Prodrugs of Nucleoside and Nucleotide Analogs for the Treatment of HCV: An Overview of Concepts and Chemistry

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What is a Prodrug?

• A compound that undergoes biotransformation prior to eliciting its pharmacological effect
  – The administered therapeutic agent is inactive but is transformed into one or more active metabolites
  – Usually implemented to address a deficiency associated with the active agent
    • Chemical or biotransformation of a structural molecular functionality that produces the actual active agent
    • Attachment of a promoiety to a pharmacologically active agent such that the prodrug can overcome the barrier that hinders the optimal use of the active agent

The Prodrug Concept

Drug → Drug

Promoiety

Biological Barrier

Drug + Promoiety

Biotransformation

Drug → Drug

Promoiety

Non-toxic & rapidly cleared
Key Issues Addressed by Prodrugs

• **Bioavailability**
  – Aqueous solubility
  – Poor passive intestinal absorption - lipophilicity
  – Leveraging transporter-mediated intestinal absorption
    • Peptide transporters
    • Nucleoside transporters
    • Ion transporters
    • Bile acid transporters
    • Vitamin transporters
  – Fast metabolism

• **Tissue Selectivity ("magic bullet")**
  – Passive enrichment
  – Transporter targeting
  – Targeting tissue- or cell-specific enzymes
  – Targeting surface antigens
Marketed Prodrugs

Lovastatin

Oseltamivir

Fosinopril

Adefovir Dipivoxil

Valaciclovir
Guidances for Prodrug Development

- A comprehensive understanding of the ADME/PK of the prodrug and the parent drug is critical.
- Depending on the rate of conversion and the site of metabolism, prodrugs may alter the tissue distribution, efficacy, and toxicity of the parent drug.
- The pro moiety should ideally be safe and rapidly excreted from the body.
- The choice of pro moiety should be considered with respect to the disease state, dose, and duration of therapy.
Unique Characteristics of Nucleos(t)ides

- The triphosphate metabolite of a nucleoside is the pharmacologically active agent.
- A nucleoside must be a substrate for each of the kinases needed to produce the active triphosphate metabolite.
- The first phosphorylation event is the most substrate selective.
- The triphosphate must be a substrate for the viral polymerase and it must get incorporated into the growing RNA or DNA chain – eliciting a chain termination event.
Poor Drug-like Characteristics of Nucleos(t)ides

- Nucleosides:
  - Highly polar
  - Require multiple enzyme mediated steps for activation
  - Can be enzymatically degraded – nucleosidases & nucleobase metabolizing enzymes

- Nucleotides
  - Highly polar
  - Chemically unstable
  - Require enzyme mediated activation
  - Can be enzymatically degraded – nucleosidases, nucleobase metabolizing enzymes and phosphatases
Ideal Characteristics of a Nucleos(t)ide Prodrug for the Treatment of Hepatitis

- Must have sufficient chemical stability and physical properties to be formulated for oral administration
- Must be stable to conditions of the gastrointestinal tract such that the prodrug reaches the site of absorption intact
- Prodrug must have good absorption properties
- Prodrug must not undergo appreciable enzymatic degradation during the absorption phase
- Nucleotides: Once absorbed the prodrug must have sufficient stability in the blood in order to reach the target organ
- Nucleotides: The prodrug must be transported into hepatocytes and release the free 5’-phosphorylated nucleoside
- Achieving a high liver-plasma ratio (liver targeting) is desirable.
- The intact prodrug must be biologically inert
- The promoiety byproducts should be non-toxic and rapidly cleared.
Issues Addressed by Nucleoside Prodrugs

- Poor oral bioavailability
- Formation of inactive metabolites
Nucleoside Prodrugs: NM283 (Valopicitabine)

Issues
- Poor oral bioavailability in rat
- Formation of inactive uridine metabolite

Results
- Leveraged peptide transporter
- %F = 33% (rat)
- No detection of uridine metabolite
- Human studies
  - 800 mg BID, 14 days gave \(-1.2 \log_{10} \text{ IU/mL reduction in viral load}\)
  - 800 mg BID + SOC, 28 days ->4 \log_{10} \text{ IU/mL reduction in viral load}
  - terminated for GI tox

Nucleoside Prodrugs: RG7128 (Mericitabine)

Issues
• Modest oral bioavailability in humans (~25%)
• Formation of inactive uridine metabolite in significant amounts
• Liver to plasma ratio ~1

Results
• Human oral bioavailability increased to ~75%
• Reduction in formation of uridine metabolite
• No improvement in liver to plasma ratio
• Human studies
  • 1500 mg BID, 14 days, -2.7 log_{10} IU/mL reduction in viral load
  • 1000 mg BID + SOC, 28 days, -5 log_{10} IU/mL reduction in viral load

PSI-6130
(EC_{90} = 4.5 \mu M)

RG7128
Mericitabine

Nucleoside Prodrugs: R1626 (Balapiravir)

R1479
(EC$_{50}$ = 1.28 µM)

Issues
• Poor animal oral bioavailability
• Poor exposure in humans

Results
• %F >90% dog
• >5-fold increase in oral bioavailability in rat and monkey
• Human studies
  • 4500 mg BID, 14 days, -3.7 log$_{10}$ reduction in viral load
  • 1500 mg BID + SOC, 28 days, -5.2 log$_{10}$ reduction in viral load
    81% RVR
• Terminated do to hematological tox

Toniutto, P., et al., IDRUGS, 2008, 11, 738
Nucleoside Prodrugs: TMC647078

TMC647078
(EC<sub>50</sub> = 7.3 µM)

Issues
• Exposure in animals poor
• Formation of inactive uridine metabolite

Results
• 3’-Monoester 10-fold increase in animal exposure
• Diester 24-fold increase in animal exposure
• Liver to plasma ratio ~1

Issues Addressed by Nucleotide Prodrugs

- Highly polar – do not penetrate biological membranes
- Chemically unstable
- Enzymatically degraded
Kinase Bypass

Bypass the non-productive phosphorylation step
Phosphoramidates

\[
\begin{align*}
\text{Nucleoside} & \quad \text{Carboxyesterase} \quad \text{or} \quad \text{Cathepsin A} \quad \text{Nucleoside} \\
\text{spontaneous} & \quad \text{Nucleoside} \\
\text{HINT-1} & \quad \text{Nucleoside}
\end{align*}
\]
HepDirect

Designed for liver targeting
SATE
Cyclic Phosphates

\[
\begin{align*}
&\text{BASE} \quad \text{X} \quad \text{Y} \\
&\text{EtO} \quad \text{NH} \\
&\text{n-Pr} \quad \text{S} \\
&\text{CYP450} \quad \text{CYP3A4} \\
&\text{PDE} \\
&\text{HO} \quad \text{OH} \quad \text{HO}
\end{align*}
\]
Double Prodrugs

Removal of phosphate promoiety ADAL1

Improves overall lipophilicity thus improving cellular uptake

Nucleotide Phosphoramidates: GS-7977

Issues
- Nucleoside inactive in replicon
- TP potent inhibitor of NS5B
- Long TP half-life in hepatocytes
- Not a substrate for DCK
- MP readily converted to TP

Results
- Potent inhibitor of HCV replication
- High TP levels in hepatocytes \textit{in vitro}
- High TP levels in liver on oral admin.
- High liver to plasma ratio – leverages first pass metabolism for liver targeting
- Human studies
  - Safe and well tolerated to 24 wks
  - 400 mg QD, 14 days, \( \sim 5.0 \log_{10} \) IU/mL reduction in viral load
  - + RBV, GT2,3 100% SVR12
  - + Daclatasvir, GT1, 100% SVR12

Sofia, M.J., et al., \textit{J. Med. Chem.}, 2010, 53, 7202. • +Daclatasvir, GT1, 100% SVR12
Nucleotide Phosphoramidates: PSI-353661

**Issues**
- Weak replicon potency
- TP is potent inhibitor of NS5B, IC₅₀ = 5.9 µM
- Polar molecule
- Weak kinase substrate

**Results**
- Potent inhibitor of HCV replication
- Conversion to the G-MP derivative occurs readily in hepatocytes
- Produced high levels of G-TP in human hepatocytes
- Produced a favorable ~5:1 liver to plasma ratio *in vivo* - leverages first pass metabolism for liver targeting

Nucleotide Phosphoramidates: INX-8189

Issues
- Low levels of TP detected in cells
- G-TP potent inhibitor: IC$_{50}$ = 0.13 µM
- Natural guanosine derivative poor cellular uptake

Results
- > 100-fold improvement in replicon potency
- Conversion to the G-MP derivative occurs readily in hepatocytes
- Substantial levels of G-TP produced in cells.
- Human studies
  - Monotherapy: 25 mg QD,
    -1.3 log10 IU/mL decline in viral load

Vernachio, J.H., et al., ACC, 2011, 55, 1843
Nucleotide Phosphoramidates: IDX184

(EC$_{50} = 3.5$ µM)

Issues
• Low levels of TP detected in cells
• G-TP potent inhibitor: IC$_{50} = 0.13$ µM
• Natural guanosine derivative poor cellular uptake

Results
• P450 dependent and independent cleavage
• High liver TP levels observed in cynomolgus monkeys on PO dosing
• 10 mg/kg PO dose to HCV infected chimpanzees resulted in -2.3 log$_{10}$ IU/mL decline in viral load after 3 days
• Human studies
  • 25-100mg QD, 3days, max -0.74 log$_{10}$ IU/mL decline in viral load
  • 50 – 200 mg QD + SOC, 14 days, -2.7 to -4.1 log$_{10}$ decline in viral load

Zhou, X.J., et al., AAC, 2011, 55, 76
Cyclic Phosphates: PSI-352938

Issues
• Weak replicon potency
• TP is potent inhibitor of NS5B, $IC_{50} = 5.9 \ \mu M$
• Polar molecule
• Weak kinase substrate

Results
• High live G-TP levels in rat and dog after PO dosing
• Observe circulating levels of intact prodrug
• CYP3A4 mediated cleavage mech.
• Human studies
  • 100 – 300 mg QD, 7 days, $-4.0 - 4.6 \ log_{10} \ IU/mL$ decline in viral load
  • 300 mg, QD, +SOC, 14 days gave $-5.5 \ log_{10} \ IU/mL$ viral load decline
• Clinical hold – liver enzyme elevation

HepDirect:

(EC$_{50}$ = 1.23 µM)

Issues
• Poor oral bioavailability in rat
• Low levels of TP after i.p. dosing
• Formation of inactive uridine metabolite

Results
• 10-fold increase in TP levels on i.p. dosing
• HCV-infected chimpanzees
  • 10 mg/kg QD, -1.3 to -1.5 log$_{10}$ IU/mL decline in viral load vs
    -1.0 log$_{10}$ IU/mL decline with 16 mg/kg NM283

Carroll, S.S., et al., AAC, 2011, 55, 3854
Other Nucleotide Prodrugs

Developing a Prodrug: *In Vitro* and *In Vivo* Characterization

- Physico-chemical characterization – solubility, chemical stability, and lipophilicity
- Hepatic/microsomal/tissue homogenate/ intestinal fluid/ plasma stability
- Caco-2 and PAMPA permeability
- Absorption mechanism(s)
- Are the active drug or prodrug substrates for efflux pumps?
- Understand the disposition of both the active parent and prodrug compounds
Summary

The application of a prodrug strategy is a well established approach to improve the physicochemical, biopharmaceutical or pharmacokinetic properties of a pharmacologically potent compound increasing its developability.

In no therapeutic field has the application of prodrug technology seen as wide and impactful an application as has been seen in the development of nucleoside and nucleotide HCV therapies.
References