Review Article

Indian Journal of Science and Research

Jain Y *et al.,* Ind. J. Sci. Res. 2022, 2(2), 38-43

ISSN 2583 – 2913

CURRANT REVIEW ON SYNTHESIS OF PRODRUG AND ITS APPLICATION

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Abstract*:* Prodrug design is a well-known molecular modification method that tries to increase the solubility and pharmacokinetic aspects of medications while lowering their toxicity by optimising their physicochemical and pharmacological properties. Carrier would alter the physical properties of a medicine in order to boost its fat or water solubility or to enable site-directed delivery. Despite the fact that multiple studies on diverse prodrugs have been conducted, there have been surprisingly few reviews on different carriers that can be employed for prodrug synthesis published too far. One of the most significant roadblocks to drug development is a lack of solubility. The goal of this review is to describe current developments and application of prodrug approach. The primary chemical carriers will be reviewed, as well as instances of effective techniques, with an emphasis on the field's progress in the last ten years.

Key words: Prodrug, Synthesis, Application, Review

Introduction: During the last two decades, there has been a steady improvement in the physicochemical, biopharmaceutical and/or pharmacokinetic properties of pharmacologically active compounds by the implementation of a prodrug strategy. It is estimated that currently about 10% of worldwide marketed drugs can be classified as prodrugs. Moreover, in 2008, one third of all approved small molecular weight drugs were prodrugs [1]. The first compound fulfilling the classical criteria of a prodrug was acetanilide, introduced (under the name of Antifebrin®) into the medical practice by Cahn and Hepp in 1867 as an antipyretic agent. In the body, acetanilide is hydroxylated (aromatic hydroxylation) to biologically active acetaminophen (paracetamol), the compound endowed with both antipyretic and analgesic activity. Acetaminophen can be also formed in the process of O-dealkylation of another analgesic drug – phenacetin (acetophenetidin), introduced into clinical use in 1887 by von Mering. Acetanilide and phenacetin were not originally designed as prodrugs, but their prodrug nature was de termined later on. Another example of a historical prodrug is aspirin (acetylsalicylic acid), synthesized in 1897 by Felix Hoffman (Bayer, Germany), and introduced into medicine.

However, the prodrug concept was intentionally used for the first time in the middle of the 20th century by the Parke-Davis company during studies on modification of chloramphenicol structure in order to improve the antibiotic's bitter taste and poor solubility in water. As a result of this work, two prodrug forms of chloramphenicol were synthesized: chloramphenicol sodium succinate with a good water solubility, and chloramphenicol palmitate used in the form of suspension in children [2].

Prodrug concept: The basic aim of prodrug design is to mask undesirable drug properties, such as low solubility in water or lipid membranes, low target selectivity, chemical instability, undesirable taste, irritation or pain after local administration, presystemic metabolism and toxicity [3]. In general, the rationale behind the use of prodrugs is to optimize the absorption, distribution, metabolism, excretion, and unwanted toxicity (socalled

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Published on Web 30/06/2022, www.ijsronline.org

ADMET properties) of the parent drugs . Classically, the term prodrug, relates to biologically inert derivatives of drug molecules that undergo an enzymatic and/or chemical conversion in vivo to release the pharmacologically active parent drug. The active drug is released from its inactive form before, during or after absorption of the prodrug. Some drugs are released only after reaching targets of their actions . A prodrug should increase the bioavailability and therapeutic effectiveness of a parent drug. Although the term prodrug is now standard, prodrugs have been also referred to as reversible or bioreversible derivatives or biolabile drug-carrier conjugates. According to Testa there are three basic, overlapping objectives in prodrug research:

1. Pharmaceutical: to improve solubility, chemical stability, and organoleptic properties; to decrease irritation and/or pain after local administration, to reduce problems related with the pharmaceutical technology of the active agent.
2. Pharmacokinetic: to improve absorption (oral and by non-oral routes), to decrease presystemic metabolism, to improve time profile, to increase organ/ tissue-selective delivery of the active agent.
3. Pharmacodynamic: to decrease toxicity and improve therapeutic index, to design single chemical entities combining two drugs (co-drugs strategy). It should be noted that strategies to improve oral bioavailability and achieve brain- and tumor-specific targeting have been the most important developments in the prodrug design during the last decade.

Structure and classification of prodrugs

There are two main classes of prodrugs:

(1) carrierlinked prodrugs

(2) bioprecursor prodrugs.

In the carrier-linked prodrugs, the active molecule (the drug) is temporary linked to a carrier (also known as a promoiety) through a bioreversible covalent linkage. Once in the body, the carrier-linked prodrug undergoes biotransformation, releasing the parent drug and the carrier. Ideally, the carrier should be nonimmunogenic, easy to synthesize at a low cost, stable under the conditions of prodrug administration, and undergo biodegradation to nonactive metabolites [4]. In so-called co-drugs (mutual prodrugs, multiple prodrugs), a prodrug is formed from two pharmacologically active agents coupled together into a single molecule, and act as promoieties of each other. Examples of co-drugs include sulfapyridine – 5-aminosalicylic acid, indomethacin – paracetamol, L-DOPA – enthacapone, gabapentin – pregabalin, 5-fluorouracil – cytarabine, 5-fluorouracil – dexamethasone triamcinolone, ampicilin – sulbactram, sulfamethoxazole – nalidixic acid [5].

The major groups of carrier-linked prodrugs are esters and amides; other groups include phosphates, carbamates, carbonates, oximes, imines and N-Mannich bases. Bioprecursors do not contain a promoiety but result from a molecular modification of the active compound itself. The bioprecursor prodrug is transformed metabolically or chemically by hydration (e.g., lactones such as some statins), oxidation (e.g., dexpanthenol, nabumetone) or reduction (e.g., sulindac, platinum (IV) complexes) to the active agent.

Based on the site of conversion into the pharmacologically active agent, the prodrugs can be additionally classified into two groups:

• Type I – metabolized intracellularly. Type IA prodrugs (e.g., acyclovir, cyclophosphamide, 5-fluorouracil, L-DOPA, zidovudine) are metabolized at the cellular targets of their therapeutic actions. Type IB prodrugs (e.g., carbamazepine, captopril, molsidomine, primidone) are converted to parent drugs by metabolic tissues, namely by the liver.

• Type II – metabolized extracellularly. Type IIA – in the milieu of the gastrointestinal fluid (e.g., loperamide oxide, sulfsalazine). Type IIB – within the circulatory system and/or other extracellular fluid compartments (e.g., aspirin, bambuterol, fosphenytoin). Type IIC – near or inside therapeutic target/cells (ADEPT, GDEPT).

Some of the prodrugs, called mixed-type prodrugs, belong to more than one class [6].

Esters of active agents with carboxyl, hydroxyl or thiol functionalities, and phosphate esters of active agents with hydroxyl or amine functionalities are the most commonly used prodrugs. Approximately half of the prodrugs currently available on the market are activated via enzymatic hydrolysis by ubiquitous esterases (acetyl cholinesterases, butyryl cholinesterases ,carboxylesterases , aryl esterases) which are present throughout the body . Following the process of esterification, a desired chemical and biological stability of prodrug esters can be achieved by an appropriate manipulation of their electronic and steric properties . An ideal ester prodrug should have chemical stability across a pH range, high aqueous solubility, exhibit good transcellular absorption, be resistant to hydrolysis during the absorption phase, and undergo rapid and quantitative breakdown to yield high circulating concentrations of the active component post absorption [7].

The main rationale underlying synthesis of prodrug esters is to (1) enhance lipophilicity, and thus the passive membrane permeability, of water soluble drugs, (2) increase aqueous solubility (phosphate esters). To increase the lipophilicity of hydroxyl group bearing pharmacologically active compounds, they can be acylated with aliphatic or aromatic carboxylic acids. Esterification of the hydroxyl group of the parent drug with carboxylic acid derivatives bearing additional functional groups, such as hydroxyl, amino or car Pharmacological boxyl, provides prodrug molecules with increased aqueous solubility (hydrophilicity) [8].

Some esters are not suitable substrates for endogenous esterases, sulfatases or phosphatases, which, in turn, impose unfavorable, too slow kinetics of the prodrug hydrolysis. To optimize the rate of enzymatic hydrolysis, knowledge on the mechanisms of the specific processes involved is highly desirable. An increase in the rate of alkaline hydrolysis can be achieved by the attachment of electron-withdrawing groups, while in the case of acidic hydrolysis, tethering of electron-donating groups to the carboxylate part of esters is recommended. If the esters are too reactive, bulky substituents causing steric hindrance to hydrolysis or esters of long-chain fatty acids can be employed [9].

Prodrug approaches for enhancing administration, permeability, absorption, and distribution of drugs

Prodrugs with increased aqueous solubility

Poor aqueous solubility is considered as a serious problem limiting the therapeutic use of numerous drugs and drug candidates. Among various strategies used to overcome this drawback is the prodrug approach. One frequently employed means of improving the aqueous solubility of a drug is by the use of esters and amides of phosphoric acid, due to the ionic nature of the phosphate group. It should be noted that phosphate derivatives display high chemical stability, often even higher than the parent compound. Under physiological conditions phosphate prodrugs undergo rapid biotransformation by endogenous phosphatases, such as alkaline phosphatase, of the intestine, plasma, and the liver. Another approach to increase aqueous solubility is esterification with amino acids. Examples of such prodrugs are valacyclovir (Valtrex®) and valgancyclovir (Valcyte®), which are valine esters of the antiviral drugs acyclovir and gancyclovir, respectively. Both prodrugs are also good substrates of small peptide transporters (PEPT1) present in the intestine epithelium, a property that further increases their bioavailability [10].

A hemisuccinate group can be conveniently used to increase water solubility, as it contains a free carboxylic group, which is suitable for the formation of dissociated salts. A fine example of the utilization of hemisuccinate esters is cinazepam, a novel benzodiazepine anxiolytic drug suitable for intravenous injections [11].

Prodrugs with increased lipid solubility: In order to improve lipophilicity, and thus passive transport through biological membranes, compounds containing polar or ionizable groups can be converted into ester prodrugs [54]. Examples of ester prodrugs with improved lipophilicity designed for oral administration. Prodrugs with increased lipophilicity are also designed for topical administration. For example, esters of ketolac (a non-steroidal anti-inflammatory drug with potent analgesic activity) and fatty acids (stearic, linoleic, oleic) allow the drug to accumulate in the skin with concomitant low skin permeation, leading to increased therapeutic efficiency and reduced side effects of the parent drug [12].

Additional examples are latanoprost and travoprost, prodrugs of prostaglandin F2 (PGF2) analogs, applied as eye drops for the treatment of glaucoma. Naturally occurring prostaglandins, including PGF2, are molecules which are relatively polar and hydrophilic due to their carboxylic acid moiety and several hydroxyl groups. They penetrate through biological membranes poorly. This problem has been solved by a modification of the chemical structure of PGF2analogs. Latanoprost (Xalatan®) and travoprost (Travatan®), isopropyl esters of latanoprost acid and travoprost acid, respectively, penetrate easily through the corneal epithelium due to their increased lipophilicity, where they are then hydrolyzed to active carboxylic acids. The free acids of latanoprost and travoprost activate prostanoid FP receptors and reduce intraocular pressure by enhancing the uveoscleral and the trabecular meshwork outflow pathways [13]

Phosphatase (Phosphoesterases) : bioconversion of phosphate prodrugs

While the charged character of the phosphate group poses a problem for drugs with intracellular targets, it can also be highly beneficial in the design of prodrugs, specifically to increase the solubility of poorly soluble therapeutics. After the administration, the phosphate promoiety is cleaved by alkaline phosphatase, a nonspecific esterase found in all tissues throughout the entire body, including the liver, kidneys, and apical membrane of enterocytes. The use of phosphate esters

has resulted in several successfully marketed prodrugs such as fosamprenavir. [14]

Amprenavir is an anti-HIV agent and being a protease inhibitor (in contrast to the above discussed nucleoside analogues) does not need to be phosphorylated to exert its therapeutic effect. In this case, a phosphate group is attached to its secondary alcohol group to produce fosamprenavir – a prodrug with approximately 10 times higher aqueous solubility as a calcium salt than the parent amprenavir.Other examples of water-soluble phosphate prodrugs are fosphenytoin, fosaprepitant and ceftaroline fosamil. With fosphenytoin, a phosphate ester is attached to an acidic NH-group of phenytoin via an oxymethylene linker. Fosaprepitant employs a direct amide N-phosphate strategy, and ceftaroline fosamil is a phosphonate prodrug of a primary amine. [15]

Enzyme prodrug therapies

Cytosine deaminase

Historically, development of EPT can be traced back to the 1980s and the first example of use of cytosine deaminase (CDase) to achieve a localized conversion of 5-fluorocytosine (5FC) to a potent anticancer agent, 5-fluorouracil (5FU). In humans, 5FU has a highly variable absorption; coupled a rather narrow therapeutic window, it makes safe dosing of 5FU challenging. Inspired by the use of 5FC as a drug against the CDase-expressing fungi, [16] performed immobilization of CDase at the tumor site, first by injection and subsequently through immobilization of the enzyme-containing reservoirs enclosed within dialysis bags. Specific success of these reports was that quantification of 5FC and 5FU in the mice blood and at the site of tumor provided direct evidence for a higher concentration of the anticancer drug at the tumor site compared to the systemic (blood) concentration.

Phosphatases and the origin of EPT

Second and possibly independent origin of development of EPT relates to the phosphate prodrugs discussed above. While phosphoesterases are quite well distributed in the body, a member of this enzyme family, alkaline phosphatase, was in fact the first enzyme used in the antibody-directed enzyme prodrug therapy, ADEPT. The latter term was independently coined by Senter and Bagshawe [17] to describe a two-step process whereby first, an antibody-enzyme conjugate is administered to the patient such as to achieve a predominant association of the conjugate with a specific antigen, e.g. achieve targeting to the tumor cells. The second step is administration of the prodrug, which is done after the clearance of the free (un-associated) antibody-enzyme conjugate from the blood stream. In such a case, enzyme mediated prodrug activation is achieved only by the antigen-bound enzyme which ensures a highly localized drug delivery.

Viral thymidine kinase A highly promising strategy to EPT is to engineer prodrug bioconversion using enzymes of non-mammalian origin or those that have low abundance in the human body. In such a case, non specific, systemic prodrug activation is suppressed to low levels and this enhances the site-specific nature of prodrug activation. This strategy was highly important for the development of ADEPT and GDEPT. Examples of prodrugs used for this methodology are nucleoside analogues and specifically those that are good substrates for the viral thymidine kinase[18]

The mechanism of antiviral activity of acyclovir and many other nucleoside analogue type drugs is that in their triphosphorylated form, these drugs become substrates for nucleic acid polymerases. Once incorporated into the de novo synthesised chain of nucleic acid, these drugs become “chain terminators” by virtue of lacking the 3’-hydroxyl group for chain extension. Of the three steps of phosphorylation, the first step is the rate limiting. Utility of acyclovir as an antiviral drug is highly facilitated by that the targeted pathogen, herpes simplex virus (HSV), uses its own, viral thymidine kinase, which is brought into the cell upon viral infection. Viral thymidine kinase is markedly more effective than the human analogue towards phosphorylation of acyclovir and this means that acyclovir phosphorylation is significantly more effective within the virus-infected cells than in the healthy ones. Correspondingly, concentration of acyclovir triphosphate in healthy cells during the antiviral treatment is rather low and toxicity of antiviral treatment is also low.

β-Lactamase

Another class of non-mammalian enzymes with a high historical importance in the context of EPT is bacterial lactamases. An aspect that contributed to the development of lactamase-based prodrug therapy is that corresponding (pro) drugs, cephalosporins, are market-validated drugs with well established methods of their synthesis. ADEPT or EPT made great use of the old chemistry and incorporated the cephalosporin platform into a prodrug strategy[19]The synthesis of cephalosporins and their analogues is well established and can be accomplished starting from a marketed antibiotic drug, cephalotin .

Nitroreductase

In the late 1960s, [20] various nitrogen-mustard analogues were synthesized and tested against different cancer cell lines. Compound CB1954 received plenty of attention due to its impressive toxicity profile in rat Walker 256 carcinoma cells documented both in vitro and in vivo. Interestingly, this effect was not confirmed in human cancer cell lines. Analysis of this phenomenon revealed that mechanism of action of CB1954 involves an initial activation by a nitroreductase enzyme into an extremely potent DNA-DNA double strand cross linking agent . This enzyme is far less active in healthy mammalian cells. [21] Delivering or expressing this protein at the nominated site of action would therefore lead to a site-specific prodrug activation, making nitroreductase a prime candidate for the use in EPT and specifically GDEPT.

Self immolative linkers

Enzymatic conversion of prodrugs is critically dependent on accessibility of the scissile bond to the enzyme. This is easy to appreciate on an example of propofol (an anesthetic) and its two clinically tested prodrugs, a phosphate ester and a phosphonoxymethyl propofol . The two prodrugs are similar in that in both cases drug release is initiated by the cleavage of a phosphoester bond. However, the two molecules differ in that phosphonoxymethyl propofol has an additional oxymethylene spacer between the scissile phosphoester and the releasable drug. Bioconversion of the phosphonoxymethylene prodrug proved to be markedly faster compared to the phosphate prodrug – reflecting the steric hindrance effect exerted by two iso-propyl ortho-substituents (in the phosphate prodrug) which is overcome by placing the scissile bond to a position more accessible by the enzyme[21]

Long circulating prodrugs

Prodrug designs presented above are elaborate and successful but are not without shortcomings. Specifically for the prodrugs with relevance to EPT and in particular for glucuronides, a significant limitation lies in that these small, water-soluble molecules have a short half-life. Short body residence time for prodrugs means that only a minor fraction of the drug is recovered from the administered prodrug. It also means that to maintain the concentration of the active drug within the therapeutic window for extended times; such prodrugs would necessitate frequent drug administration or even infusion. Potential methods to overcome this may be found in the existing toolbox of biomedical engineering and specifically, using technologies behind therapeutic agents with extended blood residence time.

Conclusion

This method may allow promising active prototypes or medicines with therapeutic benefits constrained by poor solubility to be saved from being discarded. The prodrug selectivity, toxicity, and optimal bioconversion profile may be determined by the logical selection of the appropriate pro-moiety and the kind of linkage (e.g., ester, amide, carbamate, and phosphate). The FDA has approved a number of prodrugs in the recent decade, albeit not all of them are targeted to promote solubility. Many prodrugs, such as tedizolid phosphate, ceftaroline fosamil, and fospropofol disodium, were created specifically for this purpose. As a result, the prodrug method is a useful tool in rational drug design for improving drug water solubility.

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