Drug Delivery to the CNS: Barriers that May Influence Efficacy in Treating Tuberculosis in the Brain

William F. Elmquist
Pharmaceutics

TB-Meningitis
Rockville MD
22-23 May 2017

Blood Brain Barrier
endothelial cell layer
basement membrane
capillary lumen
astrocyte endfeet
tight junctions
luminal
abluminal
efflux diffusion influx

TB-Meningitis

Systemic Pharmacokinetics

University of Minnesota
Brain Barriers Research Center

William F. Elmquist
Pharmaceutics

University of Minnesota
Translational research in CNS Drug Delivery
- 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

Pharmacodynamics (events at the target)

Pharmacokinetics (conc-time in blood)

Information

Balance

Flow
Overview

CNS 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”

Systems model

System - structure function

Black box models
Examine drug pharmacokinetics / delivery to CNS sites across several scales

Quantitative – Qualitative Oscillations in the Thought Cycle
Genetic Factors
- drug targets
- drug transporters
- drug metabolizing enzymes

Environmental Factors
- induction
- inhibition

Physiological Factors
- age, disease, etc.

Understanding Sources of Variability in Drug Response

Variability Cycle

Connect - Disconnect of the PK-PD Relationship
“Locations” of Variability in Drug Efficacy In CNS Tuberculosis

Presystemic bioavailability questions ("traditional" bioavailability)

Targeted Bioavailability

Site-specific bioavailability questions (drug targeting)
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

**Presystemic bioavailability questions** ("traditional" bioavailability)

**Site-specific bioavailability questions** (drug targeting)

**Targeted Bioavailability**
Importance of Transporters in the CNS Disposition of Drugs


illustration by Naba Bora, Medical College of Georgia.
GLUT1

P-gp
(p-glycoprotein)

Co-localization
GLUT1 - P-gp

Modified from:
Loscher, Aug. 2005
Compartmental model for solute exchange in the brain

Therapeutic decisions limited by available data at specific sites

- **input**
- **output**
- **exchange**

**plasma**
- brain capillaries
- choroid plexus
- arachnoid membrane

**cerebrospinal fluid**
- IT, ICV

**extra-cellular fluid**
- neuronal and glial cell membranes
- ependyma
- pia mater

**intracellular fluid**

To know, is to measure.
Modeling limited by available mechanistic data at specific sites

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

- **choroid plexus and arachnoid**
  - CSF flow
  - Surface area
  - Permeability
  - Transporters
  - Metabolism
  - Regional variability

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

- **neuronal and glial cell**
  - Convection
  - Diffusion
  - Transporters
  - Permeability
  - Metabolism
  - Receptors
  - Regional variability

- **extra-cellular fluid**
  - Convection
  - Diffusion
  - Transporters
  - Permeability
  - Metabolism

- **intracellular fluid**
  - Convection
  - Diffusion
  - Permeability

- **ependyma pia mater**
  - Convection
  - Diffusion
  - Permeability
  - Regional variability

- **cerebrospinal fluid**
  - Convection
  - Diffusion
  - Permeability
  - Regional variability

- **brain capillaries**
  - ECF production
  - Surface area
  - Permeability
  - Transporters
  - Metabolism
  - Regional variability
Simplified Quantitative Analysis of Drug Transfer In CNS

Drug “Binding” – Plasma and Brain

Plasma

Cu,plasma

Cplasma

BBB

PS

CL_{eff}

CL_{in}

[CL_{in}, CL_{eff}, PS]

Brain

Cbrain

Cu,brain

CSF

Ccsf

CL_{bulk}

CL_{meta}

CL_{bulk}

CL_{meta}
Kinetics of distribution - Rate and Extent

Rate (onset) - described by maximum concentration (Cmax)

Extent (exposure) - described by area under the curve (AUC)

Ratio of areas gives tissue partition coefficient

\[ K_p = \frac{AUC_{\text{brain}}}{AUC_{\text{plasma}}} \]

\[ K_{p, uu} = \frac{AUC_{\text{brain-unbound}}}{AUC_{\text{plasma-unbound}}} \]
Simplified Quantitative Analysis of Drug Transfer In CNS

Extent - partitioning of free concentration

$$K_{p,\text{free}} = \frac{CL_{in}}{CL_{out}}$$

Sum of clearances in each direction

$$K_{p,\text{free}} = \frac{PS + CL_{\text{uptake}}}{PS + CL_{\text{efflux}} + CL_{\text{metabolism}} + CL_{\text{bulk}}}$$
Simplified Quantitative Analysis of Drug Transfer In CNS

Extent - partitioning into a specific brain region

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

Tight-junction opening

Substrate for Influx Transporter

Brain Metabolism

Fluid flow

Active transport CL depends on both capacity and affinity

\[ CL_{\text{act}} = \frac{T_{\text{max}}}{Km + C} \]
Representative Case Study:
The Treatment of Glioblastoma with Inhibition of P53 Degradation – MDM2 Inhibitor

Minjee Kim,
Jann Sarkaria
Choice of PDX Glioma Model

<table>
<thead>
<tr>
<th>GBM Line</th>
<th>MDM2 amplification</th>
<th>MDM2 expression</th>
<th>p53 status</th>
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<tbody>
<tr>
<td>10</td>
<td>no</td>
<td>low</td>
<td>wildtype</td>
</tr>
<tr>
<td>12</td>
<td>no</td>
<td>low</td>
<td>mutant</td>
</tr>
<tr>
<td>102</td>
<td>yes</td>
<td>low</td>
<td>wildtype</td>
</tr>
<tr>
<td>108</td>
<td>yes</td>
<td>high</td>
<td>wildtype</td>
</tr>
<tr>
<td>143</td>
<td>yes</td>
<td>high</td>
<td>wildtype</td>
</tr>
</tbody>
</table>

![Graph showing neurosphere count and apoptosis percentages for different GBM lines.](image)
Efficacy of SAR405838 depends on tumor location

Heterotopic Xenograft

Orthotopic Xenograft

Vol (mm³)

Study Day

GBM108

Placebo

SAR405838

50mg/kg qd

p = 0.005

n = 7

n = 8

Survival

Study Day

GBM108

Placebo

SAR405838

50mg/kg qd

P = 0.59

n = 11

n = 12
SAR405838 Concentration vs. Time Profiles in Plasma and Brain

Plasma

Influence of Efflux Transporters at the BBB On Brain Penetration of SAR405838
## SAR405838 - Plasma and Brain Distribution Kinetics

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>Mdr1a/1b⁻/⁻</th>
<th>Bcrp1⁻/⁻</th>
<th>Mdr1a/1b⁻/⁻ Bcrp1⁻/⁻</th>
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</thead>
<tbody>
<tr>
<td>$T_{1/2}$ (Hr)</td>
<td>3.26</td>
<td>4.18</td>
<td>3.02</td>
<td>5.08</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (Hr)</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>4651</td>
<td>3582</td>
<td>3976</td>
<td>6164</td>
</tr>
<tr>
<td>$AUC_{\text{inf pred}}$ (hr*ng/ml)</td>
<td>61195</td>
<td>41382</td>
<td>47804</td>
<td>65867</td>
</tr>
<tr>
<td>$Vz/F$ (ml/kg)</td>
<td>1922</td>
<td>3642</td>
<td>2281</td>
<td>2779</td>
</tr>
<tr>
<td>$\text{CL/F}$ (ml/hr/kg)</td>
<td>409</td>
<td>604</td>
<td>523</td>
<td>380</td>
</tr>
<tr>
<td>$AUC_{\text{brain}}$ (hr*ng/ml)</td>
<td>1335</td>
<td>63234</td>
<td>1956</td>
<td>65442</td>
</tr>
<tr>
<td>$K_{p,\text{brain}}$ ((\frac{AUC_{\text{brain}}}{AUC_{\text{plasma}}}))</td>
<td>0.022</td>
<td>1.53</td>
<td>0.041</td>
<td>0.994</td>
</tr>
</tbody>
</table>

### Distribution Advantage

$$\text{Distribution Advantage} = \frac{K_p \text{ knockout mice}}{K_p \text{ wild-type mice}}$$
Blood brain barrier integrity in orthotopic tumors. Near-moribund mice with orthotopic GBM108 tumors were injected with TexasRed-3 kDa dextran conjugate 10 min before euthanasia and processed for cresyl violet and fluorescent microscopy on serial histology sections. Accumulation of TR-dextran within the tumor reflects disruption of the BBB. Results presented are representative of five mice analyzed. Scale bar = 500 µm.
Heterogeneous Breakdown of Tumor BBB

orthotopic GBM108 parental line
G108-VEGFA Cell Line Generation

![Graph showing expression of VEGFA in G108 cells](image)

- **Expression of VEGFA in G108 cells (ng/μg of cell protein)**
  - G108-Parental
  - G108-EV
  - G108-VEGFA

- **Comparison of Texas-Red and Cresyl violet staining**
  - GBM108-vector
  - GBM108-VEGFA
Spatial Distribution of SAR405838 (MALDI-MSI)

GBM108-Empty vector

GBM108-VEGFA
Orthotopic Survival

Vector

Survival vs Days

Placebo
SAR405838

VEGFA

Survival vs Days

Placebo
SAR405838

45 day
Conclusions for SAR405838 Study

- SAR405838, a potent MDM2 inhibitor, is subject to BBB efflux

- This preclinical study indicates enhanced delivery of SAR405838 will improve its efficacy

- Strategies to overcome limited delivery of drug across BBB will result in better treatment for brain tumors
The role of LAT1 in $^{18}$F-DOPA uptake in malignant gliomas

Ryan S. Youland · Gaspar J. Kitange · Timothy E. Peterson · Deanna H. Pafundi · Judi A. Ramiscal · Jenny L. Pokorny · Caterina Giannini · Nadia N. Laack · Ian F. Parney · Val J. Lowe · Debra H. Brinkmann · Jami N. Sarkaria

Fig. 4 LAT1 expression correlates with $^{18}$F-DOPA SUVmedian in newly diagnosed human astrocytoma. Biopsy samples were taken from regions of high (red outlined region) and low (blue outlined region) $^{18}$F-DOPA uptake (a). Samples were then stained for LAT1 using immunofluorescence (green, Cy5-Lat1; blue, DAPI-nuclei). Regions of low (b) or high (c) $^{18}$F-DOPA uptake demonstrated corresponding low and high LAT1 expression, respectively.
Discordance in tumor delineation by 18F-FDOPA PET and MRI.

Volumes defined for:

A) FDOPA positivity (yellow) by PET
B) T1 contrast enhancement (red) on T1 contrast enhanced images
C) FLAIR positive (blue)

outlined for a single patient
<table>
<thead>
<tr>
<th>Structure</th>
<th>Structure Volume (cc) from Eclipse</th>
<th>PET Volume outside of T1-GAD (cc) from Eclipse</th>
<th>PET Volume outside of FLAIR (cc) from Eclipse</th>
<th>MR Volume outside of PET (cc) from Eclipse</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1-GAD</td>
<td>10.4</td>
<td>N/A</td>
<td>N/A</td>
<td>4.9</td>
</tr>
<tr>
<td>FLAIR</td>
<td>32.0</td>
<td>N/A</td>
<td>N/A</td>
<td>21.7</td>
</tr>
<tr>
<td>PET</td>
<td>19.5</td>
<td><strong>13.8</strong></td>
<td><strong>9.1</strong></td>
<td>N/A</td>
</tr>
</tbody>
</table>

Regions of tumor with intact BBB protected from treatment by efflux transporters and TJ

RT_FDOPEA02
Grade IV
Total Multi-focal
FLAIR contour in blue
T1-GAD contour in red
PET contour in yellow

Orthogonal views with crosshairs turned on, for reference
**Regions of tumor with intact BBB protected from treatment by efflux transporters and TJ**

**T1-GAD contour includes post-op cavity (not just enhancement)**

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<th>Structure Volume (cc) from Eclipse</th>
<th>PET Volume outside of T1-GAD (cc) from Eclipse</th>
<th>PET Volume outside of FLAIR (cc) from Eclipse</th>
<th>MR Volume outside of PET (cc) from Eclipse</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1-GAD</td>
<td>47.7</td>
<td>N/A</td>
<td>N/A</td>
<td>33.2**</td>
</tr>
<tr>
<td>FLAIR</td>
<td>54.3</td>
<td>N/A</td>
<td>N/A</td>
<td>36.4</td>
</tr>
<tr>
<td>PET</td>
<td>21.7</td>
<td>7.1</td>
<td>3.8</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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**Orthogonal views with crosshairs turned on, for reference**

**RT_FDOPA05**
Grade IV Total Single

**FLAIR contour in blue**
**T1-Gad contour in red**
**PET contour in yellow**
Screen capture of biopsy planning using the registered $^{18}$F-DOPA PET and T1-CE MRI in the Stealth™ Neuronavigation System for blue needle locations at:

A) a T1 contrast enhancing, PET positive (M+P+) location

B) a non-contrast enhancing but PET positive (M-P+) location.
Region specific disease, requires region specific consideration of drug delivery
Questions:

1) In the tuberculomas, is there a change in drug penetration as they encapsulate?

2) Is there active disease in the peripheral regions that show edema?

3) What is the integrity of the BBB at different sites within an infected brain?

4) Are drug concentrations in each region adequate to treat disease?

5) Are there significant differences in delivery limitations amongst drugs used in the necessary combinations in different regions of disease?
Rifampin Concentrations in Various Compartments of the Human Brain: A Novel Method for Determining Drug Levels in the Cerebral Extracellular Space

THOMAS MINDERMANN,1* WERNER ZIMMERLI,2 AND OTMAR GRATZL1

1Neurological Surgery and Infectious Diseases; 2University Hospitals Basel, 4031 Basel, Switzerland

Rifampicin Spatial Differences in Distribution within the Human Brain
Determination of $[^{11}\text{C}]]$Rifampin Pharmacokinetics within
*Mycobacterium tuberculosis*-Infected Mice by Using Dynamic Positron
Emission Tomography Bioimaging

Vincent P. DeMarco,$^{a,b,c}$ Alvaro A. Ordonez,$^{a,b,c}$ Mariah Klunk,$^{a,b,c}$ Brendan Prideaux,$^{d}$ Hui Wang,$^{e}$ Zhang Zhuo,$^{e}$ Peter J. Tonge,$^{e}$
Robert F. Dannals,$^{f}$ Daniel P. Holt,$^{f}$ Carlton K. K. Lee,$^{c}$ Edward A. Weinstein,$^{a,b,g}$ Véronique Dartois,$^{d}$ Kelly E. Dooley,$^{b,g}$
Sanjay K. Jain$^{a,b,c}$
MALDI-MSI for Rifampicin Distribution in the Lung

Determination of $[^{11}\text{C}]$Rifampin Pharmacokinetics within *Mycobacterium tuberculosis*-Infected Mice by Using Dynamic Positron Emission Tomography Bioimaging

PET scan for distribution of 11C-Rifampicin in *M. tuberculosis* Infected Mouse Model

DeMarco et al. 2015

A

Liver

![Liver PET scan graph](image)

\[ AUC_{\text{infected}} = 0.061 \text{ ng*hr/ml} \]
\[ AUC_{\text{control}} = 0.067 \text{ ng*hr/ml} \]

B

Blood

![Blood PET scan graph](image)

\[ AUC_{\text{infected}} = 0.0056 \text{ ng*hr/ml} \]
\[ AUC_{\text{control}} = 0.0061 \text{ ng*hr/ml} \]

C

Brain

![Brain PET scan graph](image)

\[ AUC_{\text{infected}} = 0.0012 \text{ ng*hr/ml} \]
\[ AUC_{\text{control}} = 0.0008 \text{ ng*hr/ml} \]

D

Lung

![Lung PET scan graph](image)

\[ AUC_{\text{infected}} = 0.0034 \text{ ng*hr/ml} \]
\[ AUC_{\text{control}} = 0.0054 \text{ ng*hr/ml} \]
Radiosynthesis and Bioimaging of the Tuberculosis Chemotherapeutics Isoniazid, Rifampicin and Pyrazinamide in Baboons

Li Liu,† Youwen Xu,‡ Colleen Shea,‡ Joanna S. Fowler,‡ Jacob M. Hooker,*,† and Peter J. Tonge*†

*Journal of Medicinal Chemistry. 2010, 53, 2882–2891
DOI: 10.1021/jm901858n

(a) 11C PET

(b) 

\[
\text{[^{11}C]PZA} \quad \text{[^{11}C]INH} \quad \text{RIF}
\]
Table 1. LogD and PPB Determination

<table>
<thead>
<tr>
<th></th>
<th>LogD</th>
<th>PPB,(a) %</th>
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</thead>
<tbody>
<tr>
<td>RIF</td>
<td>1.67</td>
<td>27.32</td>
</tr>
<tr>
<td>INH</td>
<td>nd(^b)</td>
<td>94.63</td>
</tr>
<tr>
<td>PZA</td>
<td>−0.41</td>
<td>91.32</td>
</tr>
</tbody>
</table>

\(a\) Value expressed as % of free fraction in plasma. \(^b\) Octanol–water partitioning was highly variable.
Talazoparib – Pgp substrate - poor penetration into the brain
The sweet spot
Make things as simple as possible, but not simpler. 
Albert Einstein