Cerebral Vasomotor Responses after Recombinant Interleukin 2 Infusion

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ABSTRACT

The effects of systemic human recombinant interleukin 2 (rIL-2) infusion upon both the vasoconstrictor effect of hypocapnia and the endothelium-dependent vasodilator effect of acetylcholine (Ach) were examined in anesthetized rats equipped with cranial windows. Prior to the functional studies, each of six animals received an i.v. infusion of rIL-2 (6 \times 10^7 IU/kg) every 8 h for 3 days. At the same time, six control animals received infusions of equivalent volumes of sterile water. Eight h after the final infusion, each animal was anesthetized and equipped with a cranial window for the observation of pial arterioles overlying the left frontoparietal cortex. Pial arteriolar diameters were measured before and after the topical application of Ach which in normal cerebral arterioles elicits the release of endothelium-dependent relaxing factor, causing vasodilation. When arteriolar diameters returned to baseline, they were measured again both before and during hyperventilation-induced hypocapnia. Following functional assessments, these same pial vessels were processed for study by transmission electron microscopy to determine if any observed functional changes correlated with morphological abnormality.

Results of the statistical analyses suggested that normal Ach-induced endothelium-dependent vasodilation was absent in the rIL-2-infused group. Additionally, these animals exhibited reduced reactivity to the vasoconstrictive effects of arterial hypocapnia. The control group exhibited normal responsiveness to both Ach and hyperventilation. Ultrastructural studies revealed occasional morphological alterations of both vascular smooth muscle and endothelial cells in some vessels of rIL-2-infused animals, but not in controls. These data suggest that repeated systemic rIL-2 infusion results in altered vasomotor responsiveness within the cerebral microcirculation. The data also suggest that the observed vasomotor changes are not always accompanied by overt morphological alterations of either endothelial or smooth muscle cells.

INTRODUCTION

Immunotherapy involving high-dose i.v. administration of recombinant interleukin 2, with or without lymphokine-activated killer cells, has shown some efficacy in mediating the regression of certain forms of metastatic cancer (1). Importantly, both laboratory and clinical studies have shown that rIL-2 administration is accompanied by dose-limiting toxicity characterized, in large part, by a diffuse systemic capillary leak syndrome of undetermined etiology (2, 3). This increase in vascular permeability results in profound tissue edema, hypotension, and, in some cases, respiratory distress. In addition to these side effects which are clearly related to vascular injury, a number of patients also develop clinically significant neuropsychiatric changes which appear to be dose related and, therefore, treatment limiting (4).

In order to investigate a potential link between such neuropsychiatric changes and altered vascular permeability in the brain, we initially examined blood-brain barrier status, in cats and rats, following i.v. rIL-2 infusion (5, 6). The results of those studies revealed that single rIL-2 infusions, at clinically relevant doses, induced alterations in cerebrovascular permeability to protein. We therefore sought, in the present investigation, to extend our study of the cerebrovascular consequences of rIL-2 infusion by examining two aspects of cerebral arteriolar vasomotor function following multiple i.v. infusions of rIL-2 in rats. In each animal, we examined whether rIL-2 had any effect on the cerebral endothelium-dependent vasodilator response to topically applied 10^{-7} M Ach (Sigma, St. Louis, MO) (7–9). We then examined, in the same vessels, effects on the normal vasoconstrictor response of cerebral microvessels exposed to low levels of arterial carbon dioxide (10, 11).

Our findings indicated that, after 3 days of rIL-2 infusions, pial arterioles exhibited impairment of endothelium-dependent vasodilator mechanisms as well as reduced smooth muscle-mediated responsiveness to the vasoconstrictor influence of arterial hypocapnia. These findings suggest that cerebral vasomotor function is impaired in rats administered multiple infusions of rIL-2. Such vasomotor deficits, if widespread, could have implications for the regional regulation of cerebral blood flow (12).

MATERIALS AND METHODS

Animals. Sixteen adult male Sprague-Dawley rats, 250–325 g, were used. Eight received i.v. injections of rIL-2 and 8 were infused with sterile water, the required diluent for Proleukin (see below). Prior to data analysis, two of the rIL-2-infused animals were excluded from the study because, under the influence of anesthesia, their mean arterial blood pressures had dropped below 65 mm Hg. One water-infused animal was lost due to ventilator failure while another died from an obstructed airway.

rIL-2. Recombinant human interleukin 2 (Proleukin) was kindly supplied in sterile vials by the Cetus Corp. (Emeryville, CA). Each vial contained 1.2 mg (18 \times 10^6 IU/mg, equivalent to 3 \times 10^6 Cetus units/mg) rIL-2, lyophilized, which was reconstituted prior to use with 1.2 ml sterile water for injection. The dosage used in each infusion was 6 \times 10^6 IU/kg delivered into an indwelling cannula in the external jugular vein. This dosage level was selected because it is commonly used in clinical trials (1). Control animals received equivalent volumes of sterile water.

Experimental Design. Each animal was briefly anesthetized with ether while the right external jugular vein was cannulated orthograde. The distal end of the cannula was then passed s.c. to the posterior aspect of the neck where it was exteriorized and capped. Forty-eight h after recovery from anesthesia, each animal was infused with either rIL-2 or sterile water. Eight rats received a total of nine bolus rIL-2 infusions, delivered at 8-h intervals for 3 days. An additional eight animals received sterile water infusions 3 times daily for 3 days. Eight h following the final infusion, each animal was anesthetized with sodium pentobarbital (40 mg/kg i.v.), tracheotomized, and equipped with a femoral arterial cannula for blood pressure and blood gas monitoring. Each animal was also fitted with a cranial window for visualization of the left frontoparietal pial microcirculation (see below). Following surgical manipulations, each animal was paralyzed (galamine triethiodide, 4 mg/kg i.v.) and ventilated with room air supplemented by O_2 as needed. Body temperature was maintained at 37°C with the aid of a heating pad. As described below, pial arteriolar vasomotor responses were then examined after topical Ach application and hyperventilation-induced hypocapnia. After completion of the cranial window studies, each animal was administered a lethal dose of sodium pentobarbital.
and perfused transcardially with 0.9% sodium chloride followed by fixative consisting of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 m phosphate buffer. The morphology of pial vessels was studied by transmission electron microscopy.

Vasomotor Assessments. Each animal was equipped with a cranial window modified from those used in cats (13) and rats (14). In preparation, the head was securely positioned in a rodent stereotaxic device. Following reflection of soft tissue, the left parietal bone was thinned with a razor blade and carefully removed without disturbing the underlying dura mater. With the aid of a dissection microscope, the dura was reflected revealing the left frontoparietal pial microcirculation. The cranial window, constructed of dental acrylic, bone wax, and a round glass coverslip, was applied to the remaining bone with dental acrylic. The space within the window was then filled with artificial cerebrospinal fluid (14) by way of inlet and outlet ports constructed of blunt-end 23-gauge needles embedded in the window wall. No components of the window were in contact with the brain or the dura. Vasomotor assessments were accomplished through the use of a Leitz/Wild stereomicroscope fitted with a Vickers image-splitting device (13) and were initiated when both arterial blood pressure and blood gas levels were within normal limits. Prior to the first vasomotor assessment, vessels in the field were depicted in a sketch designating arterioles selected for study. In this manner, repeated measurements of the same vessels could be performed. To initiate the assessment of endothelium-dependent relaxation, the diameters of the selected pial arterioles were measured for the first time. A solution of 10^{-6} m ACh in mock rodent cerebrospinal fluid was then superfused under the window and the diameters of the same arterioles were immediately measured again. No more than 10 min after ACh application, the window was flushed with artificial cerebrospinal fluid. Fifteen min thereafter, just prior to hyperventilation, an arterial blood gas sample was drawn and base-line diameters of the same arterioles were again recorded. The ventilatory rate was subsequently increased such that the arterial PaCO2 level was reduced by 50% within 15 min. Vascular measurements were repeated to assess the vasocostrictror response to hypocarbria. The ventilatory rate was then reduced until arterial PaCO2 values returned to normal. Finally, in each rat, if any vessel had failed to dilate after ACh application, a third vasomotor challenge was conducted to determine whether the observed impairment could be related to smooth muscle dysfunction. In that EDR requires not only the production of EDRF by endothelium but also the generation of cGMP by vascular smooth muscle (15), we wished to examine vascular responses to another agent which, like EDRF, relaxes vessels by increasing smooth muscle cGMP but does not depend on endothelial function. For this purpose, vascular responses were measured before and after topical application of sodium nitroprusside (0.25 mg/ml cerebrospinal fluid; Sigma) (16). Following the nitroprusside test, the animals were perfused.

Histological Preparation. Following brain removal from the calvaria, the cortex and pia underlying the window were dissected free from the rest of the brain. Using the sketch of the pial vasculature beneath the window, the specific vessels studied functionally were identified (17), separated from the underlying cortex, and prepared for ultrastructural analysis. Pial arterioles were also sampled from the opposite hemisphere which had remained covered by bone throughout the experiment.

Data Analysis. The number of vessels studied functionally ranged from 6 to 16 arterioles/animal, depending on the number of vessels visible in the field. The total number of vessels examined was 71 in rIL-2-infused animals and 54 in controls. For each vessel, in each animal, the percentage of change in vascular diameter (percentage of base line) observed following ACh application was calculated. Then, for each animal, a mean and standard deviation for the percentage of change in diameter exhibited by all of the measured arterioles of that animal was determined. The means for the percentage of diameter changes exhibited by rIL-2-infused animals were then averaged and, using standard deviations derived for each animal, a pooled standard deviation and a standard error for the group were also calculated. The vasomotor data gathered following ACh application in control animals were then treated in the same manner. The mean ACh-induced response of the rIL-2-infused group was compared to the responses of the water-infused group by a 2-sample t test. The same procedure was followed in determining whether vasomotor responses to hyperventilation-induced hypocapnia differed between rIL-2-infused and control animals.

Additionally, the morphology of 12 vessels/group, from controls and rIL-2-infused animals, was examined in relation to the corresponding functional analyses.

RESULTS

Following femoral artery cannulation, mean arterial blood pressure levels for both animal groups were within normal limits throughout the surgical procedures and vasomotor assessments (Table 1). Through adjustments in ventilatory rate and stroke volume, arterial blood gas values were maintained within normal limits, except during hyperventilation (Table 1). Diameters of the vessels selected for vasomotor assessments ranged from 40 to 101 μm before ACh application.

In control animals, the normal vasodilator response of cerebral arterioles to topical Ach (9) was observed (Table 1; Fig. 1). Arteriolar diameters increased by 11 ± 2.8% (SE) of base line. Similarly, hyperventilation induced the normal cerebral vasodilator response (10, 11) to arterial hypocapnia, reducing arteriolar diameter by 12 ± 2.6% of base line (Table 1; Fig. 1).

Recombinant IL-2-infused rats showed altered vasomotor responses to both topically applied Ach and hypocapnia. Individually, cerebral vessels of rIL-2-treated rats showed considerable variation in their responses to Ach; only 7 of 70 vessels studied exhibited normal Ach-induced vasodilation. Other vessels either dilated minimally, did not change their diameter, or constricted. The calculated mean response of the 70 vessels studied following Ach exposure was a decrease in pial arteriolar diameter by 0.008 ± 2.3%, effectively zero (Table 1; Fig. 1). This response differed significantly from that observed in the control group (P < 0.01). After hyperventilation, the degree of vasoconstriction induced by arterial hypocapnia was also significantly reduced (P < 0.025) as compared to the control group (Table 1; Fig. 1). Following topical application of sodium nitroprusside, every vessel exhibited the normal vasodilator response, suggesting that the observed impairment of Ach-induced EDR was not due to an impaired ability of smooth muscle to generate cGMP.

Ultrastructural examination of pial vessels studied functionally revealed that the majority of vessels from both control and rIL-2-infused animals were morphologically normal, regardless of their responses to Ach or hyperventilation. This observation is consistent with findings of others who have shown loss of EDRF in the absence of ultrastructural change (18). Several pial arterioles from rIL-2-infused animals, however, did exhibit some endothelial cell and/or smooth muscle alterations (Fig. 2). In these vessels, endothelial cells with increased cytoplasmic lucency were occasionally observed, interspersed among morphologically normal endothelia. These cells were sometimes seen in close proximity to smooth muscle cells which also exhibited morphological abnormalities in the form of large electron-lucent cytoplasmic inclusions. Such alterations of cerebrovascular ultrastructure were reminiscent of those previously reported in studies of rIL-2-induced blood-brain barrier dysfunction (5, 6). Vessels harvested from the contralateral hemisphere, protected by bone throughout the experiment, showed a similar spectrum of morphological changes. Pial vessels from control animals exhibited no morphological abnormalities of either endothelial or smooth muscle cells.
CEREBRAL VASOMOTOR RESPONSES AFTER IL-2 INFUSION

Table 1 Effect of rIL-2 on pial arterioles

All values are means ± SEM. Responses were studied in 71 arterioles of 6 rIL-2-infused rats and in 54 arterioles of 7 control rats. Statistical comparisons of responses to Ach and hypocapnia were compared between the two groups by using t tests.

<table>
<thead>
<tr>
<th>Vessel diameter (µm)</th>
<th>Ach application</th>
<th>Hyperventilation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before (base line)</td>
<td>After</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>A. Control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel diameter (µm)</td>
<td>68 ± 2.5</td>
<td>75 ± 2.6</td>
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<tr>
<td>∆ diameter</td>
<td></td>
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<tr>
<td>Due to Ach, % of base-line value</td>
<td>11 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>105 ± 5.8</td>
<td>99 ± 11.4</td>
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<tr>
<td>PaCO₂ (mm Hg)</td>
<td>36 ± 2.5</td>
<td>40 ± 2.2</td>
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<tr>
<td>PaO₂ (mm Hg)</td>
<td>100 ± 16.5</td>
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</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.02</td>
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<tr>
<td>B. rIL-2-infused group</td>
<td></td>
<td></td>
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<tr>
<td>Vessel diameter (µm)</td>
<td>71 ± 1.9</td>
<td>71 ± 1.9</td>
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<tr>
<td>∆ diameter</td>
<td></td>
<td></td>
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<tr>
<td>Due to Ach, % of base-line value</td>
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<tr>
<td>MABP (mm Hg)</td>
<td>90 ± 4.3</td>
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<td>PaCO₂ (mm Hg)</td>
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</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.02</td>
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</tbody>
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* Statistically significant as compared to Ach response in control group (P < 0.01).
* Statistically significant as compared to hyperventilation response in control group (P < 0.025).

DISCUSSION

In summary, the results of the present study suggest that, secondary to multiple rIL-2 infusions in rats, there is impairment of cerebral vasomotor responses to physiological and experimental stimuli. A number of previous clinical and laboratory studies have similarly reported IL-2-related vascular effects including permeability increases, alterations of endothelial morphology and metabolism, and endothelial lysis (1, 2, 19–21). In some cases, these effects appeared to be directly attributable to IL-2 (19, 20), whereas, in others, host lymphoid cells have been implicated in IL-2-related vascular effects (21). This latter observation is supplemented by clinical and laboratory reports that rIL-2 can elicit the production of other soluble immune factors with vasoactive properties (22–28).

If cerebral vessels, like their peripheral counterparts, can be influenced by cytokines, as the present findings suggest, the consequences may have special significance for central nervous system function. Cerebral microvessels are equipped with special adaptations which strictly regulate not only blood-to-brain passage of circulating solutes but also regional cerebral blood flow. These adaptations exist for the purpose of maintaining optimal metabolic conditions within the neuronal/glial microenvironment. When conditions are such that these features are altered, the potential exists for adverse impact on neuronal function.

Among cerebral microvascular specializations is the capacity of small resistance vessels to change caliber in response to...
vations in systemic blood pressure or arterial content of O2 and/or CO2 (10, 29). Another potential participant in the regional regulation of cerebral blood flow is an endothelium-derived vasodilator the release of which can be experimentally elicited in vivo by topical 10-7 M ACh application (7). Impairment of both endothelium-dependent and -independent cerebral vasomotor responses has been demonstrated in a variety of pathological and experimental conditions (9, 11). In that, recently, cytokine exposure has also been shown impair vasomotor responses (30), it seemed especially timely and appropriate to undertake the present study.

As to potential mechanisms underlying the presently described vasomotor dysfunction, there is a variety of possibilities. One explanation involves the potential role of free oxygen radicals, well known to be mediators of cellular injury and vasomotor dysfunction (9, 11, 31). Support for this idea lies in reports by others that cytokines can enhance endothelial cell prostanoid synthesis (32, 33), a metabolic process during which free radicals are generated (34). Additionally, cytokines have been shown to enhance adhesion between endothelial cells and leukocytes (35), well known to induce vascular damage through free radical mechanisms (36). Furthermore, there is evidence that rIL-2-inducible cytokines can elicit superoxide anion release from endothelial cells themselves (37).

An additional explanation for the observed vasomotor dysfunction involves the potential role of TNF which we have observed to circulate at elevated levels in rats infused with rIL-2 (2). In that the anti-EDRF effect of TNF has been reported in feline cerebral arteries in vitro (30), it appears feasible that circulating TNF may play a role in the presently described in vivo cerebral vasomotor deficits.

Of potential interest, an early animal study of rIL-2-related toxicity reported that a component of the rIL-2 vehicle was found to contribute to rIL-2-induced histopathology and elevated serum liver enzymes (38). The vehicle formulation was subsequently modified to reduce the concentration of the component, and, as such, there have been no recent reports of vehicle-related toxicity. Comprehensive studies in our laboratory have, similarly, shown no alterations of cerebrovascular ultrastructure or permeability (6) and no circulating TNF3 following vehicle infusion in rats. We are, therefore, confident that vehicle effects do not underlie the presently reported vasomotor deficits.

In that pial arterioles contribute significantly to total cerebrovascular resistance (12), the observed dysfunction may have implications for cerebral blood flow in rats, and experiments to investigate this possibility are currently in progress. It may be more interesting, however, to speculate as to the potential clinical significance of the present findings. Patients undergoing high-dose rIL-2 treatment commonly develop a variety of hemodynamic changes including hypotension and decreased systemic vascular resistance (1, 39). It is feasible that such changes, compounded by cerebral vasomotor deficits, could predispose the brain to suboptimal blood flow levels in some brain regions (40), with potential metabolic consequences.

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