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REVIEW ON PRODRUG AND ITS APPLICATIONS

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ABSTRACT

Enzyme prodrug therapy is a type of cancer treatment that uses enzymes to convert a prodrug, or inactive drug, into an active drug and then kills cancer cells. The various methods of treatment employed in cancer-causing tumor cells are gene-directed enzyme prodrug therapy and antibody-directed enzyme prodrug therapy. Enzyme prodrug system has been used for the treatment of colorectal cancer, glioma, pancreatic cancer, prostate cancer, ovarian, breast, and various tumor models. This article discusses prodrugs and prodrug types -2 Therapeutics that target cells or tissues like ADEPT, GDEPT, and VDEPT.

PRODRUG

A prodrug is a pharmaceutical substance that, when consumed, gets metabolized (i.e., changed into a pharmacologically active drug) by the body. [1,2] To enhance how a medication is absorbed, transported, metabolized, and eliminated, a comparable prodrug can be employed instead of delivering the drug directly (ADME). When medicine is poorly absorbed from the digestive tract, prodrugs are frequently created to increase bioavailability.[2] The prodrug can help a drug engage more selectively with cells or procedures that aren't its original target. This lessens a drug's negative or unanticipated side effects, which is crucial for therapies like chemotherapy that can have serious unwanted, and unpleasant side effects. [3,4]

RECENT DRUGS

Approximately 10% of all marketed drugs worldwide can be considered prodrugs. Since 2008, at least 30 prodrugs have been approved by the FDA. Seven prodrugs were approved in 2015 and six in 2017. Examples of recently approved prodrugs and six in 2017. Examples of recently approved prodrugs are such as dabigatran etexilate (approved in 2010), gabapentin enacarbil (2011), sofosbuvir (2013), tedizolid phosphate(2014), isavuconazonium(2015), aripiprazole lauroxil(2015), selexipag(2015), latanoprost bound(2017), Benz hydrocodone(2018), and tozinameran(2020).[1]

CLASSIFICATION

Based on how the prodrug is transformed by the body into the final active drug form, prodrugs may be divided into two primary categories.[5] Bioactivation of type I prodrugs occurs within the cells (intracellularly). These include lipid-lowering statins and phosphorylation-required antiviral nucleoside analogs. Type II prodrugs undergo extracellular bioactivation, which occurs most frequently in digestive fluids or the body's circulatory system, most frequently in the blood. Salicin, which was previously discussed, and specific antibody-, gene-, or virus-directed enzyme prodrugs used in chemotherapy or immunotherapy are examples of Type II prodrugs. Based on elements like (Type I) whether the intracellular bioactivation location is also the site of therapeutic action or (Type 2) whether or not, both primary kinds can be further divided into subgroups.

CLASSIFICATION OF PRODRUGS [5,6]

Type	Bioactivation site	Subtype	Tissue location of bioactivation	Examples
Type I	Intracellular	Type IA	Therapeutic target tissue, cells	Acyclovir, fluorouracil, cyclophosphamide, L-DOPA
		Type IB	Metabolic tissues (Liver, GI, lungs)	Carbamazepine, captopril, carisoprodol, primidone
Type II	Extracellular	Type IIA	GI fluids	Loperamide oxide, oxyphenisatin, sulfasalazine
		Type IIB	Systemic circulation and other extracellular fluid compartments	Acetylsalicilate, bacampicillin, bambuterol, dipivefrin, pralidoxime
		Type IIC	Therapeutic target tissues and cells	ADEPTs, GDEPTs, VDEPTs

ENZYME PRODRUG THERAPY

Enzyme prodrug therapy is the most promising cancer treatment therapy which kills tumor cells by using various techniques such as Antibody-directed enzyme prodrug therapy [ADEPT] and Gene-directed enzyme prodrug therapy [GDEPT].

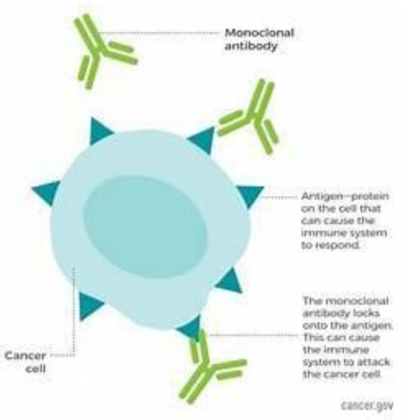
ADEPT

ADEPT is a strategy to overcome the problem of lack of tumor selectivity. An antibody designed/developed against a tumor antigen is linked to an enzyme and injected into the blood, resulting in selective binding of the enzyme in the tumor. When the discrimination between tumor and normal tissue enzyme levels is sufficient, a prodrug is administrated into the blood circulation, which is converted to an active cytotoxic drug by the enzyme, then the active drug kills the tumor cells.[4]

There are multiple steps to the ADEPT treatment process. a monoclonal antibody with an enzyme conjugates the use of an antibody (or an antibody fragment). localize at the tumor location. Either time is allowed for the unbound conjugate to be removed from the clearance speeds up the clearing of the body or its clearance. An antibody that is aimed towards either the conjugate's enzyme or antibody components. After that, a safe prodrug is given and activated on the affected area of the bound conjugate on the tumor. The present Drugs with low molecular weight can spread quickly and reach tumor areas, therefore they can the antibody-enzyme conjugate can access the targeted antibody-enzyme conjugate is still extracellular, it should be highlighted. Not internalized due to the possibility that the conjugate was quickly broken down in the lysosomal compartment of the prison. Additionally, several of the pro-drugs mentioned Utilize the external enzyme below by becoming charged and kept out of the cell until used. It is also possible to take advantage of secreted antigen if it builds up in the interstitial spaces of the tumor.to increase the conjugate's concentration toward the tumor. The ADEPT strategy has the benefit that Since it is catalytic and only requires one enzyme molecule, The prodrug should theoretically be able to produce hundreds of active molecules every second. Furthermore, since the enzyme conjugate is not active on its own (unlike Despite the immunotoxins, the unbound conjugate is permitted). before administering the prodrug, to clear.

ANTIBODIES:

Targeting is done with monoclonal antibodies due to their high specificity and ease of isolation and manipulation. Theoretically, ADEPT could utilize any antibody with the necessary selectivity. Antibodies should, however, recognize an antigen that is not easily internalized, such as chorionic gonadotropin or the human carcinoembryonic antigen (CEA).[7]



ENZYMES

Extracellular enzymes are not utilized in ADEPT. The idea that the prodrug is not activated by typical human enzymes is crucial. Therefore, the enzyme's comparable activity in humans must be quite low. It must also be functional under physiological settings and continue to function when attached to an antibody. Table I provides a summary of the enzymes that have been taken into consideration for ADEPT and the accompanying prodrugs. [8,9]

ENZYMES AND PRODRUGS USED [10]

Table I. Enzymes and prodrugs that have been proposed for targeted therapy

Enzyme	Prodrug	Drug
Glucose oxidase	Glucose	Hydrogen peroxide
Xanthine oxidase	Hypoxanthine	Superoxide, hydrogen peroxide
Carboxypeptidase G2	Benzoic acid mustard glutamates[a]	Benzoic acid mustards (various)
Carboxypeptidase A	Methotrexate-alanine	Methotrexate
β-Glucosidase	Amygdalin	Cyanide
β-Glucuronidase	Phenolmustard-glucuronide	Phenolmustard
	Epirubicin-glucuronide	Epirubicin
Cytosine deaminase	5-Fluorocytosine	Fluorouracil
β-Lactamase	Vinca-cephalosporin[a]	4-Desacetylvinblastine-3-carboxhydrazide
	Phenylenediamine mustard-cephalosporin[a]	Phenylenediamine mustard
	Nitrogen mustard-cephalosporin	Nitrogen mustards (various)
Penicillin amidase	Palytoxin-4-hydroxyphenylacetamide	Palytoxin
	Doxorubicin-phenoxyacetamide	Doxorubicin
	Melphalan-phenoxyacetamide	Melphalan
Alkaline phosphatase	Phenolmustard phosphate[a]	Phenolmustard
	Doxorubicin phosphate[a]	Doxorubicin
	Mitomycin phosphate[a]	Mitomycin
	Etoposide phosphate (etopofos)[a]	Etoposide
Nitroreductase	5-(Aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954)	5-(Aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide

a Data obtained *in vivo*.

GDEPT

GDEPT is a suicide gene therapy in which the enzyme required for prodrug conversion is produced within the target cell, using a gene delivered to it by gene therapy. When an adequate differential exists between the targeted cell and endogenous tissue, the non-toxic prodrug is administered and is subsequently converted into its toxic form within the target cell. Systems that use viral vectors to deliver the gene are known as VDEPT.

Gene technology that modifies cells for therapeutic purposes can be generically referred to as gene therapy. There are cancer cells available. by adding "suicide genes," they can become more susceptible to chemotherapy. Genes that produce alien enzymes that an approach known as may convert comparatively non-toxic prodrugs into cytotoxic drugs have been employed. Genetic prodrug therapy (GPT), virally-directed enzyme prodrug therapy (VDEPT), and less often Active treatment (GPAT). [11,12,13,14]

Fundamentals of GDEPT

• a gene that expresses a foreign enzyme that can stimulate a prodrug (or an endogenous enzyme that is only found in trace amounts in tumors);

• a prodrug or a vector that can transfer the gene to cancer cells

A variety of criteria need to be taken into account for GDEPT to succeed. The gene must first be expressed specifically in tumor cells. Second, expression needs to be as high as feasible in cancer cells. Sadly, when vectors are administered systemically, it is doubtful that expression will take place in more than 10 - 20% of tumor cells.[15] Therefore, a bystander effect is required whereby the prodrug is cleaved to an active drug which kills not only cells expressing the foreign enzyme but also tumor cells that are not expressing the enzyme. This means that expression in fewer than 100% of tumor cells can still lead to total tumor cell death.

Treatment using gene-directed enzymes (GDEPT).

Enzyme, prodrug, and gene delivery mechanism are the three main functional components necessary for suicide gene therapy to be successful (vector).

The vector's job is to deliver the gene encoding an enzyme to the intended cancer cells so that they may express it. Gene delivery techniques may be broadly categorized into two categories: viral (such as adenovirus and lentivirus) and nonviral (such as synthetic polymers and lipids, bacteria-based and cell-based). Each sort of vector has several benefits and drawbacks.

Table 2: Classification of vector types [16,17,18]

Vector type	Vector subtype	Advantages	Disadvantages
Viral vectors	Retrovirus/lentivirus	Long-term transgene expression Integrates the gene into host genome Low immunogenicity	Safety concerns (insertional mutagenesis)
	Adenovirus	Effect on dividing and nondividing cells Lower risk of host genome integration	Safety concern (high immunogenicity) Transient transgene expression
	Adeno-associated virus	Medium to high transgene expression Effect on dividing and nondividing cells No significant immunogenicity	Low DNA loading capacity Safety concerns (possibility of insertional mutagenesis)
Nonviral vectors	Synthetic polymers and lipids	Ease of preparation Lower cost Lower immunogenicity	Lower transfection efficiency
	Amino acid-based vectors	Monodisperse and uniform constructs, ability to fine tune structure	Lower transfection efficiency
	Bacteria-based vectors	Large capacity for suicide enzyme loading Bacterial minicells (BMCs) are noninfectious.	Safety concern (infection by using live bacteria)
	Cell-based vectors	Tumor tropism Self-isolated cells without the immunogenicity concerns	Low efficiency of tropism High costs Safety concern (unknown fate)

The bystander effect, a phenomenon known as the function of the enzyme expressed by the transfected cancer cells, is the process by which the nontoxic/nonfunctional prodrug is transformed into its toxic (functional) form, killing both the enzyme-producing cancer cells as well as nearby cells 'It is important to note that tumor-specific promoters can control the expression of the enzyme in transfected cells.[19,20] By limiting the production of the enzyme to just tumor cells, this regulatory component could increase the safety of the enzyme/prodrug combination. Applying cancer-specific promoters allowed for the selective expression of the suicide gene in cancer cells while preserving healthy cells. One of the most popular promoters in the area is that of human telomerase reverse transcriptase (hTERT). used in the fields and is the only transcriptional regulatory component that has effectively entered clinical trials.[21] The issue with using the hTERT promoter is its poor expression activity, though.

Several organizations have attempted to produce more precise and effective promoters to increase the activity of the expression of the promoter and also overcome the possible development of resistance by the tumor cells. A recent screening of a wide panel of normal and cancer cells revealed the promoters for Rad 51, OPN, RAN, BRMS1, and MCM5, and intriguingly, several of them displayed noticeably greater activity than the hTERT promoter. Making a chimeric promoter artificially is another way to increase promoter activity. For instance, creating chimeric promoters by the fusion of two different promoters might result in a promoter with greater activity[23,24]

GDEPT targeting systems

The delivery and selectivity of the enzyme genes must be mentioned even though the focus of this study is focused on the enzyme/prodrug systems employed in GDEPT. The most difficult obstacle to overcome before moving from an experimental strategy to a therapeutic method is likely the effective delivery of the enzyme.[25,26] Many different delivery methods are being evaluated. These include proteins, cationic amphiphiles, liposomes from adenoviruses, retroviruses, and bare DNA. The use of albumin or -fetoproteins promoters in hepatoma cells, the tyrosinase promoter in melanoma cells, the carcinoembryonic antigen (CEA) promoter in gastric cancer cells, and the osteocalcin promoter for osteosarcoma, among others, confers selectivity in several investigations.[27]

GDEPT enzymes

The enzymes employed in GDEPT must adhere to certain specifications. They must be able to catalyze certain processes, vary from any circulating endogenous enzymes, express themselves in adequate numbers, and have high catalytic activity. Enzymes of non-mammalian origin, such as bacterial cytosine deaminase (CD), bacterial carboxypeptidase G2 (CPG2), bacterial purine nucleotide phosphorylase (PNP), and bacterial nitro reductase, are examples of these enzymes. These enzymes have been postulated for GDEPT a. (NR). Different structural requirements for these exogenous enzyme substrates should apply to any human homologs. Their biggest drawback is that they may cause an immunological reaction in people.

• enzymes of human origin which are absent or are expressed only at a low concentration in normaDeoxycytidine kinase (dCK), thymidine phosphorylase (TP), and cytochrome P450, for example, are present in tumor cells (CYP). Their claimed capacity to lessen the formation of immunological responses is their primary benefit. Their presence in healthy tissues is believed to prevent the prodrugs from being specifically activated in tumors.

Additionally, the prodrug/drug system needs to adhere to several standards:

For intracellular activation, the prodrug must be able to pass the membrane of a mammalian cell. Additionally, the cytotoxicity difference between the prodrug and the equivalent drug should be as great as feasible.

The drug should be extremely diffusible or aggressively taken up by nearby cells to provide a bystander effect. The prodrug should be a suitable substrate for the expressed enzyme. The actual drug should be as cytotoxic as feasible. It takes a significant understanding of the structure-activity relationship (SAR) or, better yet, the quantitative structure-activity relationship (QSAR) for this specific type of compound to build a prodrug with minimal cytotoxicity that may unleash a highly toxic active drug.

To facilitate diffusion across cell membranes, lipophilic prodrugs are necessary. Prodrugs can also be consumed during active transportation. It appears that the most frequent prodrugs enter cells by passive diffusion.

Two classes of anticancer drugs have been used in GDEPT:

- antimetabolites
- alkylating agent

APPLICATIONS

Carboxylesterase [CE] /Irinotecan

This enzyme prodrug system has been used for the treatment of colorectal cancer,[28] glioma,[29] and various tumor models.[30,31,32]

Cytosine Deaminase [CD]/5-Fluorocytosine

Usually, 5-FU has been used for cancer chemotherapy, and its application as a prodrug in the form of and in combination with cytodomains enzyme has gained momentum in the past decades.CD/5-FC system has been used for the treatment of different types of cancer, such as colon carcinoma, glioma, and pancreatic cancer having been combined with radiotherapy, CD/5-FC has shown quite a promising results due to the radio-sensitizing effect of 5-FC on the treated cells.[33,34] In comparison to the HSV-TK/GCV, CD/5-FC has shown better results in renal and colorectal carcinoma probably due to its more potent bystander effect.[35,36] In addition, CD can be fused with E.coli uracil phosphoribosyl transferase[UPRT] and can directly convert 5-FU to 5-FdUMO resulting in improvement of activity and enhanced cancer cell killing efficiency in prostate, ovarian, colon, and breast cancer. [37,38]

Nitroreductase / CB1954

Effective use of enzyme /prodrug systems has been demonstrated in a few clinical trials used for the treatment of prostate and liver cancer.[43]

Purine Nucleoside Phosphorylase / 6-Methylpurine Deoxyriboside

The advantage of using PNP/ MEP is its high bystander activity based on gap junction-free transport of activated drugs, its effect on both proliferating and nonproliferating cells, and its unique mechanism of action that is independent of DNA synthesis. PNP catalyzes the cleavage in the glycoside bond of (deoxy) adenosine-based substrates that produce either (MEP) or 2-fluoroadenine (F-Ade). Then, these substrates are converted to their triphosphate forms by cellular monophosphate and diphosphate kinases, which can inhibit both RNA and protein synthesis.[39,40] Three studied prodrugs of this system are 6-methylpurine-2′-deoxyriboside (MEP-dR), 2-F-2′-deoxyadenosine (F-dAdo), and arabinofuranosyl-2-F-adenine monophosphate (F-araAMP). Among them, F-araAMP is clinically approved for chronic lymphocytic leukemia treatment.[41]

Horseradish Peroxidase/Indole-3-Acetic Acid

The different recombinant forms of HRP isoenzymes are produced, which have shown significant effects on breast and bladder carcinoma. Although some promising results have been obtained from HRP/IAA system.[42]

CONCLUSION

ADEPT offers a novel field of opportunities in the therapy of systemic cancer and may be a major advance for the treatment of solid tumors. Whereas GDEPT is more selective than conventional prodrug therapy, and higher drug concentrations can be generated at the tumor target. GDEPT can become a clinically effective treatment for tumors. Using this technique many severe cancer conditions can be conquered.

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