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UPDATE

Prodrug Strategy in Drug Development

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Prodrugs are chemically modified derivatives introduced in therapy due to their advantageous physico-chemical properties (greater stability, improved solubility, increased permeability), used in inactive form. Biological effect is exerted by the active derivatives formed in organism through chemical transformation (biotransformation). Currently, 10% of pharmaceutical products are used as prodrugs, nearly half of them being converted to active form by hydrolysis, mainly by ester hydrolysis. The use of prodrugs aims to improve the bioavailability of compounds in order to resolve some unfavorable characteristics and to reduce first-pass metabolism. Other objectives are to increase drug absorption, to extend duration of action or to achieve a better tissue/organ selective transport in case of non-oral drug delivery forms. Prodrugs can be characterized by chemical structure, activation mechanism or through the presence of certain functional groups suitable for their preparation. Currently we distinguish in therapy traditional prodrugs prepared by chemical derivatisation, bioprecursors and targeted delivery systems. The present article is a review regarding the introduction and applications of prodrug design in various areas of drug development.

Keywords: prodrugs, classification of prodrugs, drug development, optimization of bioavailability

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Introduction

One of the effective methods of modern research in the field of medicine is the development of prodrugs that have gained increasingly more importance in current therapy.

A prodrug refers to a pharmacologically inactive compound which is transformed into an active substance by either chemical or metabolic processes. Nowadays approximately 10% of drugs used in therapy are administered as prodrugs, and about half of these are hydrolyzed to the active form, in particular by hydrolysis of esters (figure 1) [1-10].

The actuality of prodrugs in modern therapy is demonstrated by the fact that in the last ten years several books in this field have been published and thousands of articles in scientific databases are investigating new potential molecules [11-13].

Prodrug concept

The prodrug concept has been used to improve undesirable properties of drugs since the late 19th century, but it was only at the end of the 1950s that the actual term "*prodrug*" was introduced for the first time by Adrien Albert for drugs that are inactive by themselves but which form an active derivative by biotransformation. The concept was completed by Harper in 1959 which introduced the term of drug latentiation referring to drugs that were specifically designed to require bioactivation [14].

This definition is the most appropriate even at present time and is consistent with the IUPAC definition which states that: a *prodrug is a compound* that undergoes biotransformation before exhibiting pharmacological effects [15].

In essence a prodrug is an inactive, bioreversible derivative of an active drug which undergoes enzymatic and/or chemical transformation *in vivo* in order to release the active parent drug, which can then exhibit its desired pharmacological effect [16,17].

These ideas led to the development of a relatively large number of prodrugs; the type of prodrugs depending on the specific properties of the drug that requires improvement and the type of functionality that is present in the active drug [11].

Prodrugs are inactive derivatives of active substances with optimized physico-chemical properties (higher stability, improved solubility or increased permeability), that suffer a biotransformation in the body whereby are exerting their pharmacological action [18].

Prodrugs can be defined also to be medicines which have specific protective groups, in order to prevent unwanted properties of the parent molecule. In most cases, prodrugs are simple chemical derivatives that are only one or two chemical or enzymatic steps away from the active parent drug. However, some prodrugs lack an obvious carrier or promoiety but instead result from a molecular modification of the prodrug itself, which generates a new active compound [19].

The place and speed of biotransformation are closely related to chemical structure, as well as the pharmacokinetic properties of the molecule.

The concept of prodrug has to be differentiated from drugs that are active of their own, but by biotransformation are forming one or more active metabolites and the biological effect occurs as a common result of the original drug and metabolites. These drugs are "limited" prodrugs (e.g. diazepam, carbamazepin) [11].

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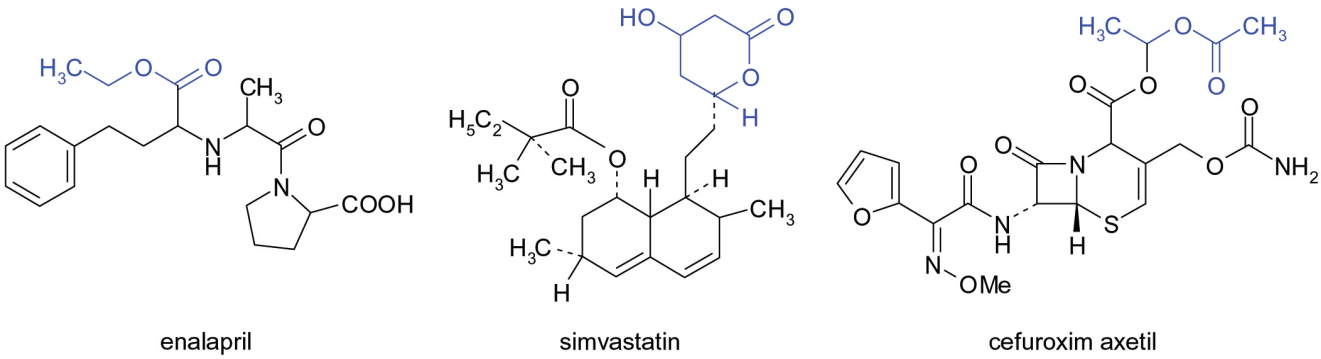

Fig. 1. The chemical structure of a few frequently used prodrugs

In some cases, a prodrug may consist of two pharmacologically active drugs that are coupled together in a single molecule, so that each drug acts as a promoiety for the other. Such derivatives are called "codrugs" (e.g. sultamicillin, sulfasalazine, benorilate, levodopa-entacapone) [11,20,21].

Recently the concept "hard-drug" and "soft-drug" were introduced. "Hard-drugs" are designed to contain structural characteristics necessary for the desired pharmacological activity in a form that is not susceptible for chemical or metabolic transformation, in order to avoid the production of toxic derivatives or to increase pharmacological efficiency. "Soft drug" are active substance with planned metabolism which after exercising its effect is metabolized into inactive and harmless metabolites and is rapidly cleared from the body; thus "soft-drugs" can be considered to be opposite to prodrugs.

The purpose of soft drugs' development is to achieve a therapeutic effect locally, eliminating systemic effects and adverse reactions (e.g. remifentanil, esmolol) [22-24].

The purpose of designing prodrugs

1. *Improving bioavailability* when the drug candidate is not drug-like due to unfavorable *physical properties* as:
   - poor water solubility,
   - low lipophilicity,
   - chemical instability,
   - unacceptable taste or smell,
   - local irritation, pain.

2. *Improving bioavailability* when the drug candidate is not drug-like, due to *pharmacokinetic properties*:
   - low bioavailability,
   - poor penetration through biological membranes,
   - increased first-pass metabolism,
   - slow absorption by parenteral route,
   - rapid absorption/elimination instead of long-lasting effect,
   - lack of specificity in certain tissues [23-26].

The specific objective of prodrug design is *to optimize unfavorable physicochemical properties, to increase chemical and/or metabolic stability, to achieve planned delivery.*

Prodrugs with optimized pharmacokinetic properties have the following advantages:

- increasing absorption from the gastrointestinal tract after oral administration,
- obtaining parenteral preparations,
- masking unpleasant tastes, odors,
- avoiding injection site irritation or pain,
- preventing rapid administration site inactivation,
- increasing passage through the blood-brain barrier,
- tissue/organ specific drug administration,
- decrease in multidrug resistance,
- side effects - and toxicity profile improved.

Prodrugs can be more effective, safer and more convenient in administration than conventional forms [26,27].

Classification of prodrugs

Prodrugs can be classified taking in consideration their chemical structure, mechanism of activation and the modified functional groups [2,28].

1. By chemical criteria can be distinguished:
   - *conventional prodrugs* - obtained by chemical derivatization; the desired objective is to optimize transport properties; they are also called "carrier-linked prodrugs" as on the parent molecule are grafted functional groups that promote absorption.
   - *bio precursors* – those substances which were not designed by conscious planning, but their activation in the body occurs through chemical reactions. Such prodrug is *lovastatin*, some vitamins (e.g. B1, B6), which after phosphorylation or oxidation (e.g. vitamin D) are exercising their physiological role [11,29].
   - *drug delivery systems:*
     – drug - polymer conjugates, where the drug is binding to a macromolecule that favors its transport
     – drug - antibody conjugates, target delivery is performed by antibody.

2. By activation mechanism can be distinguished:
   - drugs which suffer enzymatic activation – can be planned; problems may occur due to biological variability between species, genetic polymorphism, drug interaction potential.
   - drugs which suffer non-enzymatic activation – it is spontaneous, however inadequate chemical stability can create problems in conservation before use.

Classification by activation mechanism is based on the

types of reactions that result the active form, as:

- hydrolysis (ester, amide, imide, ether etc.);
- oxidation;
- reduction;
- other reactions [2,28].

A recently proposed more systematic approach to prodrugs classification is on the basis of their two cellular sites of conversion: intracellular (e.g. antiviral nucleoside phosphorylated and statins) and extracellular in digestive fluids or the systemic circulation (e.g. valganciclovir, fosamprenavir, and antibody-, gene-, or virus-directed enzyme prodrugs).

Both major types can be further categorized into subtypes, based on factors such as (type I) whether the intracellular bioactivation location is also the site of therapeutic action, or (type II) whether or no bioactivation occurs in the gastrointestinal fluids or in the circulation system.

This new classification system of prodrugs can help in the understanding of a drug product's pharmacokinetics, safety and efficacy [30,31].

Optimization of bioavailability

The purpose of prodrug synthesis in most cases is increasing bioavailability. In drug development there are some important physicochemical properties as appropriate solubility, adequate lipophilicity, good permeability, which are strongly influenced by acid-base properties of the molecules [24,28].

Prodrugs with improved lipophilicity

In many medications a *carboxyl* functional group exists as indispensable function for their pharmacological activity. However, its presence causes too high polarity for oral administration, as in the small intestine at pH 5-7 it is largely ionized, which prevents the passage of molecules through membranes by passive diffusion.

Esterification of these groups with short or long aliphatic alcohol is the most widely used method [3-24,32-34].

ACE inhibitors are mostly ethyl ester prodrugs (enalapril, trandolapril, quinapril, benazepril). Ethyl esters considerably increase lipophilicity, thus increasing absorption (figure 2) [35].

Methyl ester occurs more rarely, because by hydrolysis toxic methyl alcohol is released. Therefore this method of design of prodrugs is used only in case of low dose medicines, respectively in the case of esters with very short du-

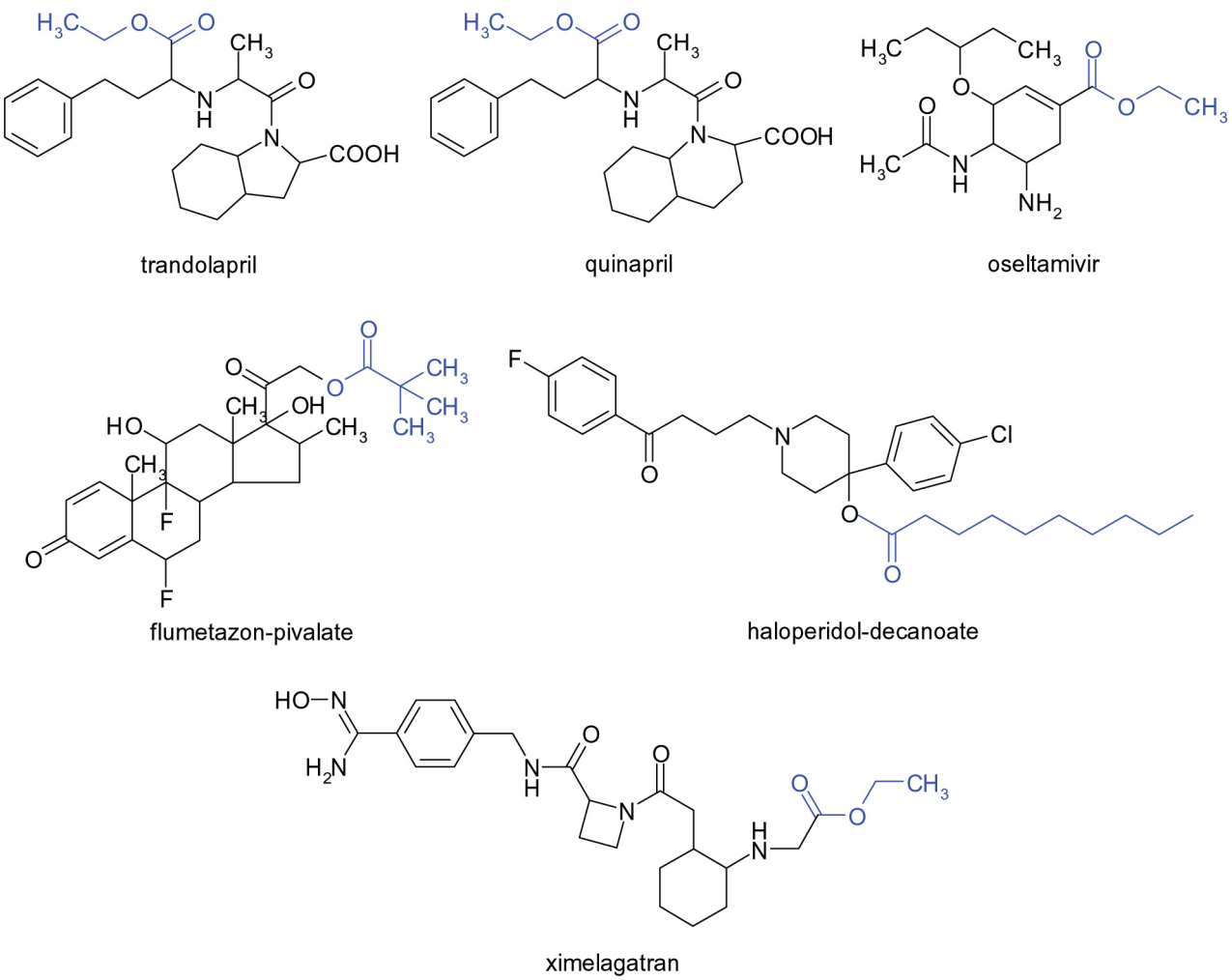

Fig. 2. Examples of ester prodrugs

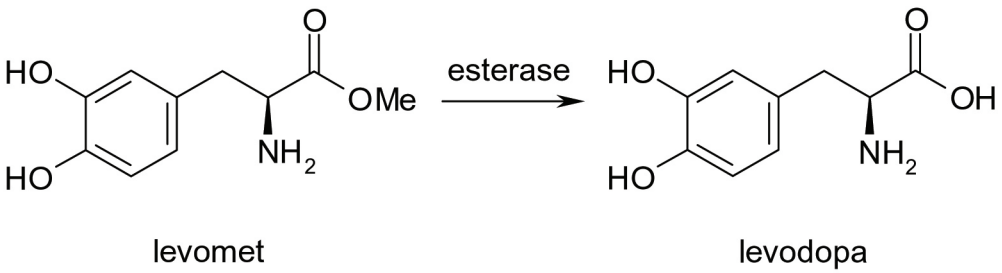

Fig. 3. Biotransformation of Levomet

ration of action. For example, several ester type prodrugs have been prepared for levodopa, but only methyl ester is found in therapy (Levomet) (figure 3).

There are compounds where the methyl ester is not a prodrug, the ester function being essential for pharmacological effect, while the free acid is without any therapeutic effect. Metabolic or chemical hydrolysis of methyl esters is in general very fast, for this reason methyl esters have short duration of action (e.g. cocaine or the beta-blocker esmolol with ultra-short effect) [11,24].

Ester type prodrugs can also be obtained from drugs with *alcoholic* or *phenolic hydroxyl* groups by esterification with short carboxylic acids (e.g. acetic acid, propionic acid, pivalic acid etc.) or long-chain acids (e.g. heptanoic acid, decanoic acid, palmitic acid etc.). Aromatic acids are less commonly used (e.g. benzoic acid) [23].

The examples below demonstrate that by development of prodrugs with increased lipophilicity, penetration through skin can be greatly improved; long lasting or even depot effect can be achieved [23].

In the series of local anti-inflammatory corticosteroids several prodrugs are used (clobetasol propionate, flumetasone pivalate, clobetasol butyrate), where the group C_{21}-OH or C_{17}-αOH is converted to ester. Terbutaline is a selective β_2-agonist bronchodilator, administered orally in high doses; while its prodrug form bambuterol (where dimethyl-carbamic acid ester of the phenolic hydroxyl group is formed) with enhanced lipophilicity and lower rate of hydrolysis under the action of cholinesterase enzyme, has a prolonged effect, therefore it is sufficient to use a dose of 20 mg once daily.

In the group of classical antipsychotics depot-acting preparations have been obtained by esterification with fatty acids. The prodrugs fluphenazine enanthate, haloperidol decanoate and zuclopenthixol decanoate oily solutions are administered once or twice monthly. Ultra lipophilic esters are deposited in fat stores from where are gradually released and converted into the active form, the effect lasting even 14-28 days; thus patient adherence is improved.

Optimization of lipophilicity can be also achieved by developing eters, acid amides, imines but the proportion of these prodrugs is very small compared to the esters [36].

Prodrugs with improved aqueous solubility

Since the 1990s once with the appearance of combinatorial chemistry and high throughput screening methods the number of drug candidates with visible poorly water solubility has increased. These compounds generally have high molecular weight, are lipophilic, their absorption being limited by inadequate solubility. It has been managed to solve this problem by using prodrug strategy [37].

Solubilization in water can be increased by the insertion of *polar structures*, thus enabling oral or parenteral administration.

The solubility increasing polar groups can be non-ionizable groups but which are easily degrading in the body. An often presented example is the non-steroidal anti-inflammatory sulindac, which is the sulfoxide derivative of the active form. The active metabolite is formed by reduction of a more polar, more soluble prodrug (figure 4) [28].

Another possibility is binding of the pharmacon to a *hydrophilic polymer* directly or by a linker; PEG,

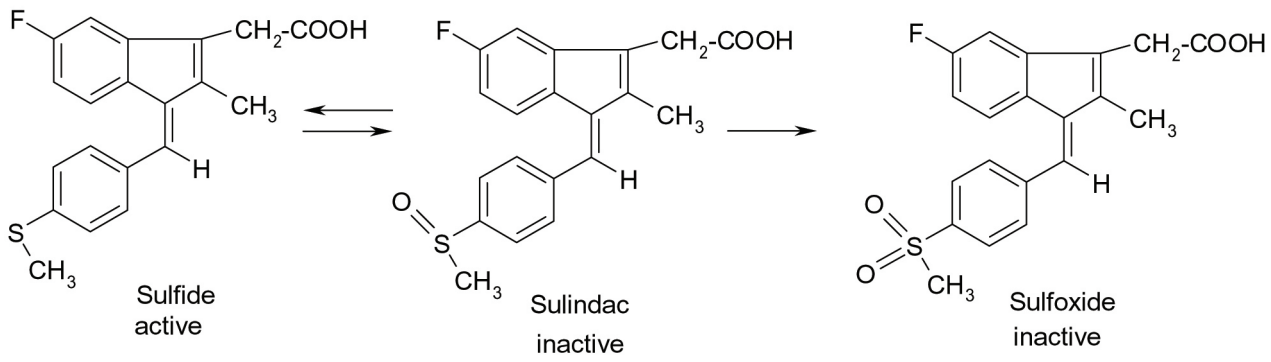

Fig. 4. Metabolism of Sulindac

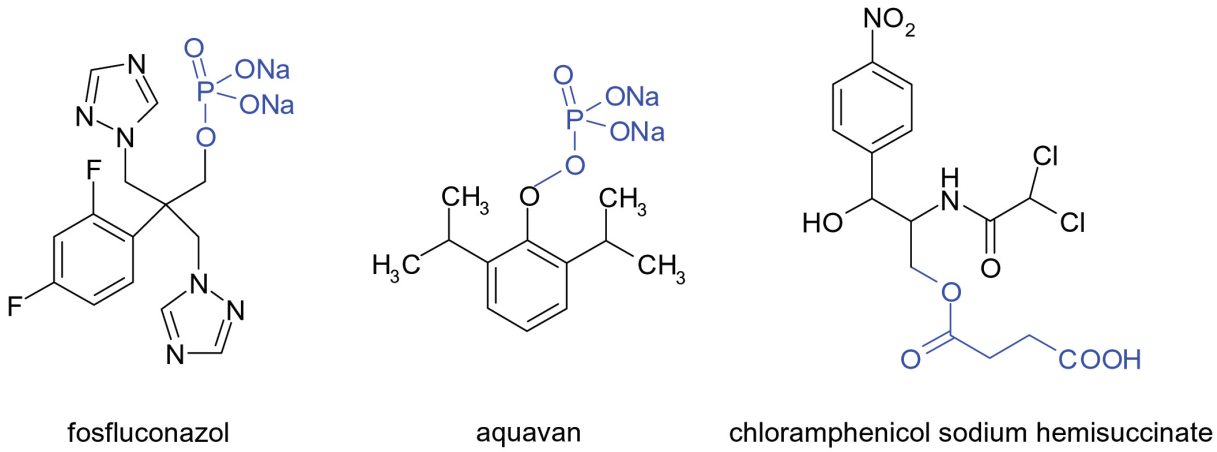

Fig. 5. Examples of promoieties for improved aqueous solubility

polyglutamate acid, dextran, chitosan are most commonly used [38].

Insertions of ionic groups such as *phosphate esters* have greater applicability. The prodrugs of phosphate esters are advantageous because, by forming salts, they have a good solubility, are rapidly dissolved in gastro-intestinal tract and are hydrolyzed in the presence of alkaline phosphatase enzyme present on the mucosal surface. An already activated form diffuses into the cell (e.g. fosfluconazol, fosphenytoin). Fospropofol (Aquavan) is the phosphoryl oxymethyl ether of propofol, it is slightly soluble in water, does not cause irritation at the injection site compared to propofol, the parent molecule used as an o/w emulsion injection (figure 5) [11,39,40].

Obtaining parenteral preparations of sparingly soluble active substances can be accomplished by esterification with *dicarboxylic acids*, this method being used for a long time. It is known that the sodium salt of chloramphenicol hydrogen succinate is one hundred times more soluble than chloramphenicol itself, so it is suitable for the preparation of parenteral solution.

It should be observed that parenteral prodrugs may experience problems concerning un-corresponding stability by forming precipitates; some prodrugs cannot be heat sterilized due to decomposition [11,23,24].

Targeted drug delivery

The efficacy criterion in certain therapy is site-specific drug delivery, when organ/tissue specific drugs are administered and enriched in the targeted organ. This is a great challenge for researchers in the pharmaceutical industry, prodrug synthesis playing an important role in this field. Based on research results we would like to emphasize two directions of utilization of prodrugs: tumor targeting and antigen targeting [11,41,42].

Prodrugs in cancer therapy

The effectiveness of cancer chemotherapy would increase if the active substance would reach the targeted tumor cell without damaging body cells. Therefore delivery to target sites with the help of prodrugs is a priority in drug research.

Tumor specificity may be achieved in many ways, such as by use of enzymes or transporters, or the development of prodrug-antibody which is selectively recognized by tumor cells. It is an advantage if the preparation can be administered also orally [43].

An example of success is capecitabine, a prodrug of 5-fluorouracil (5-FU), that requires a cascade of three enzymes for the bioconversion to the active drug. The first degradation takes place in the liver by carboxyl esterase, when pentyl alcohol, of lipophilic character, is eliminated. This is followed by deamination by cytidine deaminase enzyme present in both the liver and tumor cells, followed by selective release of 5-FU in the tumor cells under the action of thymidine phosphorylase, which shows much higher activity in tumor cells than in normal cells. The prodrug is absorbed rapidly and almost completely from the gastrointestinal tract and provides high concentration of 5-FU in targeted tumor cell. Capecitabine is used orally in metastatic colon cancer and in combination therapy in other types of cancer (figure 6) [43-45].

Prodrug-antibody conjugates

A new and promising direction in target drug delivery is coupling prodrugs with monoclonal antibodies (mAB) [41]. The conjugate formed in this way is selectively coupled to cancer cell, because the antibody is recognized only by a specific, typical antigen.

Gemtuzumab ozogamicin (Mylotarg) was the first targeted chemotherapeutic drug used to treat leukemia. It was withdrawn from the market in 2010 due to adverse reactions, but it is under further development [46-48].

Many other conjugates are under research in preclinical and clinical phases [49].

Prodrug activation in tumor cell can be achieved also with exogenous enzyme coupled to monoclonal antibody (ADEPT – Antibody Directed Enzyme Prodrug Therapy). The mechanism of action takes place in two steps. In the

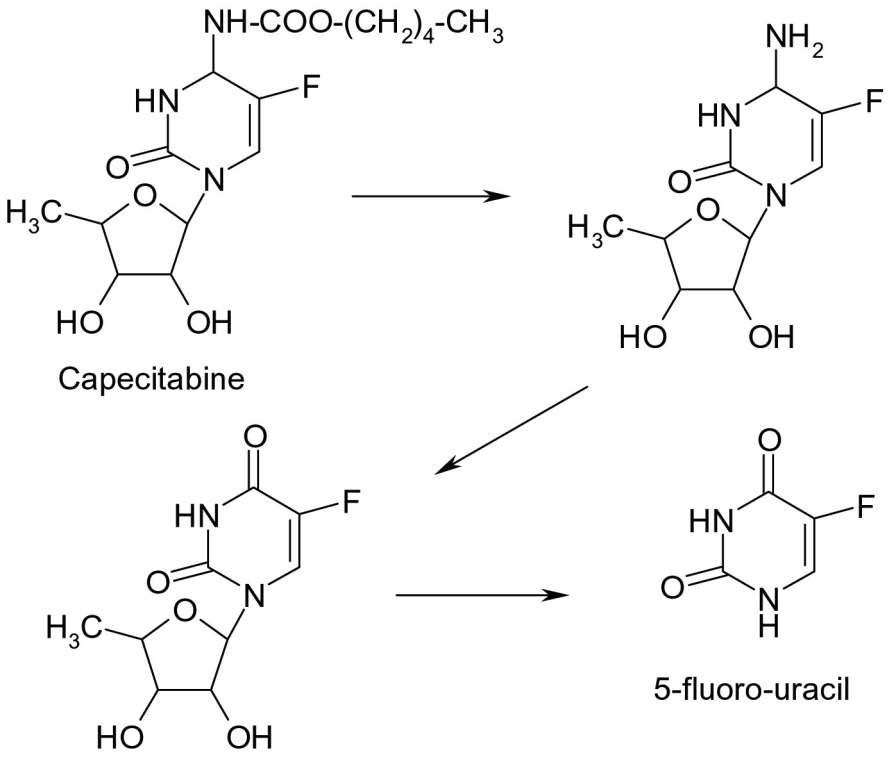

Fig. 6. Metabolic conversion of Capecitabine

first phase the exogenous enzyme is coupled to antibody and administered by infusion, leaving enough time for its localization and accumulation on the tumor cell. In the second phase the inactive prodrug, a selective substrate of the enzyme is administered, being activated by this to cytostatic drug [50-53].

Gene therapy may be basically defined as a technology aimed at modifying the genetic component of cells to achieve therapeutic benefits. In cancer gene therapy, for therapeutic gain, both malignant and nonmalignant cells can be targeted. The toxin gene therapy and enzyme-activating prodrug therapy are the two approaches targeting malignant cells. The toxin gene therapy works by transecting genes that express toxic molecules. The enzyme-activating prodrug therapy works by transferring genes able to express that can activate specific prodrugs selectively. GDEPT (Gene-Directed Prodrug Therapy) is a two-step for solid tumors. In the first step, the gene for a foreign enzyme (bacterial, viral, or yeast) is administered and targeted in a variety of ways to the tumor for expression. In the second step, a prodrug is administered that is activated to the cytotoxic drug selectively by the foreign enzyme expressed in the tumor [54].

Conclusions

Numerous prodrugs designed to overcome formulation, delivery, and toxicity barriers to drug utilization have been used in the latest years. Although the development of a prodrug can be very challenging, the prodrug approach represents a feasible way to improve the erratic properties of investigational drugs or drugs already on the market. Prodrug strategy is an effective method to improve bioavailability of medicines. By synthesis the prodrugs pharmacokinetic properties can be optimized and new compounds for oral or parenteral administration can be obtained.

The challenge in modern therapy is obtaining target drug delivery, especially in cancer therapy, where nowadays extensive research on the use of prodrugs is being conducted.

In prodrug development (even in early stages of development) full careful chemical and pharmacological characterization should be considered, as toxic active intermediaries may occur. A disadvantage in prodrug development is that it requires long and expensive syntheses.

The prodrug strategy is one of the most promising approaches to enhance the therapeutic efficacy and/or reduce the adverse effects of the pharmacologically active agents via different mechanisms, including increased solubility, stability, improved permeability and bioavailability, prolonged biological half-life time, and tissue-targeted delivery.

Despite the remarkable progress made in the field of prodrug design, more studies are clearly needed, especially at early stages of the drug discovery, for prodrugs to achieve the desired state of art and take their place in modern pharmacotherapy.

Conflicts of interest

The authors report no conflicts of interest.

References

1. Testa B, Mayer JB. Hydrolysis in Drug and Prodrug Metabolism: Chemistry, Biochemistry and Enzymology, Wiley-VCH, 2003.
2. Ettmayer P, Amidon GL, Clement B et al. Lessons learned from marketed and investigational prodrugs. J Med Chem. 2004;47(10):2393-2240.
3. Testa B. Prodrug research: futile or fertile. Biochem Pharmacol. 2004;68(11):2097-2106.
4. Rautio J, Kumpulainen H, Heimbach T, et al. Prodrugs: design and clinical applications. Nat Rev Drug Discov. 2008;7:255-270.
5. Rautio J, Laine K, Gynther M, et al. Prodrug approaches for CNS delivery. AAPS J. 2008;10:92-102.
6. Jilani JA, Idkaidek NM, Alzoubi KH. Synthesis, In Vitro and In Vivo Evaluation of the N-ethoxycarbonylmorpholine Ester of Diclofenac as a Prodrug. Pharmaceuticals (Basel). 2014;7(4):453-63.
7. Wohl AR, Michel AR, Kalscheuer S, et al. Silicat esters of paclitaxel and docetaxel: synthesis, hydrophobicity, hydrolytic stability, cytotoxicity, andprodrug potential. J Med Chem. 2014;57(6):2368-2379.
8. Liu KS, Hsieh PW, Aljuffali IA, et al. Impact of ester promoieties on transdermal delivery of ketorolac. J Pharm Sci. 2014;103(3):974-986.
9. Diez-Torrubia A, Cabrera S, de Castro S, et al. Novel water-soluble prodrugs of acyclovir cleavable by the dipeptidyl-peptidase IV (DPP IV/CD26) enzyme. Eur J Med Chem. 2013;70:456-468.
10. Lai L, Xu Z, Zhou J, et al. Molecular basis of prodrug activation by human valacyclovirase, an alpha-amino acid ester hydrolase. J Biol Chem. 2008;283(14):9318-9327.
11. Rautio J, Mannhold R, Kubinyi H, Folkers G. Prodrugs and Targeted Delivery: Towards Better ADME Properties, Volume 47, Wiley-VCH Verlag Gmbh & Co KGA, Weinheim, 2011.
12. Chiodo F, Marradi M, Calvo J, et al. Glycosystems in nanotechnology: Gold glyconanoparticles as carrier for anti-HIV prodrugs. Beilstein J Org Chem. 2014;10:1339-1346.
13. Vivekkumar K Redasani, Sanjay B. Bari. Prodrug Design: Perspectives, Approaches and Applications. Elsevier London, 2015.
14. Albert A. Chemical aspects of selective toxicity. Nature. 1958;182:421-422.
15. Wermuth CG, Ganellin CR, Lindberg P, et al. "Glossaryis of terms used in medicinal chemistry (IUPAC Recommendations 1998)". Pure and Applied Chemistry. 1998;70(5):1129.
16. N'Da DD. Prodrug strategies for enhancing the percutaneous absorption of drugs. Molecules. 2014;19(12):20780-20807.
17. Forde E, Devocelle M. Pro-moieties of antimicrobial peptide prodrugs. Molecules. 2015;20(1):1210-1227.
18. Wermuth CG, Aldous D, Raboisson P, et al. The practice of Medicinal Chemistry, fourth edition, Academic Press, London, 2015;657-692.
19. Vert M, Doi Y, Hellwich K, et al. Terminology for biorelated polymers and applications (IUPAC Recommendations 2012)". Pure and Applied Chemistry 2012;84(2):377-410.
20. Das N, Dhanawat M, Dash M. Codrug: An efficient approach for drug optimization. Eur J of Pharm Sci. 2010;41:571-588.
21. Leppänen J, Huuskonen J, Nevalainen T, et al. Design and synthesis of a novel L-dopa-entacapone codrug. Med Chem. 2002;45(6):1379-1382.
22. Bodor N, Buchwald P. Soft drug design: General principles and recent applications. Medicinal Research Reviews. 2000;20(1):58-101.
23. Takácsné Novák K. A prodrug stratégia a gyógyszerkutatásban: bevált módszerek és új irányok. Gyógyszerészet. 2013;57:451-459.
24. Keserű GyM. A gyógyszerkutatás kémiája. Akadémiai Kiadó, Budapest, 2011;539-564.
25. Zawilska JB, Wojcieszak J, Olejniczak AB. Prodrugs: a challenge for the drug development. Pharmacol Rep. 2013;65(1):1-14.
26. Stella VJ. Prodrugs: some thoughts and current issues. J Pharm Sci. 2010;99:4755-4765.
27. Stella VJ, Burchardt RT, Hageman MJ, et al. Prodrugs: Challenges and Rewards. Part 1. Springer, New York, 2007.
28. Stella VJ, Nti-Addae KW. Prodrug strategies to overcome poor water solubility. Adv Drug Deliv Rev. 2007;59(7):677-694.
29. Kokil GR, Rewatkar PV. Bioprecursor prodrugs: molecular modification of the active principle. Mini Rev Med Chem. 2010;10:1316-1330.
30. Wu KM. A new classification of prodrugs: regulatory perspective. Pharmaceuticals. 2009;2:77-81.
31. Wu KM, Farrelly J. Regulatory perspectives of type II prodrug development and time-dependent toxicity management: Nonclinical pharm/tox analysis and the role of comparative toxicology. Toxicology. 2007;236:1-6.
32. Balendiran GK, Rath N, Kotheimer A. Biomolecular chemistry of isopropyl fibrates. J Pharm Sci. 2012;101(4):1555-1569.
33. Lesniewska MA, Ostrowski T, Zeidler J, et al. Ester groups as carriers of antivirally active tricyclic analogue of acyclovir in prodrugs designing: synthesis, lipophilicity-comparative statistical study of the chromatographic and theoretical methods, validation of the HPLC method. Comb Chem High Throughput Screen. 2014;17(7):639-650.
34. Chanteux H, Rosa M, Delatour C, et al. In vitro hydrolysis and transesterification of CDP323, an $\alpha4\beta1/\alpha4\beta7$ integrin antagonist ester prodrug. Drug Metab Dispos. 2014;42(1):153-161.
35. Swaan PW, Stehouwer MC, Tukker JJ. Molecular mechanism for the relative binding affinity to the intestinal peptide carrier. Comparison of three ACE-inhibitors: enalapril, enalaprilat and lisinopril. Biochim Biophys Acta. 1995;1236(1):31-38.
36. Liu KS, Hsieh PW, Aljuffali IA, et al. Impact of ester promoieties on transdermal delivery of ketorolac. J Pharm Sci. 2014;103(3):974-986.
37. Karaman R. Computer-assisted design for atenolol prodrugs for the use in aqueous formulations. J Mol Model. 2012;18(4):1523-1540.
38. Wang H, Xie H, Wu J, et al. Structure-based rational design of prodrugs to enable their combination with polymeric nanoparticle delivery platforms for enhanced antitumor efficacy. Angew Chem Int Ed. 2014;53(43):11532-11537.
39. Lang BC, Yang J, Wang Y, et al. An improved design of water-soluble propofol prodrugs characterized by rapid onset of action. Anesth Analg. 2014;118(4):745-754.
40. Wozniak KM, Vornov JJ, Mistry BM. Gastrointestinal delivery of propofol from fospropofol: its bioavailability and activity in rodents and human volunteers. J Transl Med. 2015;13:170.
41. Sharma SK, Bagshawe KD. Antibody-directed enzyme prodrug therapy (ADEPT) for cancer. Springer 2010;393-405.
42. Tietze L, Krewer B. Antibody-directed enzyme prodrug therapy: a promising approach for a selective treatment of cancer based on prodrugs and monoclonal antibodies. Chem Biol Drug Des. 2009;74:205-211.
43. Mazzaferro S, Bouchemal K, Ponchel G. Oral delivery of anticancer drugs II: the prodrug strategy. Drug Discovery Today. 2013;18(1-2):93-98.
44. Tietze LF, Krewer B. Antibody-directed enzyme prodrug therapy: a promising approach for a selective treatment of cancer based on prodrugs and monoclonal antibodies. Chem Biol Drug Des. 2009;74:205-211.
45. Schmoll HJ, Twelves C, Sun W, et al. Effect of adjuvant capecitabine or fluorouracil, with or without oxaliplatin, on survival outcomes in stage III coloncancer and the effect of oxaliplatin on post-relapse survival: a pooled analysis of individual patient data from four randomised controlled trials. Lancet Oncol. 2014;15(13):1481-1492.
46. Loke J, Khan JN, Wilson JS, et al. Mylotarg has potent anti-leukaemic effect: a systematic review and meta-analysis of anti-CD33 antibody treatment in acute myeloid leukaemia. Ann Hematol. 2015;94(3):361-373.
47. Gamis AS, Alonzo TA, Meshinchi S, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children's Oncology Group trial AAML0531. J Clin Oncol. 2014;32(27):3021-3032.
48. Rowe JM, Löwenberg B. Gemtuzumab ozogamicin in acute myeloid leukemia: a remarkable saga about an active drug. Blood. 2013;121(24):4838-4841.
49. Singh Y, Palombo M, Sinko PJ. Recent Trends in Targeted Anticancer Prodrug and Conjugate Design. Curr Med Chem. 2008;15(18):1802-1826.
50. Aloysius H, Hu L. Targeted prodrug approaches for hormone refractory prostate cancer. Med Res Rev. 2015;35(3):554-585.
51. Schellmann N, Deckert PM, Bachran D, et al. Targeted enzyme prodrug therapies. Mini Rev Med Chem. 2010;10:887-904.
52. Osipovitch DC, Parker AS, Makokha CD, et al. Design and analysis of immune-evading enzymes for ADEPT therapy. Protein Eng Des Sel. 2012;25(10):613-623.
53. Zhou X, Wang H, Shi P, et al. Characterization of a fusion protein of RGD4C and the β-lactamase variant for antibody-directed enzyme prodrug therapy. Onco Targets Ther. 2014;7:535-541.
54. Zhang J, Kale V, Chen M. Gene-directed enzyme prodrug therapy. AAPS J. 2015;17(1):102-110.