**Definition:**
- The term prodrug was first introduced in 1958 by Adrien Albert (1907-1989, Australian Medicinal Chemist).
- According to his definition: therapeutic agents which are inactive per se but are transformed into one or more active metabolites.
- A few decades later, he apologized for having invented such an inaccurate term, because “prodrug” would have been a more descriptive term. However, by that time, the original version was used too widely to be changed.
- Approximately 20% of all small molecular drugs approved during the period 2000 to 2008 were prodrugs.
- In 2008 alone, 33% of all approved small-molecular-weight drugs were prodrugs.
- Major objectives of prodrug design is to improve **bioavailability** and **tissue selectivity**.

**Drug Disposition:** absorption, distribution, metabolism, elimination

**Drug transport:**
- **Passive diffusion**: most common
  - Diffusion rate is directly proportional to the gradient but also depends on the molecule's **lipid solubility, size, degree of ionization (pKa)**.
- **Permeability**: The ability of a drug to pass across biological membrane.

**Active transport:**
- Ions and some molecules (amino acids and some carbohydrates) move through cell membranes by active transport.
- **Endocytosis**
- **Facilitated diffusion**

---

**A. Absorption:** from administration to blood circulation

**Major site of first-pass elimination**
- Stomach
- Intestines
- Portal vein
- Liver
- Blood circulation

**at organ level**
- Oral
- Intraperitoneal, ip
- Intravenous, iv
- Intramuscular, im
- Subcutaneous, sc

**Bioavailability** is a subcategory of absorption and is the fraction (%) of an administered drug that reaches the systemic circulation.

**First-pass effect** is the fraction of drug lost during the process of absorption which is generally related to the liver and gut wall.

**Factors affecting oral bioavailability**

\[
F_{\text{max}} \quad \text{maximum achievable oral bioavailability}
\]

\[
F_a \quad \text{Fraction of the dose absorbed after oral administration}
\]

\[
F_g \quad \text{Fraction of the dose escapes intestinal metabolism}
\]

\[
F_h \quad \text{Fraction of the dose escapes hepatic metabolism}
\]

\[
E_h = 1 - F_h \quad \text{hepatic extraction ratio}
\]

\[
E_h = \frac{CL_h}{Q_h} \quad CL_h \quad \text{hepatic drug blood clearance}
\]

\[
Q_h \quad \text{hepatic blood flow}
\]

\[
F_{\text{max}} = F_a \cdot F_g \cdot (1 - CL/Q_h)
\]

\[
CL - CL \quad F_g \sim 1
\]

Fraction of drug excreted renally is small, and drug is equitably distributed between blood and plasma.

Hepatic metabolism is primary clearance mechanism.

- CL: total blood clearance
- CLH: hepatic clearance
- Qh: hepatic blood flow

\[
F_{\text{max}} = F_a \cdot (1 - CL/Q_h)
\]

Qh is a known constant, the rat liver blood flow is ~ 70 ml/min/kg. If absorption is complete (Fg ~ 1), a parent drug series has a rate blood clearance > 50 ml/min/kg, then its maximum oral bioavailability is limited to???
B. Distribution: The movement of a drug to and from the blood and various tissues of the body and the relative proportions of drug in the tissues.

- Drug distribution is related to vascular permeability, regional blood flow, cardiac output and perfusion rate of the tissue and the ability of the drug to bind tissue and plasma proteins
  - it is the unbound fraction which exhibits pharmacologic effects. It is also the fraction that may be metabolized and/or excreted
  - Protein binding can influence the drug’s biological half-life. The bound portion may act as a reservoir or depot from which the drug is slowly released as the unbound form.

C. Metabolism

- Phenytoin

D. Elimination

- Biliary Excretion: Once a substance has been excreted by the liver into the bile, and then into the intestinal tract, it can be eliminated from the body in the feces, or it may be reabsorbed.

- Renal Excretion

- Glomerular Filtration: filtration moves drugs from blood to urine, protein-bound drugs are not filtered
- Passive Reabsorption: Lipid-soluble drugs move back into the blood. Polar and ionized drugs remain in the urine.
- Active transport: Tubular "pumps" for organic acids and bases move drugs from blood to urine

The Early Times of Prodrug

The first intentionally designed prodrug is most probably methenamine (or hexamine), which was introduced 1899 by Schering. Methenamine releases 6 equiv. of the antibacterial formaldehyde along with 4 equiv. of ammonium ions in acidic urine and serves as a good example of a site-selective prodrug. It is typically used long-term to treat chronic urinary tract infections and to prevent the recurrence of infection.

Yet a lot of prodrugs were invented unintentionally

However, sometimes unintentionally developed prodrugs can reveal a less appealing truth of the drug under the development

Prodrug in 21st century: property-based drug design became an essential part of the drug discovery and development process

Classification of Prodrugs

Different activation methods

- Non-enzymatic pathway
- Endogenous enzymes: Oxid/reductases; Transferases; Hydrolases
- Non-human enzymes: ADEPT; VDEPT, GDEPT (antibody-, gene-, and virus-directed enzyme prodrug therapy)

Rationale for prodrug design

1. Improved Bioactivity
   1-1. Improved aqueous solubility
   1-2. Improved passive absorption
   1-3. Improved transporter-mediated absorption
   1-4. Protection against fast metabolism and slow-release prodrugs

2. Tissue-selective Delivery
   2-1. Passive enrichment in target tissue
   2-2. Targeting specific transporters
   2-3. Targeting tissue- or cell-specific enzymes
   2-4. Targeting surface antigens (not included in this GM)
   2-5. Enzyme-prodrug cancer therapy
Prodrug Strategies

1. Prodrugs for improved bioavailability
   Functional groups amenable to pro-drug design

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Prodrug</th>
<th>Parent drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhance lipophilicity</td>
<td>Ester</td>
<td>Carboxyl, hydroxy, thiol</td>
</tr>
<tr>
<td>Enhance lipophilicity (more stable)</td>
<td>Carbonates, Carbamates</td>
<td>Carboxyl, hydroxy, amine</td>
</tr>
<tr>
<td>Enhance lipophilicity</td>
<td>Oximes</td>
<td>Ketone, amidine, guanidine</td>
</tr>
<tr>
<td>Enhance aqueous solubility</td>
<td>Phosphate</td>
<td>Hydroxyl, amine</td>
</tr>
<tr>
<td>Enhance carrier-mediated absorption</td>
<td>Amides</td>
<td>Carboxyl, amine</td>
</tr>
</tbody>
</table>

1.1 Improved Aqueous Solubility

- Phenytoin
  - Trade name: dilantin

- Fosphenytoin
  - Trade name: cerebyx

- Phenylacetic acid
  - Vitamin B₁
  - FeCl₃
  - H⁺

- Fosphenytoin synthesis

- O₂, K₂CO₃
  - H₂O, rt

- PCl₃
  - DCM, rt

- AgOPO(OBn)₂

- Pd/C

- H₂, EIOAc

- then NaOH

- J. Pharm. Sci. 1984, 1068

- Water solubilities

<table>
<thead>
<tr>
<th>Prodrug</th>
<th>Solubility (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>25</td>
</tr>
<tr>
<td>2'-PEG₅₀₀₀</td>
<td>666</td>
</tr>
<tr>
<td>2'-PEG₁₀₀₀₀₀</td>
<td>200mg/ml</td>
</tr>
<tr>
<td>2'-PEG₂₀₀₀₀₀₀</td>
<td>125mg/ml</td>
</tr>
</tbody>
</table>

- Not only solubilizing taxol, but to afford controlled release of the drug over various periods of time

- Exclusively at 2' position

- PEG-paclitaxel


- in vivo activity against the murine leukemia cell line P388/O (Intraperitoneal injection)

- the molecular weight of PEG must be of certain magnitude so as to maintain a

- t₁/₂ (circulation) > t₁/₂ (hydrolysis)

Prodrug Strategies

Jieyu Gu

- non-steroidal anti-inflammatory agent
- Bioprecursor prodrug that is reversibly reduced to the active sulphone form after oral absorption
- ~100-fold increase in aqueous solubility
- irreversibly metabolised to sulindac sulphone which has been suggested to possess anti-proliferative effects against tumours

\[
\begin{align*}
\text{F} & \quad \text{Me} \\
\text{CHO} & \quad \text{EtCOONa} \\
\text{rt to 140 °C} & \\
\text{H}_2\text{Pd/C} & \quad \text{EtOH, r.t.} \\
\text{1) toluene, reflux} & \\
\text{2) KOH, EtOH/H}_2\text{O, reflux} & \\
\text{3) H}^+ & \\
\text{COOH} & \quad \text{MeS} \\
\text{NaIO}_3 & \quad \text{MeOH/H}_2\text{O/Acetone} \\
\text{US6025394, Feb 15 2000} & \\
\text{H}_2\text{N} & \quad \text{HN} \quad \text{S} \\
\text{O} & \quad \text{Me} \\
\text{H}_2\text{N} & \quad \text{HN} \quad \text{N} \\
\text{O} & \quad \text{Me} \\
\text{O} & \quad \text{TBU} \\
\text{ampicillin} & \quad \text{trade name: Principen} \\
\text{bioavailability: 32-55%} & \\
pivampicillin & \quad \text{trade name: Prondocillin/Miraxid} \\
\text{bioavailability: 87-94%} & \\
\text{penicillin amidase} & \\
\text{fermentation} & \\
\text{Pennicillin G} & \quad \text{Chemical synthesis:} \\
\text{Classics I, Chapter 3} & \\
\end{align*}
\]

J. Med. Chem. 2004, 4881

CN104250232, Dec 31 2014
Prodrug Strategies

Jieyu Gu

**Synthesis from academic labs**

- asymmetric allylic alkylation
- Trost's synthesis
  - *ACIE.* 2008, 3759
- asymmetric Diels-Alder
  - Corey's synthesis
  - *JACS.* 2006, 6310
- asymmetric conjugate addition
  - Fukuyama's synthesis
  - *ACIE.* 2009, 1070
- asymmetric ring opening
  - Hayashi's synthesis
  - *OL.* 2016, 3426
  - Ma's synthesis
  - *ACIE.* 2010, 4656
- asymmetric Diels-Alder
  - Shibasaki's synthesis
  - *JACS.* 2006, 6312
- asymmetric ring opening
- Xu's synthesis
  - *JACS.* 2018, 10619

**Gilead Process route**

- shikmic acid
  - RO-64-0802
  - oseltamivir
  - trade name: Tamiflu
- 3-pentanone, TsOH
- MeCl, Et$_3$N
- 80% yield

- 1) ETOH, SOCl$_2$
- 2) 3-pentanone, TsOH
- 3) MeCl, Et$_3$N
- 96% yield
- Na$_3$N, NH$_4$Cl, DMF then Ac$_2$O
- 44% yield
- 1) Ra-Ni, H$_2$
- 2) 85% H$_3$PO$_4$
- 71-75% yield


**Name Reaction**

- 97% yield
- 10:1
Prodrug Strategies

- for the treatment of chronic hepatitis B
- bioconversion by esterases and phosphodiesterases
- the oral bioavailability of ~10% for adefovir increased to 30-45% for adefovir dipivoxil

**Adefovir**

![Adefovir Structure]

**Adefovir Dipivoxil**

Trade name: Hepsera

**Melagatran**

Anticoagulant bioavailability: 3-7%

**Ximelagatran**

Trade name: Exanta (withdrawn from market) bioavailability: 20%

- Amidoximes can be used as bioprecursors for amidines
- Amidoximes are less basic and thus unprotonated under physiological conditions, thereby enhancing intestinal absorption.
- Reductases in the kidneys, liver, brain, lungs, and gastrointestinal tract are responsible for the rapid conversion of the inactive amidoximes to amidines

**1.3 Improved Transporter-mediated Absorption**

- Important: one has to be aware of potential drugdrug interactions due to saturation/inhibition of intestinal transporters

**Acyclovir**

Trade name: Zovirax

Oral bioavailability: 12-20%

**Valacyclovir**

Trade name: Valtrex

Oral bioavailability: 54%

- bioconversion by intestinal and hepatic esterases
- transported predominantly by intestinal dipeptide transporter hPEPT1


**WO2019231935, Dec 05, 2019**
1.4 Protection Against Fast Metabolism and Slow-release

Metabolically labile but important pharmacophoric elements can be masked or capped to avoid rapid metabolism.

- bioconversion by esterases
- transported by both MCT1 (monocarboxylate transporter type 1) and SMVT (sodium-dependent multivitamin transporter)
- oral bioavailability improved from 25% to 84% in monkeys

- a long-acting β adrenoceptor agonist (LABA) used in the treatment of asthma
- prolongs duration of drug action
Prodrug Strategies

For Bambuterol

\[
\text{HOCHO} + \text{Cl}_{2}N_{\text{Me}}\text{Me} \rightarrow \text{Et}_{3}\text{N} \rightarrow \text{EtOAc, rt to 60 °C}
\]

CN 110835306, Feb 25, 2020

For Ixazomib citrate

\[
\text{Cl} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{BH} \quad \text{O} \\
\text{Cl} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{BH} \quad \text{O} \\
\text{Cl} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{BH} \quad \text{O} \\
\text{Cl} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{BH} \quad \text{O}
\]


- treatment of multiple myeloma, a type of white blood cancer
- likely increased stability of oxidatively unstable boronic acid

Ixazomib citrate

trade name: Ninlaro

CN109942462A, Jun 28, 2019

For Clopidogrel

\[
\text{MeO} \quad \text{Cl} \\
\text{O} \quad \text{Cl} \\
\text{N} \quad \text{H} \quad \text{O} \quad \text{BH} \\
\text{MeO} \quad \text{Cl}
\]

clopidogrel

cytochromes P450 3A

a small portion

- highly unstable
- antiaggregating medicine
- irreversible binds to platelet ADP receptors via a covalent S-S bridge

CN106496259A, Mar 15, 2017
2. Prodrugs for improved tissue selectivity

2.1 Passive Enrichment in the Target Tissue

Brain targeting: After brain penetration, a lipophilic prodrug is converted there into a more hydrophilic molecule that remains locked in. The same conversion taking place in the rest of the body results in increased peripheral elimination.

- Selexipag: trade name: Uptravi
  - non-prostanoid prostacyclin receptor agonist for pulmonary hypertension
  - N-acetylsulfonamide prolonged duration of action and reduced side effects because of the reduction in peak-trough fluctuations

\[
\begin{align*}
\text{H}_2\text{N} & \xrightarrow{\text{H}_2, \text{PtO}_2, \text{EtOH}} \text{HN} \\
\text{H}_2\text{N} & \xrightarrow{\text{acetone}} \text{HN} \\
\text{Ph} & \xrightarrow{1) \text{BrCH}_2\text{CO}_2\text{tBu, aq KOH, benzene}} \text{Ph} \\
\text{Ph} & \xrightarrow{2) 1\text{N NaOH, MeOH:\text{Ph}}} \\
\text{Ph} & \xrightarrow{1) \text{CDI, THF}} \text{Ph} \\
\text{Ph} & \xrightarrow{2) \text{MeSO}_2\text{NH}_2, \text{DBU}}
\end{align*}
\]

- For synthesis see GM: Amphetamines and trace amines (Harwood, 2020)

Bioorg. Med. Chem. 2007, 6692

2.2 Targeting Tissue-Specific Transpoters

- substrate for the neutral amino acid transporters present at the blood-brain-barrier
- For synthesis see GM: Amphetamines and trace amines (Harwood, 2020)
- Levodopa
- DOPA decarboxylase
- Dopamine
- Can not across blood-brain-barrier

2.3 Targeting Cell/Tissue-Specific Enzymes

- Acyclovir triphosphate incorporated into viral DNA, viral DNA synthesis is inhibited
- HSV
- DNA polymerase
- Acyclovir triphosphate
- Acyclovir + ATP
- Thymidine kinase
- Acyclovir phosphate
- Human enzymes

2.4 Enzyme - Prodrug Cancer Therapy

- Selective activation of prodrugs in tumor tissue could also be done by exogenous enzymes in a two-step approach:
  1) Prodrug-activation enzyme gene or functional protein is delivered selective to tumor issues
  2) Administration of a nontoxic prodrug that is activated by the exogenous enzyme

- A prodrug approach should be explored when development of an innovative and very promising agent is precluded by a major pharmacokinetic or pharmaceutical defect. However, the prodrug approach should not be misunderstood as a universal solution to all barriers to a drug’s usefulness.

- For prodrug strategies to be successful, analysis of parent-drug properties and the proper identification of barriers are crucial.

- Different prodrug prototypes of high diversity (different attachment sites, linkers, promoieties, hydrolytic, oxidative, reductive activation, chemical vs enzymatic activation) should be considered. The feasibility of these prototypes should subsequently be evaluated with appropriate in vivo pharmacokinetic experiments.
well as one or more of the -O-P-O- atoms. Linkages of this type are well documented in the prior art and include without limitation the following: amides (-CH₂-CH₂-N(H)-C(O)) and -CH₂-O-
N=CH-; and alkylphosphorus (-C(J)₂-P(=O)(OJ)-C(J)₂-C(J)₂-). J is as described above.

Synthetic schemes for the synthesis of the substitute internucleoside linkages described above are disclosed in, for example, U.S. Patent Nos. 5,466,677; 5,034,506; 5,124,047;
5,278,302; 5,321,131; 5,519,126; 4,469,863; 5,455,233; 5,214,134; 5,470,967; 5,434,257.
Additional background information relating to internucleoside linkages can be found in, for example, WO 91/08213; WO 90/15065; WO 91/15500; WO 92/20822; WO 92/20823; WO
91/15500; WO 89/12060; EP 216860; PCT/US 92/04294; PCT/US 90/03138; PCT/US
91/06855; PCT/US 92/03385; PCT/US 91/03680; U.S. Application Nos. 07/990,848;
07/892,902; 07/806,710; 07/763,130; 07/690,786; Stichack et al., Nucleic Acid Res., 1989, 17,
Tet Lett 1991 22 7295 7299

Example 6: Preparation of Antiviral Compound for Nasal Administration.

An antisense agent per one of examples 1-3, or an antiviral nucleoside, nucleoside
mimetic or dimer according to one of examples 4 or 5 may be prepared according to one of the
methods known in the art. See, e.g. U.S. Patent Nos. 6,551,578, 6,554,497, 6,485,707, 6,468,507,
6,464,959, 6,294,153, 6,214,805, 6,087,343, 5,985,320, and 5,744,166. Administration is via
dropper, spray, and the like. Dosing is as per examples 1-5 above.