The effect of intravenous interleukin-2 on brain water content

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Parenteral treatment with interleukin-2 (IL-2) is effective against certain advanced cancers outside the central nervous system. Prior to commencement of Phase II trials in patients with brain tumors, the neurological and neuroradiological features of 10 patients treated with intravenous administration of repeated doses of IL-2 were studied. Three patients had malignant gliomas, and seven patients had extracranial cancer without evidence of intracranial metastasis. All were treated with intravenous doses of $10^5$ U/kg three times daily for up to 5 days. The patients with gliomas received cranial computerized axial tomography (CT) scans before IL-2 therapy was initiated and during the later stages of treatment. The patients with extracranial cancer underwent T2-weighted magnetic resonance (MR) imaging before and later during therapy.

After two to 11 doses of IL-2, the patients with gliomas had marked neurological deterioration that was associated with a mild to marked increase in peritumoral edema and mass effect visible on CT scans. With cessation of treatment and appropriate supportive care, all returned to their pretreatment state. The patients with extracranial cancer were either neurologically unchanged or underwent minor transient changes in mental status (lethargy and confusion). In these patients, the MR signal intensity was quantified and compared in eight anatomic regions of interest. In six of the seven patients, there were increases in gray and white matter signal intensity consistent with increased cerebral water content. The percentage changes (means ± standard error of the means) were 12.6% ± 7.3% in the gray matter and 17.0% ± 6.2% in the white matter.

This study demonstrates that treatment with a high parenteral dose of IL-2 is not tolerated by patients with gliomas due to increased cerebral edema. In patients with extracranial cancer but no brain disease, parenteral IL-2 induces an increase in the cerebral water content of both gray and white matter.

KEY WORDS • interleukin-2 • immunotherapy • cerebral edema • blood-brain barrier • cancer • brain neoplasm

SINCE an important advantage of immunotherapy is its potential for high tumor specificity, it has been investigated in various forms to determine antitumor activity in patients with brain tumors. However, numerous efforts with adoptive agents such as autologous lymphocytes, alpha-interferon, and levamisole have shown limited efficacy.10-12,22 Recently, methods have been developed to generate lymphokine-activated killer (LAK) cells that destroy fresh autologous tumor cells but not normal cells by incubating human peripheral blood lymphocytes with the lymphokine interleukin-2 (IL-2).19 In vitro, LAK cells are cytotoxic against rodent and human gliomas,4 and in vivo the administration of IL-2 alone or in conjunction with LAK cells prolongs survival in rodents with primary central nervous system (CNS) cancers.21,22 Adoptive immunotherapy is currently being evaluated for treatment of malignant brain tumors in several clinics, but has not yet been shown to have antitumor activity.

The best route of administration of LAK cells and IL-2 for the treatment of brain tumors is unknown. The effectiveness of intratumoral administration of LAK cells and IL-2 might be limited by the absence of LAK cell migration15 or IL-2 diffusion through brain parenchyma. To maintain optimum antitumor activity, LAK cells require continued exposure to IL-2 and,
although IL-2 levels in cerebrospinal fluid after intravenous administration are as high as 9 U/ml, it is unknown if these levels are attained in brain tissue. Previous reports emphasize the minimal toxicity of single-dose intratumoral administration, but no efficacy has been shown. Furthermore, this form of regional therapy, although theoretically advantageous for certain tumors and drugs, has not yet been shown to be curative for tumors in any organ.

Intravenous delivery of anticancer drugs is less invasive and often more effective than arterial or intratumoral administration. Although the penetration of LAK cells and IL-2 across the blood-tumor barrier has not been demonstrated, intravenous injection assures delivery to all intratumoral vessels, and potentially to all regions of tumor. For these reasons, parenteral administration of IL-2 and LAK cells may be efficacious; however, the tolerance to such therapy of patients with brain tumors remains unknown. The dose-limiting toxicity of IL-2 therapy for extracranial cancer includes increased vascular permeability which occurs in the lungs, kidneys, and other non-CNS organs, and it has been postulated that this vascular leak syndrome is the cause of transient mental status changes observed in many patients treated with IL-2. Alterations in brain water content induced by IL-2 treatment have not been determined in patients or animal models, but such information might implicate IL-2-elicited changes in causing cerebral vessel permeability. To assess toxicity before Phase II trials, and to determine if IL-2 induces alterations in cerebral water content, the clinical and neuroradiological effects in cancer patients of high intravenous doses of IL-2 were studied.

**Clinical Material and Methods**

**Patient Population**

Ten patients received IL-2 as part of National Institutes of Health Protocol 86-C-197 (Table 1). The recombinant IL-2 used in this trial was produced in Escherichia coli bacteria transfected with the gene for IL-2 isolated from the Jurkat cell line. After a pretreatment enhanced computerized tomography (CT) scan, three patients with persistent or recurrent malignant gliomas were admitted to an intensive care unit where they received intravenous IL-2 (10^5 U/kg/dose) three times daily after pretreatment T2-weighted magnetic resonance (MR) imaging. Follow-up T2-weighted MR images with similar technique were obtained after six to 11 doses of IL-2. Daily body weights, blood gas measurements, and serum chemical data were obtained in all patients. None of these 10 patients were taking steroids of any kind prior to or during treatment with IL-2. All IL-2 doses, regardless of total dose, were mixed in 50 ml of 5% salt-poor albumin.

**Computerized Tomography**

Pretreatment CT scans were performed after intravenous administration of contrast material on a third-generation CT scanner (GE 8800 or 9800). Subsequent CT scans were obtained during treatment at the time of neurological deterioration and were performed without intravenous infusion of contrast medium due to the prerenal azotemia that occurs in a large number of patients treated with IL-2. A neurosurgeon (S.C.S. or J.T.A.) and a neuroradiologist (N.J.P.) reviewed these scans to assess treatment-related abnormalities. The amount of cerebral edema was evaluated by the area of peritumoral hypodensity, the degree of compression of the adjacent ventricle, and the degree of shift of the midline structures.

**Magnetic Resonance Imaging**

The T2-weighted MR images were obtained on a 0.5-tesla unit. In each patient, the parameters (time of repetition, echo time, and background noise) were the same for the pre- and posttreatment images. Analysis of the MR images was done in an axial plane that passed through the centrum semiovale and the top of the lateral ventricles. A 0.3- or 0.4-sq cm circular region of interest was placed in eight anatomic areas to quantify changes in the MR signal intensity of the pre- and posttreatment images in a blinded manner. These areas

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>No. of IL-2 Doses</th>
<th>Neurological Change</th>
<th>CT/MR Study†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>glioma</td>
<td>2</td>
<td>stupor, hemiplegia</td>
<td>CT</td>
</tr>
<tr>
<td>2</td>
<td>glioma</td>
<td>8</td>
<td>stupor, hemiplegia</td>
<td>CT</td>
</tr>
<tr>
<td>3</td>
<td>glioma</td>
<td>11</td>
<td>lethargy, hemiparesis, seizure</td>
<td>CT, MR</td>
</tr>
<tr>
<td>4</td>
<td>colon cancer</td>
<td>6</td>
<td>none</td>
<td>MR</td>
</tr>
<tr>
<td>5</td>
<td>colon cancer</td>
<td>6</td>
<td>lethargy, confusion</td>
<td>MR</td>
</tr>
<tr>
<td>6</td>
<td>melanoma</td>
<td>11</td>
<td>none</td>
<td>MR</td>
</tr>
<tr>
<td>7</td>
<td>renal cancer</td>
<td>6</td>
<td>none</td>
<td>MR</td>
</tr>
<tr>
<td>8</td>
<td>melanoma</td>
<td>9</td>
<td>lethargy</td>
<td>MR</td>
</tr>
<tr>
<td>9</td>
<td>renal cancer</td>
<td>7</td>
<td>none</td>
<td>MR</td>
</tr>
<tr>
<td>10</td>
<td>melanoma</td>
<td>7</td>
<td>none</td>
<td>MR</td>
</tr>
</tbody>
</table>

* Number of doses of interleukin-2 (IL-2) (10^5 U/kg/dose three times daily for 5 days) the patient had received when follow-up imaging was performed.
† Follow-up computerized tomography (CT) or magnetic resonance (MR) imaging.
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were the right and left sides of the frontal cortex, the frontal white matter, the parietal cortex, and the parietal white matter. In each patient, five values were recorded in each anatomic region of interest, and the mean value was determined.

Each patient was analyzed as his or her own control. The change in signal intensity was determined by the following formula:

\[
\frac{\text{posttreatment signal intensity} - \text{pretreatment signal intensity}}{\text{pretreatment signal intensity}} \times 100.
\]

Results

Glioma Patients

In the three glioma patients, IL-2 treatment was associated with profound neurological deterioration. Except for minor weakness and reflex asymmetry, all patients had normal neurological examinations before treatment. However, after two and eight doses, respectively, two patients (Cases 1 and 2) became stuporous and hemiplegic; after 11 doses, the third patient (Case 3) became lethargic and hemiparetic. At the time of neurological worsening, unenhanced CT scans were obtained (Fig. 1) and IL-2 therapy was stopped. In Cases 1 and 3, complete or nearly complete recovery occurred over the next few days; the only intervention was the administration of mannitol. In Case 2, mannitol alone produced no improvement; the patient was treated with dexamethasone and made a complete recovery over the subsequent 2 months. In no patient was there a significant change in arterial blood gas values or electrolyte levels; however, two patients (Cases 1 and 3) had a weight gain of 5 kg by the time of neurological worsening, and the third patient (Case 2) had gained 0.5 kg.

At the time of neurological decline, CT scans demonstrated an increase in both cerebral edema and mass effect from the area of the brain containing the tumor (Fig. 1). After treatment of Case 1, subfalcian herniation occurred and the lateral ventricle on the side of the tumor was compressed. Despite return to her pretreatment neurological baseline level, continuing edema was observed on a CT scan 1 month after treatment. In Case 2, a less severe increase in edema and mass effect from the tumor site was noted. Despite the CT appearance, the patient suffered the worst and most prolonged neurological decline of the series. In Case 3, increased edema (also seen on MR imaging) and mass effect from the area of brain containing the tumor were also demonstrated.

There was no significant tumor response in the three glioma patients. Two patients (Cases 1 and 3) died 5
and 8 months after treatment, respectively. The remaining patient (Case 2) who was not taking dexamethasone had a decrease in tumor enhancement 3 months after treatment; however, she died after an automobile accident 5 months later.

**Extracranial Cancer Patients**

In the seven patients with extracranial cancer, with the exception of mild nonfocal neurological signs, there was no significant change in the neurological examination. One patient (Case 5) was mildly lethargic and disoriented toward the end of treatment and two other patients (Cases 8 and 9) were only mildly lethargic.

In six of seven patients, IL-2 treatment was associated with an increase in MR signal intensity in both the gray and white matter (Table 2). In 45 of 56 comparisons (eight in each patient), MR signal intensity increased after treatment with intravenous IL-2. The percentage change varied from 3% to 48%. The mean change (± standard error of the mean) was an increase of 12.6% ± 7.3% in the gray matter and an increase of 17.0% ± 6.2% in the white matter.

### Discussion

In this study, the effect of intravenous IL-2 therapy on cerebral water content was examined. Water flux into brain tissue results from the interplay between hydrostatic and osmotic pressures. At the arterial end of the capillary system, hydrostatic forces predominate and cause bulk flow of water and electrolytes into the interstitium. At the venous end of the capillary system, osmotic forces predominate and draw fluid back into the vascular space. Hydrostatic forces are largely a function of blood pressure, and osmotic forces are largely a function of the permeability of the cerebral vessels to solutes in the blood, particularly large proteins such as albumin. Excessive water flux into the brain (cerebral edema) is generally caused by alterations in vessel permeability (vasogenic edema) or injury to brain cells (cytotoxic edema). In vasogenic edema, increased vessel permeability results in flux of solutes and then water into the brain interstitium. In cytotoxic edema, injured cells draw water from the interstitium; additional water then moves from the vascular space into the hypertonic extracellular space.

An increase in cerebral edema occurred, causing neurological deterioration in three patients with gliomas who were treated with high-dose intravenous IL-2. One possible explanation is that IL-2-induced increases in tumor vessel permeability altered osmotic pressures in the arterial end of the capillary system to create additional water flux into the interstitium. In studies of 9L gliosarcoma-bearing rat brain, an elevation in the permeability of tumor and peritumoral tissue vessels to 14C-aminoisobutyric acid as compared to controls has been demonstrated. All animals treated with high doses of intraperitoneal IL-2 had a 46% increase in the permeability of tumoral vessels and a 245% increase in the permeability of peritumoral vessels. These data correlate well with the clinical observations reported here in which the region of tumor involvement developed increased swelling and mass effect. Water flux into the tumor-bearing hemisphere may have occurred secondary to an increase in vessel permeability that allowed movement of osmotically active solutes across the blood-tumor barrier. In glioma patients, this additional water could produce increased edema and new or increased neurological deficits.

A second possible explanation relates to the increase in total body water content that is commonly caused by treatment with intravenous IL-2. Most cancer patients become hypotensive during therapy, probably due to decreased systemic vascular resistance and movement of intravascular fluid into the interstitium because of leaky blood vessels. Treatment requires infusion of large amounts of fluid which causes an increase in body weight of 6% to greater than 18%. Two of the three glioma patients in this study had a weight gain of 5 kg during treatment. It is possible that this increase in total body water resulted in fluid movement across an impaired blood-tumor barrier to cause increased cerebral edema, mass effect, and neurological deterioration. However, it should be noted that the patient with the greatest neurological deficit had only gained 0.5 kg at the time of deterioration.

A third, but unlikely, possible explanation is that increased hydrostatic pressures contributed to the increase in cerebral water content. The systemic arterial pressure decreases in almost all patients who receive IL-2, and none of the three glioma patients had increased systemic blood pressure during IL-2 treatment.

The poor tolerance of glioma patients to high-dose intravenous IL-2 administration limits its potential use as a treatment for malignant primary brain tumors. Steroid administration could be used to diminish the increased cerebral edema; however, in animal models, treatment with dexamethasone markedly decreases the antitumor efficacy of LAK cells. An alternative is to lower the IL-2 dose; however, experience in animals has demonstrated that lower doses are associated with reduced tumor responses. Since IL-2 has an extremely rapid total body clearance, pharmacokinetic advantage may be obtained by arterial infusion; however, it is unknown whether IL-2 delivery to brain tissue by an

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**TABLE 2**

*Mean change in MR image brain signal intensity after intravenous IL-2 therapy*  

<table>
<thead>
<tr>
<th>Brain Tissue</th>
<th>Case No.</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>gray matter</td>
<td>-16</td>
<td>+3</td>
<td>+42</td>
<td>+27</td>
<td>+4</td>
<td>+4</td>
<td>+24</td>
<td></td>
</tr>
<tr>
<td>white matter</td>
<td>+3</td>
<td>+12</td>
<td>+48</td>
<td>+21</td>
<td>+6</td>
<td>+3</td>
<td>+26</td>
<td></td>
</tr>
</tbody>
</table>

* Each number represents the percentage change in magnetic resonance (MR) signal intensity from the pretreatment to the posttreatment MR imaging. IL-2 = interleukin-2.
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intracarotid injection would be more efficacious than by intravenous injection. Similarly, the efficacy of intraventricular and intratumoral infusion remains to be shown and may be limited by problems of LAK cell transport to tumor cells, and by limited IL-2 diffusion through brain tissue to maintain LAK cells in a maximally tumoricidal state. In addition, it has recently been observed that repeated intratumoral or intracavitary doses of IL-2 consistently elicit regional brain swelling and focal neurological deficits (D Barba, unpublished data).

Intravenous IL-2 therapy was also associated with a variable increase in the signal intensity of normal gray and white matter. Signal intensity is proportional to unbound parenchymal water, and was increased by a mean of 12.6% and 17.0% in the gray and white matter, respectively. The increases ranged from 3% to 12% in Cases 4, 5, 8, and 9, and from 21% to 48% in Cases 6, 7, and 10. In animal models, IL-2-related changes in brain vessel permeability that could lead to increased brain water have been studied. Alexander, et al., observed no alteration in the permeability of normal rat brain to 14C-aminoisobutyric acid after 5 days of intraperitoneal therapy. However, Rosenstein, et al., studied the penetration of 125I-labeled albumin into normal mouse brain after intraperitoneal IL-2 injections and observed after 6 days an eightfold increase in the amount of intracerebral albumin as compared to control findings. Ellison, et al., performed anatomic studies in eight cats that received intravenous horseradish peroxidase (HRP) followed by a single dose of IL-2 (10^5 U/kg). They reported blood-brain barrier disruption in all animals, as demonstrated by HRP penetration into normal brain parenchyma. Similar increases in permeability, rather than changes in hydrostatic pressures, may have contributed to the increased water content in our patients.

A second possible explanation for the increased intracerebral water content is that IL-2 had a cytotoxic effect on the parenchyma of the brain. This could cause cellular swelling that would increase the cerebral water content and MR signal intensity. It has been demonstrated that IL-2 penetrates into the cerebrospinal fluid to levels of 3 to 10 U/ml. If comparable tissue levels occur in the brain, it is unknown whether cytotoxic effects on glial or neuronal cells would occur.

A third possible explanation is that the IL-2 excipient caused an increase in vessel permeability. Ellison, et al., have reported that a single intravenous dose of IL-2 excipient increases HRP penetration into normal dog brain. However, Alexander, et al., studied the effect of intraperitoneal IL-2 excipient on the penetration of 14C-aminoisobutyric acid into normal rat brain, and observed no change in vessel permeability. This problem might be resolved with MR imaging of human or subhuman primates before and after treatment with IL-2 excipient.

The significance of the increased brain signal intensity in the patients with extracranial cancer is unknown.

Denicoff, et al., reported a high incidence of reversible alterations in mental status in patients treated with high-dose intravenous IL-2, and proposed that these changes were due to alterations in the blood-brain barrier that allowed penetration of encephalopathic solutes into the brain. Our data do not support this relationship between altered permeability and mental status changes, as the increases in MR signal intensity in our patients were not related to encephalopathy. The patients with the largest changes in signal intensity (Cases 6, 7, and 10) showed no changes in mental status. In the patients with minor changes in mental status (Cases 5 and 8), there were only minor increases in MR signal intensity (3% to 12%). Therefore, although flux of water and possibly solute into the interstitium occurred, it was not in sufficient amounts to cause an alteration in the neurological examination.

In summary, adoptive immunotherapy with IL-2 and LAK cells has been demonstrated to have antitumor activity in patients with certain systemic tumors such as melanoma and renal-cell cancer. However, cancers of the brain present special and difficult problems such as poor tolerance of cerebral tissue to increased interstitial water content and poor penetration of many antitumor agents across the blood-tumor and blood-brain barriers. Due to increased brain edema and production of clinically significant deterioration in neurological function, the administration of high-dose intravenous IL-2 will not be a potential treatment for glioma patients unless the lymphocyte-activating function of IL-2 can be separated from the activity that produces increased vascular permeability. Furthermore, intravenous IL-2 administration causes an increase in gray and white matter water content of patients with no detectable CNS tumors.

References

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