Platform Technology for Improving Ocular Drug Delivery

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Topical administration to retina: Cornea as a barrier

RPE as a barrier

- Blood retinal barrier (oBRB)
  - Formed by the retinal pigment epithelium (RPE) to limit transport of molecules from choroidal circulation into the retina
  - Tight junctions are expressed between the RPE cells
  - Evidence also suggests the expression of P-gp on the basolateral side of RPE, limiting transport of substances from blood into the eye

- Inner blood retinal barrier (iBRB)
Circumvention of efflux by prodrug derivatization
Transporter targeted drug delivery to retina: systemic administration

- D: Drug
- ILM: Internal limiting membrane
- P: Prodrug
- RPE: Retinal pigmented epithelium

Drug at the site of administration
Prodrug at the site of administration
Drug at the site of action
Prodrug at the site of action
Carrier protein
Barriers to drug transport
Technology

• Present invention provides di- and tri-peptide mono- and di-ester prodrugs which have sufficient physicochemical properties to be formulated into pharmacologically active compositions such as aqueous solutions, e.g., eye drops.

• These compounds can be effectively transported into the ocular tissues by evading the efflux by efflux proteins (e.g., P-gp, MRPs) and utilizing nutrient transporters (e.g., peptide, aminoacid and vitamin trasporters).

• These compounds can effectively reach the anterior segment and/or posterior segment following topical or systemic administrations.
ACYCLOVIR-PEPTIDE ANALOGS

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Assignee: The Curators of the University of Missouri, Columbia, MO (US)

Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 706 days.

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Related U.S. Application Data

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U.S. Cl. 814/424 1/2 514/2

Field of Classification Search 814/1 514/2

See application file for complete search history.

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ABSTRACT

Dipeptide and tripeptide ester derivatives of acyclovir and its analogs are disclosed which are useful to treat herpes virus infections. Also disclosed is a method for preparing a therapeutic agent for targeted delivery to eukaryotic comprising linking the therapeutic agent to one or more groups of the formula -X-Y-Z-R, wherein each X, Y and Z is independently Met, Val, Thr, Tyr, Trp, Ser, Ala or Gly; each R is independently H or an branching group, and each n is independently 0 or 1.

26 Claims, 18 Drawing Sheets
United States Patent

Mitra et al.

Title: Peptidyl Prodrugs That Resist P-Glycoprotein Mediated Drug Efflux

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Assignee: The Curators of the University of Missouri, Columbia, MO (US)

Abstract: This patent describes peptidyl prodrugs that resist P-glycoprotein mediated drug efflux. The prodrugs are intended to improve the absorption, bioavailability, and therapeutic index of drugs. They are designed to be converted to active forms within the body, thus increasing their effectiveness.

Claims:

9 Claims, 9 Drawing Sheets
Structures

Acyclovir

R-promoiety

Substitutions of R:

- Valine
- Valine-Valine
- Glycine-Glycine
- Glycine-Valine
- Tyrosine-Valine
- Valine-Tyrosine
Time course of corneal permeation of cumulative amount of ACV, L-Val-ACV, VVACV across rabbit cornea.
Transport Characteristics of Prodrugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>$P_{app} \times 10^6$ cm/sec (± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACV</td>
<td>4.24 (± 1.41)$^a$</td>
</tr>
<tr>
<td>VACV</td>
<td>12.1 (± 0.44)$^*$</td>
</tr>
<tr>
<td>VVACV</td>
<td>9.91 (± 2.40)$^*$</td>
</tr>
<tr>
<td>GVACV</td>
<td>12.4 (± 1.42)$^*$</td>
</tr>
<tr>
<td>YVACV</td>
<td>7.19 (± 1.38)$^*$</td>
</tr>
<tr>
<td>VYACV</td>
<td>8.34 (± 1.12)$^*$</td>
</tr>
</tbody>
</table>

$^a$ control

$^*$ p<0.05
Anterior chamber levels of ACV following systemic administration of ACV, VACV, and VVACV
Regeneration of ACV from Val-Val-ACV upon subsequent hydrolysis to VACV in isolated rabbit cornea

Possible mechanisms of enzymatic hydrolysis of di-peptide prodrug, Val-Val-ACV to Acyclovir in cornea. Val-Val-ACV prodrug is sequentially hydrolyzed via Val-ACV to yield the parent drug ACV. The hydrolysis is mainly enzymatic and not chemical as Val-Val-ACV is relatively more stable in IPBS (pH 7.4, $t_{1/2} \approx 108$ hrs.)
Val-Val-Acyclovir

Acyclovir Monophosphate

Cellular Kinases

ViralDNA Thymidine Kinase

Acyclovir Diphosphate

Esterases

ViralDNA Thymidine Kinase

Acyclovir Triphosphate

Esterases

Monophosphate

Cellular Kinases

Acyclovir

ViralDNA Thymidine Kinase

Acyclovir

Dipeptidase s

Val-Val-Acyclovir

No phosphorylation

Val-Acyclovir

Gets incorporated in viral DNA and causes chain termination

Inhibits Herpes virus DNA polymerase
Stereoisomeric prodrugs of ACV

- Systemic drug delivery (intravenous or oral) is potentially an effective route to treat various systemic as well as ocular disorders. However, drugs administered by this route must cross the intestine to reach the systemic circulation and subsequently the blood ocular barriers (BOB) to reach the inner ocular tissues.

- Limited therapeutic efficacy of ACV against herpes infections following oral administration is due to its poor permeability across oral mucosa. Several studies proved that oral bioavailability of ACV can be improved by prodrug derivatization.
Stereoisomeric prodrugs of ACV

- Amino acid and dipeptide prodrugs of ACV that have been evaluated before were found to be metabolized rapidly and completely to the parent drug during first pass following oral administration.

- However, regenerated ACV has to cross Blood Aqueous Barrier (BAB) to permeate into the anterior chamber ocular tissues. So there is a necessity to design prodrugs such that their hydrolytic rate can be modulated to generate higher amounts of intact prodrug in the systemic circulation resulting from improved oral bioavailability.

- Hence we synthesized stereoisomeric dipeptide prodrugs of ACV (L-val-L-val-; L-val-D-val-; D-val-L-val-; D-val-D-val-).
Stereoisomeric prodrugs of ACV

Apparent permeability of ACV Stereoisomeric prodrugs across Caco-2. Each value is represented as mean±S.D. (n = 4).

<table>
<thead>
<tr>
<th>Drug</th>
<th>K×10^3 (h⁻¹)</th>
<th>t½ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL-ACV</td>
<td>-</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>LD-ACV</td>
<td>688.23 ± 48.68</td>
<td>1.01±0.07</td>
</tr>
<tr>
<td>DL-ACV</td>
<td>110.06 ± 4.52</td>
<td>6.27±0.25</td>
</tr>
<tr>
<td>DD-ACV</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Stability of ACV prodrugs in rat intestinal homogenate—first-order degradation rate constants and half lives of all prodrugs.

“ND” Represents no degradation during the time of study. Symbol “-” represents the whole prodrug degraded in less than first sampling time point (5 min). Each value is represented as mean±S.D. (n = 3).
Corneal absorption of ACV prodrugs following oral administration

Corneal uptake of ACV following oral administration of LDACV and LACV in rats. (Each value represents mean ± SD).

Corneal concentrations of LDACV and ACV following oral administration of LDACV at a molar dose equivalent to 30 mg/kg of acyclovir in rats (■-ACV, ■-LDACV). Values are represented as mean ± SD, n = 3–4) [R-right eye, L-left eye].
Conclusions

- The dipeptide prodrugs of ACV were highly permeable across cornea as compared to ACV.
- Studies conducted in our laboratory have shown that these dipeptide ester prodrugs are less toxic (no cytotoxicity detected within the concentration range studied) as compared to TFT and ACV.
- The dipeptide prodrug Val-Val-ACV has also shown excellent in vivo activity against rabbit stromal keratitis, which is not adequately treated by current antiviral therapeutic regimens. Val-Val-ACV has been shown to be as efficacious as TFT at a much lower molar concentration in the rabbit stromal keratitis model.
- The stereoisomeric prodrug L-val-D-val-ACV exhibited good enzymatic stability and improved ocular absorption following oral administration.
- This technology can be utilized to improve ocular bioavailability of various drugs following both topical and oral administration in order to treat several ocular infections and disease conditions.
Thank you

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Questions