Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

ENZYME ACTIVATING PRODRUG THERAPHY IN CANCER

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ABSTRACT

The selective activation of prodrug in tumor tissues by exogenous enzyme for cancer therapy can be accomplished by several ways, including gene-directed enzyme prodrug therapy, virus-directed enzyme prodrug therapy, and antibody-directed enzyme prodrug therapy. The central part of enzyme/prodrug cancer therapy is to deliver drug-activating enzyme gene or functional protein to tumor tissues, followed by systemic administration of a prodrug. In this article, disadvantages and advantages associated with each approach and future perspective for improving current systems are discussed.

Keywords: Enzyme prodrug therapy, Gene-directed enzyme prodrug therapy, Virus-directed enzyme prodrug therapy, and Antibody-directed enzyme prodrug therapy.

INTRODUCTION

Almost all drugs possess some undesirable physicochemical and biological properties. Their therapeutic efficacy can be improved by minimising the undesirable properties. This can be achieved through Biological, Physical, Chemical, approaches. The Biological approach is to alter the route of administration. The physical approach is to modify the design of dosage form. The third and best approach is chemical approach by design and development of new drugs, design of hard and soft drugs, design of prodrugs [1].

TYPES OF PRODRUGS

- Carrier prodrugs- prodrug+carrier molecule, Example: becampicillin.
- Bioprecursor prodrugs-active drug within their chemical structure, Example: levodopa.

APPLICATIONS OF PRODRUG APPROACH
Pharmaceutical applications

Improvement of taste, Improvement of odour, Change of physical form, Reduction of GI irritation, Reduction of pain on injection, Enhancement of drug solubility and dissolution rate, Enhancement of chemical stability of drug.

Pharmacokinetic applications

Enhancement of bioavailability, Prevention of presystemic metabolism, Reduction of toxicity, Site specific drug delivery, Prolongation of duration of action.

CANCER

Cancer is a disease characterized by uncontrolled proliferation and spread of abnormal forms of the body's own cells. It is the second most cause of death in the developed nations and one in three people will be diagnosed with cancer during their lifestyle. In the U.K over 365000 new cases were reported and mortality in 2006 was in excess of 154000.cancer is responsible for approximately ¼ of all deaths in the U.K., with lung and bowel cancer comprising the largest category, closely followed by breast and prostate cancer. Statistics from most other countries in the developed world tell much the same story. At first sight, incidence figures for the past 100 years or so give the impression that the disease is increasing in developed countries, but cancer is largely a disease of later life and with advances in public health and medical science, many more people now live to an age where they are more liable to contract cancer [2].

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Asian J. Pharm. Res. Vol 2, Issue 1, 40-50, 2012.

Cancer chemotherapy: general principles

The term 'cancer' refers to a malignant neoplasm (new growth).Cancer cells can manifest: Uncontrolled proliferation (no differentiation), Invasiveness, The ability to metastasise. Most anticancer drugs are antiproliferative and will also affect rapidly dividing normal cells, and are thus likely to depress bone marrow, to impair healing, to depress growth, to cause sterility and hair loss, and to be teratogenic. Most cause nausea and vomiting [3].

Pathophysiology of cancer

A normal cell turns into a cancer cell because of one or more mutations in its DNA, which can be inherited or acquired, usually through exposure to viruses or carcinogens (e.g. tobacco products, asbestos).a good example is breast cancer; women who inherit a single defective copy of either of the tumour suppress of genes BRCA1, BRCA2 have a significantly increased risk of developing breast cancer. However, carcinogenesis is a complex multistage process, usually involving more than one genetic change as well as other, epigenetic factor (hormonal, co-carcinogen and tumour promoter effects, etc.) that do not themselves produce cancer but which increase likelihood that the genetic mutations will eventually result in cancer [4].

Classification

Cancers are classified by the type of cell that the tumor resembles and is therefore presumed to be the origin of the tumor. These types include Solid tumors eg: breast cancer, ovarian cancer etc. Tumors in blood eg: polycythemia, leukemia, hodkings disease etc., But tumours are generally classified as benign tumours and malignant tumours.

Benign tumors

Edge is well defined, Usually it is covered by sheath, Can be removed by surgery, Chemotherapy is not required, If untreated leads to malignant cancer.

Malignant tumors

Edge is not well defined, No specific shape but it will be proliferating which looks like a web of crab hence it is called as cancer, Surgery is difficult, Chemotherapy is required, Metastasis is seen which may produce secondary tumors.

The Goal of Cancer Treatments

- Curative
- Total irradication of cancer cells
- Curable cancers include testicular tumors, Wills tumor
- Palliative
- Alleviation of symptoms
- Avoidance of life-threatening toxicity

- Adjuvant therapy
- Attempt to eradicate microscopic cancer after surgery

STRATIGIES FOR ENZYME/PRODRUG FOR CANCER THERAPY

The selective activation of prodrug (s) in tumor tissues by exogenous enzyme(s) for cancer therapy can be accomplished by several ways, including gene-directed enzyme prodrug therapy (GDEPT), virus-directed enzyme prodrug therapy (VDEPT), and antibody-directed enzyme prodrug therapy (ADEPT). The central part of enzyme/prodrug cancer therapy is to deliver drug-activating enzyme gene or functional protein to tumor tissues, followed by systemic administration of a prodrug. Although each approach (GDEPT, VDEPT, and ADEPT) has been tested in clinical trials, there are some potential problems using the current delivery systems. In this article, disadvantages and advantages associated with each approach (GDEPT, VDEPT, and ADEPT) and future perspective for improving current systems is discussed [5].

Chemotherapy is an important treatment for cancer patients. However, its success is limited by several drawbacks, including insufficient drug concentrations in tumors, systemic toxicity, lack of selectivity for tumor cells over normal cells, and the appearance of drug-resistant tumor cells. A number of strategies have been used to overcome these problems, including alternative formulations (e.g., liposomes), resistance modulation (e.g., PSC833), antidotes/toxicity modifiers (e.g., ICRF-18), and gene therapy.

One promising area for improving tumor selectivity is enzyme prodrug therapy. Enzyme-activating prodrug therapy is a two-step approach. In the first step, a drug-activating enzyme is targeted and expressed in tumors. In the second step, a nontoxic prodrug, a substrate of the exogenous enzyme that is now expressed in tumors, is administered systemically. The net gain is that a systemically administered prodrug can be converted to high local concentration of an active anticancer drug in tumors.

To be clinically successful, both enzymes and prodrugs should meet certain requirements for this strategy. The enzymes should be either of nonhuman origin or human protein that is absent or expressed only at low concentrations in normal tissue. The protein must achieve sufficient expression in the tumors and have high catalytic activity. The prodrug should be a good substrate for the expressed enzyme in tumors but not be activated by endogenous enzyme in nontumor tissues. It must be able to cross the tumor cell membrane for intracellular activation, and the cytotoxicity differential between the prodrug and its corresponding active drug should be as high as possible. It is preferred that the activated drug be highly diffusible or be actively taken up by adjacent non

expressing cancer cells for a "bystander" killing effect, the ability to kill any neighboring non expressing cells . In addition, the half-life of active drug should be long enough to induce a bystander effect but short enough to avoid the drug leaking out into the systemic circulation.

Currently, delivery methods for an enzyme/prodrug strategy can be divided into two major classes: (a) delivery of genes that encode prodrug-activating enzymes into tumor tissues (GDEPT, VDEPT, etc.); and (b) delivery of active enzymes on to tumor tissues (ADEPT). The aim of this review is to summarize some of the areas of recent progress in enzyme-activating prodrug therapy and discuss areas of future development.

GENE DIRECTED ENZYMATIC PRODRUG THERAPHY (GDEPT)

GDEPT, also known as suicide gene therapy, is a technique that involves physical delivery of a gene for a foreign enzyme to tumor cells where a systemically administered nontoxic prodrug can be activated after expression of the enzyme. Many GDEPT studies have used liposomal gene delivery, but the challenge of vector delivery is common for all areas of gene therapy and has been exhaustively reviewed elsewhere [6-8]. An early example of GDEPT is the combination of HSV-TK and GCV. GCV is an antiviral drug, which is phosphorylated by HSV-TK and then by cellular kinases to produce GCV triphosphate, which disrupts DNA synthesis during S phase, leading to cell death. A second early example is the combination of the bacterial CD and the antifungal drug 5-FC, which was effective to kill tumor cellsis,after the conversion by CD to active 5-FU [9- 13].

GDEPT can also be used to improve the selectivity of currently used agents. CYP-based prodrug activation systems are one example that shows promise for clinical use. Members of the CYP enzyme super family convert the hemotherapeutic agents cyclophosphamide and IFA to active alkylating agents that cause cell death. The expression of CYP is generally high in liver but lower in tumor cells [22], providing a potential mechanism for intrinsic drug resistance. Tumor cells are highly sensitive to cyclophosphamide or IFA after the delivery of the CYP2B1 gene into tumor cells both in vitro and in vivo [23]. To additionally improve and increase the efficiency of this system, a two enzymes/one prodrug system was developed. In this study, CYP2B1 was co expressed with the RED gene and used to activate prodrug cyclophosphamide in tumor cells

The rationale is that CYP-catalyzed prodrug activation is dependent on electron reduction from the flavoenzyme RED, an enzyme widely expressed in many cell types, including tumor cells. Despite the significant level of endogenous basal expression, the authors found

that RED gene transfer greatly enhanced the cyclophosphamide sensitivity of tumor cells transfected with CYP2B1. Thus, endogenous RED levels in tumor tissues may be an important determinant for the sensitivity of tumor cells to CYP/cyclophosphamide gene therapy. This result also suggested that CYP-based cancer gene therapy might be particularly effective for tumors that express a high endogenous level of RED

Recently, an approach to improve the CPA/MTX-_- peptide system was described. CPA is a zymogen that becomes catalytically active after its propeptide is removed by trypsin. Activated CPA converts MTX-_-peptide prodrug into active MTX that inhibits dihydrofolate reductase and causes cell death. Because trypsin is localized in the small intestine but is absent in tumors, the prodrug activation by CPA is limited to the intestine, causing local toxicity and low drug concentration in tumors. An additional advantage of this system is the potential use for other antifolates, such as the _-peptide conjugates of thymidylate synthase inhibitors.

VIRUS DIRECTED ENZYMATIC PRODRUG THERAPY (VDEPT)

VDEPT is a pharmacologically oriented gene therapy strategy that uses viral vectors to deliver a gene that encodes an enzyme that is capable of converting a systemically administered nontoxic prodrug into a cytotoxic agent within tumor cells [24]. The NTR/CB1954 combination was an initial example of VDEPT in which colorectal and pancreatic cancer cells were found to be sensitized to CB1954 after retroviral transduction and expression of the E. coli NTR gene [25]. Currently, several viruses have been used for VDEPT, including retroviruses, adenoviruses, HSV (26), adeno-associated virus [27–29], lentivirus, and EBV [30]. Over the years, many drug-activating enzyme gene/prodrug combinations have been delivered into tumors in vitro or in vivo by VDEPT, the majority using CD/5-FC or HSV-TK/GCV with the involvement of retroviral and adenoviral vectors. These examples were reviewed elsewhere [31]. Several recent illustrations of VDEPT are described below and are also listed in Table 2 to highlight novel therapeutic strategies.

Recently, recombinant retroviruses were used to individually deliver six different cyclophosphamide- or IFA-metabolizing human CYP genes to 9 L gliosarcoma cells. It wasfound that CYP2B6 and CYP2C18 transfection yielded pronounced cytotoxicity after cyclophosphamide treatment, with more efficient prodrug activation and cytotoxicity observed after transfection with RED. An adenoviral vector was used. Despite extensive use of retroviral and adenoviral vectors to deliver prodrug-activating enzyme genes, both vectors have some disadvantages, which limit the use of VDEPT. The major

disadvantage associated with a retroviral vector is that recombinant retroviruses only target dividing cells, whereasmost human tumor cells are slowly dividing, yielding a low transduction rate (2–10%). When this strategy uses HSV-TK/GCV or CD/5-FC to generate antimetabolites, which also require cell division for activity, it is not surprising that the results are less than dramatic. However, this drawback could be beneficial in some case. Brain tumors, where only tumor cells are proliferating, allow for a high tumor: normal transfection differential for retroviral delivery [37]. The low retrovirus titer, leading to decreased infection efficiency, is another drawback. Some researchers have been trying to increase retrovirus titer for VDEPT. It was found that prolonged low speed centrifugation during viral preparation was a simple way to concentrate recombinant retrovirus 100-fold. Another disadvantage is that retroviruses produced from murine or dog cells are all sensitive to human serum when applied in human subjects, whereas viral particles generated from human cells are more resistant.

To solve this problem, efforts have been made to develop a variety of packaging cell lines that produce high-titer recombinant retroviruses resistant to human serum. In addition, a soluble protein called Gal 1–3Gal was found to protect retroviruses from human serum when coadministered with retroviruses. The other disadvantage as associated with retroviral vectors include immunogenicity, risk of insertional mutagenesis, risk of reversal to wild-type virus, envelope-induced complement-mediated inactivation, difficulties in producing high-titer viruses, and only targeting dividing cells.

Compared with retroviruses, adenoviruses have some advantages, including higher titers capable of generating infections in both dividing and non dividing cells. The disadvantages of adenoviral vectors include immunogenenicity, reversal to wild type, and short periods of gene expression in dividing cells. In addition to adenoviral and retroviral vectors, an EBV-based viral vector has been used to deliver exogenous enzyme-encoding CD or NTR into EBV-positive B-cell lines to activate 5-FC or CB1954, respectively [30]. Both enzyme systems were effective to kill tumor cells in vitro in a prodrug dependent manner.

GENE PRODRUG ACTIVATION THERAPHY (GPAT)

GPAT is a variation of GDEPT, which uses known transcriptional differences between normal and tumor cells to drive the selective expression of a drug-metabolizing enzyme to convert a nontoxic prodrug into a toxic moiety. The goal of GPAT is tumor-selective therapy, and this strategy has been used for breast and pancreatic cancer therapy, TREs are placed upstream of the enzyme gene, driving selective expression [38]. A number of tumor-specific TREs have been used, including genes that are amplified in tumor cells compared with normal cells or genes that express tumor-associated antigens, such as CEA for colorectal cancer or N-myc for neuroblastoma. Alternatively, TREs of tissue-specific genes can also be used for GPAT (Table 3).

GPAT has been applied clinically. Thus far, there are 20 Phase I trials for GPAT in progress worldwide. HSV-TK and CD systems are the main activating enzymes used in these clinical trials. The first targeted gene therapy trial for breast cancer and the first to use the CD system in human subjects were performed by Panhda et al. Because over expression of erbB2 was found in 20–50% of breast carcinoma, the erbB2 promoter was used to drive the tumor-specific expression of the E. coli CD gene. In a Phase I clinical trial, the therapy was performed in 12 breast cancer patients who received both intratumoral injections of a plasmid construct containing the therapeutic cassette of the E. coli CD gene driven by the erbB2 promoter and systemic administration of the prodrug 5-FC. The expression of the therapeutic construct was observed in the majority of injected nodules, with expression limited to erbB2- positive tumor cells, and no expression was detected in adjacent normal tissues, indicating excellent tumor selectivity. In addition, there was a reduction in tumor size after plasmid injection and systemic prodrug administration in 4 of 12 patients (33%), without causing local or systemic complications. Results such as these provide additional encouragement for the development of GPAT strategies.

OTHER GENETIC APPROACHES

In addition to the above approaches, genetically modified cells have been used to expresdrug-activating enzyme genes in tumors. In this approach, drug-activating enzyme genes are stably transfected into cells that are additionally encapsulated by cellulose sulfate. The engineered cells are then introduced into tumors by injection in an immunoprotected environment to produce enzymes in tumors [42]. This method was developed as a novel approach that combines gene/cell therapy with chemotherapy. It was also considered as a safe and easy application for clinical use, because delivery of suicide gene-transfected/encapsulated cells is a feasible clinical approach without involving direct gene therapeutic interference in patients.

Using this approach, CYP2B1 was delivered into mice for tumor therapy. In these studies, encapsulated human embryonal kidney epithelial 293 cells expressing CYP2B1, under the control of the cytomegalovirus immediate early promoter, were administered into mice by two routes: (a) to deliver the capsules directly into the tumors in nude mice; and (b) to implant capsules adjacent

to pre-established pancreatic tumors in nude mice . Low doses of the prodrug IFA were administered to tumor-bearing mice every 3rd day for 2 weeks in both studies. Tumor regression was achieved after 3 weeks, with no tissue reaction or pancreatitis observed 7 days after injection. A similar result was observed when Feline kidney cellswere used for CYP2B1 expression. Human breast cancer cells (MDA-MB-361) have also been used to express enzymatically active surface-tethered bacterial CPG2(Q)-3 [43]. In this study, engineered breast cancer cells were mixed with nonexpressing cells, and the resultant mixtures were injected into nude mice that had a breast carcinoma xenograft. After 4 days, the prodrug CMDA was administrated into those mice.

Expression of the drug-activating enzyme was able to convert the prodrug into the cytotoxic moiety in vivo, resulting in either cures or tumor regression in all surface-tethered CPG2 (Q)-3-expressing groups. Furthermore, CPG2 activity was not detected in blood samples, indicating there was no significant shedding of the enzyme into the blood circulation, and high level of selectivity for the surface-tethered approach was achieved. This method was developed as a novel approach that combines gene/cell therapy with chemotherapy. It was also considered as a safe and easy application for clinical use, because delivery of suicide gene-transfected/encapsulated cells is a feasible clinical approach without involving direct gene therapeutic interference in patients.

ANTIBODY DIRECTED ENZYMATIC PRODRUG THERAPHY (ADEPT)

It is a strategy in which a tumor-associated monoclonal antibody is linked to a drug-activating enzyme to createa systemically administered conjugate that only targets tumor tissues. Nontoxic prodrug is then administrated systemically and is converted by the pretargeted enzyme localized on the tumor surface into a toxic drug, resulting in cytotoxic effects in tumor cells [44–49].

The ideal drugs for ADEPT are small molecules that can diffuse within the tumor tissues, including both antigen-positive and antigen-negative tumor cells, and cause a bystander effect. When ADEPT is applied clinically, the interval between enzyme and prodrug administrations should be optimized so that the conjugate is only accumulated in tumors rather than in blood and normal tissues, to avoid systemic toxicity. ADEPT has been used to deliver many drug-activating enzyme genes to tumors in vitro and in vivo, and recent examples are described below and are also listed in Table 4.

There are a number of general considerations for ADEPT. The target antigen should be either expressed on the tumor cell membrane or secreted into the extracellular matrix of the tumor [51], and the use of a high affinity monoclonal antibody is essential. The enzyme should be able to exert its optimal activity at a pH close to that of the tumor extracellular fluid.

Because antibody-enzyme conjugate may be immunogenic, circulating host anticonjugate antibodies may interfere with treatment. Therefore, the drug chosen should be dose dependent and cell cycle independent [44]. Ideally, the enzyme system should not have a human homologue to avoid prodrug activation outside the tumor site [46]. Because the interval between enzyme and prodrug administrations is important for ADEPT, some studies were performed to explore the optimal interval in animals. In human subjects, 7 days were needed for adequate clearance of antibody-enzyme conjugate from the plasma before the prodrug may be administrated safely, to avoid activation of prodrug in plasma and subsequent systemic toxicity.

Like GDEPT and VDEPT, there are many clinical limitations associated with ADEPT. In poorly vascularized tumors, delivery of the large conjugate is restricted, and it is not possible to deliver antibody/enzyme conjugate to all of the tumor cells [52]. Because the enzyme level is low, it is very difficult to generate adequate quantities of active drug to reach the lethal concentration. Furthermore, the binding of the conjugate to the cell surface is limited by antigen heterogeneity.

Other drawbacks of ADEPT include cost and difficulties with development and purification of antibodies, immunogenicity of antibodies, accessibility of tumor to the enzyme/antibody conjugate, and the conversion of prodrugs in nontumor tissue. The main problem with ADEPT is the immunogenicity of the antibody enzyme conjugate, which limits multiple cycles of its application . To solve this problem, several solutions have been tried, including the use of humanized proteins and concomitant administration of immunosuppression [44].

Because of the problems mentioned above, many ways have been tried to improve ADEPT. The first way to improve ADEPT is to use a three-phase system to speed up the removal of enzymes from the circulation without affecting the enzyme activity in tumor tissues [44, 48]. In this approach, a galactosylated anticonjugate antibody was applied after the administration of conjugate and prodrug as a clearing agent that reacted with the conjugate in the plasma, thus decreasing its blood levels, but retaining enzymatic activity in tumors [44]. A second way to improve ADEPT is to use a conjugate containing an enzyme and a partial fragment of antibody, which would be cleared more rapidly from the circulation, with the prodrug given earlier, whereas the enzyme level within the

tumor is at the peak concentrations [49]. The third way to improve ADEPT is to combine ADEPT with an antivascular agent, a drug that selectively inhibits tumor blood flow and causes extensive necrosis.

ADVANTAGES AND DISADVANTAGES OF GDEPT, ADEPT, VDEPT

All three enzymatic-prodrug strategies have practical advantages for optimizing the treatment of human cancer. GDEPT and VDEPT have an advantage over ADEPT in that most enzymes need cofactor(s) that is present only inside the cells.

Therefore, enzymes delivered by ADEPT may need to gain access inside the cells before they can optimally activate prodrugs. This requirement is limited by the poor penetration of large-sized antibody-enzyme conjugates. In GDEPT, gene-encoding enzymes can be specifically delivered to target tissues by the use of tissue-specific elements, to drive the expression of the enzyme within the target cells. Despite this idea, some theoretical risks for GDEPT, including insertional mutagenesis, anti-DNA antibody formation, local infection, and tumor nodule ulceration, restrict its use.

The other limiting factors include immunogenicity in ADEPT and difficulties with the selective delivery and expression of genes in GDEPT. Regarding VDEPT, most viral vectors are engineered to be replication deficient. However, there is a slight risk of reversion to wildtype virus. Furthermore, retrovirus vectors are inserted into the host-cell DNA, which may cause mutagenesis of the host's genome. Another drawback associated with retroviral vectors is that they only target dividing cells. Even in a rapidly growing tumor nodule, only 6–20% of cells are in a proliferating state and in S phase [53]. Thus, the majority of the tumor would not be sensitive to killing mediated by retroviral VDEPT. On the basis of these variables, the choice for GDEPT, VDEPT, or ADEPT should depend on the clinical scenario and is determined by how developers view the risks associated with each approach. Recent clinical trials of enzyme/prodrug therapy are summarized in Table 5

FUTURE PERSPECTIVE

There are three major aspects that need to be improved for enzyme/prodrug combination therapy in the future.

Improved Prodrugs

The design, synthesis, and clinical trials of prodrugs were recently reviewed elsewhere [56]. One limitation for the prodrug/enzyme approach is that only a small part of the tumor cells become activation competent with current strategies. To overcome this problem, the design of appropriate prodrugs that can diffuse efficiently and can kill activation incompetent cells via a bystander effect is necessary. Because hypoxia and lower pH is a common environmental feature in solid tumors, there is a

need to design prodrugs, which can be activated under these conditions. Currently available systems, including HSV-TK/GCV and CD/5-FC, are dependent on ongoing DNA replication in proliferating cells. Because the majority of tumor cells are in a nonproliferating state, these two commonly used systems are not very effective in killing tumor cells. The ideal active drugs should be effective against both dividing and nondividing cells. Unfortunately, most of the prodrugs used now are antimetabolites and target only dividing cells for cytotoxicity. Alkylating agents derived from the prodrugs CB1954 or IFA are not cell phase specific [57] and may represent a prototype for the development of other novel prodrugs in this class. In a series of CB1954 derivatives, evaluated in a Chinese hamster cell line transfected with the E. coli NTR, 4 of 20 analogues were more potent cytotoxic agents than the parent compound .CBI-TMI, a potent minor groove alkykating agent, was synthesized and tested in human ovarian carcinoma cells, where this novel drug gave a 10–21-fold increase in cytotoxicity in the presence of E. coli B NTR. However, all of these new prodrugs have the additional hurdle of FDA approval for use in humans, as well as evaluation in combination with enzyme system.

In addition to alkylating agents, other classes of prodrugs have been developed as well. Some effort was made to improve the water solubility, stability in blood, and susceptibility to enzymatic cleavage for camptothecin, an antitumor alkaloid which acts by inhibiting the activity of topoisomerase I [58]. The other efforts were aimed at the synthesis of a series of new prodrugs of daunorubicin and doxorubicin to find a better substrate for enzyme _-glucuronidase [59].

Improved Enzymes

The techniques used to improve enzymes to activate prodrugs are reviewed elsewhere [60]. Use of substrates for human enzymes may allow prodrug activation in nontumor tissues. One solution to this problem is to develop a mutant form of human enzymes by site-directed mutagenesis to avoid immune response against nonhuman protein and improve the kinetics of the enzymes for the prodrugs [60,61] or make the prodrug a highly specific substrate for the enzyme. In addition, because certain prodrugs may be activated by a cascade of several enzymes, the cotransfection of genes for each member of the pathway is an alternative to increase the yield of active drugs. Finally, use of enzymes from different species may provide another way to improve enzymatic activity, e.g., yeast CD is more efficient at converting 5-FC to 5-FU than bacterial CD after retroviral infection in murine squamous carcinoma cells and in a mice model of squamous cell cancer of the head and neck. However, this does not avoid the concern over immunogenicity of nonhuman proteins.

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Improved Methods to Deliver Prodrug Activating Enzymes into Tumour Tissues

Using current delivery systems, only 10–55% of cells can be targeted, depending on the tumor and the delivery route. The physical/chemical delivery methods, such as electroporation, direct intracellular injection, and calcium phosphate coprecipitation have been successful in vitro, but clinically, they are only suitable for transfection of tissues that can be removed from the body and then easily returned. Therefore, the improvement in design of delivery vectors of therapeutic genes into tumor cells and

development of nonviral vectors are expected [62]. Because of the risks of VDEPT and multiple steps involved to generate functional enzymes in GDEPT, developing nonviral vectors that are able to deliver active enzymes rather than genes into tumors are beneficial for clinical application. Because antibody-enzyme conjugates are large molecules that are difficult to penetrate into tumors using ADEPT, developing a novel approach that is quick, efficient, and involves a small molecule as a targeting agent is needed.
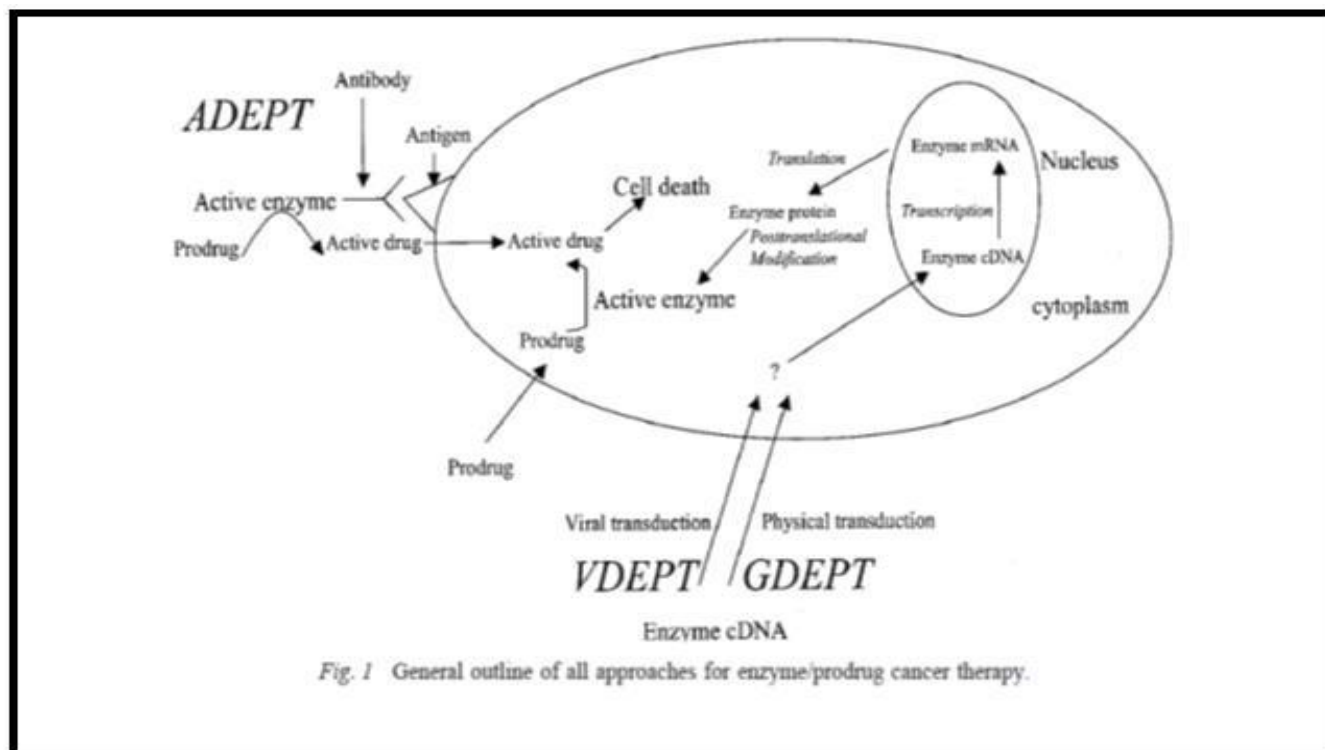

Fig. 1 General outline of all approaches for enzyme/prodrug cancer therapy.

Table 1: Selected examples of GDEPT

Enzymes	Prodrugs	Model systems
Human βglucuronidase	HMR 1826	Tumor cells and xenograft model in nude mice [14]
Bacterial nitroreductase	CB1954	Chinese hamster and 3T3 cells [15]
Carboxypeptidase	MTX-_-peptide	Cos-1 cells [16]
CYP2B1 and p450 reductase	Cyclophosphamide	Rat 9L gliosarcoma cells [17]
Rabbit CYP4B1	2-AA or 4-IMa	Human, rat glioma cells and in nude mice tumor Model [18]
Thymidine phosphorylase	5-FU or 5_-DFURa	LS 174T human colon carcinoma cells [5]
Rabbit and human carboxylesterase	Irinotecan	Glioblastoma and rhabdomyosarcoma cells and preclinical mouse xenograft model [19]
E. coli _-galactosidase E. coli _-galactosidase	Anthracycline	Human melanoma cells [20]
Cytosine deaminase	5-FC	Murine fibroblast cells [13]
Thymidine kinase	GCV	Cisplatin-resistant human ovarian carcinoma cells [21]

Table 2. Selected examples of VDEPT

Viral vectors	Enzymes delivered	Prodrugs	Model system
Adenovirus	Herpes simplex virus thymidine kinase	GCV	Mouse prostate cancer cell line and clinical trials
			Human lung adenocarcinoma cell
	Human carboxylesterase	Irinotecan	Ovarian tumor cells and animal model of
	E. coli nitroreductase	CB1954	disseminated intraperitoneal carcinoma [32-34]
Retrovirus	E. coli nitroreductase	CB1954	Colorectal, pancreatic, ovarian cancer cells and
	Yeast ytosinedeaminase	5-FC	xenografts of human ovarian & pancreatic
	Human CYP and p450 reductase	Cyclophospha mide	cancer
		and IFA	SCC VII murine squamous carcinoma cells and
			YCD-expressing tumors
			Gliosarcoma cells and in vivo tumor model [34-36]
EBV	Nitroreductase	CB1954	EBV-positive B-cell lines [30]

Table 3. Selected examples of GPATs

Promoters	Enzyme genes	Enzyme genes
Ornithine decarboxylase	Rabbit carboxylesterase	Neuroblastomacellserexpressing N-myc [41]
Regulatory elements of MUC1 and erbB2	Herpes simplex virus thymidine kinase	MUC1-positive cells [39]
CEA tumor antigen	Herpes simplex virus thymidine kinase	CEA-producing gastric cancer cell lines [7]
fetoprotein enhancer	Herpes simplex virus thymidine kinase	Hepatocellular carcinoma cell lines [7]
erbB2	Cytosine deaminase	Breast and pancreatic tumor cells [7]
Human tyrosine promoter	Purine nucleoside phosphorylase	Melanoma cell lines [7]

Table 4. Examples of ADEPT in enzyme/prodrug cancer therapy

Therapy route	Enzymes	Antibodies	Prodrugs	Model systems
In vitro	β-glucosidase	Bladder cancer-associated monoclonal antibody	Amygdalin	HT 1376 bladder cancer [46]
	Human βglucuronidase	Humanized CEA-specific binding region	A series of new prodrugs of Anthracyclines	Murine L 1210 tumor cell Line [47]
	Human βglucuronidase	Single-chain anti-CD20 Antibody	Doxorubicin	Fused protein [49]
	Human βglucuronidase	Humanised Fab fragments of the anti-CEA Mab	Doxorubicin	Fused protein [50]
In vivo	Carboxy-peptidase G2	Anti-CEA antibody	CMDA	Xenograft of human colon Carcinoma [45]

Table 5. Examples of recent clinical trials of enzyme/prodrug therapy

Approach	Enzyme	Prodrug	Efficiency
GPAT	CD cDNA directed by erbB-2 promoter	5-FC	Plasmid construct was intratumorally injected in 12 breast cancer patients [38].
ADEPT	CPG2 linked to F(ab_)2 fragment of murine A5B7 monoclonal antibody	CMDA	The concentration of active drug (CJS11) in plasma of 10 patients with colorectal carcinoma was evaluated. On biopsies, CPG2 activity was only localized in metastatic tumor [52,54].
VDEPT	Adenoviral transduction of HSV-TK	GCV	Recombinant adenovirus containing HSV-TK was injected into the pleural cavity of 21 patients with mesothelioma. HSV-TK was detected in tumors of 11 patients [32].
	Retroviral transduction of HSV-TK	GCV	Intraprostatic injection of recombinant adenovirus containing HSV-TK was administered to 18 patients with local recurrence of prostate cancer. Fall in serum PSAa by 50% was observed for 6 weeks to 1 year in 3 patients [55].
			Gene therapy in combination with surgery was applied to 48 patients with GBM.aNo significant side effects were observed, and the 12-month survival rate was 27% [55].

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