Toxicity studies of retroviral-mediated gene transfer for the treatment of brain tumors

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Retroviral-mediated transfer of the herpes simplex virus thymidine kinase (HSVtk) gene into malignant tumors confers drug susceptibility to the antiviral drug ganciclovir. The authors have recently shown that in situ transduction of the rat 9L brain tumor following HSVtk-producer cell implantation led to tumor regression after ganciclovir administration in treated rats. A wide spectrum of potential adverse effects may, however, be associated with the application of this approach to treat brain tumors, including dissemination of the retroviral vector to nontumoral tissues within or outside the central nervous system, proliferation of the injected murine vector-producer cells at the injection site, immune-mediated responses to the implantation of xenogeneic cells, and damage to the brain from toxic by-products of the HSVtk-ganciclovir interaction. These possibilities were investigated using intracerebral and systemic injections of retroviral vector-producer cells carrying the HSVtk or the lacZ gene in mice, rats, and nonhuman primates.

Using the lacZ gene as a reporter gene, no evidence of β-galactosidase activity consistent with vector transduction was detected in any major body organ in the treated mice or rats. Similarly, the HSVtk gene transfer did not result in toxicity, with or without ganciclovir administration. In studies using rat and monkey models, no proliferation of the vector-producer cells occurred after intracerebral injection. Vector-producer cell survival was limited to 7 to 14 days. High-dose steroid therapy did not appear to extend the survival of these xenogeneic cells in rats. No significant inflammatory response was observed in the meninges or brain parenchyma. Endothelial cells were occasionally transduced in brain capillaries adjacent to the injected site of the vector-producer cells. Injection of producer cells into brain tissue elicited mild edema and reactive gliosis surrounding the injection site, which were probably the cause of a transient toxic response arising 4 to 5 days following injection of the producer cells; short-term administration of dexamethasone eliminated that response.

No neurological deficits were observed in the rats or primates treated with the HSVtk vector-producer cells, with or without ganciclovir. In primates injected with producer cells, magnetic resonance imaging before, during, and after ganciclovir administration showed minimal and localized breakdown of the blood-brain barrier without significant edema or mass effect. Similarly, histological examination of the monkeys' brains showed no damage to neurons, astroglia, or myelin. Long-term clinical (>9 months) and radiological (3 months) assessment of the primates has revealed no evidence of toxicity. The results of these studies indicate that intratumoral implantation of HSVtk-producer cells can be attempted for the treatment of brain tumors, without anticipating significant adverse toxicity to normal brain or remote proliferating tissues.

**KEY WORDS** • retroviral vector • gene transfer • brain neoplasm • ganciclovir • thymidine kinase • toxicity • mouse • rat • primate

The transfer of drug-susceptibility genes by retroviral vectors into malignant tumors has recently been proposed as a novel approach for the treatment of cancer. The potential of the herpes simplex virus thymidine kinase (HSVtk) gene to confer sensitivity to the antiviral drug ganciclovir and subsequently to lead to tumor regression has been shown in various animal tumor models. We have recently demonstrated the efficacy of this approach in eradicating brain tumors by direct intratumoral injection of HSVtk vector-producer cells and systemic ganciclovir therapy. Producer cells are derived from murine fibroblasts (NIH3T3 cells) that have been engineered to produce replication-incompetent murine retroviral vectors. These producer cells continuously produce retroviral vectors in the vicinity of the injection site within
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the tumor. The vectors integrate the foreign genes into
the genome of the tumor cells only during active de-
oxynucleic acid synthesis. The resultant gene pro-
duct will only then be expressed by the host tumor cell.
The survival of these murine producer cells when in-
jected into the brain and the host immune response
with and without ganciclovir therapy are unknown.

A wide spectrum of potential adverse effects may be
associated with the clinical application of this approach
to treat brain tumors. First, an immune-mediated
meningoencephalitis may complicate inoculation of xen-
genetic cells into the brain. Second, there may be local
transduction of normal slowly proliferating brain tissue,
such as endothelial and glial cells, that could lead to
deleterious side effects with exposure to ganciclovir.
Third, escape of the retroviral vector into the systemic
circulation may lead to integration of the HSVtk gene
into normal proliferating tissues, such as the bone mar-
row and epithelium of the gastrointestinal tract, and
produce organ damage. We evaluated these potential
toxicities in murine, rat, and nonhuman primate models
and examined the survival of murine producer cells
in rat and monkey brains, with and without dexa-
methasone therapy.

Materials and Methods

Vectors and Cell Cultures

The 3-galactosidase (G1B5V/S.29) and HSVtk (G1-
TkSVNa.53) gene vectors were used in our studies,* and
the G1 backbone of these vectors was derived from the
Mooney murine leukemia virus. The G1TkSVNa.53
vector contains the herpes simplex thymidine kinase
 gene just downstream of the 5' long terminal repeat,
which it uses as its promoter. The simian virus 40 early
promoter serves as an internal promoter for the neo-
mycin phosphotransferase gene NeoR, which confers
resistance to the neomycin analog G418. In the 3-gala-
tosidase vector, the lacZ gene replaces the HSVtk gene.
The HSVtk and 3-galactosidase vectors are packaged
by the amphotropic retroviral-producer cell lines
FAT 2.4 and PA317, respectively. The G1B5V/S.29-
 producer cells used for these studies had a NeoR tit-
er of 0.5 x 10^9 to 1.0 x 10^10 colony-forming units
(CFU)/ml. The G1TkSVNa.53-producer cell line gen-
erated an HSVtk titer of 0.5 x 10^9 CFU/ml. Both cell
lines were negative for replication-competent virus by
S+/L- assay.

The cloned vector-producer cell lines were main-
tained in Dulbecco's modified Eagle's medium with
10% fetal bovine serum, 2 mM L-glutamine, penicillin
(50 U/ml), streptomycin (50 µg/ml), and Fungizone
(ampotericin B, 25 µg/ml). The vector-producer cells
were grown in T-175 flasks. For in situ gene transfer,
the medium was removed and the cells were rinsed with
saline. The monolayer was then incubated in 0.05%
trypsin ethylenediamine tetra-acetic acid for 5 to 10
minutes at 37°C. The cells were collected in Hanks'
balanced salt solution, washed twice, and resuspended

at 5 x 10^5 to 1 x 10^6 cells/ml for injection. In some
experiments, NIH3T3 cells that had been pretransduced
with the 3-galactosidase gene (3T3 3-galactosidase non-
 producer cells) were used. These non-producer cells
were obtained by transferring supernate from a confluent
tissue culture of 3-galactosidase-producer cells onto
an NIH3T3 cell culture. The transduced cell lines were
then selected in G418 medium (1.0 mg/ml) for 7 days.

Ganciclovir Administration

Ganciclovir was administered intraperitoneally into
mice (150 mg/kg twice a day) and rats (15 mg/kg twice
a day) and intravenously into primates (10 mg/kg/day
in a single dose). The primates dose was equivalent to
that used in human patients treated for cytomegalovirus
retinitis.

Histochemical Staining

Expression of 3-galactosidase was detected using an
X-gal histochemical stain. Staining the brain with X-
gal turns 3-galactosidase-expressing cells blue when an
indolyl is liberated from X-gal by the action of 3-ga-
tactosidase, and subsequent oxidation and self-cou-
pling forms an indigo blue derivative. Thus, the vector-
containing cells can be discriminated from unmodified
cells with light microscopy. Tissue sections were either
stained with hematoxylin and eosin (H & E) alone or
counterstained with H & E following X-gal staining to
enhance detection of blue-staining cells.

Protocols and Results

Toxicity Studies in Mice

Intraperitoneal Administration of Vector-Producer
Cells. The potential toxic effects of the direct injection
of vector-producer cells into the peritoneal cavity were
evaluated using adult C57BL/6 mice, each weighing 16
to 20 gm. Twelve mice received an intraperitoneal
injection of 5 x 10^4 HSVtk vector-producer cells and
12 received an intraperitoneal injection of 5 x 10^5
3-galactosidase-producer cells. The mice were observed
for 7 days, with no evidence of toxicity, and then six
mice in each group received ganciclovir (150 mg/kg
intraperitoneally twice a day) for 7 days. Six mice from
each group were sacrificed on Day 15 after cell injection.
Microscopic histological sections of the lungs, spleen,
thymus, liver, small and large intestine, and bone mar-
row revealed no evidence of necrosis, inflammation, or
X-gal-positive staining cells (Table 1).

Intravenous Administration of Vector-Producer
Cells. The toxicity of intravenous injection of vector-
producer cells was also evaluated using C57BL/6 mice.
These cells are trapped in the lungs and may result in
transduction of the respiratory epithelium and diffuse
destruction of lung parenchyma after ganciclovir ad-
ministration. Twelve mice received 5 x 10^6 HSVtk
vector-producer cells via the lateral tail vein, and an
intravenous injection of 5 x 10^6 3-galactosidase vector-
producer cells was given to 12 control mice. The mice

* Vectors generously provided by Genetic Therapy, Inc.,
Gaithersburg, Maryland.

† Ganciclovir supplied by Syntex Laboratories, Inc., Palo
Alto, California.
were observed for 7 days for evidence of toxicity and then received ganciclovir (150 mg/kg intraperitoneally twice a day) for 7 days. Six mice from each group were sacrificed 15 days after cell injection, and their organs, including the lungs, spleen, thymus, liver, small and large intestine, and bone marrow, were evaluated histologically for evidence of toxicity. This examination revealed no evidence of necrosis, inflammation, or increased X-gal staining of the tissues (Table 1).

**Long-Term Toxicity Studies in Mice.** Four groups of six mice each underwent intraperitoneal or intravenous injections of HSVtk and β-galactosidase vector-producer cells as described above, and three mice in each group received ganciclovir. These mice were followed to assess long-term toxicity. No evidence of toxicity has been observed during an assessment of more than 7 months (Table 1).

**Toxicity Studies in Rats**

**Intracerebral Injection of Vector-Producer Cells.** Fischer 344 rats, each weighing 230 to 350 gm, were anesthetized with intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg) and placed in a stereotactic apparatus.† With the β-galactosidase gene as a marker, 10 rats received an injection of 3 × 10⁶ G1BGSvN.29 vector-producer cells into the deep white matter of the right frontal lobe (depth 3.5 mm). Five control rats received a stereotactic injection of NIH3T3 cells transduced in vitro with G1BGSvN.29T3 cells (β-galactosidase-expressing 3T3 cells that do not produce infectious retroviral vectors). All cell suspensions were injected in 50 μl over a period of 15 minutes, after which time the needle was retracted slowly over 5 minutes. On Day 5, 9, or 14 after cell injection, the rats were sacrificed and their organs, including the brain, heart, lungs, liver, spleen, thymus, kidneys, and small and large intestine, were harvested for histological examination. Expression of the G1BGSvN.29 vector was detected by X-gal staining.

The life span of the β-galactosidase-producing murine cells in the brain, without ganciclovir administration, was found to be limited to 7 to 14 days (Table 2). Mild edema and reactive gliosis occurred only at the injection site in the brain. When β-galactosidase-producer cells were injected, transduction of endothelial cells at the injection site was occasionally evident; however, no increased number of X-gal-positive staining cells were seen in the brain remote from the injection site or in peripheral organs. No evidence of local or remote transduction was seen when 3T3 β-galactosidase non-producer cells were injected.

**Intracerebral Injection of HSVtk Vector-Producer Cells With Ganciclovir Treatment.** This experiment was conducted to evaluate whether the combination of HSVtk-producer cell injection into the brain followed by ganciclovir treatment results in toxicity. Ten Fischer 344 rats were inoculated with 3 × 10⁶ G1TkSVNa.53 vector-producer cells mixed with 3 × 10⁶ β-galactosidase-transduced murine fibroblasts (for localization of the injection site by X-gal staining) into the deep white matter of the cerebral hemisphere. Ten control rats were injected with 3 × 10⁶ β-galactosidase-producer cells. The cells were injected in 50 μl of balanced (Hank’s) salt solution. Five days after cell injection, the rats were treated with ganciclovir (15 mg/kg twice a day) for 7 days and sacrificed 3 days later for histological examination of the brain. A mild transient toxic response was evident during the initial 24 to 48 hours after cell injection (Table 2). Evidence of toxicity consisted of weakness, weight loss, somnolence, and dehydration, and was easily reversible by subcutaneous saline administration for 48 hours. A similar response was observed during the 1st day of ganciclovir therapy.

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<table>
<thead>
<tr>
<th>No. of Mice</th>
<th>Injected Cells</th>
<th>Route of Cell Injection</th>
<th>No. Receiving Ganciclovir†</th>
<th>No. Sacrificed‡</th>
<th>Results§</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>acute toxicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5 × 10⁶ HSVtk-producer cells</td>
<td>intraperitoneal</td>
<td>6</td>
<td>6</td>
<td>no pathology</td>
</tr>
<tr>
<td>12</td>
<td>5 × 10⁶ β-galactosidase-producer cells</td>
<td>intraperitoneal</td>
<td>6</td>
<td>6</td>
<td>no pathology; no increased number of X-gal-positive staining cells</td>
</tr>
<tr>
<td><strong>long-term toxicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5 × 10⁶ HSVtk-producer cells</td>
<td>intraperitoneal</td>
<td>3</td>
<td>—</td>
<td>at 7 mos no evidence of clinical toxicity in any mouse</td>
</tr>
<tr>
<td>6</td>
<td>5 × 10⁶ β-galactosidase-producer cells</td>
<td>intraperitoneal</td>
<td>3</td>
<td>—</td>
<td>at 7 mos no evidence of clinical toxicity in any mouse</td>
</tr>
<tr>
<td>6</td>
<td>5 × 10⁶ HSVtk-producer cells</td>
<td>intravenous</td>
<td>3</td>
<td>—</td>
<td>at 7 mos no evidence of clinical toxicity in any mouse</td>
</tr>
<tr>
<td>6</td>
<td>5 × 10⁶ β-galactosidase-producer cells</td>
<td>intravenous</td>
<td>3</td>
<td>—</td>
<td>at 7 mos no evidence of clinical toxicity in any mouse</td>
</tr>
</tbody>
</table>

* HSVtk = herpes simplex virus thymidine kinase. † Ganciclovir (150 mg/kg) administered twice a day for 7 days, starting on Day 7 after cell injection. ‡ In acute toxicity study, mice were sacrificed on Day 15 after cell injection. § In acute toxicity study, the lungs, spleen, thymus, liver, small and large intestine, and bone marrow were evaluated by light microscopy.

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| TABLE 1 |

Toxicity studies of HSVtk- and β-galactosidase-producer cells in mice*

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† Stereotactic apparatus manufactured by David Kopf Instruments, Tujunga, California.
Toxicity of retroviral-mediated gene transfer

**TABLE 2**

Toxicity studies of intracerebral murine HSVtk- and β-galactosidase-producer cells in rats*

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Injected Cells</th>
<th>No. Receiving Ganciclovir†</th>
<th>No. Sacrificed</th>
<th>Results‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3 × 10⁶ β-galactosidase-producer cells</td>
<td>0</td>
<td>Day 5, 9, or 14 after cell injection</td>
<td>Life span of murine cells 7 to 14 days; mild edema and reactive gliosis at injection site in brain; transduction of endothelial cells occasionally seen at the injection site; no increased number of X-gal-positive staining cells in remote organs</td>
</tr>
<tr>
<td>5</td>
<td>3 × 10⁶ HSVtk-producer cells</td>
<td>10</td>
<td>Day 3 after termination of ganciclovir</td>
<td>Mild toxic response (weakness, somnolence, dehydration) for the initial 24 to 48 hrs after cell injection and in some animals for the 1st day of ganciclovir treatment; moderate edema around injection site</td>
</tr>
</tbody>
</table>

* HSVtk = herpes simplex virus thymidine kinase.
† Ganciclovir (15 mg/kg) administered twice a day for 7 days, starting on Day 5 after cell injection.
‡ Brain, heart, lungs, liver, spleen, thymus, kidneys, small and large intestine, and bone marrow were evaluated by light microscopy using X-gal staining.

The frequency and severity of this response were similar in the animals receiving HSVtk and those receiving β-galactosidase vector-producer cells. It may be an immune response against the xenogeneic cells. Histological evaluation showed moderate edema around the injection site (Fig. 1A). In subsequent experiments involving more than 150 rats, the administration of steroids (dexamethasone, 0.5 mg/kg, in drinking water calculated as 20 ml water consumption/rat/day) completely abolished this toxic response in all rats.

**Toxicity Studies in Nonhuman Primates**

**Intracerebral Injection of HSVtk Vector-Producer Cells With or Without Ganciclovir Treatment.** The survival duration and proliferation potential of murine vector-producer cells within the brain of immunosuppressed primates and the toxicity from the intracerebral injection of HSVtk vector-producer cells alone or with subsequent ganciclovir therapy were studied in five *Macaca mulatta* monkeys, each weighing 6 to 8 kg (Table 3). To more closely mimic the clinical situation of a patient with a malignant brain tumor, the monkeys were treated with high-dose steroids (dexamethasone, 2 mg/kg/day intramuscularly) starting 7 days before surgery, then continued for 3 weeks, gradually tapered over a 1-week period, and then discontinued. Antibiotic therapy (cefotaxime, 30 mg/kg, twice a day) was administered intramuscularly starting on the day of surgery and continued for 10 days. Under general anesthesia with intravenous Pentothal (thiopental) and 0.75% to 2.0% isoflurane and aseptic conditions, the monkeys received a stereotactic intracerebral injection of 1.0 × 10⁶ HSVtk-producer cells mixed with 1.0 × 10⁶ β-galactosidase-producer cells (total volume 250 μl) into the deep white matter of the right frontal lobe. One monkey received bilateral injections: the HSVtk/β-galactosidase mixture as described into the right frontal lobe and 1.0 × 10⁶ NIH3T3 cells pretreated with the β-galactosidase vector into the left frontal lobe. A venous port was installed in two monkeys for subsequent ganciclovir therapy via the right internal jugular vein. The proximal catheter was placed in the right atrium, and the distal access port was positioned subcutaneously in the interscapular region of the back. Ganciclovir was administered to the two monkeys with the venous access port as a slow intravenous infusion (10 mg/kg in 50 ml normal saline over 30 minutes daily) for 14 days. Magnetic resonance (MR) imaging, including T₁- and T₂-weighted and gadolinium-enhanced T₁-weighted studies, was obtained 5 and 90 days after cell injection in the monkeys not receiving ganciclovir and 14 days after cell injection (7 days after initiation of ganciclovir therapy) in the monkeys treated with ganciclovir. Physical and neurological examinations were performed twice a day on each monkey. Repeat blood samples for routine chemistry and hematological analysis were obtained from all animals before and during the experiment. Cerebrospinal fluid (CSF) was taken by cisternal puncture from all monkeys on Day 10 after cell injection and was assayed for routine chemical and bacteriological analysis. Two monkeys that received intracerebral injections of the HSVtk-producer cells without ganciclovir were kept alive for long-term observation (Table 3).

No proliferation of the producer cells within the brain was observed in either the ganciclovir-treated or non-treated monkeys (Table 3). This was determined by histological examination and MR imaging of the monkeys’ brains. In one animal that received β-galactosidase-producer cells as a marker, producer cells were found 15 days after cell injection (Fig. 1B). No β-galactosidase-producer cells were found in either of the
two animals examined 3 weeks after cell injection, including one monkey that did not receive ganciclovir. This suggests that the xenogeneic producer cells can survive in the brain up to 15 days, but not beyond 3 weeks. This time frame is similar to that observed in the rat brain.

Toxicity Evaluation. Toxicity was assessed by clinical, radiological, and laboratory (blood chemical, bacteriological, and histological) evaluation of the monkeys. Neurological examination of all monkeys before and after cell injection and during ganciclovir therapy was unchanged from baseline. No motor or behavioral changes were observed at any phase of the experiment. Two monkeys treated with HSVtk-producer cell injections without ganciclovir therapy are being followed for potential long-term toxicity. To date, 270 days after injection of the murine cells, no ill-effects or any changes from baseline neurological status have been observed.

In the ganciclovir-treated and nontreated monkeys, images obtained 7 days after cell injection showed the localized injection site as an isointense lesion (Fig. 2A). The area of injection became hypointense 7 days after initiation of ganciclovir therapy (Fig. 2B and C), measuring a few millimeters in diameter with enhancement of the surrounding rim following gadolinium infusion. No edema or mass effect was observed in any of the monkeys. This radiological appearance is compatible with a localized breakdown of the blood-brain barrier secondary to the mechanical effect of cell injection and ganciclovir-induced lysis of the producer cells. On MR images obtained 90 days after cell injection, only a small nonenhancing hypointense region was demonstrated, with no evidence of mass effect at the site of injection (Fig. 2D). This radiological appearance is compatible with a localized encephalomalacia at the injection site.

Since the xenogeneic cells may cause inflammatory response by leaking into the subarachnoid space (reactive meningitis), CSF samples were collected by cisternal puncture from all five monkeys 14 days after...
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![Image of brain scans showing different conditions](image)

**FIG. 2.** A: Gadolinium-enhanced T1-weighted magnetic resonance (MR) image of a monkey brain obtained 7 days after injection of herpes simplex virus thymidine kinase (HSVtk) vector-producer cells showing an isointense lesion with a peripheral enhancing rim (arrow) at the injection site. B: Gadolinium-enhanced MR image of a monkey brain obtained 14 days after cell injection and 7 days after initiation of ganciclovir therapy showing a hypointense lesion with pronounced enhancement of the rim (arrow) at the injection site. C: Brain specimen from the monkey imaged in B 24 hours later, showing no edema or mass effect at the injection site (arrow). D: Gadolinium-enhanced MR image of a monkey brain obtained 90 days after cell injection (but with no ganciclovir therapy) revealing a small, nonenhancing, hypointense lesion at the cell injection site (arrow).

**TABLE 3**

<table>
<thead>
<tr>
<th>No. of Monkeys</th>
<th>Injected Cells</th>
<th>No. Receiving Ganciclovir</th>
<th>Death/Sacrifice</th>
<th>Results§</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10⁷ HSVtk-producer cells &amp; 10⁶ β-galactosidase-producer cells</td>
<td>2</td>
<td>Day 7 (1) or 14 (1) after initiation of ganciclovir</td>
<td>no evidence of CNS toxicity; local transduction of endothelial cells at the injection site; limited demyelination around the implanted cells after GCV therapy; no clinical or radiological evidence of producer cells; life span of xenogeneic producer cells 2 wks</td>
</tr>
<tr>
<td>2</td>
<td>10⁷ HSVtk-producer cells &amp; 10⁶ β-galactosidase-producer cells</td>
<td>0</td>
<td>—</td>
<td>no evidence of CNS toxicity; local transduction of endothelial cells at the injection site; no meningeal irritation and no neurological deficits during 4 mos after cell injection; no clinical or radiological evidence of proliferation of producer cells; life span of xenogeneic producer cells 2 wks</td>
</tr>
<tr>
<td>1</td>
<td>10⁷ HSVtk-producer cells &amp; 10⁶ β-galactosidase-producer cells (rt hemisphere); 10⁷ JTV3 β-galactosidase non-producer cells (lt hemisphere)</td>
<td>0</td>
<td>Day 21 after cell injection</td>
<td>no evidence of CNS toxicity; local transduction of endothelial cells at the injection site; no meningeal irritation and no neurological deficits during 4 mos after cell injection; no clinical or radiological evidence of proliferation of producer cells; life span of xenogeneic producer cells 2 wks</td>
</tr>
</tbody>
</table>

*HSVtk = herpes simplex virus thymidine kinase; CNS = central nervous system.
†Ganciclovir (10 mg/kg) administered once a day for 14 days, starting on Day 7 after cell injection.
‡Examination included magnetic resonance imaging at various phases, cerebrospinal fluid sampling, blood chemistry, bacteriology, hematology, and histology of the brain and of major organs.

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injection of the cells. All samples contained normal protein and glucose levels, without pleocytosis. Bacteriological cultures were negative. Table 4 summarizes the CSF characteristics of the samples.

Mild reactive gliosis without evidence of edema or pathological changes in neighboring brain tissue occurred at the cell injection site in both the ganciclovirtreated and nontreated animals. Specimens stained for myelin showed localized demyelination limited to the injection site which did not increase in size when ganciclovir was given (Fig. 1C). A few endothelial cells at the injection site stained for β-galactosidase activity, demonstrating evidence of transduction with the β-galactosidase gene (Fig. 1D).

Complications. The only complications observed were infectious, which were believed to result from the profound immunosuppression induced by high-dose steroid administration. These included two fatal septic complications. One monkey that had undergone implantation of the venous access port into the right heart developed acute bacterial endocarditis, Staphylococcus aureus sepsis, and septic shock, resulting in death 3 days after cessation of prophylactic antibiotic therapy and 7 days after initiation of ganciclovir therapy. An MR image obtained 24 hours before death, as well as the macroscopic and microscopic appearance of the brain, showed no evidence of central nervous system (CNS)-related toxicity. The second monkey, which received bilateral cell injections (HSVtk-producer on one side and β-galactosidase-transduced cells on the other side) but no ganciclovir therapy, died on Day 21 postoperatively (1 day before planned sacrifice). Necropsy revealed bilateral interstitial pneumonia due to cytomegalovirus as well as systemic mycosis (Candida albicans cultured from all harvested organs) as the cause of death. No clinical or histological perturbations of the CNS were observed.

Discussion

The application of retroviral-mediated gene transfer for the treatment of CNS malignancies has the potential of toxic side effects. We investigated various aspects of potential CNS and systemic toxicities of the HSVtk ganciclovir system in normal mice, rats, and nonhuman primates. Assessment of transduction of normal proliferating tissues, using the β-galactosidase gene, showed no evidence of spread of the G1BqSN.29 vector beyond the injection site. Similarly, no pathological changes were detected in remote tissues when HSVtk-producer cells were injected intraperitoneally, intravenously, or intracerebrally and followed with ganciclovir administration. Since any vector particles that might be released from the area of injection would be quickly bound by the thousands of amphotropic vector receptors present on each host cell, these observations were anticipated. Theoretically, even if all of the vector particles produced were able to cross the blood-brain barrier and escape into the systemic circulation, there would be few vector particles relative to the number of receptors in any organ and risk of injury to non-CNS organs would be minimal. The only evidence of transduction of normal tissue was limited to endothelial cells within the injection site. Furthermore, transduction and subsequent damage to cerebral blood vessels within or adjacent to a brain tumor may be desirable as selective destruction of these vessels may decrease blood supply to the tumor and contribute to its complete eradication.

No spontaneous formation of replication-competent retrovirus has ever been demonstrated in over 200 lots of supernatant from the vector-producer cells used in this experiment (data not shown). Thus, no replication-component virus is expected to originate from the vector-producer cells in vivo. Even if it were to do so, the human complement system is expected to inactivate such a retrovirus.

The random nature of retroviral integration into cells allows the potential of an untoward insertional event. Because of the inactivation of these vectors by human complement, the probability for insertional mutagenesis in normal cells is extremely small and cells undergoing such an unlikely event would be eliminated by the administration of ganciclovir. No such mutagenesis has been observed in the monkeys injected with HSVtk vector-producer cells during an observation period of 270 days; the animals remain under evaluation for the development of such an event.

Based on histological examination of the rat and primate brains injected with β-galactosidase-producer and β-galactosidase-transduced cells, intracerebral survival of the murine vector-producer cells appears to be limited to less than 3 weeks. This suggests that sustained proliferation of these cells in vivo will not occur, but the survival period is sufficient for transduction of surrounding tumor cells.

The by-products resulting from the interaction between cells containing the HSVtk gene and ganciclovir may not cause any pathological changes in normal tissues. No damage was observed, even in those tissues that received high concentrations of HSVtk-producer cells before ganciclovir administration, including brain and lung tissue. Localized breakdown of the blood-brain barrier was the only change observed by MR imaging following injection of the producer cells into the primate brain. This focus of contrast material was enhanced by ganciclovir administration, but resolved completely within 90 days after cell injection. No edema

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**Table 4**

Characteristics of cerebrospinal fluid in the primate study group

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Protein (mg/dl)</th>
<th>Glucose (mg/dl)</th>
<th>Fl-galactosidase Cells (µl)</th>
<th>Ganciclovir Treatment*</th>
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<tbody>
<tr>
<td>1†</td>
<td>79</td>
<td>70</td>
<td>9</td>
<td>+</td>
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<tr>
<td>2</td>
<td>8</td>
<td>66</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>3‡</td>
<td>5</td>
<td>119</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
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<td>−</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>59</td>
<td>0</td>
<td>−</td>
</tr>
</tbody>
</table>

* Symbols: + = ganciclovir administered; − = no ganciclovir treatment.

† Death from Staphylococcus aureus sepsis and bacterial endocarditis.

‡ Death from systemic mycotic infection.
Toxicity of retroviral-mediated gene transfer

or mass effect from those cells occurred before or after ganciclovir administration.

Intracerebral injection of the murine cells may be accompanied by leakage of these cells into the subarachnoid space and an inflammatory reaction. In addition, an immune-mediated response to the cells can be associated with localized or diffuse encephalitis. There was no evidence of meningitis or encephalitis demonstrated in the behavior, the histology, or the CSF analysis of the treated primates.

The complications encountered in the primate experiment included two deaths. Both were related to septic complications, including opportunistic infections (cytomegalovirus and Candida albicans), secondary to the immunosuppression and central line placement. No CNS toxicity or other complications were observed during repeated neurological assessment, MR imaging studies, and macroscopic and microscopic examination of the brains.

Injection of HSVtk retroviral vector-producer cells into the brain using murine producer cells and concomitant ganciclovir therapy is not associated with significant toxicity to the brain or remote organs. Investigation of this gene transfer approach to treat malignant brain tumors in humans is not expected to be associated with significant toxicity.

References


Manuscript received December 3, 1992.

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