

isolated from the western yew, *Taxus brevifolia*, which is insoluble in water. It is normally administered intravenously by dilution into saline of the drug dissolved or suspended in polyoxyethylated castor oil. This carrier has been reported to induce an anaphylactic reaction in a number of patients (Sarosy and Reed (1993)) so alternative carriers have been proposed, such as a mixed micellar formulation for parenteral administration, described by Alkan-Onyuksel, et al., *Pharm. Res.* 11(2), 206–212 (1994).

Gene transfer is rapidly becoming a useful adjunct in the development of new therapies for human malignancy. Tumor cell expression of histocompatibility antigens, cytokines, or growth factors (e.g., IL-2, IL-4, GM-CSF) appears to enhance immune-mediated clearance of malignant cells in animal models, and expression of chemoprotectant gene products, such as p-glycoprotein in autologous bone marrow cells, is under study as a means of minimizing marrow toxicity following administration of otherwise lethal doses of chemotherapeutic agents.

Theoretically, the most direct mechanism for tumor cell killing using gene transfer is the selective expression of cytotoxic gene products within tumor cells. Classical enzymatic toxins such as pseudomonas exotoxin A, diphtheria toxin and ricin are unlikely to be useful in this context, since these enzymes kill only cells in which they are expressed, and no current gene transfer vector is capable of gene delivery to a sufficiently high percentage of tumor cells to make use of the above recombinant enzymes.

Another strategy that has been developed to selectively kill tumor cells involves the delivery to replicating tumor cells and expression of genes encoding toxic prodrugs such as the Herpes simplex virus thymidine kinase (HSV-tk) gene followed by treatment with ganciclovir. Ganciclovir is readily phosphorylated by the HSV-tk, and its phosphorylated metabolites are toxic to the cell. Very little phosphorylation of ganciclovir occurs in normal human cells. Although only those cells expressing the HSV-tk should be sensitive to ganciclovir (since its phosphorylated metabolites do not readily cross cell membranes), in vitro and in vivo experiments have shown that a greater number of tumor cells are killed by ganciclovir treatment than would be expected based on the percentage of cells containing the HSV-tk gene. This unexpected result has been termed the “bystander effect” or “metabolic cooperation”. It is thought that the phosphorylated metabolites of ganciclovir may be passed from one cell to another through gap junctions.

Although the bystander effect has been observed in initial experiments using HSV-tk, the limitations present in all current gene delivery vehicles mean that a much greater bystander effect than previously noted will be important to successfully treat human tumors using this approach. One of the difficulties with the current bystander toxicity models is that bystander toxicity with metabolites that do not readily cross the cell membrane will not be sufficient to overcome a low efficiency of gene transfer (e.g., transfection, transduction, etc.). In the known toxin gene therapy systems, the efficiency of transduction and/or transfection in vivo is generally low. An existing protocol for treating brain tumors in humans uses retroviral delivery of HSV-tk, followed by ganciclovir administration. In rat models, using HSV-tk in this context, tumor regressions have been observed (Culver, et al., *Science*, 256: 1550–1552 (1992)). The HSV-tk approach has not proven sufficient in humans thus far, although some tumor regressions have been observed.

Similarly, the usefulness of *E. coli* cytosine deaminase (which converts 5-fluorocytosine to 5-fluorouracil and could

theoretically provide substantial bystander toxicity) in this regard remains to be established. Initial studies have shown that cytosine deaminase expression followed by treatment with 5-fluorocytosine in clonogenic assays leads to minimal bystander killing (C. A., Mullen, C. A., M. Kilstrup, R. M. Blaese, *Proc. Natl. Acad. Sci. USA*, 89: 33–37 (1992)).

Prodrug activation by an otherwise non-toxic enzyme (e.g., HSV-tk, cytosine deaminase) has advantages over the expression of directly toxic genes, such as ricin, diphtheria toxin, or pseudomonas exotoxin. These advantages include the capability to titrate cell killing, optimize therapeutic index by adjusting either levels of prodrug or of recombinant enzyme expression, and interrupt toxicity by omitting administration of the prodrug. However, like other recombinant toxic genes, gene transfer of HSV-tk followed by treatment with ganciclovir is neither optimized to kill bystander cells nor is it certain bystander toxicity will occur in vivo as has been characterized in vitro. An additional problem with the use of the HSV-tk or cytosine deaminase to create toxic metabolites in tumor cells is the fact that the agents activated by HSV-tk (ganciclovir, etc.) and cytosine deaminase (5-fluorocytosine) will kill only cells that are synthesizing DNA (Balzarini, et al., *J. Biol. Chem.*, 268: 6332–6337 (1993), and Bruce and Meeker, *J. Natl. Cancer Inst.*, 38: 401–405 (1967)). Even if a considerable number of nontransfected cells are killed, one would not expect to kill the nondividing tumor cells with these agents.

It is therefore an object of the present invention to provide vehicles that increase the efficiency of delivery of therapeutic reagents, including viral vectors, cells, nucleic acids, antibodies and other proteins, lipids, and carbohydrates, to tumors, especially brain tumors.

It is a further object of the present invention to provide vehicles that are useful for direct delivery into tumors of drugs in solid or liquid form, as well as genetic material, including genetic material contained within cells.

SUMMARY OF THE INVENTION

The major problem with current direct delivery techniques of therapeutic reagents into solid tumors, especially of cells or large volumes of recombinant DNA reagents or drugs, has been resistance of the tissues to the influx of the fluid and/or cells, resulting in low quantities of the fluid and/or cells penetrating into and remaining in the tumor tissue to be treated. Increased penetration and/or reduced backflow and diversion through the point of entry, so that more material is introduced into and remains in the tumor, is obtained through the use of a viscous vehicle, most preferably having a similar density to tissue, for the material to be delivered. Preferred materials include solutions or suspensions of a polymeric material which gel or solidify at the time of or shortly after injection or implantation. In the preferred embodiment, the solution is injected via a catheter into regions of the tumor to be treated.

DETAILED DESCRIPTION OF THE INVENTION

The general criteria for vehicles for delivery to solid tumors are that the materials must not inactivate the chemotherapeutic agent and must impart to the delivered material a density or viscosity similar to that of tissue.

Materials to be Delivered

As used herein, chemotherapeutic agents include synthetic organic or inorganic drugs, biologically active materials which replace or supplement a normal function such as