Interleukin-1β and adverse effects on cerebral blood flow during long-term global hypoperfusion

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Object. The effects of interleukin (IL)-1β on the cerebral vasculature are complex and incompletely understood. Many pathophysiological states in which inflammatory cascades have been implicated also have varying degrees of cerebral hypoperfusion. The purpose of this investigation was to examine the long-term effects of this proinflammatory cytokine and its antagonist on cerebral blood flow (CBF) following global cerebral hypoperfusion.

Methods. Sprague–Dawley rats were randomly assigned to 12 groups and given continuous intracerebroventricular (ICV) infusions of IL-1β, the IL-1 receptor antagonist (IL-1ra), or saline vehicle (control). Global cerebral hypoperfusion was produced by occlusion of both carotid arteries and one vertebral artery. Cerebral blood flow was measured at baseline and again after initiation of the infusions by performing a 133Xe clearance study. Prolonged ICV administration of IL-1β resulted in a significant decrease in CBF compared with that in controls. Prolonged administration of the antagonist IL-1ra resulted in significant increases in CBF compared with that in both IL-1β–treated animals and controls.

Conclusions. This experiment demonstrates that long-term treatment with the proinflammatory cytokine IL-1β adversely affects CBF.

KEY WORDS • cerebral blood flow • cytokine • interleukin-1β • rat

INTERLEUKIN-1β is a proinflammatory cytokine that is produced in the brain in response to pathological stressors such as stroke, trauma, infection, and SAH. In many cases, this response is delayed. For example, Legos and colleagues demonstrated in the rat that an increase in IL-1β expression peaked approximately 3 days after focal ischemia.

Several investigators have reported that the short-term effect of central administration of IL-1β is vasodilation with an increase in CBF. Monroy, et al. measured CBF indirectly by performing transcranial Doppler ultrasonography following a single ICV injection of IL-1β and observed that CBF was significantly elevated over the 3-hour measurement period. Plata-Salaman and associates found similar results in a spinal cord blood flow model, noting that spinal cord blood flow was significantly increased 30 minutes after an injection of IL-1β. Shibata, et al. measured the diameters of cerebral arterioles in pigs for 30 minutes after the topical application of IL-1β and reported arteriolar dilation. The vasodilatory effect of IL-1β was blocked by intravenous administration of indomethacin. Also supporting this short-term effect, Osuka, et al. measured the diameters of canine basilar arteries on angiograms obtained after the administration of IL-1β and found a significant increase in vessel diameter, which reached a maximum 2 to 3 hours posttreatment. The acute vasodilatory effect that is supported by these experiments is not limited to IL-1β; others have shown acute vasodilatory effects of other cytokines including tumor necrosis factor–α and IL-1α.

In contrast to the well-studied short-term effects of IL-1β, the effect of prolonged IL-1β administration has not been investigated. There are reasons to suspect that the prolonged effects may be at variance with the short-term vasodilatory actions. Interleukin-1β has been associated with vasospasm in both animal and human studies. It is also relevant to consider that IL-1β is a cytokine with a broad spectrum of activities and that many of the downstream effects of its activation occur over a prolonged interval. For example, IL-1β promotes the expression of IL-6; this expression peaks hours after IL-1β administration. Furthermore, it is well established that IL-6 is a potent vasoconstrictor, even over short time intervals. The relationship between the direct effect of IL-1β on CBF and the effect of downstream cytokines such as IL-6 on CBF are not well understood.

Given these concerns, this experiment was designed to study the effects of long-term IL-1β use on CBF. The aim was to examine this effect in healthy rats as well as in those subjected to chronic global hypoperfusion. To control for endogenous IL-1β activity, the CBF response to IL-1ra was also examined.
Materials and Methods

Experimental Design

This experiment was conducted in accordance with the guidelines proposed by the Institutional Animal Care Committee of the Mayo Clinic. Eighty-four male Sprague–Dawley rats (Simonsen Laboratories, Inc., Gilroy, CA), each weighing between 345 and 455 g at the start of the experiment, were randomly assigned to 12 groups of seven rats each. The groups differed with respect to surgical preparation (three-vessel occlusion or sham procedure) as well as the type of ICV infusion (IL-1β, IL-1ra, or saline) (Table 1). Animals were denied access to food and water for 12 hours prior to the surgical procedure.

Animal Preparation

All rats were anesthetized with an intraperitoneal injection of pentobarbital (65 mg/kg). A femoral arterial line and a venous line were placed. Throughout the procedure, blood pressure was continuously measured by means of a strain gauge (Statham Instruments, Oxnard, CA) attached to the femoral artery catheter and recorded on a polygraph (model 78; Grass Instrument Co., Quincy, MA). Core temperature was continuously measured with a rectal temperature probe, and the animals were maintained at a normothermic level (37 ± 0.5°C) by use of a heating pad (K-Pad; German-Rupp Industries, Bellville, OH). Arterial blood gas analysis, including monitoring of PaO2, PaCO2, and pH, was performed immediately after each CBF measurement.

The anterior neck of each rat was opened under sterile conditions and the CCA, internal CA, and external CA on the left side were exposed. A polyethylene catheter was introduced into the left external CA in a retrograde fashion, with the tip of the catheter projecting to the CA bifurcation. The contralateral CCA was also exposed in all rats. After this exposure and catheter placement, an initial baseline CBF measurement was made in all rats, as described later in this paper.

At the conclusion of the anterior part of the operation, the rats were placed prone and a second baseline CBF measurement was made. Following this, the intravascular catheters were removed and all wounds were closed using 4-0 Vicryl sutures (Ethicon, Somerville, NJ). All procedures were performed using sterile procedures and with the aid of an operating microscope (Olympus, Tokyo, Japan).

Three-Vessel Occlusion

Six groups of rats underwent three-vessel occlusion at the time of the initial operation and three groups underwent only the operation described earlier. In the rats undergoing three-vessel occlusion, both CCAs were ligated with 4-0 silk sutures (Ethicon) during the anterior part of the procedure. During the posterior part of the procedure, the posterior lateral mass of C-1 on the left side was drilled off by using a high-speed air drill (Hall Surgical, Santa Barbara, CA), exposing the VA. This artery was coagulated using bipolar forceps and divided. The remainder of the surgical procedure was identical in all groups.

Intracerebroventricular Infusion

The osmotic minipumps were filled with 200-μl saline solutions containing either IL-1β (Research Diagnostics, Inc., Flanders, NJ) at a concentration of 0.672 μg/200 μl, IL-1ra (R & D Systems, Minneapolis, MN) at a concentration of 6.72 μg/200 μl, or saline alone. The minipump infusion rate was 0.25 μl/hour, resulting in a continuous ICV infusion of IL-1β at a rate of 1 ng/hour over the 2- or 4-week interval or a continuous infusion of IL-1ra at a rate of 10 ng/hour over the same interval. Doses were selected on the basis of prior studies15,16,41,48,55 in addition to the manufacturer’s recommendations.

Measurements of CBF

Cerebral blood flow was measured at baseline before vessel occlusion, following the initial surgical procedure (three-vessel occlusion or sham operation) and at 2 or 4 weeks postoperatively by using a 133Xe washout technique.15 Regional CBF was measured using xenon-133 and a cadmium telluride detector system ( Radiation Monitoring Devices, Inc., Watertown, MA), in which a measurement volume of 0.5 mm3 was directed at the left parietal cortex. The window discriminator was set at 76 keV and at 200 keV to minimize the Compton scatter. The resultant counts were recorded on a strip chart recorder. The 1-minute initial slope index was used to calculate the CBF. The partition coefficient (λ) used for xenon-133 was 0.63.2

Convalescence of the Animals

Following the initial surgery and the CBF measurements, the animals convalesced for either 2 or 4 weeks. Animals were housed individually in transparent cages with unrestricted access to food and water for 12 hours prior to the surgical procedure.

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>No. of Rats</th>
<th>Occlusion</th>
<th>ICV Infusion</th>
<th>Duration of Treatment (wks)</th>
<th>Preop CBF (ml/100 g/min)</th>
<th>Postop CBF (ml/100 g/min)</th>
<th>Final CBF (ml/100 g/min)</th>
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<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>yes</td>
<td>saline</td>
<td>2</td>
<td>87.1 ± 15.1</td>
<td>42.1 ± 7.7</td>
<td>56.4 ± 15.6</td>
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<tr>
<td>2</td>
<td>6</td>
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<td>saline</td>
<td>4</td>
<td>84.5 ± 6.3</td>
<td>45.8 ± 7.8</td>
<td>70.2 ± 13.5</td>
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<td>3</td>
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<td>yes</td>
<td>IL-1β</td>
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<td>35.2 ± 6.6</td>
<td>47.8 ± 5.9</td>
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<td>82.1 ± 10.0</td>
<td>33.3 ± 11.0</td>
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<td>5</td>
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<td>IL-1ra</td>
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<td>73.2 ± 8.0</td>
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<td>77.0 ± 8.0</td>
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<td>6</td>
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<td>yes</td>
<td>IL-1ra</td>
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<td>81.8 ± 10.4</td>
<td>43.0 ± 13.6</td>
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<td>7</td>
<td>no</td>
<td>IL-1β</td>
<td>4</td>
<td>87.0 ± 12.4</td>
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<td>49.4 ± 17.4</td>
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<tr>
<td>8</td>
<td>5</td>
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<td>IL-1ra</td>
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<td>83.8 ± 11.6</td>
<td>78.8 ± 12.7</td>
<td>83.4 ± 9.6</td>
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<td>saline</td>
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<td>81.0 ± 12.8</td>
<td>77.6 ± 14.1</td>
<td>64.4 ± 5.9</td>
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<td>10</td>
<td>6</td>
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<td>IL-1β</td>
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<td>IL-1ra</td>
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<td>76.2 ± 11.9</td>
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<tr>
<td>12</td>
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<td>no</td>
<td>saline</td>
<td>2</td>
<td>84.5 ± 11.1</td>
<td>74.5 ± 6.8</td>
<td>66.5 ± 11.3</td>
</tr>
</tbody>
</table>

* Data are expressed as means ± SDs.

TABLE 1: Final CBF measurements by experimental group*
Interleukin and cerebral blood flow

water in a room maintained between 21.5 and 22.5°C with a 12-hour light/dark cycle. The rats were observed and weighed daily.

Statistical Analysis

The mean values and SD were determined for each continuous variable. Differences between groups were analyzed by an analysis of covariance model with a $2 \times 3 \times 2$ factorial design. This model was used to test the effects of treatment, occlusion, and the duration of the experiment on CBF at the final CBF measurement, while controlling for the baseline CBF. For the purpose of the statistical analysis, there were two levels of occlusion (occluded and not occluded), three levels of treatment (IL-1β, saline, and IL-1ra), and two levels of duration of the experiment (2 and 4 weeks). Statistical significance was determined at a level of probability lower than 0.05.

Results

The mean CBF values for each group are provided in Table 1. Eleven rats died during the surgical procedure and were excluded from this analysis. Three-vessel occlusion resulted in an immediate 50.9 ± 12.8% (mean ± SD) reduction in CBF. This effect diminished over the 2- or 4-week duration of the experiment. An increase in CBF was noted in groups with vessel occlusion at the 2-week measurement compared with the baseline postocclusion measurement, and at the 4-week measurement compared with the 2-week measurement. The ischemia-by-time relationship was significant for the final CBF measurement ($p = 0.009$), that is, CBF tended to return to baseline over time in rats subjected to vessel occlusion.

The effect of treatment (IL-1β, IL-1ra, or saline) on CBF was highly significant and is presented in Table 2. The IL-1β treatment groups had a significantly lower CBF compared with the saline treatment groups ($p = 0.015$) and the IL-1ra treatment groups ($p < 0.001$). The IL-1ra treatment groups had a significantly higher CBF than either the IL-1β ($p < 0.001$) or saline treatment groups ($p < 0.001$). The probability values that were presented were derived from an analysis of a covariance model controlling for baseline CBF, occlusion, and time. In the analysis of the effect of treatment, treatments were pooled across the occlusion status (occluded and nonoccluded) and time (2 and 4 weeks) because there was no evidence of a significant interaction between these variables and the treatment status. The determination of significance was not affected by the presence or absence of three-vessel occlusion, as demonstrated by the highly nonsignificant treatment–occlusion interaction ($p = 0.98$). The determination of significance was not affected by the time (2 or 4 weeks) of the final measurement, as demonstrated by the highly nonsignificant treatment–time interaction ($p = 0.65$). Similarly, the determination of significance was not affected by the choice to use the preoperative CBF ($p = 0.52$) or the postoperative CBF ($p = 0.39$) as a baseline measurement.

Table 2: Effect of IL-1β and IL-1ra on CBF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Rats</th>
<th>CBF (ml/100 g/min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>26</td>
<td>55.6 ± 13.5</td>
</tr>
<tr>
<td>saline</td>
<td>26</td>
<td>64.1 ± 13.4</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>21</td>
<td>79.5 ± 10.7</td>
</tr>
</tbody>
</table>

*p Values are expressed as means ± SDs; $p = 0.015$ for IL-1β treatment group compared with saline treatment group; $p < 0.001$ for IL-1β treatment group compared with IL-1ra treatment group; and $p < 0.001$ for saline treatment group compared with IL-1ra treatment group.

Discussion

In this model an immediate reduction in CBF of 50.9 ± 12.8% was achieved by occlusion of both CCAs and a single VA. These results are similar to those reported by de la Torre and Fortin who used their three-vessel occlusion model in Sprague–Dawley rats. De la Torre and Fortin achieved a three-vessel occlusion by ligating both CCAs via an anterior neck exposure and by ligation one subclavian artery via a thoracotomy. In contrast, we occluded the VA from a posterior approach to avoid performing a thoracotomy. Our approach to the VA was similar to that described by Pulsinelli and Buchan; however, we chose to drill off the bone of C-1 over the VA so that we could confirm visually that the artery had been occluded. Although the reduction in CBF tended to diminish over the 2- or 4-week duration of the experiment, the three-vessel occlusion method is a reproducible model of chronic global hypoperfusion.

Because of its size (17.5 MW) and structure, peripherally injected IL-1β cannot cross the blood–brain barrier passively. Following ICV injection, however, IL-1β rapidly diffuses through brain tissue. Interleukin-1β follows a logarithmic disappearance pattern following a single central injection. The use of an osmotic minipump allows for a theoretically continuous infusion over the 2- or 4-week interval and allows the agent to achieve a steady-state concentration in the CSF.

Our data indicate that prolonged administration of IL-1β decreases CBF and that IL-1ra increases CBF compared with controls. These effects were noted at both 2 and 4 weeks postoperatively in both animals that were subjected to three-vessel occlusion and those that were not. The decrease in CBF that was seen in animals not subjected to three-vessel occlusion and receiving saline highlights the importance of proper controls in these types of experiments. The relative increase in CBF that was seen in response to IL-1ra was most likely a result of the antagonism of endogenous IL-1β action. Levels of endogenous IL-1β were not measured during the course of this experiment. Future experiments in which the expression of endogenous IL-1β is compared at baseline and in the hypoperfused state may help us understand the role of this cytokine under these conditions.
These data support a growing body of evidence that links IL-1β with vessel narrowing or states of reduced CBF. Many experiments have demonstrated an association between vasospasm and IL expression. Shimokawa, et al.,53 have shown that long-term treatment with IL-1β induces vasospastic responses in the coronary vessels of pigs. These investigators found that the vasospastic response is partially mediated by platelet-derived growth factor53 and by basic fibroblast growth factor.21 Aihara and associates measured mRNA expression of cytokine genes and the calibers of basilar arteries in dogs following experimental SAH. They found that levels of IL-1α and IL-6 mRNA peaked concurrently with the most severe vessel contraction. These authors did not study IL-1β levels. Onda, et al.,38 also looked at mRNA expression in an experimental canine SAH model. After identifying DV5 27, which was the most highly upregulated gene following hemorrhage, these authors performed a second experiment in which they examined the differential expression of this gene in human umbilical smooth-muscle cells in response to various stimuli. They found that DV5 27 was strongly upregulated by the addition of IL-1β. Interestingly, no upregulation effect was noted for IL-6.

Based on their findings many authors have supported a strong relationship between vasospasm and an inflammatory response following SAH.1,7,11,12,25,37,41–43,60,61 An association between cytokines and vasospasm has also been suggested in analyses of humans following SAH. In their report on patients who underwent neurosurgery after SAH, Fassbender, et al.,12 found that increased expression of IL-1β in the spinal fluid was associated with significantly increased velocities on transcranial Doppler ultrasonography examination as well as significantly worse overall outcomes. Nam and coworkers36 extracted leukocytes from patients following SAH and then activated these cells with lipopolysaccharide. These investigators found that patients, in whom a higher activation index of IL-1β was found after administration of lipopolysaccharide, were significantly more likely to experience vessel narrowing postoperatively. Kikuchi, et al.,24 failed to detect IL-1β on CSF assays performed on a small number of patients with SAH. This group did, however, report an elevation in IL-6, a cytokine that is associated with IL-1β action.9,10,17,25,28,31,36,39,47,48 Gaetani, et al.,34 have also reported a significant elevation of IL-6 levels in the CSF of seven patients with SAH in whom vasospasm developed, compared with seven patients with SAH in whom vasospasm did not occur. Osuka, et al.,39 have reported that IL-6 levels, but not IL-1β levels, were significantly elevated in the CSF samples of five patients with SAH who had symptomatic vasospasm compared with 16 patients with SAH without vasospasm.28. It is possible that IL-1β may effect significant changes, with differences in concentration that small studies are unable to detect. Another association between IL-1β and vasospasm is seen in the circumstantial relationship between tirilazad mesylate, which is known to inhibit IL-1β, and better outcome following SAH.13,23 Interleukin-1β has also been associated with an increase in the volume of infarction in models of acute cerebral ischemia. Relton and Rothwell48 were the first to demonstrate that a single ICV injection of IL-1β exacerbated neuronal cell death in rats following acute middle cerebral artery occlusion, a finding that has been confirmed by others.55 Furthermore, endogenous IL-1β is produced rapidly following acute cerebral ischemia in rodents.18,58,59,60 Conversely, the administration of IL-1ra before a period of acute ischemia is associated with significantly decreased neuronal death in numerous studies,4,15,20,30,46,55,57,66 suggesting a role for endogenous IL-1β in the pathogenesis of acute cerebral ischemia. The role of IL-1β and IL-1ra in more prolonged, but less severe states of hypoperfusion has not been previously examined.

Conclusions

The proinflammatory cytokine IL-1β adversely affected CBF at 2 and 4 weeks of the experiment. Future studies are needed to evaluate the potential therapeutic role for antagonists of IL-1β in patients suffering from injuries to the central nervous system that are associated with reductions in CBF. The applicability of this model is limited by the relatively modest reductions in CBF that were achieved by performing three-vessel occlusion.

Acknowledgments

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Interleukin and cerebral blood flow


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