibitor (large dashed line) shows that SC-236 (100 mg/kg SC) increases the tolerance of aortic occlusion by approximately 11.3 minutes. The horizontal bars represent standard errors at the ET$_{50}$ required to produce paraplegia.
Since SC-236 is relatively insoluble in aqueous solution, a quantal analysis curve for DMSO, the vehicle required to solubilize SC-236, was compared with a quantal analysis curve for normal saline (0.9% saline), the more commonly used vehicle in the rabbit spinal cord ischemia model. The comparison was made to determine whether DMSO itself affects the behavioral response. When behavioral analysis was assessed 18 hours after aortic occlusion, the ET$_{50}$ of the DMSO-treated group (18.84 ± 3.19 minutes) was not significantly different from that of the saline-treated group (22.97 ± 1.87 minutes). By 48 hours after occlusion, the ET$_{50}$ for the DMSO-treated group had increased slightly to 20.24 ± 2.09 minutes, whereas that for the saline group remained unchanged (Table 1). Nonetheless, no significant statistical differences (P > 0.05) in the ET$_{50}$ values for the different vehicle-treated groups were noted at either time point.

SC-236 treatment (100 mg/kg) significantly prolonged the ET$_{50}$ of the group to 30.04 ± 3.55 minutes (P < 0.01) at 18 hours after occlusion, a shift of approximately 59% compared with the DMSO group, whereas effects of the lower doses were less pronounced (Figure; Tables 1 and 2). In addition, the effect of the higher dose (100 mg/kg) of SC-236 was durable since there was still a significant difference (P < 0.05) in the ET$_{50}$ between the DMSO-treated (20.24 ± 2.09 minutes) and drug-treated (30.04 ± 3.55 minutes) groups by 48 hours after the initial occlusion (Tables 1 and 3).

To determine whether the neuroprotective effects of SC-236 could be attributed to the induction of hypothermia or hyperthermia, we determined whether the dose of SC-236 (ie, 100 mg/kg SC) after a baseline body temperature was established with the use of a digital rectal thermometer. Baseline temperature was 102.7 ± 0.3°F. At 1, 2, and 18 hours after SC-236 SC-236 treatment (100 mg/kg) significantly prolonged the ET$_{50}$ of the group to 30.04 ± 3.55 minutes (P < 0.01) at 18 hours after occlusion, a shift of approximately 59% compared with the DMSO group, whereas effects of the lower doses were less pronounced (Figure; Tables 1 and 2). In addition, the effect of the higher dose (100 mg/kg) of SC-236 was durable since there was still a significant difference (P < 0.05) in the ET$_{50}$ between the DMSO-treated (20.24 ± 2.09 minutes) and drug-treated (30.04 ± 3.55 minutes) groups by 48 hours after the initial occlusion (Tables 1 and 3).

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administration, body temperature was 102.8±0.6°F, 102.8±0.5°F, and 103.0±0.3°F, respectively.

**Discussion**

Recent evidence has provided some insight into the role of inflammatory mediators after an ischemic event. Current hypotheses have proposed that the inflammatory reaction is initiated at the level of the microvasculature and within various cell types. For instance, COX-2 mRNA and protein levels have been shown to be significantly increased within neurons and vascular cells after cerebral ischemia and other insults that result in neurodegeneration. Moreover, COX-2 protein appears to be expressed not only within ischemic cells but also in cells located in the “penumbra” and in normal cells around the infarct zone. A role for COX-2 in the pathophysiology of the peri-ischemic zone as the infarct enlarges in the later stages of ischemic injury is supported further by the observation that administration of a COX-2 inhibitor reduces ischemia-induced delayed neuronal damage. Although this mechanism requires further investigation, it has been suggested that COX-2 activity is driven by inducible NO synthase production of NO, which in turn supports the production of COX-2 products. Consistent with this, Nogawa et al showed that COX-2 mRNA expression in the brain peaked 12 hours after middle cerebral artery occlusion, at a time when inducible NO synthase also reached peak expression.

In the present study SC-236 served as a pharmacological tool to determine whether COX-2 is involved in the ischemic cascade and resulting behavioral deficits after aortic occlusion in the rabbit spinal cord ischemia model. The results of our study identified inhibition of COX-2 as an effective means to generate a neuroprotective effect (improved behavior) after the onset of spinal cord ischemia, supporting earlier findings that implicated COX-2 involvement in the progression of the ischemic cascade in cerebral ischemia. Furthermore, our results are in agreement with the study of Resnick et al, who showed that the selective COX-2

### TABLE 3. Comparison of Effects of Vehicle or SC-236 Treatment on Clinical Outcome in Rabbits 48 Hours After Aortic Occlusion

<table>
<thead>
<tr>
<th>Duration of Ischemia, min</th>
<th>Saline</th>
<th>DMSO (10 mg/kg)</th>
<th>SC-236 (50 mg/kg)</th>
<th>SC-236 (100 mg/kg)</th>
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<tr>
<td>10</td>
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Results are behavioral readings for each group of rabbits and are the number of rabbits either functional (F) or paraplegic (P) for each treatment group. Rabbits were treated subcutaneously with either saline (n=9), DMSO (n=14), SC-236 (10 mg/kg; n=14), SC-236 (50 mg/kg; n=12), or SC-236 (100 mg/kg; n=13) administered 5 minutes after the start of occlusion. Ellipses indicate that an animal was not tested for that particular group or duration of ischemia.
inhibitor SC58125 improved functional outcome after experimental spinal cord injury induced by a different method.

The mechanism responsible for COX-2–mediated neurodegeneration or COX-2 inhibitor neuroprotection in the rabbit spinal cord ischemia model is not clearly understood. In the present study relatively high doses of SC-236 were required to a statistically significant increase in ET50 values. Since there is no information available on the pharmacokinetics of SC-236 in rabbits, it is possible that high doses of SC-236 were required because of poor penetration of SC-236 into the CNS, the spinal cord in particular. Although COX-2 production of prostaglandin, particularly prostaglandin E2, which regulates many physiological processes including vasomotility, platelet aggregation, and immunomodulation, has been proposed to perpetuate the ischemic cascade, administration of COX-2 inhibitors after an acute noxious stimulus did not appear to alter prostaglandin synthesis in the rat spinal cord. Thus, further studies are required to understand COX-2 inhibitor effects in the spinal cord after ischemia and to determine whether the neurodegenerative effects of COX-2 are mediated through the synthesis of prostaglandin E2 or other mediators. The present study focused on the behavioral effects of COX-2 inhibition after ischemia. Since SC-236 was found to be neuroprotective in the rabbit spinal cord ischemia model, histological and neuroanatomical studies are required to determine which neuronal populations of spinal cord neurons mediate the neuroprotective effects of COX-2 inhibitors. Additionally, since COX-2 inhibitors may alter a variety of physiological responses, detailed studies aimed at identifying physiological parameters affected by SC-236 should be conducted in the future. However, in the present study we found that SC-236 did not alter body temperature, suggesting that the neuroprotective effects of SC-236 are not related to the induction of hypothermia or hyperthermia.

Conclusion
We have demonstrated that the selective COX-2 inhibitor SC-236 is neuroprotective in a reversible spinal cord ischemia model using clinical rating scores as the primary end point. COX-2 inhibitors have been used clinically for various indications and have been shown to be safe, effective, and have been shown to be safe, effective, and easily administered for the treatment of a variety of diseases. Our results suggest that selective COX-2 inhibitors also may be of therapeutic value in the treatment of spinal cord and cerebral ischemia.

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References


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