Factors Influencing Drug Delivery to the Brain: Multiple Mechanisms at Multiple Barriers

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Pharmacokinetics
((conc-time in blood))

Pharmacodynamics
((conc-time at the target))

Target biology

Pharmacodynamics

Pharmacokinetics

CNS drug development: Must keep in mind the big questions!

Why Does a Drug Work??

Why Doesn’t a Drug Work??

Why does this one work, and that one doesn’t??

Connect - Disconnect of the PK-PD Relationship

CNS

Blood

Pharmacodynamics
(effects at the target)

Pharmacokinetics
(conc-time at the target)

Pharmacokinetics
(conc-time in blood)

Balance

Information flow

CNS

Blood

Information flow
CNS Pharmacokinetics and Pharmacodynamics (Drug Delivery) in the Era of Systems Biology

Dose and dosing regimen

"Traditional PK/PD"

Mechanisms of delivery and action

"the Big Picture"

"Molecular pharmacokinetics"

Black box models

System - structure function

Systems model

Connect - Disconnect of the
PK-PD Relationship

Understanding Sources of Variability in Drug Response

Variability Cycle

Genetic Factors

- drug targets

- drug transporters

- drug metabolizing enzymes

Environmental Factors

- induction

- inhibition

Physiological Factors

- age, disease, etc.
Mechanisms that influence the fraction of the drug in the systemic circulation that is available for distribution to target tissue and the exposure of the tissue to the drug:

- Distribution of blood flow
- Ratio of total clearance to a distributional clearance

Distributional clearance - membrane permeability, competing carrier-mediated transport (influx or efflux), protein-binding, intracellular metabolism, tissue transit time, capillary structure

Total clearance - will affect the availability of the drug in the blood to distribute to the tissue.
Examine a location considering the interplay between external factors and mechanisms.

**Physicochemical Properties**

**Physiology / Pathology**

**Drug Metabolism**

**Membrane Permeability**

**Drug Transport**

**Protein Binding**

**Gene Regulation**

**Receptor Affinity**

**Protein Expression**

**Targeted Bioavailability**

**Pharmacological / Toxicological Response**

**External factors**

**Dosage Regimen**

**Patient**

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**One "Location": Blood-brain Barrier**

Importance of Transporters in the CNS Disposition of Drugs

GLUT1

P-gp (p-glycoprotein)

Co-localization GLUT1 - P-gp

Loscher, Aug. 2005

Decisions limited by available data at specific sites

Compartmental model for solute exchange in the brain
Modeling limited by available mechanistic data at specific barriers

- **plasma**
  - Brain capillaries
  - ECF production
  - Surface area
  - Permeability
  - **Transporters**
  - Metabolism
  - Regional variability

- **choroid plexus**
  - Arachnoid

- **extra-cellular fluid**
  - Neuronal and glial cell
  - Convection
  - Diffusion
  - **Transporters**
  - Permeability
  - Metabolism
  - Receptors
  - Regional variability

- **cerebrospinal fluid**
  - Ependyma
  - Pia mater
  - Convection, Diffusion
  - Permeability, Regional variability

**Simplified Quantitative Analysis of Drug Transfer In CNS**

**Drug “Binding” – Plasma and Brain**

- **Plasma**
  - \( C_{plasma} \)
  - \( C_{plasma} \) to CSF

- **Brain**
  - \( C_{brain} \)
  - \( C_{brain} \) to CSF

- **CSF**
  - \( C_{csf} \)
  - \( C_{csf} \) to plasma

- **BBB**
  - \( CL_{eff} \)
  - \( CL_{in} \)

- **BCSFB**
  - \( [CL_{in}, CL_{eff}, PS] \)
Simplified Quantitative Analysis of Drug Transfer In CNS

**Extent - partitioning into brain parenchyma**

\[ K_{p,\text{free}} = \frac{PS + CL_{\text{uptake}}}{PS + CL_{\text{efflux}} + CL_{\text{metabolism}} + CL_{\text{bulk}}} \]

Tight-junction opening

Substrate for Influx Transporter

Tight-junction opening

Inhibition of Efflux transporter

**Drug Targeting Index** (a measure of delivery)

\[ DTI = \frac{\frac{AUC_{\text{target, intervention}}}{AUC_{\text{blood, intervention}}}}{\frac{AUC_{\text{target, control}}}{AUC_{\text{blood, control}}}} \]

A neutral intervention would lead to a targeting index of unity, where positive effect would lead to a DTI greater than one, and a negative effect would result in a DTI less than one.

The critical issue in the quantitative assessment of drug targeting is the need to measure drug concentrations at the target site.
Many processes can be occurring simultaneously!
Case Study:

Tyrosine Kinase Inhibitors for Glioblastoma Multiforme (Glioma)

“Molecularly-Targeted” Agents. Can they find the target?

Numerous clinical trials with targeted tyrosine kinase inhibitors for glioma have failed.

Is there a PK-PD disconnect for these drugs in glioma?

**consilience**: to give a purpose to understanding the details

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**Figure 1.** Schematic representation of main oncogenic signaling molecular pathways and corresponding single and multtargeted drugs under development.

**Targets**

**Mol Cancer Ther 2007;6(7)**

**Drugs**

**TYROSINE KINASE RECEPTORS**

- SUNITINIB
- BOSUTINIB
- SCH78273
- VATALANIB
- AMG 706
- A7790476
- GW574844
- AVS170102
- XL820
- SL99
- CHIR-256

**RAS/MAPK PATHWAY**

- RAS
- RAF
- MAPK

**Pi3k/AKT/mTOR PATHWAY**

- Pi3k
- AKT
- mTOR

**CELL MEMBRANE**

- PD1
- PI3k
- PIK3C
- PI3CA
- PIK3R1
- PIK3R2
- PIK3R3
- PIK3R4
- PI4K1B
- PI4K1C
- PHF1
- PHF2
- PIK3R1
- PIK3R2
- PIK3R3
- PIK3R4
- PI4K1B
- PI4K1C
- PHF1
- PHF2
- PIK3R1
- PIK3R2
- PIK3R3
- PIK3R4
- PI4K1B
- PI4K1C
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- PHF2
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- PHF1
- PHF2
- PIK3R1
- PIK3R2
- PIK3R3
- PIK3R4
- PI4K1B
- PI4K1C
- PHF1
- PHF2
Target Locations

MRI-Detectable Portion of Tumor

Actual Tumor

1033 days

Kristin R. Swanson, Ph.D., University of Washington, 2008

Like Fighting a Forest Fire

Tumor Initiation

left front

fire start

propagation axis (wind)

right front

Where is the drug needed?

Factors enhancing infiltration of tumor

Intact BBB

head of the fire

Broken BBB: MRI contrast
Invasive Cell Migration
Localized??

Locations

Normal Brain
Invading cells
Tumor core

Glioma cell
Glioma Invasive “Stem” Cell

"Molecularly-Targeted" Agents.
Can they find the target in the brain?

In Vivo Methods

Mouse models:
Wild type: FVB
Transgenic: Abcb1a/b-Abcg2 (-/-)

Blood
Brain
Quantitative Analysis of Drug Targeting

Multiple transporters

Blood

CL\textsubscript{10}

CL\textsubscript{out}

CL\textsubscript{lin}

Brain - glioma Target Site

Other Tissue

Quantitative Analysis of Drug Targeting

Dasatinib – Brain/Plasma ratios in triple knockouts

\((\text{Abcb1a/b-Abcg2 (-/-)})\)

The brain-to-plasma ratio of dasatinib in wild-type and triple-knockout FVB Mice

\((* \ p<0.05, \ n=4)\)

* Chen Y. et al., JPET September 2009 vol. 330 no. 3 pgs. 956-963
Brain concentration of dasatinib in WT mice with and without genetic deletion of efflux transport.

Chen Y. et al., JPET September 2009 vol. 330 no. 3 pgs. 956-963

Brain concentration of dasatinib in WT mice with and without pharmacologic inhibition of efflux transport.

Chen Y. et al., JPET September 2009 vol. 330 no. 3 pgs. 956-963
Gefitinib Brain-to-Plasma Ratios at 90 min Postdose

Brain-to-Plasma ratios

WT  P-gp  BCRP  TKO

***


Lapatinib Brain Distribution

Steady-State Brain/Plasma Ratios

WT  P-gp  BCRP  TKO

0.3 mg/hr/kg  3.0 mg/hr/kg

~ 40-fold  ~ 40-fold

data from: Polli et al., An Unexpected Synergist Role of P-Glycoprotein and Breast Cancer Resistance Protein ... DMD 37:439-442, 2009
Steady-State Brain to Plasma Ratios

Sorafenib - Influence of Multiple Transporters on Brain Distribution

- WT
- P-gp KO
- Bcrp KO
- TKO

Bcrp / P-gp and Erlotinib Brain Penetration

- Wild-type
- TKO

42-fold increase

Alzet pump IP, 24 hour infusion, n = 3
Erlotinib Brain-to-Plasma Concentration Ratio at Different Locations in Rat Brain Tumor

Influence of Elacridar on Erlotinib Brain Penetration in Different Brain Regions

Targeted bioavailability ↑↑↑
Differences in Brain Distribution Enhancement between Genetic Knockouts and Pharmacological Inhibition

Generally knockouts show greater enhancement

1) accessibility of inhibitors?
   (dose, potency, parenchymal concentrations)

2) locations of transporters?
   (BBB vs parenchyma)

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11C-elacridar
(GF120918)

Dorner, 2009
Evaluation of Limiting Brain Penetration Related to P-glycoprotein and Breast Cancer Resistance Protein Using $[^{13}C]$GF120918 by PET in Mice

Kazutoshi Kanemura,1,2* Hidekazu Tanaka,2 Tatsuo Inoue,2 Yuki Fujii,3* Takeshi Hasegawa,4 Takahito Tanaka,5 Kazuhiko Yamanaka,6 Hidehito Kiyosue,2 Satoshi Yake,7* Masaki Kurosawa,1 Toshimizu Fukumura,1 and Min Zhang1

**Figures and Text:**

- **BCRP KO** vs. **P-gp/BCRP KO**
- **Wild-type** vs. **P-gp KO**
- **P-gp/Bcrp** vs. **WT Bcrp**

**Equations and Data:**

- $E_{D_{50}} = 1-2 \text{ mg/kg}$
- $K_1 = \frac{C_b}{C_{pdt}}$

**Graphs:**

- Scatter plot showing the relationship between $E_{D_{50}}$ and GF120918 dose (mg/kg). The graph includes data points for brain and brain/blood with a trend indicating a dose-dependent response. The ED50 is highlighted as 1-2 mg/kg for GF120918.
In Vitro- In Vivo Correlations, What about BCRP vs P-gp?

Kusuhara and Sugiyama

Kp (brain) Ratio \( (\frac{K_p}{\text{brain}})_{\text{KO}}/\frac{K_p}{\text{brain}}_{\text{WT}}) \) vs CFR for BCRP and MDR1

Data from Enokizono et al., DMD 2008

Is BCRP Important in the BBB? - Does BCRP KO tell the story?

Kp ratio brain

Data from Enokizono et al., DMD 2008
Simple Kinetic Model to Explain Non-Additive Increases in Brain Distribution of Dual P-gp / BCRP Substrates

Kusuhara and Sugiyama

Relative Distributional Clearances

\[
K_{p, \text{brain \_ratio}}(\text{P-gp}) = \frac{K_{p, \text{brain \_P-gp}} \text{ in } P\text{-gp}^+ \text{ mice}}{K_{p, \text{brain \_normal}} \text{ in normal mice}} = 1 + \frac{PS_{\text{P-gp}}}{PS_{\text{BCRP}}}
\]

\[
K_{p, \text{brain \_ratio}}(\text{BCPR}) = \frac{K_{p, \text{brain \_BCR}} \text{ in } BCR\text{-p}^+ \text{ mice}}{K_{p, \text{brain \_normal}} \text{ in normal mice}} = 1 + \frac{PS_{\text{BCRP}}}{PS_{\text{P-gp}}}
\]

\[
K_{p, \text{brain \_ratio}}(\text{DKO}) = \frac{K_{p, \text{brain \_P-gp\text{-BCR}} \text{ p}^+ \text{p}^+ \text{ mice}}{K_{p, \text{brain \_normal}} \text{ in normal mice}} = 1 + \frac{PS_{\text{P-gp}} + PS_{\text{BCRP}}}{PS_{\text{P-gp}} + PS_{\text{BCRP}}}
\]
A hypothetical common substrate drug with following kinetic parameters; $PS_{2,u} = 0.5$, $PS_{BCRP} = 2$, $PS_{P-gp} = 5$

- **Wild type P-gp TKO BCGRP**  
  - $PS_{luminal} = 7.5$  
  - $K_{p,brain} ratio(P-gp) = 3$

- **P-gp TKO**  
  - $PS_{luminal} = 7.5$  
  - $K_{p,brain} ratio(BCRP) = 1.4$

- **P-gp TKO/BCRP**  
  - $PS_{luminal} = 7.5$  
  - $K_{p,brain} ratio(DKO) = 15$


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**Influence of Relative Distributional Clearances on the Brain-to-Plasma Ratios of Dual Substrates**

- **Dual - Pgp - dominant**
  - $PS_{2,u} = 0.5$
  - $PS_{P-gp} = 5$
  - $PS_{BCRP} = 2$

  - e.g., dasatinib, erlotinib, gefitinib, lapatinib (affinity and capacity)
Influence of Relative Distributional Clearances on the Brain-to-Plasma Ratios of Dual Substrates

- Dual - BCRP - dominant
  - $PS_{2,u} = 0.5$
  - $PS_{P-gp} = 2$
  - $PS_{BCRP} = 8$

Influence of Relative Distributional Clearances on the Brain-to-Plasma Ratios of Selective Substrates

- Selective - BCRP
  - $PS_{2,u} = 0.5$
  - $PS_{P-gp} = 0$
  - $PS_{BCRP} = 5$

Selectives e.g., dantrolene

Duals e.g., sorafenib
Influence of Relative Distributional Clearances on the Brain-to-Plasma Ratios of Selective Substrates

Relative Increase in Brain-to-Plasma Ratio

Wild type  P-gp  BCRP  TKO

Selective - Pgp  e.g., quinidine

PS_{2,u} = 0.5
PS_{P-gp} = 5
PS_{BCRP} = 0

Influence of Relative Distributional Clearances on the Brain-to-Plasma Ratios of Dual Substrates

Relative Increase in Brain-to-Plasma Ratio

Wild type  P-gp  BCRP  TKO

Increased Passive Permeability!
Integrity of Tight Junctions in WT vs Triple Knockout Mice

![Graph showing Brain Space % (Percent of Total Brain Volume) vs Various Conditions]

**Brain Space %**

\[ \text{Brain Space} = \left( \frac{\text{Amount in brain}}{\text{Plasma Conc}} \right) \times 100 \]

10 min post bolus, n = 4, each time point

De Novo Induction of Genetically Engineered Brain Tumors in Mice Using Plasmid DNA

**Fig. C.20 A.** Tumors were induced by injection of oncogenic PEI/DNA complexes into the lateral ventricle of neonatal mice. This is a co-transfection of four plasmids (250 ng each, 2 µl volume). Grey arrows mark the transposon termini, similar to a retroviral LTR. SB transposase gene is encoded on the Luc vector to facilitate integration. **B.** Survival of mice from A. C. Mice were imaged to detect luciferase expression as a measure of tumor burden. D-Luciferin was injected to mark tumor growth via bioluminescence.

John Ohlfest, Brain Tumor Program, U of MN
Characterization of the NRAS/shP53/EGFRvIII model

Large tumor in the right hemisphere invading the left hemisphere

20x increase magnification showing infiltrating tumor in normal brain

John Ohlfest, Brain Tumor Program, U of MN

Genetically-Engineered Mouse model of glioma

Induce tumor

FVB Wild-type

Induce tumor

FVB Mdr1a/b (-/-)
Bcrp1 (-/-)
**Experimental Design**

- WT or TKO mice
- 15 mg/kg Dasatinib
- Twice daily, 7 days

**GFP-Guided Dissection Strategy**

- Normal Brain
- Rim
- Core
Dasatinib Regional Brain Distribution

Regional Effect on Signaling

- AKT
- pAKT
- SrC
- pSrC
- Actin
Less Efflux = Superior Efficacy

Conclusions:

1) multiple mechanisms at multiple barriers may limit glioma treatment to invasive brain tumor stem cells (barrier 1 (BBB); barrier 2 (BTSC))

2) several “molecularly-targeted” drugs are substrates of critical transport systems that are in both barriers

3) "molecularly-targeted" drugs may be effective in glioma if delivery issues are overcome and allow personalized therapy depending on individual tumor

4) need dirty drug (s), sharp needle
Overview

Major Challenges (Opportunities) in Describing the Kinetics of Drug Distribution in the CNS *(systems biology to do list)*

1) limited knowledge of biochemical, anatomical, and physiological variables that influence drug transport and delivery in the CNS  
   *(to do: integrate locations and mechanisms)*

2) develop methods to measure time and space dependent changes in drug concentration in and around the target site  
   *(to do: use complementary existing technologies and strive to develop new measurement techniques)*

Overview

Major Challenges (Opportunities) in Describing the Kinetics of Drug Distribution in the CNS *(to do list) continued*

3) design appropriate experimental and mathematical models that incorporate critical transport or transformation mechanisms, allowing predictions of concentration-time and space profiles leading to the site of action  
   *(to do: make correlations between animal models and human application)*

4) incorporate pharmacokinetic and pharmacodynamic information to help design and implement more effective treatments for CNS diseases  
   *(to do: educate the many disciplines involved in the discovery, development and eventual use of new treatments)*
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Consilience: Beware of the simplified boxes.

Make things as simple as possible, but not simpler.

Albert Einstein