The role of Binding Kinetics in Drug Action

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Mastering Medicinal Chemistry
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www.irnd3.org
Take home message

• Binding kinetics influence
  – Molecular mechanism of action (MMOA)
    • efficacy
    • safety
    • therapeutic index.
Part 1. How do medicines work?

Part 2. The impact of MMOA on PK/PD relationships.

Part 3. Practical application at iRND3.
Drug action begins with binding
‘Corpora non agunte nisi fixata’
A substance will not work unless it is bound
-Paul Ehrlich, 1913
Molecular Mechanism of Action (MMOA)

- MMOA: mechanism through which specific molecular interactions between the drug and its target result in an effective and safe pharmacological response.
  - Includes binding kinetic and conformational changes that specifically provide a therapeutically useful response.

MMOA-pharmacological hot spot
Aspirin and Ibuprofen: Two Medicines, One Target, Different Molecular Mechanisms, Different Uses

• Aspirin has anti-platelet activity whereas NSAIDs do not
  - Effective for prevention of atherothrombotic disease

• Both bind to the active site of cyclooxygenase 1 and 2
  - Aspirin irreversible inactivation via acetylation of Ser530
  - Ibuprofen and other NSAIDs are reversible

• Irreversible action of aspirin in platelets leads to long lasting anti-thrombotic effects
  - Platelets do not have the capacity to resynthesize new protein

Substrate - arachidonic acid

Aspirin

Ibuprofen
• Communication of information as an analogy of MMOA

  – Proximity is rarely sufficient for effective sharing of specific information
  – MMOA is a language to communicate specific information.

  ‘Pharmacological hot spots’
Why is molecular mechanism important?

- Physiology
  - Uses molecular mechanism to provide selective and safe responses
  - spatial and kinetic control of physiologic responses

- Drug Discovery
  - flood the system with drug.
  - maintain drug concentrations above IC$_{50}$ per dosing interval.

Approved molecular medicines have evolved with specific, selective MMOAs
The optimal MMOA depends on the potential for mechanism-based toxicity

**No Mechanism based toxicity**
- hit target as hard as possible
- results in lower concentrations of drug
- increase therapeutic index
  - full agonist
  - irreversible inhibitors
  - noncompetitive inhibitors
  - insurmountable antagonists
  - slow dissociation
  - PAMs (positive allosteric modulators)

**Mechanism-based toxicity**
- Molecular mechanism can provide opportunity to differentiate efficacy from mechanism-based toxicity.
  - partial agonists
  - rapidly reversible inhibitors
  - uncompetitive
  - functionally selective receptor modulators
  - use dependent channel blockers

Swinney, NRDD, 3, 801, 2004
Swinney ARMC, 46, 301, 2011
Many drug class evolved to have optimal MMOA

No mechanism based toxicity evolve to slow off rates

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Fast-off, surmountable</th>
<th>Slow-off insurmountable</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARBs</td>
<td>losartan</td>
<td>Candesartan/valisartan</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>Diphenhydramine</td>
<td>desloratadine</td>
</tr>
<tr>
<td>Antimuscarinics</td>
<td>Ipratropium</td>
<td>tiotropium</td>
</tr>
</tbody>
</table>

With mechanism based toxicity evolve to fast off rates

<table>
<thead>
<tr>
<th>Cyclooxygenase inhibitors</th>
<th>kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>aspirin</td>
<td>irreversible</td>
</tr>
<tr>
<td>indomethacin</td>
<td>Slow reversible</td>
</tr>
<tr>
<td>ibuprofen</td>
<td>Fast reversible</td>
</tr>
<tr>
<td>COX2 selective</td>
<td>COX2 irreversible</td>
</tr>
<tr>
<td>COX2 selective</td>
<td>COX2 reversible??</td>
</tr>
</tbody>
</table>
Two types of kinetic responses

Responses:

1) equilibrium

2) non-equilibrium
Equilibrium vs non-equilibrium in drug action

Kinetic vs thermodynamic control in chemistry

Diels alder

\[ \text{thermodynamic product} \quad \leftrightarrow \quad \text{Kinetic product} \]

Electrophilic addition

\[ \text{Kinetic product} \quad \text{thermodynamic product} \]

\[ \text{HBr} \quad 71\% \quad 15\% \quad 29\% \quad 85\% \]

T = 0 °C
T = 40 °C
Outcome controlled by competing rates and relationship to equilibrium

• Definition of ‘fast’ and ‘slow’ with regard to binding kinetics is only relative to a competing rate
  – A turtle could be fast in comparison to a snail

  ![turtle](turtle.png) ![snail](snail.png)

• For example: a 10-min dissociation half-life is...
  – ...very fast when the competing rate is elimination of medicine from the body with a half-life measured in hours
  – ...very slow when the competing rate is opening and closing of a channel with a half-life measured in milliseconds
### Kinetic vs thermodynamic control

<table>
<thead>
<tr>
<th></th>
<th>Kinetic</th>
<th>Thermodynamic</th>
</tr>
</thead>
<tbody>
<tr>
<td>competition</td>
<td>Rate-dependent</td>
<td>Concentration-dependent</td>
</tr>
<tr>
<td>behavior</td>
<td>Switch-like</td>
<td>adjustable</td>
</tr>
<tr>
<td></td>
<td>Non-equilibrium</td>
<td>equilibrium</td>
</tr>
</tbody>
</table>

Swinney, Let Drug Design Disc, 2006, 3, 569-574
Competing rates define a response

**PD outlast PK**

- $k_{\text{elim}} \uparrow$
- $E + I \leftrightarrow EI$
- $k_{\text{off}}$

$k_{\text{off}}$ slower the $k_{\text{elim}}$

**insurmountable**

- $E + I \leftrightarrow EI$
- $E + S \rightarrow ES$
- $k_{\text{off}}$
- $k_{-s}$

when $k_{\text{off}} < k_{-s}$

inhibition will be functionally irreversible

**use-dependence**

- $RI \leftrightarrow R$
- $R'$
- $k_{\text{act}}$
- $k_{\text{act}}$

when $k_{\text{off}} < k_{\text{act}}$

the block will accumulate
PART 2

MMOA effects dose-response (PK/PD) relationships.
Dose response curves (IC$_{50}$) can shift between in vitro and phenotypic assays

Fractional occupancy = Drug/(Drug + $K_I$)

In vitro activity

Fractional activity

[Inhibitor]
0.01 0.1 1 10 100 1000

In vitro- purified protein- target
Functional- cells, tissues, animals- phenotypic
Competition may cause a shift in dose response curves under equilibrium conditions

- $IC_{50}$ relationship to affinity ($K_i$) depends on the binding mechanism
  - $IC_{50}$ is an operational term
  - Competition shifts dose response curve
  - $IC_{50} = K_i (1 + S/Km)$

\[ E + S \rightleftharpoons ES \rightarrow \text{product} \]

\[ k_{on} \quad \downarrow \quad k_{off} \]

E:I
Slow binding kinetics can limit competition in non-equilibrium systems

- Irreversible
- Slow dissociation kinetics in non-equilibrium system
- Functionally irreversible (insurmountable)

\[
\begin{align*}
E + S & \rightleftharpoons ES & \text{product} \\
\text{k}_{\text{on}} & \downarrow & \text{k}_{\text{off}} & \text{k}_{\text{off}} \text{ very slow} \\
E:&I
\end{align*}
\]

Mutations to EGFR kinase increase affinity for ATP
- Decrease effectiveness of inhibitors because of equilibrium competition
- Next generation inhibitors limit competition with irreversible or slowly reversible binding kinetics

Yun et al PNAS 105, 2070 (2008)
Both biochemical efficiency and PK effect PK/PD relationships
Many medicines do not have a shift in activity. They have good biochemical efficiency ($K_i/EC_{50}$).

The shift in activity between binding and function ($K_i/EC_{50}$) is called Biochemical Efficiency-(BE)

- Good BE is a property of many best in class medicines (>90% of drugs in study with BE > 0.4)
- Molecules that couple more efficiently to the desired response will have a greater therapeutic index.
- Enables efficacy at lower drug concentrations

Swinney CTMC 6, 461 (2006)
Part 3: Practical application at iRND3

- We look to identify compounds with time-dependent activity.
- Difficult to predict
- Establish simple screen
- Shift in activity with preincubation
Four point MMOA assay

Determines **time-dependence** with and without preincubation of inhibitor with enzyme prior to starting the enzyme reaction
Determines **competition** of inhibitor with substrate at two substrate concentrations

Enzyme-TbGSK3β kinase
Substrate- ATP
Inhibitors-
**Tideglusib**
Time dependent
ATP competitive no preincubation
Non-competitive preincubation

**GW8510**
Not time-dependent
ATP competitive

Identify compounds with different MMOAs

Swinney, ZT et al PLOS NTD, 2016
Evidence for irreversibility
Preincubation of enzyme and tideglusib followed by a 1 to 100 dilution
Tideglusib inhibits TbGSK3β
GW8510 does not

Tideglusib
• Irreversible inhibitor of human GSK3β
• Developed for Alzheimer’s
• One year of dosing in phase II trials
• Well tolerated
• Did not meet efficacy end points
The effect of equilibrium and non-equilibrium kinetics on drug action

E + I ↔ EI

**equilibrium**
- reversible
- Fast kinetics
- occupancy dependent on substrate competition
- \( \text{occupancy} = \frac{[\text{drug}]}{[\text{drug}] + K_I(1 + S/K_m)} \)

E*I

**nonequilibrium**
- irreversible
- Slow kinetics
- occupancy less dependent on substrate competition
- \( \text{occupancy} = \frac{[\text{drug}]}{[\text{drug}] + K_I} \)

**biochemical**
- surmountable
- Minimize mechanism-based toxicity

**functional**
- insurmountable
- Good biochemical efficiency
- Minimize off-target toxicity

**clinical**
- improved therapeutic index
Take home message

- Binding kinetics influence
  - Molecular mechanism of action (MMOA)
    - efficacy
    - safety
    - therapeutic index.
Institute for Rare and Neglected Disease Drug Discovery, aka iRND3

Non-profit 501c3 drug discovery organization
Well equipped laboratory Mountain View, CA, USA
Experience drug discovery team with many years of Pharma experience
www.irnd3.org

Mission
iRND3's mission is to discover new medicines for rare and neglected diseases

Vision
-to utilize our understanding of how successful new medicines are discovered to implement and execute drug discovery strategies that supply our growing pipeline of new candidate medicines for rare and neglected diseases.

-to evolve an innovative, socially responsible operational model for successful collaborations between the non-profit, private and public to translate scientific discoveries to a continuous source of new medicines.
Medicines with slow or irreversible binding kinetics.

They are discovered in many therapeutic areas

- Slow dissociation reversible ($t_{1/2}$)
  - Amlodipine (77 min) hypertension
  - Aprepitant (154 min) emesis
  - Buprenorphine (166 min) pain
  - Candesartan (11.5 h) hypertension
  - Darunavir (>240h) antiviral
  - Desloratadine (>6 h) antihistamine
  - Efavirenz (4.1 h) antiviral
  - Lapatinib (300 min) anticancer
  - Maraviroc (10.5 h) antiviral
  - Olmesartan (72 min) hypertension
  - Oseltamivir (33-60 min) antiviral
  - Saxagliptin (5.1 h) diabetes
  - Telaprevir (2.9 h) HCV (phase III)
  - Tiotropium (34.7 h) COPD

- Irreversible
  - Aspirin; anti-platelet
  - Azacitidine; anticancer
  - Cefditoren; antibiotic
  - Clavulanic acid, Sulbactam, tazobactam; β-lactamase inhibitors
  - Finasteride; BPH
  - Formestan; anticancer
  - Omeprazole, Lansoprazole; GERD
  - Orlstat; obesity
  - Penicillin; antibiotic
  - Procarbazine; lymphoma
  - Selegiline, Tranylcypromine; depression
  - Ticlopidine, clopidogrel, prasugrel; anti-platelet
  - Vigabatrin; epilepsy