Capping Drugs: Development of Prodrugs

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It is well documented that till recent times drugs derived from plants were used to relieve patients from suffering. But at the turn of the last century, with the improvement in purification methods using chromatographic techniques, single compounds with well-defined structure became available for testing and treatment. Simultaneously, progress in organic synthesis allowed the synthesis of a plethora of pure compounds some of which became readily available for use as drugs. In fact today more than half of the drugs used in practice are of synthetic origin.

A drug can be defined as a chemical used for treating, curing or preventing disease in human beings or in animals. In the process of treatment, drugs are also used for medical diagnosis and for restoring, correcting, or modifying physiological functions. Conventional drugs suffer from many drawbacks in their performance.

Site specificity: Most of the drugs do not specifically attack the affected parts of the body. Orally or intravenously administered drugs need to necessarily travel through blood stream to the site of requirement. In the process, they may cause toxic side effects. For example almost all the presently available anticancer chemotherapeutic agents are cytotoxic in nature i.e., they attack growing cells. Since cancer cells grow at a faster rate than the normal cells, the anticancer drugs act as chemotherapeutic agents. However, they are not target specific, and therefore are also toxic to normal cells.

Permeability: Orally administered drugs must cross the cell membrane barrier twice. When a drug is taken, it must initially cross the cell membrane barrier to get into the blood stream for transportation. After reaching the affected part of the body, it must cross the cell membrane again, this time of the affected cell.

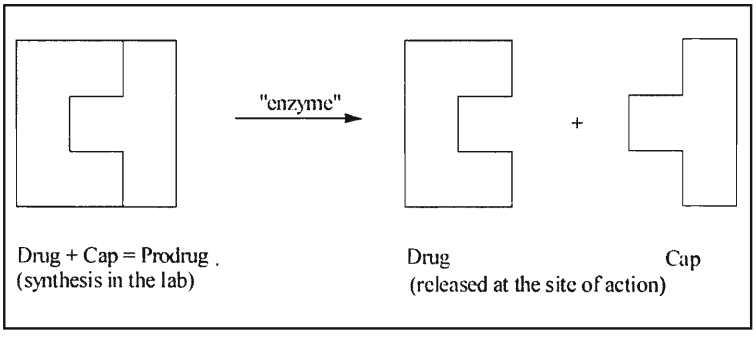
Keywords
Prodrugs: characteristics and development.

Naturally, drugs cannot be effective if their permeability properties into or out of the specific cells do not meet the desired levels.

Resistance: The drugs must be resistant to degradation from different body parts and fluids. It is also desirable to retain the drug molecule in the specific parts of the body for a longer duration so that its effective activity profile is completely realized.

The Prodrug Concept

Albert and his coworkers were the first ones to suggest the concept of prodrug approach for increasing the efficiency of drugs in 1950. They described prodrugs as pharmacologically inactive chemical derivatives that could be used to alter the physicochemical properties of drugs, in a temporary manner, to increase their usefulness and/or to decrease associated toxicity. Subsequently such drug-derivatives have also been called 'latentiated drugs', 'bioreversible derivatives', and 'congeners', but 'prodrug' is now the most commonly accepted term. Thus, prodrug can be defined as a drug derivative that undergoes biotransformation enzymatically or nonenzymatically, inside the body before exhibiting its therapeutic effect. Ideally, the prodrug is converted to the original drug as soon as the derivative reaches the site of action, followed by rapid elimination of the released derivatizing group without causing side effects in the process. The definition of the prodrug indicates that the derivatizing group is covalently linked to the drug molecule. However, the term prodrug has also been used for salts formed by the drug molecules.



Scheme 1. As shown in the figure, a derivative of a known, active drug (D) can be capped to furnish a prodrug (PD). The PD enhances delivery characteristics and/or therapeutic value of the drug by transforming into the active drug via an enzymatic or a chemical process to remove the cap P at the site of action to regenerate D.

Characteristics of a Prodrug

In recent years numerous prodrugs have been designed and developed to overcome barriers to drug utilization, such as low oral absorption properties, lack of site specificity, chemical instability, toxicity, bad taste, odour, pain at application site, etc. It has been suggested that the following characteristics of a prodrug must be improved for site-specific drug delivery.

1. The prodrug must be readily transported to the site of action.

2. The prodrug must be selectively cleaved to the active drug utilizing special enzymatic profile of the site.

3. Once the prodrug is selectively generated at the site of action, the tissue must retain the active drug without further degradation.

Development of Prodrugs

Prodrugs, which were developed by taking specific administration properties into consideration, are discussed here.

To improve membrane transport: Barbiturates (*Figure* 1) are a group of compounds responsible for profound sedative-hypnotic effect. They are weakly acidic in nature and are converted to the corresponding sodium salt in aqueous sodium hydroxide. The sodium salt is extensively employed for intravenous anaesthetic properties. Barbituric acid is the parent member of this group of compounds. Various barbiturates differ in the time required for the onset of sleep and in the duration of their effect. Hexobarbitone was found to be an effective drug but its membrane permeability was found to be low. However N-methylhexobarbitone a simple

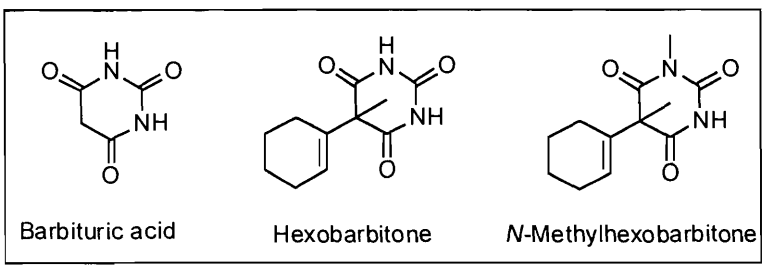


Figure 1. Hypnotic and sedative agents.

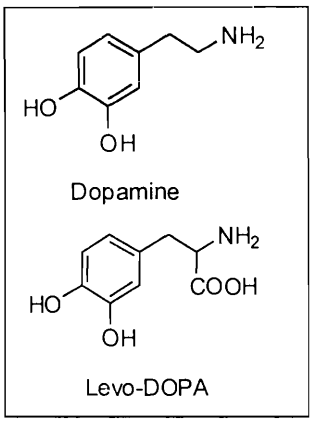


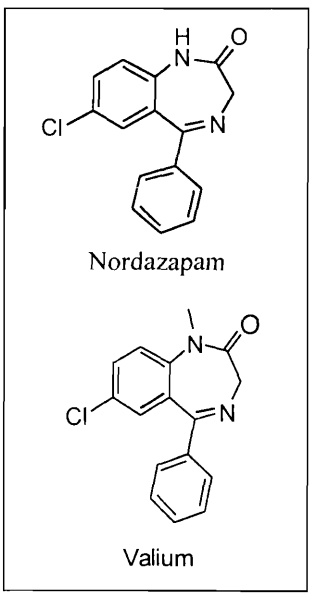
Figure 2: Drug and prodrug for Parkinson's disease.

derivative of the parent drug was found to have better permeability characteristics. After intake, the N-methyl group is cleaved in the liver to release the physiologically active drug.

Similarly, membrane transportation characteristics of the neurotransmitter dopamine (*Figure* 2) used for the treatment of Parkinson's disease can be improved by administering its prodrug L-3,4-dihydroxyphenylalanine (Levo-DOPA; *Figure* 2). This derivative has better blood-brain permeation characteristics since it uses amino acid channels for transportation. Once inside the cell, decarboxylase enzyme removes the acid group to generate dopamine.

Prolonged Activity: Nordazapam (*Figure* 3) is a drug used for sedation, particularly as an anxiolytic. It is also used as a muscle relaxant. However, it loses activity too quickly due to metabolism and excretion. A prodrug introduced to improve the retention characteristics is valium (*Figure* 3). Due to presence of N-methyl group the prodrug resists quick degradation. Slow release of the nordazapam in the liver by demethylation prolongs body retention characteristics.

Figure 3. Anxiolytic and muscle relaxing agents.



Decreased or lack of secretion of the enzyme insulin in pancreas leads to diabetes. Insulin is responsible for degradation of carbohydrate molecules to smaller units and is important in the catabolic process. Chronic diabetic patients take bovine insulin supplement through intravenous injections. Retention time of insulin in the blood is about six hours. So, patients need to administer required dose of insulin frequently. It is desirable to increase the retention characteristics of the enzyme so to make it effective for prolonged periods. It was found that 9-fluorenylmethoxycarbonyl (Fmoc) protection (*Figure* 4) of the hydroxy/amino groups of the enzyme makes it inactive and also increase its retention in blood for prolonged periods. The Fmoc group binds to the enzyme covalently and in the process makes it inactive as well as reduces its rapid degradation by natural body process. However, at the pH of about 7.4 prevalent in the blood serum the protected enzyme gets hydrolyzed slowly and irrevers-

ibly back to the enzyme and Fmoc protecting group. The hydrolysis process was found to be slow and constant, which means that the release of enzyme is also slow and regulated. The hydrolysis rate can be fine tuned by selecting derivatives of Fmoc protecting group or number of Fmoc groups. It was shown that insulin having two Fmoc protecting groups was ten times more stable and more effective than the parent enzyme. It should be noted that the hydrolysis of the protecting group takes place in the blood without mediation from other enzymes.

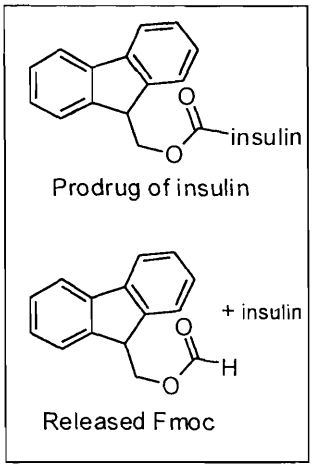
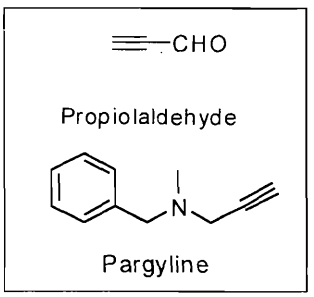


Figure 4. Fmoc protected prodrug of insulin.

Masking from other Enzymes: Sometimes drugs are highly toxic when administered directly. Suitable modification of the drug molecule to an inactive agent reduces toxicity. For example propiolaldehyde (*Figure* 5) is used in the aversion therapy on patients addicted to alcohol. However, it is a highly irritating chemical and causes allergic reactions. As an alternative, closely related compound, pargylene (*Figure* 5), which is converted to propiolaldehyde by oxidative enzymes only in liver, is used for alcohol deaddiction.

Tissue Specific Prodrug Design: In this approach of prodrug design the site-specific drug delivery can be achieved by the tissue activation, which is the result of an enzyme unique to the tissue or present in higher concentration. For example glycosidase enzymes are present in much higher concentration in bacteria associated with colon. This aspect can be utilized in the design of colon specific drug delivery. Glycosides are hydrophilic in nature and are poorly absorbed in small intestine. Once they reach the colon, bacterial glycosides release the free drug to be absorbed in that region. Dexamethasone and prednisolone (*Figure* 6) are corticosteroids used for anti-inflammatory properties. They are steroid drugs and are hydrophobic in nature. They are absorbed efficiently in intestinal tract and as such do not reach colon area for treatment. However, when produgs dexamethasone-21-β-glucoside and prednisolone-21-β-glucoside were used they were absorbed in colon more efficiently compared to their parent drugs. The prodrugs are hydrophilic in nature and therefore are absorbed poorly in intestine. The glucosidase enzymes

Figure 5. Prodrug for anti-alcoholic addiction.



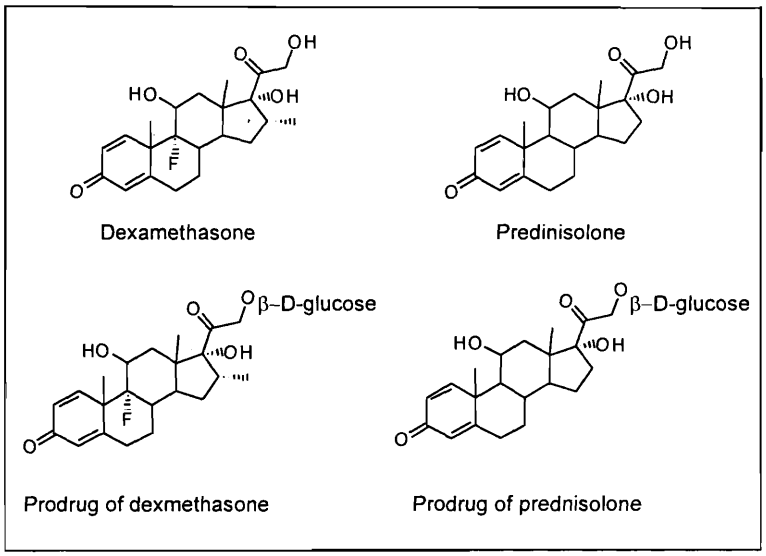


Figure 6. Antiinflammatory corticosteroid drugs and their prodrug derivatives.

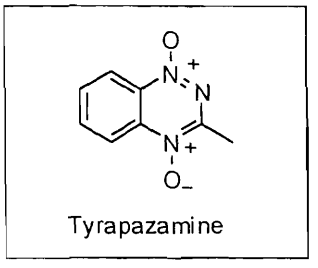
present in the bacteria located in colon release the parent hydrophobic drugs for absorption in the area.

Prodrug Design Based on Site-specific Conditions: A variety of conditions such as pH, oxygen content, which are site specific and are different from other parts of the body, can be effectively utilized for prodrug design. This aspect is an important feature in the research on prodrugs targeted at cancer cells. The cancer cells grow at a much faster rate than normal cells. Tumor cells associated with cancer can be differentiated from normal cells. The blood vessels in the tumor tissue often lack regularity and systematic connectivity leaving unvascularized zones, especially in the interior areas leading to unstable blood flow. Cells that do not have blood supply die as a result of lack of oxygen supply and also the intermediate regions get deficient supply of oxygen. This area is called hypoxia[1] region.

Lack of oxygen in hypoxia cells or the bio-reductive conditions prevalent in them can be utilized for specific prodrug development wherein the active drug can be selectively released under bio-reductive conditions. The bio-reductive enzymes present in the cell perform one electron reduction. In normal cells oxygen reverses this reduction process. However, in hypoxia cells, due to

Hypoxia region is a major problem in cancer treatment. The hypoxic cells are more resistant to damage by radiation therapy since the radioactive agents do not reach the cells in sufficient quantity. Moreover, oxygen is a radiation sensitizer and low oxygen concentration in hypoxia region greatly reduces the efficiency of treatment. Due to low oxygen supply the metabolic activity in the hypoxia cells are lower than normal cells. Many anti-tumor chemotherapeutic agents that target fast growing cells by interfering in the metabolic activity,become ineffective against hypoxia cells. In addition, it is also known that hypoxia cells are primarily responsible for mutation and metastasis condition of cancer. Therefore, it is desirable to target hypoxia cells.

near absence of oxygen, further reduction takes place to generate drug from prodrug moiety. For example, Tyrapazamine (*Figure* 7) has been developed as a cytotoxic agent. It has two N-oxide moieties, which on one electron reduction twice gets converted to highly reactive diradicals. The diradicals are responsible for cleavage of DNA. Even though such diradicals are generated in the normal cells, they get reconverted to N-oxides due to the presence of oxygen. Whereas in hypoxia cells the diradicals have longer lifetime to interact with DNA molecules and further cleave them. Such a cleavage of single or double strand DNA leads to destruction of cells. Thus, the N-oxide prodrug was found to be highly effective in hypoxia cells.



Tyrapazamine

Figure 7. Prodrug directed to hypoxia cells.

Enzyme Specific Prodrug Design: As described previously certain enzymes express predominantly at the affected parts. Prodrug development can take advantage of this aspect so that the over expressed enzymes at the affected parts of the body can be induced to release the drug at the site. This condition is of particular importance in targeting cancer cells. Due to differing physiological conditions enzyme groups such as glucuronidases, proteases[2], receptors show activity in excess in cancer cells compared to normal cells. Several prodrugs have been developed taking advantage the excessive activity of the above enzymes in tumor tissues.

Scheeren and his group clearly demonstrated that the cytotoxic activity of important antitumor drugs could be enhanced and restricted to tumor-affected tissues by making peptide derivatives. They prepared derivatives of doxorubicin (*Figure* 8) and paclitaxel (*Figure* 9) wherein active sites are blocked by strategically attaching suitable polypeptide to the drug but separated by spacer. The spacer was used to expose the polypeptide chain open for plasmin activity. Both the prodrugs were found to be inactive and stable under biological pH conditions but they were readily cleaved with the release of parent drugs in the presence of plasmin enzymes present in tumor cells. The prodrugs were synthesized by blocking important functional group in the molecule with a polypeptide-capping agent to make them inactive. The spacer

[2] Protease enzymes are responsible for breakdown of proteins. They scissor the protein chain at amide bonds linking specific amino acids. It was found that even though protease enzymes are ubiquitous in all cells and blood serum, their activity is excessively associated with tumor metastases. Primary malignant cells are encapsulated in extra cellular matrix made of proteins. In order to proliferate and to reach metastases the primary tumor has to break through the matrix through protease activity. It was shown that serine protease plasmin plays an important role in tumor invasion to other areas. In blood circulation plasmin activity is restricted by the presence of antiplasmin enzymes.

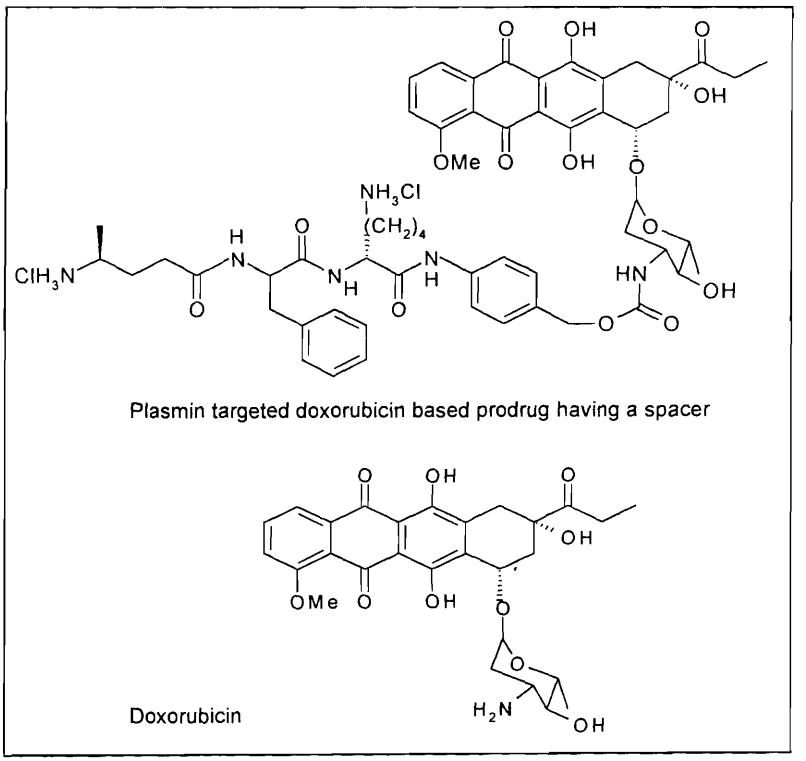
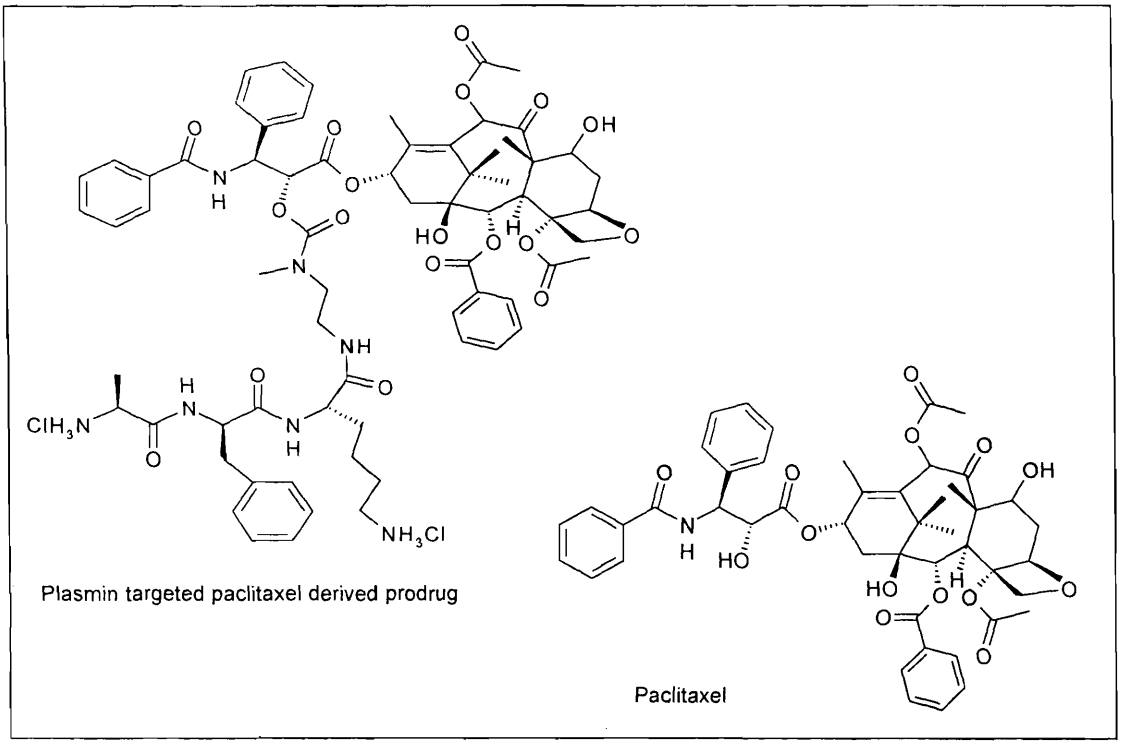


Figure 8. Plasmin targeted anticancer prodrug and its parent drug.

group was designed to self eliminate after hydrolysis of the polypeptide chain by the enzyme.

Conclusion

It is clear from the foregoing, that the development of prodrugs promises to be a very effective method for treatment of diseases in the future. This approach has several advantages over conventional drug administration. Site specificity is central to the prodrug development strategy. Even though at present prodrugs are not prevalent in clinical use, in future there will be prodrugs for every known drug to make them effective in treatment. Drug discovery and prodrug development appear to be complementary for the generation of target specific medicines of future. At present the research in this area is at a nascent stage due to lack of information regarding all the enzymes or receptors most suitable for targeting purposes. As the unravelling of the micro-



biological details of the affected targets become clear, prodrug development will surely decrease side/toxic effects of drugs and also trigger development of more potent primary drugs.

Figure 9. Plasmin targeted paclitaxel prodrug and parent natural product.

Acknowledgement

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Suggested Reading

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