Limited efficacy of gene transfer in herpes simplex virus–thymidine kinase/ganciclovir gene therapy for brain tumors

PIOTR HADACZEK, PH.D., HANNA MIREK, PH.D., MITCHEL S. BERGER, M.D., AND KRYSZTOF BANKIEWICZ, M.D., PH.D.

Molecular Therapy Laboratory, Brain Tumor Research Center, Department of Neurological Surgery, University of California, San Francisco, California

Object. Low efficacy of gene transfer, transient gene expression, and toxicity of viral vectors are the major hurdles in successful anticancer gene therapy. The authors conducted in vitro (U87MG cell line) and in vivo (xenograft, tumor-bearing rodent model) studies to address the stability of transduction by using the adenoassociated virus serotype-2 (AAV2)–thymidine kinase (TK) vector over time.

Methods. Standard methods for cell growth and a ganciclovir (GCV) cytotoxicity assay were applied. The AAV2-TK was infused into implanted tumors in athymic rats via convection-enhanced delivery (CED). Thymidine kinase expression was evaluated through immunohistochemical analysis, and the distribution volumes of the transduced tumors were calculated. Twenty-four hours following the viral infusions, animals were treated with GCV (50 mg/kg intraperitoneally every day for 10 days; six rats) or phosphate-buffered saline (six rats).

A rapid decrease in TK expression over time was observed both in vitro and in vivo. A large volume of the tumor (up to 39%) was transduced with AA2-TK following CED. Administration of GCV resulted in limited therapeutic effects (survival of 25.8 compared with 21.3 days).

Conclusions. Rapid elimination of TK expression from dividing tumor cells and focal transduction of the brain tumor were most likely responsible for the limited bystander effect in this approach. Immediate administration of GCV is crucial to assure maximal efficacy in the elimination of cancer cells. In addition, the complete or diffused transduction of a brain tumor with TK may be required for its total eradication.

Key Words • adenoassociated virus • thymidine kinase • ganciclovir • convection-enhanced delivery • glioma • gene therapy

The era of gene therapy has opened new directions for the treatment of tumors in humans. Initial enthusiasm accompanying the first experiments and clinical trials receded when practical gene therapy approaches revealed limitations in this new field of medicine. Researchers in several studies have addressed restrictions associated with the low efficacy of gene transfer, transient gene expression, and toxicity of viral vectors. A very detailed knowledge of the molecular mechanisms through which viral vectors transduce target cells as well as effective ways of delivering them to the target tissues are the most critical factors limiting beneficial effects of gene therapy. Therefore, additional translational in vitro and in vivo experiments should mark directions for clinical trials.

Transduction of tumors with the HSV-TK gene, which activates the nucleoside analog prodrg GCV, has been one of the most effective means of treating experimental brain tumors. The HSV-TK phosphorylates GCV to its monophosphate form (that is, GCV-MP), which is further phosphorylated by cellular kinases to GCV-DP and GCV-TP, which competes for mammalian DNA polymerases, resulting in inhibition of DNA synthesis. This therapy has the potential for selective killing of dividing cancer cells, as AAV-2 selectively infects tumor cells expressing heparin sulfate proteoglycan binding site and neurons, leaving other cell types such as astrocytes and endothelial cells unaffected. This approach has the capability of killing only dividing TK-positive cancer cells on GCV administration, leaving other postmitotic and TK-transduced cells such as neurons unaffected.

Adenoassociated virus–based vectors are favorable candidates as they derive from a nonpathogenic virus. They have been shown to infect a wide range of tissues and trigger few cytokotic or adverse immune reactions in animal models. In addition, the small size of the AAV particle (20 nm) allows one to use a CED method to achieve widespread distribution of AAV-2 in the brain. Infusions delivered while maintaining a pressure gradient over time have been shown to distribute molecules to large areas of brain tissue (up to several square centimeters) in both rats and rhesus monkeys. We designed a series of in vitro and in vivo experiments to address the stability of transduction by using the adenoassociated virus serotype-2 (AAV2)–thymidine kinase (TK) vector over time.
Gene therapy limitations in treating gliomas

with AAV2-TK to test the transduction efficiency of the AAV-2 vector in vitro and in vivo, to investigate the therapeutic potential of a HSV-TK/GCV system on U87MG cells in vitro, and to analyze the potential efficacy of this system to cure U87MG-derived tumors in athymic rats.

Materials and Methods

We used U87MG human glioblastoma multiforme cells for all experiments. The cells were maintained in complete minimum essential medium consisting of Eagle minimum essential medium supplemented with 10% fetal bovine serum, nonessential amino acids, and antibiotic agents (streptomycin 10,000 μg/ml and penicillin 10,000 U/ml). Incubation conditions were 37°C in an atmosphere of 95% air and 5% CO2. For in vitro experiments U87MG cells were infected with AAV2-TK.

Adenoassociated Virus Vector Production

Recombinant AAV2-TK vector was produced in a derivative of human embryonic kidney 293 cells propagated in roller bottles. Following expansion, cells were cotransfected with three plasmids that have been described previously (helper plasmid 19, containing AAV rev and p19; pladeno-5, an adenoviral helper plasmid; and pTK1, a plasmid containing the HSV-TK gene under control of the cytomegalovirus promoter and flanked by AAV-2 inverted terminal repeats). After 16 to 24 hours, the transfection buffer was replaced with serum-free medium and the roller bottles were incubated for another 48 hours to allow for vector amplification. Following this incubation period, the cells were harvested, homogenized by microfluidizer, and clarified by centrifugation. The AAV2-TK vector was isolated from impurities in the clarified cell lysate by cation exchange chromatography. The vector was further purified by anion exchange chromatography to reduce the level of residual cellular/plasmid DNA and protein impurities. The flowthrough from the anion exchange column was concentrated and dialyzed. The concentrated vector was sterile filtered (0.22 μm), aseptically filled into 1-ml Hanks solution for 5 minutes. The needle was kept in place for 1 additional minute before removal.

Expression of TK Within Brain Tumor in Vivo

Twenty-four rats underwent implantation of U87MG cells transduced with AAV2-TK (18 hours before implantation). Forty groups were created (six animals per group) to study TK expression in the in vivo model. Animals were killed 7, 14, 21, and 25 days postsurgery. The rats received lethal injections of sodium pentobarbital (50 mg/kg) and were transcardially perfused with 0.9% PBS followed by 350 ml 4% paraformaldehyde in 0.01 M PBS buffer. The brains were removed, quickly frozen in liquid nitrogen, and placed in a small-animal stereotactic frame (David Kopf Instruments, Tujunga, CA). A sagittal incision was made through the skin, and a burr hole was placed in the skull by using a twist drill. The coordinates were 0.5 mm posterior to the bregma and 3 mm to the left of the midline. A 25-gauge needle was inserted 4.5 mm below the dura mater. All cell implantations were performed using a 10-μl Hamilton syringe (Hamilton Co., Reno, Nevada) secured to the stereotactic frame. Rats were injected intracranially with 500,000 cells in 10 μl Hanks solution for 5 minutes. The needle was kept in place for 1 additional minute before removal.

Bystander Effect In Vivo

The U87MG cells were transduced with AAV2-TK (as previously described). Twenty-four hours later, the transduced cells were trypsinized and mixed with TK-negative cells at different ratios (1:1 and 1:9). The mixtures were seeded onto 12-well plates for subsequent GCV treatment (10 μM). Nontransduced U87MG cells (negative control) and TK-positive cells (positive control) were included in this experiment. Living cells from the respective wells were trypsinized and counted once every 24 hours over a 6-day period, and the cell growth curve was determined for each culture.

Animals and Tumor Implantation

The human glioblastoma multiforme cell line U87MG was used to establish an animal brain tumor model for our study. All the experiments were performed on male athymic rats (homozygous Rowett nude rats; National Cancer Institute, Bethesda, MD), weighing between 80 and 200 g; these procedures were approved in accordance with the regulations of the Committee on Animal Research, University of California, San Francisco. The rats were anesthetized with isoflurane (Baxter, Deerfield, IL) via inhalation (3 L mixed with O2) and placed in a small-animal stereotactic frame (David Kopf Instruments, Tujunga, CA). A sagittal incision was made through the skin, and a burr hole was placed in the skull by using a twist drill. The coordinates were 0.5 mm posterior to the bregma and 3 mm to the left of the midline. A 25-gauge needle was inserted 4.5 mm below the dura mater. All cell implantations were performed using a 10-μl Hamilton syringe (Hamilton Co., Reno, Nevada) secured to the stereotactic frame. Rats were injected intracranially with 500,000 cells in 10 μl Hanks solution for 5 minutes. The needle was kept in place for 1 additional minute before removal.

Distribution of AAV2-TK Within Rat Brain Tumors

Six rats with implanted U87MG tumors were chosen to study AAV2-TK distribution within the tumor mass. The AAV2-TK was continually administered to each brain tumor via CED. A 27-gauge needle fused with a Teflon tube (0.02 in) and connected to a programmable microinfusion pump (Bioanalytical Systems, West Lafayette, IN) was used for delivery. The loading chamber (Teflon tubing, 0.0625-in outer diameter × 0.02-in inner diameter) was filled with 1.6 × 106 particles of AAV2-TK in a total volume of 20 μl PBS and heparin. The needle was placed in the center of the tumor mass (anteroposterior 0.5 mm, 3 mm to the left of the midline, and 4 mm below the dura mater), and the infusion of GCV was administered at a rate of 329 μl/minute for 40 minutes. Seventy-two hours after surgery the animals were killed for analysis. After removal and fixation, the brains were washed in PBS and transferred to 30% sucrose in 0.1 M PBS solution for cryopreservation. The brains were cut into 40-μm serial coronal sections on a cryostat. Frozen sections were collected in a series in anti-freeze solutions and stored at −70°C. Every 10th section was stained for immunohistochemical analysis. Sections were washed in PBS and incubated in 1% H2O2 for 20 minutes to block endogenous peroxidase activity. After washing in PBS, the sections were incubated in blocking solution (1% normal goat serum and 0.01% Triton X-100 in PBS) for 30 minutes, followed by incubation in primary an-
tibody solution (a rabbit polyclonal HSV-TK antibody; 1:10,000, a gift from Dr. William Summers) overnight at room temperature. After washing the tissue sections, they reacted for 1 hour with goat biotinylated anti–rabbit immunoglobulin G secondary antibody (1:300; Vector Laboratories, Inc., Burlingame, CA). Antibody binding was visualized with streptavidin–horseradish peroxidase (1:300; Vector Laboratories, Inc.) and 3’3’-diaminobenzidine staining (Vector Laboratories, Inc.). A coverslip was placed on the sections, which were then examined using light microscopy.

Image Analysis

The tumor volumes as well as the distribution of AAV2-TK within a tumor mass were analyzed using a Macintosh-based image analysis system (NIH Image, version 1.61; National Institutes of Health, Bethesda, MD). Images of stained tissue slices were captured using a charge-coupled device and commercially available software (Adobe Photoshop; Adobe Systems, Inc., San Jose, CA). The area of distribution of infused viral particles in each tissue section was automatically determined using a threshold of 50% of the maximal stained optical density (NIH Image). The sum of the areas of infusion was used to determine the volume of distribution in each tumor and was estimated in serial sections (every 10th) according to the method of Cavalieri under ×1.25 magnification. Similarly, total volumes of implanted tumors were calculated.

Experimental Design of GCV Treatment for Survival Study

Twelve rats underwent intracranial implantation of U87MG cells.
After 15 days, AAV2-TK was administered via CED to each brain tumor (number of viral particles, volumes, and conditions described earlier). Vectors were coinfused with heparin (1000 U/ml) to increase distribution within the tumor mass. Previously, we showed that heparin enhanced AAV-TK gene transfer in the rat brain.24 Twenty-four hours after infusions, six animals were slated for GCV treatment (50 mg/kg GCV intraperitoneally every day for 10 days) and six control rats were left untreated (that is, PBS was administered). Animals demonstrating symptoms and evidence of distress due to brain tumors were killed. A survival curve was drawn for each group, and statistical significance was calculated using an analysis of variance test.

**Results**

Transgene Expression and Persistence

To determine the efficiency and persistence of AAV2-TK transduction, we infected U87MG cells with our vector in vitro and counted TK-immunoreactive cells at different time points postinfection. We observed a radical decrease in immunoreactivity over time. Twenty-four hours after infection, almost 100% of the U87MG cells appeared to be TK positive. On Days 3, 5, and 7, this percentage of TK-positive cells progressively decreased to 79, 40, and 16%, respectively. At the end of Weeks 2 and 3 (Days 14 and 21), only small percentages of cells stained positive for TK (4.7 and 1.6%, respectively). We performed a parallel experiment in vivo with previously transduced U87MG cells that had been implanted into rat brains 18 hours after transduction with AAV2-TK. Animals were killed at various time points. Immunochemical reactions against TK protein on paraffin-embedded tumor sections were analyzed. We observed similar rapid loss of TK immunoreactivity within the tumor mass. On Days 7, 14, 21, and 25, the percentages of TK-positive cells were 12.5, 3.6, 1.2, and 0.5%, respectively. A comparison of HSV-TK expression between in vitro and in vivo models is featured in Fig. 1. Examples of immunohistochemical staining of U87MG cells expressing TK from both models are presented in Fig. 2a–d.

Sensitivity of U87MG Cells Transduced With AAV2-TK to GCV at Varying Time Points In Vitro

To test the cytotoxicity of GCV in HSV-TK–expressing U87MG cells, we transduced cells with AAV2-TK at varying time points prior to GCV treatment. As illustrated in Fig. 3, cells infected 1, 7, and 14 days before introducing GCV (10 μg/ml) were exquisitely sensitive to GCV. The most prominent decrease in cell survival was observed in cells transduced 1 day before GCV addition. Cells that had been infected 7 and 14 days earlier remained sensitive to GCV, although the number of surviving cells was greater. Nevertheless, the majority of U87MG cells were killed by Day 8. In turn, when the cells were transduced 21 and 30 days prior to GCV introduction, the induction of cytotoxicity was much less efficient and 7 days of GCV administration did not reduce the cell number below the starting cell count of 400,000 cells/well.

Bystander Effect In Vitro

To evaluate the bystander effect in vitro, TK-positive cells were mixed at different ratios with nontransduced U87MG cells. Powerful bystander killing was observed when TK-positive cells made up 10 to 50% of the initial cell population. With only 10% of the TK-positive U87MG cells, GCV treatment caused a significant decrease in cell growth when compared with control (nontransduced cells) cultures (Fig. 4).

Distribution of AAV2-TK Within Tumors

Using CED and coinfusion of viral particles with heparin directly into the U87MG tumors on Days 18 and 22, we were able to transduce a significant volume of tumor tissue. Twenty-two days after implantation the mean tumor volume was 233 mm³; however, 4 days earlier (Day 18) this volume was only 108 mm³. After infusion of AAV2-TK particles in a total volume of 20 μl, we noted a mean TK-transduced area of 42 mm³. In 18-day-old tumors this area...
gene in tumor cells renders transgene from rapidly dividing tumor cells.

Survival Analysis

All rats in the survival study demonstrated neurological symptoms of intracerebral progressive tumor growth (ca-chexia, poor grooming, hunched back, huddling in the corner, and decreased movement) and were killed just after the first symptoms became clearly observable. The administration of GCV resulted in a therapeutic effect. The mean survival time for rats in the GCV-treated group was 25.8 days, whereas those in the control group survived only 21.3 days.

Discussion

In gene therapy trials, HSV-TK is used as a suicide gene. Transduction of malignant cells with this gene combined with GCV administration has become an experimental method for treating solid tumors. A phosphorylated form of GCV, GCV-TP, kills only rapidly dividing tumor cells, whereas normal brain tissue should not be affected by the treatment, thus making this system very specific and especially suitable for brain tumors. In virtually all cases, the efficacy of such therapies has been limited by low levels of transgene transduction. Poor distribution of recombinant viral vectors throughout the tumor mass is a limiting factor for successful tumor eradication. The choice of a vector that could give high and stable expression of HSV-TK in tumor cells is another key component of this suicide-based approach. Adenovirus-based viral vectors seem to hold considerable promise for gene therapy of tumors in humans given that they show both low toxicity and low immune response. One of the most attractive features of recombinant AAV vectors is the ability to be stably maintained in nondividing host cells; however, stable gene expression is transient in malignant cells such as the U87MG cells used in this experiment. To further address therapeutic efficacy of the suicide gene approach in brain tumors, an animal model with xenograft tumor-bearing rats was used to analyze TK expression dynamics within U87MG tumors as well as the efficacy of GCV treatment on growing tumors.

Expression of the HSV-TK gene in tumor cells renders them sensitive to GCV. Thus, the level and pattern of TK expression may determine the efficacy of HSV-TK–mediated cytotoxicity. We analyzed the persistence of TK protein expression in U87MG cells transduced with AAV2-TK. The protein product of the AAV-1 locus. Rather, these recombinant vectors can persist as episomes or, alternatively, can integrate into the cellular genome at sites other than chromosome 19 (a site-specific manner at the AAV-1 locus). The effectiveness of the AAV2-TK/GCV strategy critically depends on transduction of a sufficient number of tumor cells to achieve total eradication of the tumor mass.

![Graph demonstrating bystander effect in vitro.](image-url)

**FIG. 4.** Graph demonstrating bystander effect in vitro. Previously transduced U87MG cells (transduction with AAV2-TK 24 hours prior to the experiment) were mixed at two different ratios (1:1 and 1:9) with controls (nontransduced cells) and seeded into a 12-well plate for subsequent GCV treatment. Note that an observable bystander effect was observed even when the initial cell culture population consisted of only 10% of TK-positive cells. Uniform and dispersed distribution of TK-positive cells within the culture is crucial for the bystander effect to take place.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Treatment</th>
<th>Survival Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1328</td>
<td>GCV</td>
<td>25</td>
</tr>
<tr>
<td>1331</td>
<td>GCV</td>
<td>25</td>
</tr>
<tr>
<td>1334</td>
<td>GCV</td>
<td>26</td>
</tr>
<tr>
<td>1335</td>
<td>GCV</td>
<td>30</td>
</tr>
<tr>
<td>1364</td>
<td>GCV</td>
<td>26</td>
</tr>
<tr>
<td>1366</td>
<td>GCV</td>
<td>23</td>
</tr>
<tr>
<td>1332</td>
<td>PBS</td>
<td>22</td>
</tr>
<tr>
<td>1337</td>
<td>PBS</td>
<td>25</td>
</tr>
<tr>
<td>1363</td>
<td>PBS</td>
<td>21</td>
</tr>
<tr>
<td>1365</td>
<td>PBS</td>
<td>20</td>
</tr>
<tr>
<td>1371</td>
<td>PBS</td>
<td>21</td>
</tr>
<tr>
<td>1372</td>
<td>PBS</td>
<td>19</td>
</tr>
</tbody>
</table>

* Mean survival time in GCV-treated rats was 25.8; in PBS-treated rats, 21.3 days.

**TABLE 1**

Survival time in GCV-treated and control rats harboring U87MG-derived intracranial tumors*

---

* P. Hadaczek, et al.
Data from our previous studies in nonhuman primates have demonstrated that CED can result in a much wider tissue distribution of AAV-mediated gene expression when compared with the injection technique. Additionally, we found that blocking receptors in the neuronal membrane by heparin (a soluble receptor analog for AAV) coinfusion permits extended distribution of AAV-based vectors, resulting in transduction of a significantly larger area of the rat brain. Both CED and heparin coinfusion were used to achieve an efficient way of delivering the TK gene into tumor. By infusing $1.6 \times 10^{10}$ particles of AAV2-TK in a total volume of 20 μl, we obtained a mean transduction area of approximately 43.6 mm² within the tumor tissue. In the group of rats bearing end-stage tumors (22 days from implantation), this number accounted for only 18% of a total tumor mass (233 mm³). Nonetheless, 4 days earlier (18-day-old tumors), the AAV2-TK–transduced portion of a tumor mass was significantly larger (39%), clearly indicating the importance of early introduction of AAV2-TK vector delivery into growing tumors.

To verify the efficacy of the HSV-TK/GCV gene therapy approach, we designed a survival study involving the administration of GCV against intracranially growing tumors. Analysis of our results demonstrated that intraperitoneal injection of GCV produced a therapeutic effect and prolonged survival time in the treated rats. Despite a statistically significant difference in survival between GCV-treated and control animals (25.8 compared with 21.3 days, $p < 0.05$), we concluded that even if an extensive tumor area was transduced with AAV2-TK vector, we would be unable to eradicate the tumors. Based on the results of our in vivo transduction efficacy experiment, we assumed that more than 39% of a tumor mass was transduced with the suicide vector (probably more than 50%, as in our survival study we transduced tumors on Day 16). As it has been already shown by others, data from our in vitro studies confirmed that complete transduction of tumor cells is not required for complete tumor cell killing. In fact, as few as 4.7% transduced cells (14 days posttransduction) were sufficient successfully to kill all growing in vitro tumor cells.

J. Neurosurg. / Volume 102 / February, 2005
This bystander effect involves spreading of phosphorylated GCV to neighboring TK-negative cells. Such powerful action is most likely due to the fact that TK-positive cells are evenly dispersed among other nontransduced cells, and thus toxic molecules can be distributed. This very potent bystander killing was confirmed by an in vitro experiment in which TK-positive cells were mixed with nontransduced U87MG cells at two different ratios. When TK-positive cells comprised 50% of the initial cell population, the addition of GCV produced massive inhibition of the cell line growth almost identical to that with the positive control (that is, U87MG cells transduced with AAV2-TK). Even when the cell culture consisted of only 10% of TK-positive cells (1:9 ratio), the inhibition of cell growth was substantial. Nonetheless, the bystander effect was not that efficient in our in vivo model. Even if as much as 39% of the tumor volume was transduced by AAV2-TK, tumors were not completely eradicated and the animals had to be killed because of tumor growth progression. Through the CED technique, AAV2 particles were delivered into the central portion of a growing neoplastic mass, locally transducing only the core of the tumor and leaving its peripheries unaffected. So even if the central mass was most likely eradicated by GCV treatment, peripheral cells were still dividing because they were isolated from immediate contact with the TK-positive cells. Therefore, the bystander effect could not exert its function. The unaffected tumor masses had eventually overgrown the space left by the cells killed by GCV. Note that it is often very misleading to compare in vivo and in vitro bystander effects.

In this experiment we aimed to model large human glioblastoma multiformes and deliver AAV2-TK 15 days after tumor implantation when the lesions covered approximately 50% of the striatum. To eradicate growing tumors successfully, we would probably have to distribute AAV2-TK vector within most if not all of the tumor mass. Other investigators have shown that in using a similar suicidal gene therapy system, the growth of intracranial rodent brain tumors can be halted. Nonetheless, in experiments conducted by Dewey, et al., AAV2-TK was delivered just shortly after tumor implantation (3 days) when tumors were quite small. One might assume that at such an early stage of tumor growth, infusion of AAV2-TK covered the entire area of implanted cells and subsequent treatment with GCV completely eradicated cancer cells. Brand, et al., reported that larger tumors of clinically relevant size can also be successfully treated when composed of retrovirally transduced TK-positive cells. These investigators proved that CC531 colon adenocarcinoma tumors as large as 4000 mm³ in syngenic Wag/Ola rats could be completely eradicated in an ideal condition of 100% gene transfer. Analysis of these results indicated that the HSV-TK/GCV system would probably work in humans only if maximal transduction was achieved. Furthermore, in humans, gliomas can reach a volume of a few dozen cubic centimeters, making efficient gene transfer very challenging, if not impossible.

Another possible explanation for the low efficacy of the HSV-TK/GCV approach is that there is inefficient blood-brain barrier penetration of GCV. Based on data in our study, however, we could find no TK-immunoreactive cell within the tumors of GCV-treated rats, whereas TK-immunoreactive cells were seen in control animals that had received PBS in place of GCV, thus strongly indicating that GCV was able to penetrate the blood–brain barrier and kill TK-positive tumor cells (Fig. 5c and d).

It must be emphasized that to evaluate fully any anticancer gene therapy, an appropriate animal model must be chosen. In vivo, the immune system plays a significant role in tumor destruction. Such response is characterized by an influx of immune cells—mainly CD4⁺ T cells, natural killer cells, and macrophages—activated by exposed tumor antigens from the dead cells. It has been proven that an integrated, complete immune response is necessary for prolongation of survival. This effect is mediated mainly by a tumor-specific cytotoxic and proliferative T-cell response. In our study we used immunocompromised animals, and thus we could not count on the full contribution of an active immune system. We addressed the efficacy of the HSV-TK/GCV system by using AAV2 in the absence of the immune component. It is quite possible that by eliminating it, we determined only the effectiveness of a TK gene transfer system in the limited presence of a bystander effect in vivo. Based on data from this study, it would appear that AAV2 is a suboptimal platform for the suicidal approach in treating tumors. Moreover, localized infusion of viral particles, leading only to focal as opposed to dispersed transduction with the TK gene within the tumor mass, is a major hurdle for the bystander effect and its beneficial augmentation of cytotoxicity in vivo.

Conclusions

In summary, results of our study raise the question about the usefulness of AAV-based vectors for gene therapy in human cancers. Unstable and transitional episomal expression in rapidly dividing cells seems to be a limiting factor for such application. In suicidal gene therapy systems based on the usage of HSV-TK and AAV2, immediate administration of GCV is crucial to assure maximal efficacy in the elimination of cancer cells. Based on our findings it appears that for the HSV-TK/GCV approach to work much more than 50% of the tumor must be transduced with TK. Data from further studies will determine whether complete or diffused transduction of brain tumor with TK is required for complete eradication of the tumor through GCV administration in immunocompetent animal models.

Acknowledgment

We thank Dennis Deen, Ph.D., for his critical remarks in preparation of this manuscript.

References

Gene therapy limitations in treating gliomas


Manuscript received February 18, 2004. Accepted in final form October 7, 2004.

The study was supported by the National Brain Tumor Foundation from July 2002 to June 2003 and by Accelerate Brain Cancer Cure Foundation for 2002 (K.B.).

Address reprint requests to: Piotr Hadaczek, Ph.D., Department of Neurosurgery, University of California, San Francisco, MCB, 1855 Folsom Street, Room 230, San Francisco, California 94103. email: piotr@itsa.ucsf.edu.