Therapeutic peptides

Synthetic therapeutic peptides: science and market

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Challenges for Therapeutic Peptides Part 1: On the Inside, Looking Out

FOREWORD

SPECIAL FOCUS: THERAPEUTIC PEPTIDES

Therapeutic peptides
Outline:

- What is peptide?
- What is therapeutic peptide?
- Why they are important?
- How they are used in treatment of cancer?
- What are the important medicinal chemistry considerations for their design and preparation?
- Examples of therapeutic peptides
- Future directions of the field
Peptides are short chains of amino acid monomers linked by peptide (amide) bonds, the covalent chemical bonds formed when the carboxyl group of one amino acid reacts with the amino group of another. Peptides are distinguished from proteins on the basis of size, and as a benchmark can be understood to contain approximately 50 amino acids or less.
History of therapeutic peptides

- Oxytocin (1953)
  https://www.youtube.com/watch?feature=player_detailpage&v=_GgGDLGCI4A

- Insulin (1982)
  https://www.youtube.com/watch?feature=player_detailpage&v=CuQMpN7rM-4

- 65 peptide based drugs in the past 30 years

- Market – 11.5 billion euros!
Advantages of peptide therapeutics

- Natural compounds (like insulin)
- Fast clearance and low toxicity – degradation products are amino acids.
- Small size and good tissue penetration (unlike antibodies), therefore decreasing the amount needed to reach their targets.
- Compared to small molecules, peptides tend to have higher specificity and affinity for their targets.
What are the important requirements and challenges?

- Rapidly eliminated in the body, unless chemical modifications are made
- Expensive
- Labile during storage at ambient temperatures
- Not normally orally available, requiring injection by needle and being associated with self-administration compliance issues
Table 1: Approved peptide drugs products since 2000

Second approvals of drug substances (for example Exenatide as Byetta in Europe in 2011) are not included.

<table>
<thead>
<tr>
<th>Approval year</th>
<th>Location</th>
<th>Generic name</th>
<th>Brand name</th>
<th>Company</th>
<th>Indication</th>
<th>Route</th>
<th>Dose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>EU</td>
<td>Atosiban</td>
<td>Tractocile</td>
<td>Ferring</td>
<td>Premature labour</td>
<td>IV</td>
<td>&lt;330mg</td>
</tr>
<tr>
<td></td>
<td>US</td>
<td>Bivalirudin</td>
<td>Angiomax</td>
<td>Medicines Company</td>
<td>Unstable angina</td>
<td>IV</td>
<td>250mg</td>
</tr>
<tr>
<td></td>
<td>NZ</td>
<td>VIP</td>
<td>Aripitadil</td>
<td>Senatek</td>
<td>Erectile dysfunction</td>
<td>AI**</td>
<td>25μg</td>
</tr>
<tr>
<td>2001</td>
<td>US</td>
<td>Nesiritide</td>
<td>Natrecor</td>
<td>Scios</td>
<td>Congestive heart failure</td>
<td>IV</td>
<td>1.5mg</td>
</tr>
<tr>
<td></td>
<td>US</td>
<td>Triptorelin</td>
<td>Telstar</td>
<td>Debiopharm</td>
<td>Hormone-responsive cancer</td>
<td>IM</td>
<td>3.75mg</td>
</tr>
<tr>
<td>2002</td>
<td>US</td>
<td>Teriparatide</td>
<td>Forteo</td>
<td>Lilly</td>
<td>Osteoporosis</td>
<td>SC</td>
<td>20μg</td>
</tr>
<tr>
<td>2003</td>
<td>US</td>
<td>Abarelix</td>
<td>Plenaxis</td>
<td>Praecis</td>
<td>Prostate cancer</td>
<td>IM</td>
<td>113mg</td>
</tr>
<tr>
<td></td>
<td>US</td>
<td>Enfuvirtide</td>
<td>Fuzeon</td>
<td>Roche</td>
<td>HIV-1</td>
<td>SC</td>
<td>90mg</td>
</tr>
<tr>
<td>2004</td>
<td>US</td>
<td>Ziconotide</td>
<td>Prialt</td>
<td>Elan</td>
<td>Severe and chronic pain</td>
<td>IT</td>
<td>100μg</td>
</tr>
<tr>
<td>2005</td>
<td>US</td>
<td>Exenatide</td>
<td>Byetta</td>
<td>Amylin</td>
<td>Diabetes, Type 2</td>
<td>SC</td>
<td>10μg</td>
</tr>
<tr>
<td></td>
<td>US</td>
<td>Pramlintide</td>
<td>Symlin</td>
<td>Amylin</td>
<td>Diabetes, Type 1 and Type 2</td>
<td>SC</td>
<td>15μg</td>
</tr>
<tr>
<td>2007</td>
<td>US</td>
<td>Lanreotide</td>
<td>Somatuline LA</td>
<td>Ipsen</td>
<td>Agromegaly</td>
<td>IM</td>
<td>30mg</td>
</tr>
<tr>
<td>2008</td>
<td>US</td>
<td>Degarelix</td>
<td>Firmagon</td>
<td>Ferring</td>
<td>Prostate cancer</td>
<td>SC</td>
<td>120mg</td>
</tr>
<tr>
<td></td>
<td>EU</td>
<td>Ionabant</td>
<td>Firzyr</td>
<td>Jeneris</td>
<td>Hereditary angioedema</td>
<td>ITD</td>
<td>&gt;1mg</td>
</tr>
<tr>
<td>2009</td>
<td>EU</td>
<td>Linagliptide</td>
<td>Victoza</td>
<td>Novo Nordisk</td>
<td>Diabetes, Type 2</td>
<td>SC</td>
<td>1.2mg</td>
</tr>
<tr>
<td>2010</td>
<td>US</td>
<td>Tesamorelin</td>
<td>Egrifta</td>
<td>Theratechnologies</td>
<td>Lipodystrophy in HIV</td>
<td>SC</td>
<td>2mg</td>
</tr>
<tr>
<td>2012</td>
<td>US</td>
<td>Sinapotide</td>
<td>Lucinactant</td>
<td>Discovery</td>
<td>RDS in premature infants</td>
<td>ITD</td>
<td>&gt;1mg</td>
</tr>
<tr>
<td></td>
<td>EU</td>
<td>Pasireotide</td>
<td>Signifor</td>
<td>Novartis</td>
<td>Cushing's disease</td>
<td>SC</td>
<td>600μg</td>
</tr>
<tr>
<td></td>
<td>US</td>
<td>Peginesatide</td>
<td>Omontys</td>
<td>Affymax</td>
<td>Anaemia in CKD with dialysis</td>
<td>SC</td>
<td>~20μg</td>
</tr>
<tr>
<td></td>
<td>US</td>
<td>Carfilzomib</td>
<td>Kyprolis</td>
<td>Onyx</td>
<td>Refractory multiple myeloma</td>
<td>IV</td>
<td>60mg</td>
</tr>
<tr>
<td>2012 (pending at time of press)</td>
<td>EU</td>
<td>Afamelanotide***</td>
<td>Scenessse</td>
<td>Cliniuvell</td>
<td>Erythropoietic protoporphyia</td>
<td>SC</td>
<td>16mg</td>
</tr>
<tr>
<td></td>
<td>US</td>
<td>Linacotide</td>
<td>Linzees</td>
<td>Ironwood</td>
<td>Constipation in IBS</td>
<td>PO</td>
<td>266μg</td>
</tr>
<tr>
<td></td>
<td>EU</td>
<td>Lixisenatide</td>
<td>Lixumia</td>
<td>Sanofi-aventis</td>
<td>Diabetes, Type 2</td>
<td>SC</td>
<td>10μg</td>
</tr>
<tr>
<td></td>
<td>US</td>
<td>Teduglutide</td>
<td>Cattex</td>
<td>NPS</td>
<td>Adult short bowel syndrome</td>
<td>SC</td>
<td>~5mg</td>
</tr>
</tbody>
</table>

* Where possible minimum single dose (not necessarily daily dose) is listed – some doses are weight-dependent
** Autoinjector
*** Pre-approved in Italy in 2010
Distribution of existing drugs

Figure 1. Distribution of the drugs approved by the US FDA by chemical species.
Peptides approved by the US FDA during the period 2009–2011.

In ecallantide, colors show the pairing of Cys; in Brentuximab vedotin, in black, the peptide monomethylauristatin E, in red, the Val-Cit dipeptide, which is liberated by Cathepsin B, and a self-immolative p-aminobenzyloxycarbonyl spacer; in blue, the monoclonal antibody modified. mAb: Monoclonal antibody.
What are the important requirements and challenges?

• Rapidly eliminated in the body, unless chemical modifications are made

• Expensive

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## Most important human proteolytic enzymes involved in peptide degradation

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Metalloproteases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metalloproteases</strong></td>
<td>Nepriylisin, or enkephalinase, or neutral endopeptidase⁹ Thimet oligopeptidase, or endo-oligopeptidase A, or endopeptidase 24.15, or pz-peptidase⁹</td>
</tr>
<tr>
<td><strong>Exopeptidases</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Aminopeptidases</strong></td>
<td>Leucyl-aminopeptidase Aminopeptidase M or N, or alanyl-aminopeptidase, or membrane alanine aminopeptidase⁹</td>
</tr>
<tr>
<td><strong>Dipeptidyl-peptidases and tripeptidyl-peptidases</strong></td>
<td>Dipeptidyl-peptidase I, or cathepsin C or J Dipeptidyl-peptidase IV⁹</td>
</tr>
<tr>
<td><strong>Cysteine proteases</strong></td>
<td>Prolyl tripeptyl-peptidase</td>
</tr>
<tr>
<td><strong>Peptidyl-dipeptidases</strong></td>
<td>Peptidyl-dipeptidase A, or angiotensin-converting enzyme⁹</td>
</tr>
<tr>
<td><strong>Aspartic acid proteases</strong></td>
<td>Metallo-carboxypeptidases</td>
</tr>
<tr>
<td><strong>Pepsin</strong></td>
<td>Carboxypeptidase A Carboxypeptidase B, or protaminase Carboxypeptidase N, or lysine(arginine) carboxypeptidase, or kininase I⁹</td>
</tr>
<tr>
<td><strong>Cathepsin B</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Cysteine proteases</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Cathepsin D</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Serine proteases</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Endopeptidases</strong></td>
<td></td>
</tr>
<tr>
<td><strong>α-Chymotrypsin</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Trypsin</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Thrombin</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Plasmin</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Prolyl oligopeptidase, or prolyl endopeptidase⁹</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma kallikrein</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Pancreatic elastase</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Leukocyte elastase, or neutrophil elastase, or lysosomal elastase</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Cysteine proteases</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Cathepsin B</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Clostripain, or endoproteinase Arg-C</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Calpain-1, or μ-calpain</strong></td>
<td></td>
</tr>
</tbody>
</table>
**Lead peptide chemical optimization**

- Search for the minimum active sequence from N- and/or C-terminal truncated analogues.

- Determine significance of the N- and C-terminus.

- Deletion of one or more consecutive amino acid and combinatorial deletion with two or more positions omitted independently throughout the sequence.
Lead peptide chemical optimization

• Simplification and optimization of the structure after alanine scanning (Ala-scan) and D-scanning (D-scan) to eliminate potential sites of cleavage and to determine important functional groups involved in the interaction with the target of interest.
Lead peptide chemical optimization

- Cyclization of the peptide sequence to
  - decrease the conformational flexibility of linear peptides,
  - reduce hydrogen bonding, to enhance membrane permeability
  - to increase their stability to proteolysis by endo- and exopeptidases.
**Lead peptide chemical optimization**

- **Substitution of a natural amino acid** residue by an unnatural amino acid (*D*-configuration), an N-methyl-α-amino acid to increase plasma stability of the peptide and affinity for its target.

- **Amide bond replacement between two amino acids:**
  
  - NH-amide alkylation

  - the carbonyl function of the peptide bond can be replaced by $\text{CH}_2$, C( S) (endothiopeptide, $\text{--C(S)\text{--NH--}}$) or PO$_2$H (phosphonamide, $\text{--P( O)OH\text{--NH--}}$).

  - NH-amide bond can be exchanged by O (depsipeptide, $\text{--CO\text{--O--}}$), S (thioester, $\text{--CO\text{--S--}}$) or $\text{CH}_2$ (ketomethylene, $\text{--CO\text{--CH}_2\text{--}}$).
Lead peptide chemical optimization

- **Blocking N- or C-terminal ends** by N-acylation, N-pyroglutamate, C-amidation and so on, or addition of carbohydrate chains (glycosylation: glucose, xylose, hexose and so on) to increase plasma stability.

- **N-terminal esterification** (phosphoester) or pegylation modifications to enhance plasma stability and to reduce immunogenicity.

- **Pegylation** is also designed to make the peptide larger (generally >50 kDa) to retard excretion through the kidneys (renal clearance).
**Stapled Peptides**

- **Stapled peptides** hydrocarbon-stapled α-helical peptides, that combine the broad target recognition capabilities of protein therapeutics with the robust cell-penetrating ability of small molecules.

- This novel class of peptides is locked into their bioactive α-helical fold through the site-specific introduction of a chemical brace, an all-hydrocarbon staple.

  - [https://www.youtube.com/watch?feature=player_detailpage&v=WScPbvUwDno](https://www.youtube.com/watch?feature=player_detailpage&v=WScPbvUwDno)

- Stapling can greatly improve the pharmacologic performance of peptides, increasing their target affinity, proteolytic resistance, and serum half-life while conferring on them high levels of cell penetration through endocytic vesicle trafficking.
How do they work

- https://www.youtube.com/watch?feature=player_detailpage&v=vXnW7MGcZjk
- https://www.youtube.com/watch?feature=player_detailpage&v=0xz4bIJkN90
Antimicrobial peptides

• Antimicrobial peptides are an evolutionarily conserved component of the innate immune response and are found among all classes of life.

• Fundamental differences exist between prokaryotic and eukaryotic cells that may represent targets for antimicrobial peptides.

• These peptides are potent, broad spectrum antibiotics which demonstrate potential as novel therapeutic agents.

• They have been demonstrated to kill *Gram negative* and *Gram positive bacteria*, Mycobacterium tuberculosis, enveloped viruses, fungi and even transformed or cancerous cells.
Modes of action of antimicrobial peptides

The modes of action includes

- disrupting membranes,
- interfering with metabolism,
- targeting cytoplasmic components

- The initial contact between the peptide and the target organism is electrostatic, because most bacterial surfaces are anionic and peptides are cationic. They insert into membrane bilayers to form pores by ‘barrel-stave’, ‘carpet’ or ‘toroidal-pore’ mechanisms. Alternately, they may penetrate into the cell to bind intracellular molecules which are crucial to cell living.

[https://www.youtube.com/watch?feature=player_detailpage&v=9-HegAO4T0A](https://www.youtube.com/watch?feature=player_detailpage&v=9-HegAO4T0A)

Copyright: wikipedia.com
Real Time Quantification of Cellular Uptake in Live Cells Using Bioluminescent Imaging
Measurement of Cellular Uptake

1) PAMPA (Parallel Artificial Membrane Permeability Assay)
2) Caco-2 Cells
3) Modifying the molecule with Fluorescein, Biotin

Predicts transcellular permeability of a drug

DC: Donor Compartment
AM: Artificial Membrane
AC: Acceptor Compartment

http://www.pharmacelsus.de/pdf/8_PAMPA.pdf
Measurement of Cellular Uptake
Probe Design

R₈C-c

R₈C
Proof of Concept in Cell-Free Assays

C = L-Cys

c = D-Cys

GSH = Glutathione
Cells were incubated with the test compound (2h, 1h, 30 min, 0 min), CBT added and BL was measured.
Future directions of the field

• Delivery of peptides therapeutics still suffers because they have to be injected. However, erythropoietin and insulin are blockbusters even though they cannot be taken orally.

• The majority of marketed peptide are peptide hormones or peptide derivatives that simulate the action of hormones.

• Peptides that are agonists or antagonists for receptors implicated in oncology and inflammation, peptides as antibiotics, or peptides that act as enzyme.

• Other therapeutic peptides, such as antimicrobial peptides, with broad-spectrum antimicrobial activity against bacteria, viruses and fungi, are also promised a great future.

• Hundreds of synthetic therapeutic peptides are in clinical development.
Outline

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- How they are used in treatment of cancer?
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Exam: Examples of questions

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