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The Pharmacology of Gene Therapy

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The objective for human gene therapy is to express exogenous DNA at a site in vivo for long enough, and at sufficient levels to produce a therapeutic response. The obstacles to this objective are numerous and include the formulation or packaging of the DNA, in vivo delivery, penetration of biological barriers, DNA elimination within the cell and from the tissue compartments of the whole body, control of product expression and overt toxicity. The current challenge is to resolve each of these obstacles to produce a practical and efficient gene therapy. In doing so, it is vital to understand the disposition of DNA vectors in vivo, and to know how conventional medicines may be used to modulate this disposition and to enhance the therapeutic effect of these vectors. Many of the general concepts of human gene therapy have been reviewed extensively in the literature. This review discusses some of the pharmacological aspects of gene delivery and the fate of vectors in vivo, and then highlights how drugs are being used to modulate gene therapy.

Key words: ganciclovir; gene targeting; genetic engineering; gene therapy; gene transfer; regulation of gene expression; somatic gene therapy; transfection; transgenes; vectors, genetic

The potential for using genetic technology to treat human diseases has stimulated the imagination of numerous researchers from a diverse range of disciplines. Gene therapy has been a prolific area of research over the past decade and continues to receive considerable attention. However, the use of genetic material as therapeutic agents has produced many novel and challenging obstacles that remain to be overcome. The current challenge is to resolve each of these obstacles to produce a practical and efficient means of treating human disease. Many of the limitations to using genetic material as therapeutic agents, such as poor bioavailability, stability in vivo, formulation and delivery to the site of action, apply to conventional medicines. In addition, understanding the disposition of DNA vectors in vivo, and how conventional medicines may be used to modulate this disposition may be essential in developing practical and clinically useful gene therapies. For these reasons, the pharmacologist has an important role in the development of clinically feasible gene therapies. This review addresses many of the aspects of the pharmacology of gene therapy. For more general reviews of gene therapy and current approaches to delivering genetic material to cells and tissues, the reader is referred to recent literature (1-6).

Broadly speaking, gene therapies can be divided into 3 categories based on the mode of delivery to the target tissue (7). The first category, ex vivo delivery, involves removing the target cells from the patient, transfecting the cells with a transgene and then returning them to the patient. This approach is limited to those cells that can be readily obtained and cultured, and bypasses many of the pharmacological problems associated with the other categories. The second category involves direct application of the transgene to the target tissue. Examples include attempts to delivery transgenes to the lungs of cystic fibrosis patients by intratracheal application or direct injection of genes into solid tumors. Intramuscular injection has also been attempted for the treatment of muscular dystrophy. The third category is in vivo delivery via the blood stream. This is the most versatile approach but is limited by the complex pharmacological problems associated with circulating DNA. To date, no clinical trails have commenced using delivery via the circulation (7). However, for gene therapy to become an acceptable and routine clinical procedure, this mode of delivery will need to be refined and developed.

Gene Therapies and Dose-Response Relationships

The relationship between the amount of transgene administered to a host and therapeutic response often receives little attention in preclinical gene therapy studies. There are several possible reasons for this. Firstly, for many diseases under consideration for gene therapy, good animal models are not available. Human genetic diseases can be particularly difficult to reproduce in animals, although the recent use of transgenic animals has been of assistance (8-14). Animal models may not include physiological parameters that can affect transgene expression and therapeutic response. For example, many anti-cancer gene therapies have a demonstrated efficacy in tumor transplant animal

models (15-19). However, these models often do not take into consideration parameters such as normal tumor vascularization, cellular and extracellular heterogeneity, lymph drainage and tumor growth rates that differ markedly between the animal model and primary or secondary tumors in humans. Secondly, a single dose therapeutic response in an animal model may be sufficient to warrant human studies. However, factors that can affect response such as differences in size, immunological activities (20), and metabolic rates (21,22) need to be considered (22-25). For example, differences in the immunological response between experimental animals and humans to vectors used for gene therapy have emerged as a major concern and have particularly affected the design of adenovirus-based gene therapies in humans. This is the result of most humans having been exposed to adenoviral infections and having developed neutralizing antibodies to the virus (26,27). Animal models used to assess gene therapies do not have this limitation. Thirdly, dose escalation studies aimed at determining the maximum response and toxicity of a transgene require amounts of material that are often difficult to produce. The choice of animal models and their predictive power for subsequent human studies remains an important area in gene therapy research.

A number of preclinical studies have revealed interesting insights into the relationship between gene dose and activity. In a murine tumor model designed to evaluate the effects of an adenoviral vector encoding tumor necrosis factor-a (TNF-a), Mauceri et al (28) showed a dose-dependent decrease in tumor size over the dose range of 1x107 to 5x108 plaque forming units (PFU) administered directly into the tumor (Fig. 1). Half-maximum response was seen at approximately 1.6x108 PFU. This is equivalent to a dose of 8x109 PFU/kg. In a similar study using an adenoviral based HSV-thymidine kinase (HSVtk) vector as a suicide gene, Goebel and associates (29) reported a dose-dependent reduction in tumor growth rate but only observed a reduction in the size of an established tumor at a dose of 1010 PFU (5x1011 PFU/kg). These doses suggest that very large amounts of viral vector will be required to treat human cancers where solid tumors can be as large as 1010 cells at the time of diagnosis.

A common approach to cancer gene therapy is to use suicide transgenes. These genes express an enzyme such as herpes simplex thymidine kinase that activates a pro-drug to kill the cell. A range of suicide transgenes are available but differ considerably with respect to their bystander activity (ability of transfected cells to kill adjacent non-transfected cells) (30). Suicide gene therapy introduces a further complication in that response will be dependent on selecting both the appropriate gene dose and the appropriate pro-drug dose. Sewell et al (31) studied these two parameters in a murine model and showed that both the viral titres and the pro-drug dose (ganciclovir) necessary for a therapeutic response may be considerably less than that routinely predicted from in vitro studies. Minimizing the dose of ganciclovir is clinically important since the drug exhibits considerable side-effects within its therapeutic range (32).

Figure 1. Dose-response relationship for adenovirus encoding TNF-a and tumor growth in a murine model. Mice were inoculated with the human cell line SQ-20B and then treated intratumourally with an adenovirus-based vector that expresses TNF-a. Adapted from Mauceri et al (28).

Figure 2. Compartmental model describing the intracellular disposition of transgenes and their products. The 6 compartment model shows a sequential series of events from uptake of vector from the extracellular space to secretion of the protein product. Each compartment has an elimination process representative of the degradation pathway for each component. Adapted from (38).

Some clinical assessment of gene dose-response has been reported, although mostly with respect to toxicity (33-36). Intratumor injection of adenoviral vectors encoding wild-type p53 with doses up to 1010 PFU to patients with non-small cell lung cancer is well tolerated (35) although intra- pleural doses of 1012 PFU to patients with mesothelioma induced minor side effects including temporary systemic inflammation (36). In cystic fibrosis patients, a dose escalation study over 3 orders of magnitude failed to demonstrate a therapeutic response to an adenovirus encoding the cyctic fibrosis transmembrane conductance regulator despite evidence for transgene expression in 5 of 6 patients (37).

Pharmacokinetics of DNA in Vivo

Kinetics of Cellular Uptake and Intracellular Trafficking

Two aspects of the kinetics of exogenous DNA in vivo can be considered from: (a) how DNA is taken up and processed at its target site; and (b) how it is handled by the body as a whole. The first serious attempt to understand the kinetics of gene therapy at a cellular level was reported by Ledley and Ledley in 1994 (38). They outlined the likely events that might affect transgene expression and proposed a 6 compartment linear model to describe the uptake, intracellular trafficking and expression

of exogenous genes (Fig. 2). Their classical pharmacological compartmental approach, although theoretical in nature, provided a basis for developing strategies to enhance or modulate transgene expression at a cellular level. Interestingly, their analysis suggested that promoter strength might be of limited assistance in optimizing gene expression if DNA, RNA, and protein stability are ignored.
At a cellular level, intracellular trafficking appears to be a critical rate-limiting function, especially for non-viral vectors (Fig. 2). Uptake via the endosomal system can be enhanced pharmacologically with drugs that inhibit endosomal acidification or by agents that enhance the rate of DNA release from the endosomes. This was elegantly demonstrated in vitro using the transferrin-based non-viral delivery system pioneered by Birnstiel and associates (39). Chloroquine (40,41), HA-2 derived peptides (42), and adenoviral particles (43-45) all increase the release of intact transgene from the endosomes and increase gene expression. Whether such agents will be effective in vivo in humans is yet to be established.
Another example of the use of more conventional therapies to modify the uptake and expression of transgenes was demonstrated by Patijn et al (46). They used hepatocyte growth factor (HGF) to enhance expression of a retroviral vector in the liver where expression of most vectors is small because of the non-proliferative nature of the hepatocytes. HGF induces a mitogenic response in the liver resulting in cell proliferation and a degree of hypertrophy in vivo (46-48). In this manner, it mimics the proliferation following partial hepatectomy that has been shown to enhance hepatic transgene expression (49). When administered by constant infusion over 5 days, up to 30% of hepatocytes were stably transduced following retroviral administration. By contrast, Kosai and associates (50) found that less than 1% of hepatocytes were transduced if HGF was administered intravenously as a bolus dose. Nevertheless, this may be sufficient to elicit a pharmacological response. Given the importance of transgene expression in the liver for a number of gene therapy protocols (49,51-53), these findings are significant and clinically relevant. Other mitogens such as tri-iodothryonine also have been shown to enhance liver expression (54), although at much lower levels than that reported by Patijn et al (46).

Fate of Exogenous DNA in Vivo

DNA used in gene therapy can be administered in vivo by use of viral particles, complexes with cationic macromolecules or liposomes, or simply as naked DNA (7). A number of systems have been described where expression of transgenes in peripheral tissues occurs following intravenous injection of vectors formulated as cationic complexes, as liposomes or as adenoviral particles (49,55-59). Intravenous administration is attractive pharmacologically as it is simple and because it may provide access to tissues where direct injection of genetic material is not practical. Targeting of exogenous DNA and its persistent expression at therapeutically useful levels in peripheral tissues following intravenous injection is still under development (7).
Delivery of foreign DNA to specific tissues in vivo is influenced by many factors including the structure and size of the DNA, stability against nuclease degradation, and the pharmacokinetics of the delivery system (38,60). After administration, plasmid DNA that enters the circulation is rapidly removed mainly by the non-specific scavenger receptors located in the liver (61,62). These receptors are involved in removing anionic macromolecules from the circulation (63). DNA complexed to cationic liposomes also can be rapidly cleared from the circulation via a mechanism involving Kupffer cell phagocytosis (64,65). Similarly, virions administered intravenously have been shown to distribute primarily to the reticuloendothelial system (66).
Plasmid DNA is extensively degraded in vivo although exactly where this degradation takes place remains unknown (62). The very rapid clearance of transgenes from the plasma compartment may be advantageous when DNA is delivered locally using direct injection, since it will limit the likelihood of expression of the exogenous DNA that inadvertently escapes the site of delivery. However, for systems dependent on delivery via the circulation, rapid removal by the liver will limit the extent of gene transfer to peripheral tissues, especially those with relatively small blood flows.

Figure 3. Disappearance of plasmid DNA in rats. Animals were administered 35S-DNA intra-arterially either alone (circles) or with the non-specific scavenger receptor antagonist polyinosinic acid (triangles). Blood samples were collected over 20 min and intact DNA was quantified after agarose gel electrophoresis. The clearance of plasmid from the plasma was 30 mL/min/kg which exceeded total liver blood flow. In the presence of polyinosinic acid, clearance decreased to 11 mL/min/kg.

Understanding the pharmacokinetics of exogenous DNA in vivo can assist in designing strategies that maximize gene transfer by enhancing uptake, distribution or clearance. For example, Fig. 3 shows the plasma-time profile for plasmid DNA following intravenous administration to rats (unpublished data). The half-life of the intact DNA was significantly increased if polyinosinic acid, a potent inhibitor of the hepatic scavenger receptors, was co-administered with the DNA. Other agents such as dextran

sulfate also may be used in this manner to enhance plasmid DNA half-life (62). However, disappearance of DNA from the circulation is still relatively rapid due, in part, to degradation by DNase (62). There are at least two approaches that could be used to inhibit DNA metabolism and further increase the circulation time of transgenes. Firstly, nuclease inhibitors may be developed for use in vivo although, to date, no such agents are available. Secondly, the DNA can be packaged to render it resistant to degradation. DNA condensed with polycations is not readily metabolized by nucleases due to limited access of the enzyme to the DNA. However, the degree of protection is dependent on the length of the polycation (67). Polylysines with an average length of 256 amino acids were significantly better at protecting condensed DNA from degradation compared to polylysine of only 96 amino acids (67).

Route of Administration

The route of vector administration has been shown to influence the extent of transgene expression (68-70). For DNA given by non-intravenous methods, bioavailability of the construct and the gene product may vary considerably according to how and where the vector was administered to the patient. Moreover, immunological responses in vivo to transgenes appear to be quite different depending on the route of administration (69).

Figure 4. Disposition of transgenes in vivo. Administration of vectors into the central compartment results in distribution to the target tissue where access to parenchyma tissue will require crossing the endothelial cell and basement membrane barriers. DNA also may be taken up by non-target tissues where it can be eliminated. Metabolism of DNA in the central compartment can occur by nucleases in the plasma or associated with cells lining the blood vessels.

Transgenes administered intravenously must cross the endothelial cell barrier and basement membrane before reaching target cells in the parenchyma (Fig. 4). This can be a very slow process compared to general tissue distribution, metabolism, and excretion, hindering efficient gene delivery. Alternative routes of administration may be ideal for some tissues by avoiding both rapid clearance from the central compartment and the endothelial cell/basement membrane barrier. This was recently illustrated for gene delivery to the pancreas (71), an organ that receives less than 5% of cardiac output (72). Uptake of cholecyctokinin-DNA complexes by the pancreas following intravenous administration was minimal even when hepatic clearance was blocked. However, intraperitoneal administration of the complex resulted in about 25% of the dose accumulating in the pancreas over 24 hr. Whether this was due to lymphatic drainage of the peritoneum or some other delivery mode is currently unknown. Targeting of tumors in the pancreas by intraperitoneal administration of transgenes has been recently reported by Yang et al (73). In a murine pancreatic tumor model, these investigators showed that intraperitoneal administration of HSV-thymidine kinase encoded by a retroviral vector resulted in the integration of the virus into the genome of the tumor cells. Moreover, on the addition of ganciclovir, a significant reduction in tumor growth was seen demonstrating the practical feasibility of intraperitoneal transgene administration.

Pharmacological Modulation of Therapeutic Genes

Conventional medicines are increasingly being used to overcome some of the obstacles faced with gene therapy. Drugs have been used to reduce unwanted immune responses to transgenes, regulate their expression and enhance their therapeutic effects (Table 1). However, multi-agent therapy introduces further pharmacological considerations such as potential interactions between the drug(s) and the transgene product, dosing regimes and toxicity of the combined treatment. When conventional drugs are used to supplement the effects of gene therapies, additive or synergistic responses also may need to be considered.

Immunosuppression and Gene Therapy

Immunological responses to foreign DNA vectors have presented a major problem for gene therapies that are not designed to immunize the patient against the antigen expressed from the transgene. Immunomodulation can be directed towards the vector itself (74-76) or towards the gene product (77-79). The induction of an immune response to vectors used for gene therapy can lead to a decrease in transfection efficiency by neutralizing antibodies, destruction of target cells and inflammation (80). Importantly, immune responses have been shown to limit multiple dosing with the same vector (75). However, in several animal models, transgene expression following single and multiple doses has been prolonged or enhanced by simultaneously immunosuppressing the animal (Table 1). Three classes of immunosuppressant agents have been investigated: anti-T cell antibodies, immunophilin ligands, and cytotoxic drugs. All target the T-cell response to antigen either by down-regulating essential surface receptors (antibodies), inhibiting calcineurin- dependent intracellular signaling (immunophilin ligands), or by non-specific toxicity (cytotoxic agents). The action of some of these

drugs may not be limited to their immunomodulatory activities. Russell and co-workers (56,81) showed that etoposide, a topoiso- merase inhibitor, increased transduction of cultured cells by adeno-associated virus at least 2 orders of magnitude. However, they were unable to show an effect of etoposide in vivo. To date, the combination of immunosuppressive agents and gene therapies in humans has not been critically evaluated. Sullivan et al (78) reported that immunosuppression of rhesus monkeys with cyclophosphamide and prednisone helped sustain expression of a reporter gene in the liver suggesting that this approach may be beneficial in humans.
Zhang et al (82) recently demonstrated that soluble tumor necrosis factor binding protein inhibited the inflammatory response to adenoviruses and prolonged transgene expression following intravenous or intranasal administration to mice. Their results suggest that TNF-a has a central role in early inflammation and may be an important therapeutic target for future drug development.

Table 1. Immunosuppressant agents used to enhance gene therapy

Table 2. Drug-regulatable gene expression systems used for gene therapies

Regulation of Transgene Expression
The ability to regulate transgene expression over a long period will be critical for gene therapies that require intermittent production of the gene product. In addition, regulatable expression may be necessary for gene products that have small differences between effective and toxic doses (small therapeutic window). A number of laboratories have started to develop drug-sensitive promoter systems that allow for control of transgene expression and some of these systems have been tested in vivo. Gene regulation by a secondary drug may provide a quantal response (on/off switch) or a graded response (concentration-dependent effect) depending on the system. Most notable is the tetracycline-dependent vectors originally developed by Gossen and Bujard in 1992 (83). This system is based on two regulatory elements from the tet operon of the E. coli Tn10 transposon and gives a graded response to treatment with tetracycline or its analogs (84-86). A number of investigators have constructed vectors suitable for gene therapy incorporating a tetracycline-dependent promoter and have demonstrated regulated gene expression in cultured cells (87-91) and in vivo (92-96). Harding et al (97) recently showed tertracycline-dependent transgene expression in vivo. They transfected neuronal cells of rats with an adenovirus encoding the green fluorescent protein under the control of a tetracycline-regulatable promoter. By adding and removing doxycycline from the drinking water of the animals, expression of the reporter gene could be switched on and off, respectively. Saitoh and associates (86) also showed doxycycline-dependent gene expression in vivo by implanting polymer-encapsulated neuroblastoma cells carrying a tetracycline-regulatable proopio- melanocortin gene into the subarachnoid space of the central nervous system. Upon intraperitoneal administration of doxycycline, a dose-dependent release of ACTH has observed. Together, these studies indicate the potential of tetracycine-regulatable gene expression in whole animals.
There are several other drug-dependent expression systems that have been described (Table 2). Direct engineering of the cAMP response element upstream of a transgene can provide cAMP-dependent expression (98,99). In cell culture, these vectors can be activated by agents, such as forskolin, that increase intracellular cAMP levels. Suzuki et al (100) showed enhanced expression of a cAMP-regulatable b-Gal gene in the pulmonary epithelium of mice following treatment with the cAMP analog 8-bromo-cAMP and the phosphodiesterase inhibitor theophyline. Other drug-dependent expression systems are less well studied but show the potential for different promoter sequences as switches in gene therapy.
Drug Enhancement of Gene Delivery
Drug combinations can be used to treat a range of diseases where single drug therapies have little or no effect. Often the success of multi-drug treatments is due to additive or synergistic responses and is best when the individual drugs act via different mechanisms to produce similar responses. This can lead to much lower, and therefore safer, drug doses required to achieve the same effect. The possibility that conventional drug treatments may complement gene-based treatments is important for optimizing clinically relevant gene therapies. Already, several interesting drug-gene combinations have been reported, mostly for cancer-directed gene therapies (55-62) .
Insertion of the p53 gene into rapidly proliferating tumor cells lacking functional p53 can induce apoptosis and tumor suppression in vivo. The effects of p53 gene transfer has been shown to be enhanced with cisplatin, a conventional anti-cancer drug (101-103). The mechanism of enhancement may be multifactorial. Nguyen et al (103) showed that pretreatment of H1299 cells, which lack functional p53, with cisplatin before transfection with wild-type p53 was critical for optimum effect of the drug-gene combination in vivo. By contrast, Ogawa et al (101) reported that wild-type p53 already

expressed in human colon cancer cells sensitized them to subsequent treatment with cisplatin. Clearly, the in vivo pharmacokinetic-pharmacodynamic relationship of these two therapies needs to be more thoroughly investigated.
Cisplatin also has been combined with interferon-g gene therapy in a murine ovarian carcinoma model (104). This study suggested that cisplatin increased the transfection efficiency of the transgene which was delivered as a cationic lipid complex. The combination was effective in vivo in part due to induction of inducible nitric oxide synthase. Finally, the anti-tumor activity of the HSV-tk/ganciclovir system against human or rat osteosarcomas (in a murine model) is significantly enhanced when methotrexate is co-administered (105).
Other drug-gene combinations have been reported. Bradykinin, and its analogue RMP-7, have been used to enhance the intracarotid delivery of transgene vectors leading to a significantly greater therapeutic effect (106,107). These drugs appear to act as permeabilisers of the blood-brain barrier allowing greater access of the pro-drug ganciclovir. Suicide gene therapy often relies on intracellular communication involving gap junctions for the bystander effect which permits killing of adjacent cells that are not transduced. Without this effect, suicide gene therapy would have limited anti-tumor activity. Park and associates (108) have shown that retinoic acid increases gap junction communication by inducing connexin expression. Combining retinoic acid treatment with HSVtk gene therapy augmented the bystander effect in vivo resulting in a greater efficacy of the suicide transgene (HSVtk/ganciclovir). Finally, Mhashilkiar et al (109) showed that protein kinase C inhibitors and anti-Tat intracellular antibodies cooperatively inhibit HIV replication.
In summary, the increasing use of conventional drugs to modulate the efficacy of gene therapies may be essential for the long-term clinical development of gene-based treatments. In this review, the role of drugs in optimizing the delivery and pharmacokinetics of transgenes has been demonstrated. In addition, it has been shown how conventional medicines may be useful in enhancing responses to transgenes as well as regulating their level of expression in target tissues.

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