**Prodrug: Approach to better drug delivery**

**Snehal A. Gavhane1 Sachin B. Somwanshi2**

1. Research Scholar, Department of Pharmaceutics, PRES’s, College of Pharmacy (For Women), Chincholi, Nashik, MH, India-422102

E-mail: [snehalgavhane25@gmail.com](mailto:snehalgavhane25@gmail.com)

2.Associate Professor, Department of Pharmaceutics, PRES’s, College of Pharmacy (For Women), Chincholi, Nashik, MH, India-422102

E-mail: [sachinsomwanshi27@gmail.com](mailto:sachinsomwanshi27@gmail.com)

**Abstract**

Albert was the first to introduce the idea of a prodrug in medicinal chemistry.

"A prodrug is a molecule that lacks intrinsic biological activity but can produce a biologically active drug through the various stages of its metabolism," according to the definition provided. This definition and the one adopted by IUPAC both state that a prodrug is any substance that goes through biotransformation prior to manifesting its pharmacological effects. Prodrugs can be thought of as medications that temporarily alter or remove undesired qualities in the parent molecule by adding specialised non-toxic protecting groups.

In order to be effective, a prodrug must be able to solve a crucial paradox: while it must be hydrophilic enough to satisfy solubility, bioavailability, and transport requirements, it must also be lipophilic enough to cross a membrane or metabolic barrier.

An appealing substitute is a chemical remedy that uses a prodrug strategy. By modifying physico-chemical factors that affect absorption or by focusing on certain enzymes or membrane transporters, the prodrug method has also been frequently employed to enhance drug delivery to its site of action. With this in mind, a prodrug design is a lead modification technique used to fix a flaw in a drug candidate. It may be helpful in avoiding issues with formulation and solubility, absorption and distribution, instability, site specificity of liberation, prolonged release, and toxicity, among other effects.

Keyword : prodrug, enzyme, gene, pharmacodynamic, bioconversion.

**Introduction**

Since the late nineteenth century, the prodrug notion has been utilised to enhance medications' unfavourable characteristics. Prodrugs are biodegradable, inactive derivatives of active drug molecules that must be changed in vivo in order to release the active parent drug, which can then provide the desired pharmacological action in the body. The majority of the time, they are straightforward chemical derivatives that are only a single or two enzymatic or chemical steps removed from the active parent medication. There are many prodrugs on the market that aim to remove obstacles to drug use. The prodrug technique is a workable way to enhance the unpredictable qualities of investigational medications or drugs that are already on the market, despite the fact that developing a prodrug can be highly difficult. This chapter explains the purpose of use. [1]

# **Prodrugs in medicinal chemistry and enzyme prodrug therapies**

Finding the disease and the appropriate drug target, the lead and pharmacophore, and optimising the drug lead molecule's interaction with the target are the conventional steps in developing a successful drug for the market. Despite being successful up until this point, the therapeutic development programme could yet fall short due to the molecule's subpar pharmacokinetics (PK). Each of the four constitutive barriers—absorption, distribution, metabolism, and excretion, or ADME—can contribute to PK deficiencies. Pharmacological lead is heavily optimised to increase its PK and get around these obstacles employing prodrugs in many cases of drugs that have been introduced successfully to the market. Prodrugs are by definition the precursors or derivatives of therapeutically active compounds that are bioconverted into their active state. which, whether by spontaneous mechanisms (such hydrolytic breakdown) or through a biocatalytic mechanism, are bioconverted into their active form inside the body. When a prodrug technique is used in drug delivery, the goal is usually to help the drug get past a barrier—either literal or figurative—in order to increase the amount of the drug that can be delivered. Poor water solubility, which can greatly reduce a drug's usefulness for therapeutic purposes, poor absorption from the gastrointestinal system into blood, low rates of cell penetration, etc. are a few examples of such barriers.[2]

Prodrug degradation with subsequent drug recovery and prodrug activation are the two categories into which prodrug bioconversion processes can be arbitrarily divided (Scheme 1). A prodrug molecule for the prodrug degradation class is a conjugation of the parent drug; a masking group (commonly called a "promoiety") is removed during the bioconversion reaction; and the chemical complexity of the molecule reduces. The prodrug undergoes a point chemical modification (such as the cardamine-to-carbonyl transformation) in the prodrug activation category (also known as the "bio precursor" category), which leaves the molecule's chemical complexity largely unaltered but significantly increases therapeutic activity. Alternately, prodrug activation entails a conjugation step that increases the molecule's chemical complexity (for example, phosphorylation for nucleoside analogues). These two classes are here and below, these two classes of prodrug bioconversion are denoted as “drug recovery” and “prodrug activation”, respectively.[2]

The structural characteristics of the parent drug molecule and, more particularly, the availability of suitable chemical functionalities that may be utilised to mask the drug's pharmacodynamic activity by, for example, an attachment of the modifying group, determine the design strategy for a prodrug in the first place. The second, equally crucial factor relates to the bioconversion mechanisms of medication release. Although this process could be spontaneous, the bulk of the time, enzymatic processes are used to create drugs (either by recovery or activation). The primary goal of conventional prodrugs in medicinal chemistry is normally to obtain a quantitative recovery of the drug. The medication distribution throughout the body is often given little to no thought in these applications, and the bioconversion enzyme (such as esterase and phosphoesterases) may be dispersed throughout the body. In some cases, a specific enzyme may be primarily expressed in one organ, such as the liver, and bioconversion of a prodrug may occur primarily there (for example, the first step of capecitabine's bioconversion is carried out by carboxylesterases, and HepDirect prodrugs are intended for activation by cytochromes). The presentation following just briefly discusses the design of these prodrugs for broad therapeutic use in order to provide the right context, both scientifically and historically.[2]

The creation of prodrugs for an advanced drug delivery potential known as Enzyme Prodrug Therapy (EPT) is the major topic of this research. To do this, a clever subclass of prodrugs is created so that bioconversion is carried out by a designated enzyme implanted in a specified site within the body. By doing this, the medicine can only be recovered or activated where the enzyme is. In the case of EPT, quantitative drug recovery is less crucial than it is for general medicinal prodrugs, and achieving a site-specific drug recovery is the main objective. There are several methods for localising the enzyme at the desired spot, with varying degrees of effectiveness and development from the lab to the clinic. The initial widely acknowledged success of EPT in the past was attributed to antibody-directed enzyme-prodrug therapy (ADEPT). With this injection-based method of EPT, the enzyme is coupled to an antibody, which makes it easier for the enzyme to bind to the site of action. Examples of ADEPT frequently rely on drug recovery mechanisms that extracellular prodrug bioconvert via. Encapsulated enzymes that were surgically implanted at the site of a tumour that had been removed for post-operative chemotherapy have also shown some early success. There has been a significant increase in interest in this type of EPT recently. The EPT method with the most active clinical trials right now is that of gene-directed EPT (GDEPT), also known as “suicide gene therapy”. In this instance, the cells express the enzyme for prodrug conversion upon transduction of the latter, and viral vectors are the most effective means of achieving this. Examples of GDEPT nearly completely rely on the supplied prodrugs' intracellular activation.[2]

# **Strategies for Enzyme/Prodrug Cancer Therapy**

The treatment for enzyme-activating prodrugs involves two steps. An enzyme that activates drugs is initially targeted and expressed in malignancies. The second stage involves systemically delivering a non-toxic prodrug, a substrate of the exogenous enzyme that is now produced in tumours. The ability to convert a systemically delivered prodrug into a highly concentrated local form of an active anticancer medication in tumours is the net benefit. Both enzymes and prodrugs need to meet specific criteria for this technique to be clinically effective. The enzymes should either come from sources other than humans or be human proteins that are either absent or just present in very small amounts in healthy tissues. The protein needs to express itself sufficiently in the tumours and exhibit strong catalytic activity. The prodrug ought to be an excellent substrate for the enzyme produced in tumours but not activated by the endogenous enzyme in tissues that are not cancers. For intracellular activation, it must be able to cross the cancer cell membrane, and the cytotoxicity difference between the prodrug and the equivalent active drug should be as large as possible. Additionally, the active medication's half-life should be both long enough to cause a bystander effect and brief enough to prevent drug leakage into the systemic circulation.[3]

Priority should be given to the enzyme when selecting the ideal enzyme/prodrug combination. It is anticipated that acceptable prodrugs can be developed for practically any enzyme substrate specificity based on prior experience (Connors, 1995). The bystander effect necessary (see the Bystander Effect section) would not be accomplished if the cells were killed by the activity of the enzyme alone; expression of the enzyme alone shouldn't result in cytotoxic effects. To prevent harmful activation of the prodrug in healthy tissues, the reaction route should also be distinct from any endogenous enzyme. The fundamental disadvantage of using proteins of human origin is that they may not completely prevent difficulties associated with acquired immunity, particularly after extended dosing or prolonged protein expression. The chosen prodrug should have appropriate pharmacological and pharmacokinetic qualities, be chemically stable under physiological settings, and be easily diffusible throughout the tumour (perhaps a neutral species). The released drug should be at least 100 times more toxic than the prodrug in order to achieve meaningful therapeutic benefit. Additionally, the hazardous substance should have a half-life that permits diffusion to neighbouring transfected cells (bystander effect) while guaranteeing that any drug that escapes and enters the bloodstream would be rendered inactive. Additionally, to kill a variety of cell types, the induced cytotoxicity should not depend on the cell cycle phase or proliferation independent, to kill a wide range of tumour cell populations.[4]

## **Approaches to Deliver Prodrug-activating Enzymes into Tumor Cells or Tissues**

ADEPT is a method that combines a systemically injected compound with a tumor-associated monoclonal antibody to selectively target tumour tissues. Following systemic administration, a nontoxic prodrug is transformed into a poisonous drug by a pretargeted enzyme located on the tumour surface, causing cytotoxic effects in tumour cells. Small compounds that may diffuse inside tumour tissues, including both antigen-positive and antigen-negative tumour cells, and result in a bystander effect are the best medications for ADEPT. To prevent systemic toxicity while using ADEPT clinically, the period between enzyme and prodrug administrations should be optimised. This will ensure that the conjugate accumulates only in tumours rather than in blood and healthy organs. Many deliveries have been made using ADEPT drug-activating enzyme genes to tumours in vitro and in vivo.

Use of a high affinity monoclonal antibody is crucial, and the target antigen should either be expressed on the tumour cell membrane or released into the extracellular matrix of the tumour. At a pH that is similar to the pH of the extracellular fluid found in tumours, the enzyme ought to be able to exert its best action. Circulating host anticonjugate antibodies could make treatment difficult since antibody-enzyme conjugates may be immunogenic. Consequently, the medication of choice ought to be dose dependent and cell cycle independent. To prevent prodrug activation outside the tumour site, the enzyme system should ideally not have a human counterpart.

Since the time between the injection of the prodrug and the enzyme is crucial for ADEPT, tests were conducted on animals to determine the ideal time frame. The prodrug CMDA was able to be safely administered 48 or 72 hours after antibody-enzyme administration thanks to a rapid clearance of conjugate from the circulation that was made possible by linking the enzyme CPG2 to the anti-CEA antibody A5B7. To prevent activation of the prodrug in the plasma and consequent systemic toxicity, it was necessary for 7 days for the antibody-enzyme combination to be sufficiently cleared from the plasma before the prodrug could be administered safely to human subjects. In a recent Phase I clinical trial, this CMDA/CPG2 prodrug/enzyme system was applied to 10 patients with colorectal cancer. A second clearance Ab against CPG2 was also utilised to reduce the amounts of conjugate in the circulation and other nontumor organs after the bacterial enzyme CPG2 was conjugated to the F(ab′)2 fragment of the murine A5B7 monoclonal Ab. In this trial, the active drug CJS11, a bifunctional alkylating agent produced from the prodrug CMDA by the action of CPG2 localised in tumours, was measured in plasma levels. After application of the clearing agent, CPG2 activity was detected in metastatic tumour biopsies but not in normal tissues. In this system, a half-life of 36 14 min for the active medication was observed in plasma. The application of ADEPT to increase the selectivity of the present therapy for solid tumours shows promise in this first example.

Similar to GDEPT and VDEPT, ADEPT has a lot of clinical restrictions. Delivery of the large conjugate is constrained in weakly vascularized tumours, making it impossible to reach every tumour cell with the antibody/enzyme conjugate. Due to the low enzyme level, it is highly challenging to produce enough active medication to reach the deadly concentration. Antigen heterogeneity is another factor that restricts the conjugate's ability to bind to the cell surface. Cost and challenges with antibody production and purification, immunogenicity of antibodies, tumour accessibility to the enzyme/antibody combination, and conversion of prodrugs in nontumor tissues are some further downsides of ADEPT. The primary issue with ADEPT is that it cannot be applied more than once due to the antibody-enzyme conjugate's immunogenicity. The use of humanised proteins and concurrent immunosuppressive therapy are two strategies that have been tested to address this issue.[4]

Numerous strategies have been developed to enhance ADEPT in response to the aforementioned issues. Utilising a three-phase method to hasten the clearance of enzymes from the bloodstream without impairing the enzyme activity in cancer tissues is the first step in improving ADEPT. A galactosylated anticonjugate antibody was used in this method as a clearing agent that reacted with the conjugate in the plasma, lowering its blood levels but maintaining its enzymatic activity in tumours. Use of a compound combining an enzyme and a partial antibody fragment, which would be removed from the circulation more quickly, along with the prodrug given earlier, while the enzyme level within the tumour is low, is a second strategy to enhance ADEPT at the peak concentrations . Combining ADEPT with an antivascular agent, a medication that specifically blocks tumour blood flow and results in widespread necrosis, is the third technique to enhance ADEPT. When combined with the antivascular agent 5, 6-dimethylxanthenone-4-acetic acid at 20 hours after the conjugate injection, a conjugate containing the bacterial CPG2 and the F(ab′)2 fragment of anti-CEA antibody to activate the prodrug CMDA killed a larger portion of the tumour, doubled the concentration of antibody-enzyme conjugate retained in tumour, and significantly prolonged the tumour growth inhibition calcium. Furthermore, prodrug retention within the tumour was boosted by 16-fold by 5, 6-dimethylxanthenone-4-acetic acid. The use of mutant forms of human enzymes is the fourth technique to enhance ADEPT because they prevent systemic toxicity brought on by the use of wild-type human enzymes and reduce immunological responses brought on by the use of nonhuman enzymes. The thymidylate synthase inhibitors GW 1031 and GW 1843 as well as the dihydrofolate reductase inhibitor MTX were all effectively activated by a mutant form of human CPA attached to a tumor-associated antibody, but none of these prodrugs were effective substrates for endogenous CPA. When compared to nonendogenous enzymes and endogenous enzymes, mutant human enzymes may be less immunogenic and systemically harmful. The last option to enhance ADEPT is to use recombinant DNA technology to create a fusion protein with specific properties and eliminate further antibody purification procedures, which could result in lower antibody binding of the conjugate or decreased enzymatic activity. In comparison to the prodrug alone, the growth inhibition produced by this fusion protein and doxorubicin prodrug was superior. Recently, an expression plasmid for the production of a fusion protein containing the single-chain Fv anti-CD20 mouse monoclonal antibody and human lysosomal enzyme β-glucuronidase was found to bind CD20-expressing lymphoma cells in a specific manner and was able to activate the prodrug N-[4-daunorubicin-N-carbonyl (poxymethyl)phenyl]O-b-glucuronyl carbamate at a rate similar to that of purified human β-glucuronidase.[5]

# **Bacterial-directed enzyme prodrug therapy**

A tissue phenotype that distinguishes cancer tissue from healthy tissue is related to the selectivity of bacterial proliferation within tumours. Ironically, the tumor's microenvironment, which shields it from the majority of anticancer treatments, also serves as its "Achilles heel," making it susceptible to bacterial anticancer medicines. It is widely known that distinct experimental cancers selectively accumulate different microorganisms. For instance, Salmonella strain VNP20009 has shown cancer to normal tissue ratios of 300–25,000:1. Many explanations have been put out to account for such observations. The principal causes of this characteristic are zones of necrosis, which are either the direct or indirect outcomes of cancer growth processes. Neovascularization, also referred to as the process of creating new blood vessels, is necessary for cancer growth and development. When the tumor's radius reaches a certain size, oxygen can no longer sufficiently reach the tumor's inner layers, and the cells begin to gradually lose oxygen. Low oxygen partial pressure causes more angiogenesis in the hypoxic zone. The delivery of therapeutic medicines and immune cells encounter physiological hurdles in these newly created arteries because of their aberrant structure and function. The fact that they are made up of pores with different sizes ranging from 200 nm to 2 m (depending on the tumor)[6] is one of its exploitable abnormalities. [6]

This could make it possible for germs to leave the vasculature and settle locally inside the tumour mass. Necrotic zones are pockets of dead cells that are typically, though not always, located in the centre of the tumour mass. Such areas are favourable for bacterial growth because they should offer defence from the immune system and enough nutrients (such as purines) from the dead cancer cells. In fact, some cancers (often large with substantial necrotic sections) have been anecdotally reported by surgeons to produce a decaying aroma upon surgical removal, most likely coming from infected microbes.[7]

Depending on the species, the specific site of bacterial proliferation within the tumour may change. Anaerobic bifidobacterial development was seen as several clusters within non-viable tumour regions, according to a new 3D imaging investigation. According to data by Forbes et al., salmonellae multiplied inside the necrotic regions of model tumours. Such a finding suggests that their application is restricted to big tumours. This, however, conflicts with current evidence and older data published by that show Salmonella proliferation in both normoxic and hypoxic regions. A clinical setting prefers such a capability. A good bacterial anticancer agent should target and spread inside of microscopic metastatic tumours, which by nature don't have necrotic areas. For instance, it has been demonstrated that Escherichia coli K12 MG1655 and HJ1020 with light emitting genes can target both tiny and large tumours, and even anaerobic Bifidobacterium breve has showed a comparable ability.[8]

As vehicles for cancer gene therapy, bacteria have many benefits. For instance, it is simple to alter bacteria to produce exogenous products with therapeutic value, enhance their tumour selectivity, or express prodrug activating enzymes and reporter proteins for visual confirmation of treatment site and therapeutic outcome. Different kinds of bacteria have various ways of becoming tumor-specific. Obligate anaerobes can produce spores that can only germinate in the anoxic areas of tumours, like Gram-positive Clostridium species. As a contrary, facultative anaerobes like Gram-negative Salmonella and Escherichia coli build up inside tumours for a variety of reasons, including immune system protection, positive chemotaxis towards resources within the tumour microenvironment, and trapping in the disordered vasculature of tumours. Over their viral vector cousins, bacteria have one major advantage over them: bacterial infections during cancer treatment are easily managed by antibiotics.[9]

Effective blood flow is necessary for tumour growth. Inhibiting angiogenesis is a potentially effective method of treating cancer patients. Despite the discovery of a large number of endogenous angiogenesis inhibitors, clinical testing was hampered by the necessity for high doses, production restrictions, and the relative instability of the required recombinant proteins. [10] Microvascular endothelial cells produced at the tumour site are the target of antiangiogenic therapy. Specific antiangiogenic therapy does not require the entry of therapeutic molecules into tumour cells, has minimal to no toxicity, and cannot cross the hematoencephalic barrier. Regardless of the tumor's cell type, it regulates tumour growth and prevents the development of acquired drug resistance. [11] A viable method of avoiding problems associated with systemic drug administration is the addition of genes that code for antiangiogenic proteins. Therapeutic genes that code for antiangiogenic compounds can be delivered to patients using a variety of carrier systems, such as recombinant adenoviruses or liposomes. [12] Antiangiogenic gene therapy can be applied locally or systemically. On the optimum application strategy, scientists are still at odds. Local (intratumor) administration is accompanied by a potent "bystander effect" that amplifies the antagonistic action of inserted genes, and it shouldn't be linked to possible systemic therapeutic adverse effects. [13] On the other hand, a persistent rise of endostatins in blood is made possible by systemic administration of genes coding antiangiogenic agents. [14]

**Conclusion**

With a focus on enzyme-prodrug therapy, this study aims to describe the enzymes employed to achieve prodrug bioconversion as well as the concerns linked to the manufacturing and utility of prodrugs specific to these enzymes. Given that so many marketed medicines are prodrugs, the state of the art in prodrug design already portrays this field as being highly successful. EPT, on the other hand, has had only little success since its inception a few decades ago. When compared to traditional therapy, ADEPT has some benefits, including: Since each enzyme molecule can cleave a large number of prodrug molecules, there is increased selectivity for malignant cells that takes advantage of the specificity of the Ab; internalisation of the Ab-enzyme conjugate into tumour cells is not required; there is an amplification effect; and in the majority of the examples described here, the released active drug is of low molecular weight, enabling it to.

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