The effect of interleukin-2 on the blood-brain barrier in the 9L gliosarcoma rat model

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Carbon-14-labeled aminoisobutyric acid was used to determine local blood-to-tissue transfer constants in 22 Fischer rats with intracerebral 9L gliosarcomas that received either high-dose parenteral interleukin-2 (IL-2) or a control injection. In tumor and peritumoral tissue, the transfer constants in the IL-2-treated animals (89.6 ± 14.6 and 35.8 ± 6.0, respectively, mean ± standard error of the mean) were larger (p < 0.05) than in control animals (61.4 ± 6.4 and 14.6 ± 2.2, respectively). In contrast, in normal frontal and occipital tissue contralateral to the tumor-bearing hemisphere, there was no significant difference between the transfer constants in IL-2-treated and control animals. Furthermore, treatment of animals with IL-2 excipient caused no change in permeability as compared to animals treated with Hanks' balanced salt solution.

Parenteral injection of IL-2 increases blood-brain barrier disruption in tumor-bearing rat brain but does not increase the vascular permeability of normal brain. Methods to prevent this increased tumor vessel permeability are required before parenteral IL-2 can be used safely for the treatment of primary or metastatic brain tumors.

Key Words: interleukin-2 • immunotherapy • brain neoplasm • blood-brain barrier • rat

Despite an increased understanding of the biology of malignant gliomas, current treatments only modestly influence patient survival. Due to the limited efficacy of surgery, irradiation, and chemotherapy, numerous investigators have studied alternative approaches such as attempts to increase the host immune response to gliomas by active or passive therapy; however, these efforts have had limited success. Adoptive immunotherapy refers to the transfer to a tumor-bearing host of cells with antitumor reactivity that can mediate antitumor effects. Adoptive transfer of lymphokine-activated killer (LAK) cells plus interleukin-2 (IL-2), and treatment with high-dose IL-2 alone, mediates regression of certain cancers outside the central nervous system (CNS).

In Phase I studies of patients with primary and metastatic brain tumors, markedly increased cerebral edema has been observed in the region of the tumor after high-dose intravenous IL-2 therapy. If intravenous LAK/IL-2 therapy is to be used safely for gliomas, this effect requires further study both to determine its cause and to find a means to eliminate it. For these reasons, the effect of high-dose parenteral IL-2 on the blood-brain barrier (BBB) was studied in a commonly used rodent model that allows the simultaneous study of effects on normal and tumor-bearing brain tissue.

Materials and Methods

Tumor Model and Animal Preparation

Twenty-two adult male Fischer 344 rats, weighing 175 to 250 gm each, were anesthetized with intraperitoneal chloralhydrate and placed in a stereotaxic frame.* Each animal received a 4-μl injection containing 2 × 10⁵ 9L gliosarcoma cells in Eagle's minimal medium with glutamine into the right frontal white matter. Untreated animals in this model die approximately 22 days after injection. On Days 11 through 15 after tumor implantation, the animals received either IL-2 or control injections. After the final dose on Day 15, the animals were anesthetized by inhalation of halothane, nitrous oxide, and oxygen. Insertion of femoral arteriovenous fistulas and immobilization with plaster casts were performed. One milliliter of 2% Evans blue dye was injected intravenously. Blood gas determinations were made (range: pO₂ 81.8 to 111.8, pCO₂ 31.2 to 43.8, and pH 7.31 to 7.40), and body temperature was maintained at 36° to 37°C with a heat lamp. Thirty minutes after the Evans blue dye injection and after full recovery from anesthesia, carbon-14-labeled

* Stereotaxic frame manufactured by Narishige Scientific Instrument Laboratory, Tokyo, Japan.
alpha-aminoisobutyric acid (\(^{14}\)C-\(\alpha\)AIB) was injected intravenously, and rapid serial blood sampling (0.1 cc each) was performed for 10 minutes. The animals were then decapitated and the brains were removed rapidly for analysis.

**Experimental Groups**

Two groups of animals were studied. In the treatment group, 10 animals received intraperitoneal injections of a 2 \(\times 10^6\)-U/kg dose of IL-2 in 0.5 ml of Hanks' balanced salt solution (HBSS) three times daily from Day 11 through Day 15 after tumor implantation. This dose was determined by earlier lethal-dose studies on Fischer rats with intracerebral 9L gliosarcomas treated with IL-2 three times daily on Days 11 to 15 after implantation, based on the work of Donohue and Rosenberg 2 and Ettinghausen and Rosenberg. 7 With doses of 1.0 to 7.0 \(\times 10^6\) U/kg, there was progressive dose-related weight loss, diarrhea, and leathargy (JT Alexander, et al., unpublished data). At 1.0 \(\times 10^7\) U/kg three days a time for five days, one of two animals in these earlier studies died. The highly purified recombinant IL-2 used in these trials was produced in Escherichia coli transfected with the gene for IL-2 isolated from the Jurkat cell line. 17

In the control group, 12 animals received 0.5-ml intraperitoneal injections of IL-2 excipient (four animals) or HBSS (eight animals) on the same dose schedule as the treatment group. The injections of IL-2 excipient consisted of 0.5 ml of HBSS which contained the same amount of sodium dodecyl sulfate and mannitol that would be present in a 2-million U/kg dose of IL-2. (The precise amount of sodium dodecyl sulfate and mannitol is proprietary information and is not included in this manuscript.) All animals were weighed on Days 11 to 16.

**Permeability Assessment**

Aminoisobutyric acid (AIB) is a synthetic nonmetabolized neutral amino acid which enters brain cells readily, but slowly moves across brain capillaries. Carbon-14-labeled \(\alpha\)AIB, 40 to 60 mCi/mmol, was used to determine the blood-to-brain transfer constants. The isotopic purity was greater than 99%, and pH was adjusted to 7.4. Thirty minutes after Evans blue dye injection, the animals received bolus intravenous injections of AIB. Timed arterial blood samples were rapidly drawn and centrifuged; plasma \(^{14}\)C radioactivity was measured by beta scintillation counting. The brains were extracted within 1 minute after decapitation.

Sixteen animals were injected with 10 \(\mu\)Ci of AIB; in this group, isotope detection within the brain was determined by tissue solubilization and \(^{14}\)C-beta scintillation counting. Small samples of brain tissue were removed from each of five regions: tumor, brain around the tumor, right parietal white matter, left frontal white matter, and left occipital white matter. The tumor and surrounding brain tissue were identified by Evans blue tissue staining and the gross appearance of the tumor. The samples were placed in preweighed counting vials and reweighed. Sample solubilization, tissue beta counting, integration of the plasma radioactivity-time curve, and calculation of transfer constants for the different brain regions were performed as described previously. 1

Six animals (two IL-2-treated animals, two that received excipient, and two that received HBSS) were injected with 100 \(\mu\)Ci of AIB, and isotope detection was determined by quantitative autoradiography. This was performed to identify more precise regional changes that is allowed with tissue solubilization and \(^{14}\)C-beta scintillation counting. The extracted brains were frozen in liquid Freon that was cooled to \(-40^\circ\)C. By methods described previously, 1 tissue sections were prepared for histological study and quantitative autoradiography, and measurements of unidirectional blood-to-brain transfer constants were obtained in the five brain regions cited above.

Values obtained from the 16 animals analyzed with tissue solubilization and \(^{14}\)C-beta scintillation counting were used in the statistical comparisons. The Wilcoxon two-sample rank-sum test of independent samples 3 was used to compare transfer constants from the treatment and control groups. In addition, transfer constants between the excipient- and HBSS-injected animals were compared.

**Results**

**Tissue Staining**

In all rats, tumors in the right hemisphere were deeply stained with Evans blue dye; peritumoral tissue was also stained, but less deeply. In the control (left) hemisphere, there was no staining in the IL-2-treated, control-excipient, or control-HBSS groups (Fig. 1).

**Transfer Constants**

The transfer constants are listed for all experimental groups in Table 1. As described previously for other brain-tumor models, 14 the transfer constants within the tumor are highly variable. In tumor, brain around the tumor, and right parietal white matter, the vascular permeability (transfer constant) of the IL-2-treated group was greater than that of corresponding sites in the control group (p < 0.05). In left frontal and occipital white matter, there was no significant difference between the IL-2-treated and the control groups. In the control group, there were no differences in vascular permeability in the five anatomical regions between the excipient-injected animals and the HBSS-injected animals.

**Animal Weights**

The IL-2-treated rats weighed 181 \(\pm\) 14 gm (mean \(\pm\) standard deviation) at the start of treatment and 183 \(\pm\) 13 gm at the end of treatment. Animals in the control...

FIG. 1. Macroscopic histological sections stained with hematoxylin and eosin (A and C) showing intracerebral 9L tumors in rats treated with either high-dose interleukin-2 (IL-2) (A) or excipient (C). Corresponding images of regional transfer constants are coded to a range of specific values (B and D). The tumor border is outlined by the dotted line in all images. Note the higher transfer constant in the intracerebral tumor of the IL-2-treated animal (A and B); the mean tumor rate constant in this image is $87 \, \text{mg/gm/min} \times 10^{-3}$. Also note the absence of $^{14}$C-AIB activity (that is, blood-brain barrier disruption) in the non-tumor-bearing hemisphere of both animals.

group weighed $202 \pm 22 \, \text{gm}$ at the onset of treatment and $213 \pm 25 \, \text{gm}$ at completion.

Discussion

Since immunotherapy has the potential of being highly tumor-specific, it has been used in various forms to investigate antitumor activity in brain-tumor patients; however, treatment of glioma patients with adoptive immunotherapy by transfer of autologous lymphocytes, α-interferon, Corynebacterium parvum, and levamisole has shown limited success. Recently, methods have been developed to generate lymphocytes that destroy fresh autologous tumor cells, but not normal cells, by incubating human peripheral blood lymphocytes with the lymphokine-activated IL-2. Adoptive immunotherapy with intravenous IL-2 and/or with activated lymphocytes has efficacy in vitro against rodent and human gliomas and in vivo against certain non-CNS cancers. It is currently being evaluated for treatment of malignant brain tumors in several clinical centers but has not yet shown antitumor activity in animal models or in patients.

The best route for administration of LAK cells and IL-2 for optimal efficacy against brain tumors remains unclear. The effectiveness of intratumoral administration of LAK cells and IL-2 might be limited by the absence of LAK-cell migration, or IL-2 diffusion, through brain parenchyma. In addition, LAK cells require continued exposure to IL-2 to maintain optimum antitumor activity, and increased cerebral edema has been observed in glial patients who received repeated IL-2 injections into brain-tumor cavities or directly into brain tumors (Barba D, Saris SC, Holder C, et al. J Neurosurg 70 (In press, 1989)). Furthermore, although recent studies by Jacobs, et al., Ingram, et al., and R Merchant (unpublished data, 1988) have shown minimal toxicity following single-dose intratumoral injection of LAK cells and IL-2, efficacy via this route of administration has not been demonstrated. In general, intravenous delivery of anticancer drugs is less invasive and more effective than arterial or intratumoral administration. Although penetration by LAK cells or IL-2 across the blood-tumor barrier has not been reported, intravenous injection assures delivery to all intratumoral vessels, and potentially to all regions of tumor.

We have treated patients with primary and metastatic brain tumors with high-dose intravenous IL-2 in a Phase I trial, and observed increased treatment-induced cerebral edema in the region of the tumor but not in the contralateral hemisphere (SC Saris, et al., unpublished data).
The effect of interleukin-2 on the blood-brain barrier

TABLE 1
Transfer constants for all anatomical areas in all groups*

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Tumor</th>
<th>Brain Around Tumor</th>
<th>Rt Parietal White Matter</th>
<th>Lt Parietal White Matter</th>
<th>Lt Frontal White Matter</th>
<th>Lt Occipital White Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>89.6 ± 14.6 †</td>
<td>35.8 ± 6.0 †</td>
<td>19.2 ± 4.6 †</td>
<td>11.4 ± 1.7 †</td>
<td>8.3 ± 1.8 †</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>61.4 ± 6.4 †</td>
<td>14.6 ± 2.2 †</td>
<td>7.9 ± 0.6 †</td>
<td>6.2 ± 1.3 †</td>
<td>9.5 ± 2.2 †</td>
<td></td>
</tr>
<tr>
<td>control-excipient ‡</td>
<td>72.1 ± 6.3</td>
<td>8.4 ± 3.4</td>
<td>5.3 ± 2.1</td>
<td>6.6 ± 2.9</td>
<td>4.5 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>control-HBSS ‡</td>
<td>61.3 ± 5.7</td>
<td>12.0 ± 8.1</td>
<td>6.4 ± 1.0</td>
<td>4.2 ± 0.5</td>
<td>8.6 ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

* All values represent the mean ± standard error of the mean for the transfer constants in each anatomical area for each group. There were eight rats in the interleukin-2 (IL-2) group, eight in the control group, four in the control-excipient group, and eight in the control-Hank’s balanced salt solution (HBSS) group.
† Significant difference between these two groups: p < 0.05.
‡ Quantitative autoradiographic data are included.
at nontoxic doses, IL-2 might be a means to increase the delivery of certain chemotherapeutic agents to brain tumors, but not to normal brain.

We also demonstrated the absence of increased permeability in animals injected with the IL-2 excipient, which contains a small amount of mannitol and sodium dodecyl sulfate. This finding agrees with the results of Rosenstein, et al., in mice, but it disagrees with those of Ellison, et al., who described increased cerebral vessel permeability in four of six cats after bolus intravenous injection of IL-2 excipient. If increased permeability due to excipient is observed in patients, different vehicle formulations will be needed to eliminate this undesirable side effect.

Immunotherapy with LAK cells is appealing because of their ability to recognize and selectively destroy glioma cells. However, infiltration of these cells into brain tumor (after local or intravenous administration) in sufficient numbers to cause tumor regression remains to be demonstrated. In addition, the adoptive agent (IL-2) which transforms lymphocytes into killer cells produces undesirable alterations in vascular permeability. When this side effect causes pulmonary edema or pre-renal azotemia, medical treatment that does not mitigate against the benefits of treatment can be used. However, when symptomatic cerebral edema occurs, the best treatment (steroids) diminishes the antitumor activity of the immunotherapy. These problems will need to be overcome before LAK/IL-2 immunotherapy can be safely used to treat intracranial tumors.

References


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