Intraarterial Delivery of Genetic Vectors for the Treatment of Malignant Brain Tumors

Zvi R. Cohen MD[1], Revital Duvdevani PhD[1], Dvora Nass MD[2], Moshe Hadani MD[1] and Zvi Ram MD[1]

[1]Department of Neurosurgery and [2]Institute of Pathology, Sheba Medical Center, Tel-Hashomer, and Sackler Faculty of Medicine, Tel Aviv University, Israel

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Abstract

Background: The transfer of therapeutic genes into malignant brain tumors has been the subject of intense preclinical and clinical research in recent years. Most approaches have used direct intratumoral placement of a variety of vectors and genes, such as retroviruses or adenoviruses carrying drug-susceptibility genes, modified replication-competent herpes virus, and several vectors carrying tumor suppressor genes such as the p53 gene. However, clinical results have so far been disappointing, mainly due to the limited ability to effectively distribute the genetic material into the target cell population. Accordingly, alternative delivery approaches into the central nervous system, e.g., intravascular, are under investigation. Genetic vectors administered intravascularly are unlikely to penetrate the blood-brain barrier and transfer a gene into brain or tumor parenchyma. However, intravascular delivery of vectors may target endothelial cells lining the blood vessels of the brain. Since endothelial cells participate in a variety of physiological and pathological processes in the brain, their modulation by gene transfer may be used for a variety of therapeutic purposes. Angiogenically stimulated endothelial cells within tumors replicate rapidly and hence may become targets for retroviral-mediated gene transfer.

Objective: To assess the anti-tumor effect of transferring a drug-susceptibility gene into endothelial cells of the tumor vasculature.

Methods: As a model for this approach we delivered concentrated retroviral vectors carrying a drug-susceptibility gene via the internal carotid artery of rats with malignant brain tumors. The safety and efficacy of this approach, without and with subsequent treatment with a pro-drug (ganciclovir), was evaluated.

Results: No acute or long-term toxicity was observed after intraarterial infusion of the vector. Treatment with ganciclovir resulted in variable hemorrhagic necrosis of tumors, indicating preferential transduction of the angiogenically stimulated tumor vasculature. This was accompanied by severe toxicity caused by subarachnoid hemorrhage and intracerebral hemorrhage in vascular territories shared by the tumor and adjacent brain.

Conclusion: The data indicate that endothelial cells can be targeted by intraarterial delivery of retroviral vectors and can be used for devising new gene therapy strategies for the treatment of brain tumors.

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The incurable nature of malignant gliomas has made these tumors an attractive candidate for various experimental biologic therapies, including gene therapy. Delivering genetic material into brain cells is a formidable technical hurdle for which no efficient solution currently exists. Studies in animals and humans [1–3] have shown that direct injection of bioparticles, such as vector-producer cells and free viruses, into brain or solid tumors does not result in significant penetration of the particles into the intercellular spaces or in significant transduction of target cells. Accordingly, alternative delivery systems to access the central nervous system are needed. One such access route is the vascular supply of the brain.

However, genetic vectors administered intravascularly are unlikely to penetrate the blood-brain barrier and transfer a gene into brain parenchyma. Although global distribution of small (20 nm) iron oxide particles in brain tissue has been recently described after BBB disruption and intravascular infusion of the particles [4,5], the size of these particles is significantly smaller than most currently used viral genetic vectors. Similarly, intravascular administration of viral vectors into a variety of brain tumors has shown a limited transfer of the transgene into brain tumor parenchyma.

Intravascular delivery of vectors may, however, target endothelial cells in blood vessels of the brain. Endothelial cells participate in a variety of physiological and pathological processes in the brain, and modulation of their activity by gene transfer may be used for therapeutic purposes. Transduction of endothelial cells after intratumoral implantation of cells engineered to continuously release retroviral vectors carrying the herpes thymidine kinase (HStk) gene has also been shown to contribute to tumor regression after ganciclovir therapy, probably by inducing local ischemia within the tumor [6].

The angiogenically stimulated tumor vasculature renders tumoral endothelial cells to transduction with retroviral vectors. Retroviruses may be used to transfer a "suicide" gene into endothelial cells in order to disrupt tumoral vasculature and induce tumor ischemia and regression. The HStk gene is an example of a gene used in "suicide" gene therapy. Cells transduced with the HStk gene become sensitive to the antiviral drug ganciclovir, while non-transduced cells are unaffected.

BBB = blood-brain barrier

To assess the feasibility of targeting endothelial cells of normal brain and cerebral tumors by intravascular administration of retroviral vectors carrying the HStk gene, we evaluated the safety and efficacy of this approach after a single intracarotid infusion of a high titer preparation of retroviral vectors in a rat model of brain tumor.

Materials and Methods

The experiments were approved by the Sheba Medical Center Animal Care and Use Committee and were conducted in accordance with the NIH guidelines for the use and care of experimental animals.

Animals and brain tumor model

Fischer rat 9L gliosarcoma was used for the brain tumor model. 9L cells were propagated in T-175 tissue culture flasks in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (Hyclone Laboratories, Inc., Logan, UT, USA), 2 mM L-glutamine (GIBCO BRL, Gaithersburg, MD, USA), 50 units/ml penicillin (GIBCO), 50 μg/ml streptomycin (GIBCO), and 2.5 μg/ml Fungizone (ICN Biomedicals, Inc., Costa Mesa, CA, USA).

Fischer 344 rats weighing 230–300 g were anesthetized with i.p. ketamine (90 mg/kg; Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA) and xylazine (10 mg/kg; Mobay Corporation, Shawnee, KS, USA) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Syngeneic 9L gliosarcoma cells ($4x10^4$ cells in 5 μl Hank's balanced salt solution) were injected into the deep white matter of the right cerebral hemisphere.

Surgical procedure for intraarterial infusion of vector

Rats bearing 15 day old cerebral tumors were anesthetized as previously described, and an anterior midline neck incision was performed to expose the right carotid artery. The external carotid artery was ligated distally and a PE10 tube was cannulated through the stump of the external carotid artery into the common carotid artery. A 750 μl suspension of the retroviral suspension or saline was infused over 1 minute. The stump of the external carotid artery was then ligated, the cannula removed, and the rats allowed to recover.

Retroviral vector preparation

The HStk (G1Tk1SvNa.7) vector (Genetic Therapy Inc., Gaithersburg, MD, USA) has a G1 backbone derived from the Moloney murine leukemia virus (MoMLV). Each vector contains the HStk gene just downstream of the 5' long terminal repeat and uses this LTR as its promoter. The Simian virus-40 (SV40) early promoter serves as an internal promoter for the neomycin phosphotransferase gene, NeoR, which confers resistance to the neomycin analog G418. The vector packaged by the amphotropic retroviral vector producer cell line PA317, which is derived from NIH3T3 cells, has a titer of $1.0x10^4$ to $1.0x10^5$ colony-forming units per ml on NIH 3T3 cells. The cell line was negative for replication-competent virus by S+/L- assay.

At Genetic Therapy, Inc., the retroviral supernatant was further concentrated over cesium gradient to yield a preparation of 2.1x10 CFU/ml on NIH3T3 cells. This high titer preparation was used in subsequent safety and efficacy experiments.

Histological and safety evaluation

Rats with 15 day old cerebral tumors were infused over 1 minute with retroviral vector containing 2.7x10/particles (n = 8) or saline (n = 4). The stump of the external carotid artery was ligated, the cannula removed, and the rats allowed to recover. At 48 hours post-infusion two rats from each group were sacrificed and the heart perfused with 200 ml of heparinized saline (100 U/ml) to wash away residual blood. The brain was then removed, serial thin (10 μcm) sections were obtained and subjected to histological analysis using hematoxylin & eosin and myelin stains. The remaining rats were treated with ganciclovir (15 mg/kg, twice a day) for 5 days starting 48 hours after carotid perfusion, and upon completion of ganciclovir the brains were harvested as previously described and subjected to histological evaluation.

Long-term safety studies were conducted in 12 non-tumor bearing rats. Eight rats received intracarotid infusion of the retroviral vector and four received intracarotid saline infusion. Rats were examined weekly for neurological and general toxicity. Rats were euthenized 60 days after carotid infusion and the brains were removed for histological evaluation. Systemic organs (lung, heart, liver, spleen, kidney, intestine, and gonads) were also harvested for histological evaluation.

Survival studies

Rats bearing 15 day old cerebral tumors received intraarterial infusion of 750 μl of the retroviral concentrated suspension (n = 13) or saline (n = 11). The rats were allowed to recover for 48 hours and then were started on i.p. ganciclovir (15 μg/kg, twice a day) for 5 days. Mortality was documented upon death or when animals became moribund and unable to feed. The brains of these rats were removed for macroscopic and histological evaluation.

Results

Safety studies and histological findings

No clinical toxicity or histological abnormalities were observed in tumor-bearing rats that received intracarotid infusion of either retroviral vector or saline and were sacrificed 48 hours after carotid infusion.

Non-tumor-bearing rats that received intracarotid infusion of the HStk vector and followed for 60 days prior to sacrifice showed no clinical toxicity. Histological evaluation of their brains and systemic organs showed no abnormalities.

HStk = herpes simplex-thymidine kinase

LTR = long terminal repeat

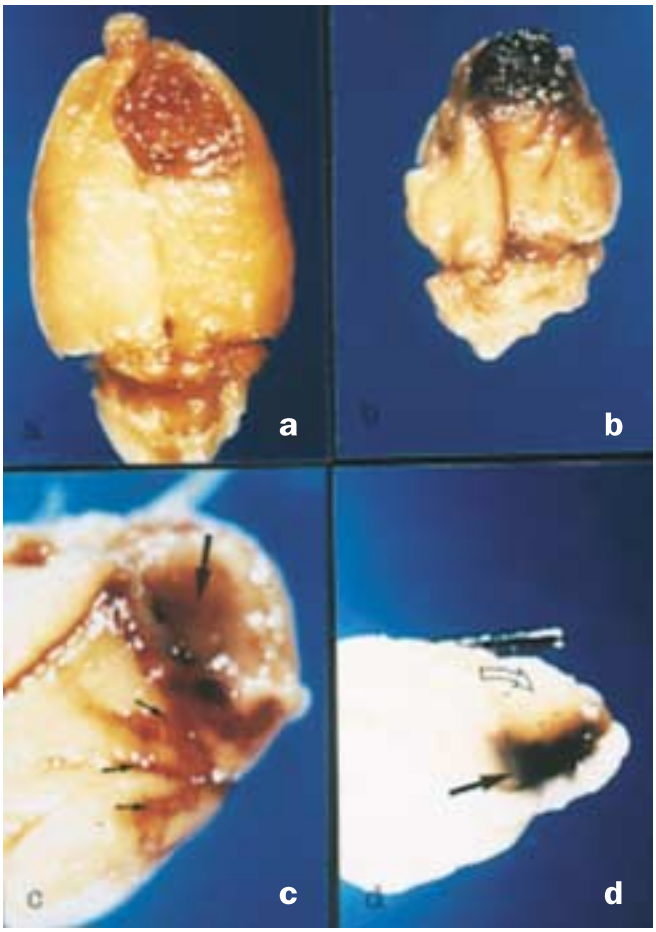

Figure 1. [a] Brain from a saline-treated rat showing no evidence of intratumoral hemorrhage after ganciclovir therapy. **[b]** HStk-treated rat showing intratumoral hemorrhage 5 days after ganciclovir administration. **[c]** Acute subarachnoid hemorrhage in a brain of an HStk-treated rat after ganciclovir therapy (short arrows). The tumor bed after removal of the tumor mass shows fresh hemorrhage (large arrow). **[d]** Intracerebral hemorrhage (arrow) adjacent to the tumor (curved arrow) in an HStk-treated rat.

Tumor-bearing rats that were treated with i.p. ganciclovir for 5 days after carotid infusion with saline showed no toxicity or histological abnormalities. However, rats receiving ganciclovir after intracarotid infusion of the HStk retroviral vector showed general signs of toxicity (eye discharge, decreased movement, and loose stool) without focal neurological deficits, starting on the second day of ganciclovir therapy. Two rats died during ganciclovir therapy (day 3 and day 5 of drug therapy). Histological evaluation of the brains of the surviving rats showed evidence of severe subarachnoid hemorrhage, intracerebral hemorrhage adjacent to the tumor mass, and intratumoral hemorrhage. The degree of tumor necrosis varied from small

SAH = subarachnoid hemorrhage
ICH = intracerebral hemorrhage

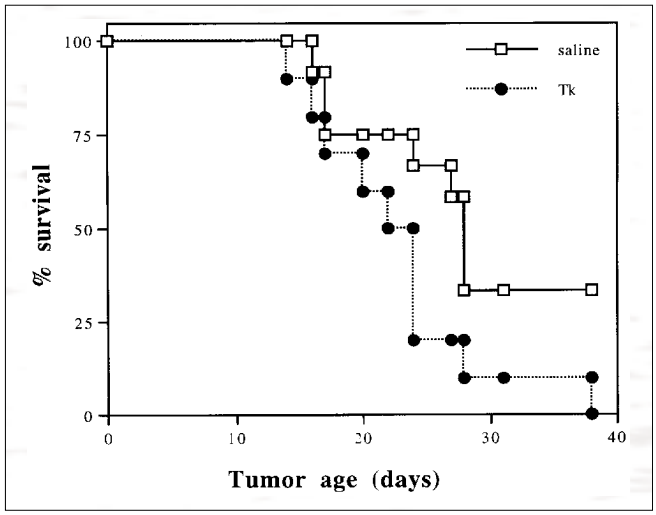

Figure 2. Survival curve of tumor-bearing rats treated intraarterially with either saline or the HStk vector and intravenous ganciclovir. Early death is seen in the HStk-treated rats due to hemorrhagic complications. (Tk = retroviral vector carrying the herpes simplex virus thymidine kinase gene).

intratumoral hemorrhage to diffuse hemorrhagic necrosis [Figure 1].

Survival studies

Saline-treated rats displayed the typical survival curve for 9L tumor, with the majority dying from their tumor within 4 weeks. However, in the HStk-treated rats, the previously observed toxic effects were seen after initiation of ganciclovir therapy, with rats dying at earlier time points than controls [Figure 2]. Histology of harvested brains displayed the same hemorrhagic complications seen in rats evaluated in the safety studies, namely SAH and ICH in brain regions supplied by the vascular territory supplying the tumor.

Discussion

The transfer of genes into the brain is limited by the lack of efficient delivery systems. Direct intraparenchymal implantation of various vector systems (genetically modified cells, viral vectors) has been shown to result in limited distribution of the particles into the intercellular space of normal brain and solid brain tumors [1,5]. *In vivo* gene transfer occurred only locally, extending from 10 to 20 cell layers surrounding the tract through which vector-producer cells were injected [3]. Although gene transfer to brain is expected to be blocked by the blood-brain barrier, intravascular injection of vector particles may target the brain endothelial cells that form the BBB. Endothelial cells constitute an important component of brain function in normal and diseased states. Endothelial cells in brain capillaries help to regulate the concentrations of ions, metabolites, neurotransmitters and drugs in the brain parenchyma [2]. Endothelial cells in the brain also participate in various disease processes, such as atherosclerosis where secretion of growth factors in response to endothelial damage may lead to the

development of lesions of atherosclerosis [7]. Angiogenesis is another condition involving endothelial cells, where neovascularization occurs in response to various growth stimuli. Tumor-associated angiogenesis [8] accompanies the rich vascularization and rapid endothelial proliferation observed in many brain tumors. Transduction of endothelial cells may be used as a relay station for local expression of the gene product and penetration of the encoded protein across the BBB into the brain; or it may be used to modulate, or affect, the function of brain endothelial cells themselves.

Our results demonstrate that a single intraarterial infusion of retroviral vector particles results in significant transduction of tumoral endothelial cells, leading to hemorrhagic necrosis of the tumors after ganciclovir therapy is initiated. However, this effect was associated with significant toxicity stemming from the side effects of tumor hemorrhage (SAH), but also from non-tumoral vascular damage to adjacent brain resulting in ICH in brain regions that are supplied by the same vascular territories as the tumor.

Retroviral vectors have been investigated in the past in *in vitro* and *in vivo* transduction experiments of endothelial cells in the peripheral circulation. Gene transfer into endothelial cells at specific sites of an artery was found to be very low even when a segment of a blood vessel was trapped between two inflated balloons before administration of the vector [9,10]. Retroviruses can only integrate into the genome of cells that are actively synthesizing DNA. The overall rate of replication of endothelial cells in adults is very low. However, focal areas in blood vessels, called high turnover regions, show a significantly higher replication rate [11], and in our study the endothelial cells in the high turnover regions in normal brain may have been the target of retroviral vector-mediated gene transfer in the perfused non-tumoral brain. Considering the inability to mechanically isolate brain vessels, as has been done in previous transduction studies of peripheral blood vessels [9,10], it is impressive that sufficient transduction of normal vasculature took place to cause ICH. This suggests that endothelial cells in normal brain can be targeted for therapeutic purposes by viral vectors. Transduction efficiency may be enhanced by repeated administration of the vector.

Endothelial cells in tumors, including those in the brain, respond to various angiogenic stimuli secreted by the tumor, which result in a rapid replication rate during the neovascularization process of growing tumors [8]. The high mitotic activity of these endothelial cells enhances their susceptibility to transduction by retroviral vectors. Our results suggest, at least in some cases, that an efficient gene transfer occurred to elicit a significant toxic effect to tumor vasculature. While the use of toxic genes, such as the HStk gene, is too risky, this approach can be exploited to target endothelial cells in tumors with other therapeutic genes that would inhibit local angiogenesis and reduce tumor blood supply.

Several issues are still unknown and will need to be addressed before an intravascular approach to deliver viral vectors can be used in clinical trials. Early inactivation of the retroviral vectors by complement or the immune system may undermine their efficacy. Although not observed in our study, release of retroviral vectors into the systemic circulation may lead to transduction of normal proliferating tissues in the body, such as the bone marrow and gonads, and result in insertional mutagenesis (activation of oncogenes or inactivation of suppresser genes) that may lead to tumorigenesis.

Endothelial cells can be targeted with retroviral vectors effectively even after only a single intraarterial infusion of the vectors. The efficacy may be augmented by continuous infusion of vectors using superselective angiographically placed catheters placed into the blood vessels feeding the tumor. Studies of intravascular transfer of selective genes should now be performed to assess the safety and therapeutic potential of this approach for initial clinical trials.

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Correspondence: Dr. Z. Ram, Dept. of Neurosurgery, Sheba Medical Center, Tel-Hashomer 52621, Israel. Phone: (972-3) 530-2650, Fax: (972-3) 530-2150 or 535-4413, email: zviram@post.tau.ac.il