Targeted Bioavailability: Mechanisms Influencing the Tissue Distribution of Drugs

June 8, 2005
The New England Drug Metabolism Discussion Group Summer Symposium
University of Massachusetts Medical School
Shrewsbury, Massachusetts

William F. Elmquist, Pharm.D., Ph.D.
Department of Pharmaceutics
University of Minnesota-Twin Cities

University of Minnesota
Outline

Background

1) Drug transport proteins in the body and the CNS drug disposition (overview)

Experimental Studies

2) Delivery of Anti-tumor Agents (Gleevec) to CNS

Summary

3) CNS drug delivery program and efflux transporters
Variability in Drug Response in the CNS

- variability in distribution
- variability in receptors
- variability in elimination and metabolism
- variability in absorption

Why study drug transporters in the CNS barriers?
Bioavailability Definitions:

FDA - "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action."


Commonly accepted definition:

Typically, one modifies this definition to limit the delivery path from the site of administration to the bloodstream, i.e., the term bioavailability is “the fraction of the oral dose that actually reaches the systemic circulation”, and “commonly applied to both the rate and extent of drug input into the systemic circulation".
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.
“Locations” of Variability in Drug Response

Presystemic bioavailability questions ("traditional" bioavailability)

Oral dosage form → Intestinal absorption → Intestinal metabolism → Systemic circulation → Tissue distribution → Cellular delivery → Target site → Drug action

Site-specific bioavailability questions (drug targeting)

Targeted Bioavailability
Mechanisms influencing intestinal absorption:
- solubility
- permeability
- transit time
- carrier-mediated influx and efflux
- stability
- dosage form performance (disintegration, dissolution)

Presystemic bioavailability questions ("traditional" bioavailability)

Site-specific bioavailability questions (drug targeting)
“Locations” of Variability in Drug Response

Presystemic bioavailability questions
(“traditional” bioavailability)

Site-specific bioavailability questions
(drug targeting)

Targeted Bioavailability

Oral dosage form

Intestinal absorption

Intestinal metabolism

Liver metabolism

Mechanisms influencing the intestinal first-pass metabolism:

- carrier-mediated transport
- phase I metabolism (e.g., CYP3A4)
- phase II metabolism (e.g., UGTs)
- enzyme induction
- enzyme inhibition
- locations of windows of absorption
- intestinal motility / transit time
- genetic expression patterns
“Locations” of Variability in Drug Response

Presystemic bioavailability questions ("traditional" bioavailability)

Site-specific bioavailability questions (drug targeting)

Mechanisms influencing the intestinal first-pass metabolism:
- carrier-mediated transport
- influx and efflux transporters
- phase I (e.g., CYP3A4)
- phase II (e.g., UGTs)
- enzyme induction
- enzyme inhibition
- blood flow
- protein binding
- genetic expression patterns

Oral dosage form

Intestinal absorption

Intestinal metabolism

Liver metabolism

Cellular delivery

Targeted Bioavailability
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, 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) vs. Site-specific bioavailability questions (drug targeting)

Targeted Bioavailability
Mechanisms that influence cellular delivery

- membrane permeability
- carrier-mediated transport (influx/efflux)
- intracellular metabolism
- receptor-mediated transport
- binding
“Locations” of Variability in Drug Response

Presystemic bioavailability questions ("traditional" bioavailability)

Site-specific bioavailability questions (drug targeting)

Oral dosage form

Intestinal absorption

Intestinal metabolism

Tissue distribution

Target site

Drug action

Mechanisms influencing the drug action at the target site

- intracellular signaling
- intracellular transport
- expression of target receptors
- receptor affinity
- availability of cofactors

Targeted Bioavailability
“Locations” of Variability in Drug Response

Presystemic bioavailability questions ("traditional" bioavailability)

Oral dosage form

Intestinal absorption

Intestinal metabolism

Liver metabolism

Systemic circulation

Tissue distribution

Cellular delivery

Target site

Drug action

Site-specific bioavailability questions (drug targeting)

Targeted Bioavailability
Examine a location considering the interplay between external factors and mechanism.
“Traditional Bioavailability” \( F = F_a \times F_g \times F_h \)

\[
\begin{align*}
F &= 0.80 \times 0.75 \times 0.5 \\
&= 0.3
\end{align*}
\]
At each location, several mechanisms at play:

Influence of fruit juices on oral bioavailability “traditional bioavailability”
The effects of fruit juices on drug disposition: a new model for drug interactions

G. K. Dresser and D. G. Bailey
Department of Medicine, London Health Sciences Centre, and University of Western Ontario, London, Ontario, Canada

\[ F = f_a \times f_g \times f_h \]

Figure 1 Proposed location and direction of active transport of P-glycoprotein and the OATP in enterocytes. Baseline conditions (A) and effects of fruit juice administration (B) are depicted. Fruit juices are proposed to have a greater inhibitory effect on OATP-mediated drug uptake than on P-glycoprotein-mediated drug efflux into intestinal lumen.
Complexity of the Transporter Problem at Various Barriers

Many processes can be occurring simultaneously!
“Targeted” Bioavailability $F = Fa \times Fg \times Fh$ and $Fd, Fc, Ft$
Importance of Transporters in the Disposition of Drugs

illustration by Naba Bora, Medical College of Georgia.
Figure 1. Model for solute exchange between plasma and the compartments of the central nervous system illustrating the components of the blood–brain barrier.

Adapted from Quentin Smith

Compartmental model for solute exchange in the brain
Fig. 1.1. Linear evolution of blood–brain barrier methodologies from vital dyes to molecular biology.
Blood-Brain Barrier (BBB)

Basolateral membrane

Apical membrane

4 cells comprising the CNS microvasculature
Cooray et al., 2002
NeuroReport

Microvessel from normal brain stained for GLUT-1 and BCRP

GLUT-1 on both inner and outer walls of the microvessel

BCRP shows a more narrow distribution
Background

Selected Drug transport systems in BBB and BCSFB

MCT1 -- monocarboxylate transporter
OATP2 -- organic anion transport protein 2
MRP -- multidrug resistant-associated protein (ABCCX)
P-gp -- p-glycoprotein (ABCB1)
BCRP -- breast cancer resistance protein (ABCG2)
Murakami, 2000

PS (in situ perf) versus logP(MW^{−\frac{1}{2}})
Barriers to Drug Delivery

Presystemic bioavailability questions
("traditional" bioavailability)

Targeted Bioavailability

Site-specific bioavailability questions
(drug targeting)
Outline

Background

1) Drug transport proteins in CNS
drug disposition (overview)

Experimental Studies

2) Delivery of Anti-tumor Agents to CNS

Summary

3) CNS drug delivery program
and efflux transporters
CNS Delivery of Anti-tumor Agents

1) Brain Around Tumor - BAT (growing edge)
2) Membrane permeability vs. efflux transport
3) Tumor vasculature
4) Primary and secondary tumors (metastases)
STI-571  (Gleevec\textsuperscript{TM})

Recently approved for chronic myelogenous leukemia (CML)

Several clinical trials currently underway for glioma
Mechanism of Action of STI-571

“Molecularly Targeted” Therapy

ATP

\textit{bcr-abl} tyrosine kinase

\textit{Substrate}

\textit{PO}_4

ATP

\textit{bcr-abl} tyrosine kinase

\textit{Substrate}

\textit{CML}

Altered cellular adhesion
Abnormal proliferation
Inhibition of apoptosis

Altered cellular adhesion
Abnormal proliferation
Inhibition of apoptosis

STI571

\textbf{X}
Kaplan-Meier survival plots

intracerebral human glioma nude mice

STI-571 txt bid PO 50 mg/kg/day

Whole-body Autoradiography of Pigmented Rats

From Novartis Pharma
Methods

- The directional flux of STI-571 was studied in MDCKII epithelial cell monolayers, using Transwell inserts. The effect of P-glycoprotein inhibition on the directional flux was also determined.
In Vitro Experiment

![Diagram of in vitro experiment showing different transport pathways in parental and MDR1-transfected cells.](image)
Figure 1. Directional flux of STI-571 Across MDCKII Monolayers  July 31, 2001
STI-571 Flux

MDR1-transfected MDCKII epithelium

Corrected flux ratio

4.5-fold

“Corrected” flux = \( \frac{(B\text{-to-}A}{A\text{-to-}B)}_{\text{mdr}} \times \frac{(B\text{-to-}A}{A\text{-to-}B)}_{\text{wt}} \)
In Vitro Experiment
In Vitro Experiment

MDR1-transfected cells
Effect of Specific P-gp inhibition on STI-571 Flux

Figure XX. Directional flux of STI-571 Across MDCKII Monolayers (effect of 1µM LY335979)
RESULTS

The directional flux of $^{14}$C-STI-571 was studied in MDCKII epithelial cell monolayers.
**Figure 6.** Brain to plasma ratio of tritiated inulin (10 min post tail vein injection) and carbon-14 STI-571 (90 min and 120 min post oral dose, 25 mg/kg) in wild-type mice. (dual label liquid scintillation counting, total levels, not corrected for residual volume (inulin space))
Figure XX. Brain to Plasma Ratio of STI-571 Radioequivalents 60 minutes post oral dose (25 mg/kg) in wild-type mice, p-gp knockout mice, and wild-type mice treated with potent p-gp modulator.
Figure 4. Brain to plasma ratio of STI-571 radioequivalents post oral dose (25 mg/kg) at various times postdose. (mean ± SD, n = 4 at each time point) corrected for residual vascular volume (inulin space).
Radiopurity of $^{14}$C-STI-571 (Gleevec™)

- Retention time of parent STI-571 by UV detection
- Tracer STI-571 injected on column
Brain/Plasma ratio KO = 11.2 fold opportunity for targeted bioavailability

**Brain/Plasma ratio WT**
**A** - Plasma STI-571 conc vs time

**B** - Brain STI-571 conc vs. time

**C** - Increase in STI-571 brain penetration in the P-gp knockout enhanced: 6-8 fold!

“targeted bioavailability”
A) mice developed neurological symptoms while on imatinib

B) pathological evidence of macrophage brain and meningeal masses

C) brain and spinal cord B-lymphoid cell staining

D) laser dissected regions compared using PCR primers specific for P210 Bcr/Abl

Wolff et al., Blood 2003

The CNS is a sanctuary for leukemic cells in mice receiving imatinib mesylate for BCR/Abl induced leukemia
from Wolff et al.,

*CSF/ Plasma STI571 concentrations in the mouse model*

<table>
<thead>
<tr>
<th></th>
<th>Drug</th>
<th>Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>ng/ml</td>
<td>µM</td>
</tr>
<tr>
<td>Plasma (average)</td>
<td>6958 (± 2082)</td>
<td>11.8 (± 3.5)</td>
</tr>
<tr>
<td>CSF (pooled)</td>
<td>45</td>
<td>0.08</td>
</tr>
<tr>
<td>Plasma/CSF ratio</td>
<td>155</td>
<td>NA</td>
</tr>
</tbody>
</table>

*(n = 9)*
Leis et al., Low penetration of imatinib (STI571) into the CSF indicates need for standard CNS prophylaxis in patients with CML lymphoid blast crisis and Philadelphia Chromosome Positive ALL. Abstract, American Society of Hematology, December 8, 2001
P-gp substrate pharmacophore model and Gleevec aligned. Blue features (hydrophobes) and green features (hydrogen bond acceptors) with vector in the direction of the putative hydrogen bond donor. (Ekins et al, Molecular Pharmacology, vol. 61, 2002)
Many processes can be occurring simultaneously!
The Effect of Bcrp1 (Abcg2) on the In vivo Pharmacokinetics and Brain Penetration of Imatinib Mesylate (Gleevec): Implications for the Use of Breast Cancer Resistance Protein and P-Glycoprotein Inhibitors to Enable the Brain Penetration of Imatinib in Patients

Pauline Breedveld,¹ Dick Pluim,¹ Greta Cipriani,¹ Peter Wielinga,² Olaf van Tellingen,³ Alfred H. Schinkel,¹ and Jan H.M. Schellens¹,45
Figure 2. Transport of imatinib by Bcrp1 in the absence and presence of pantoprazole or elacridar. A, the MDCKII parental and MDCKII-Bcrp1 cells were pre-incubated for 2 hours with 5 μmol/L LY335979 (n 2 mL OptiMEM per compartment). The indicated concentration of [14C]imatinib and 5 μmol/L LY335979 were applied at t = 0 to the apical or basal side and the amount of [14C]imatinib appearing in the opposite basal compartment (closed symbols) or apical compartment (open symbols) was determined. Samples were taken at t = 1, 2, 3, and 4 hours. Points, means of each experiment in triplicate; bars, SD. B, MDCKII parental and MDCKII-Bcrp1 cells were pre-incubated for 2 hours with 5 μmol/L LY335979 and without (control) or with indicated concentrations of pantoprazole, or were preincubated for 2 hours without (control) or with 5 μmol/L elacridar. One micromole per liter [14C]imatinib and the indicated concentration of pantoprazole or elacridar were applied at t = 0 to the apical or basal side and the amount of [14C]imatinib appearing in the opposite basal compartment (AB) or apical compartment (BA) was determined. Samples were taken at t = 1, 2, 3, and 4 hours. Points, means of each experiment in triplicate; bars, SD.
Breedveld et al. (BCRP and Imatinib CNS Penetration)

Cancer Res 2005; 65:(7). April 1, 2005

[A] Imatinib Brain penetration
- BCRP, P-gp

[B] Imatinib Brain penetration
- control mice w/inhibitor
Transport Mechanisms at the Blood-Brain Barrier

From Tsuji, 2000.
Barriers to Drug Delivery

Presystemic bioavailability questions ("traditional" bioavailability)

Site-specific bioavailability questions (drug targeting)

Targeted Bioavailability

Oral dosage form

- Intestinal absorption
- Intestinal metabolism

- Liver metabolism

Systemic circulation

- Tissue distribution

- Cellular delivery

Target site

Drug action
Program for CNS drug delivery evaluation

Biochemistry and molecular biology

In Vitro cellular barriers
  (transfected cell lines and isolated brain cellular barriers)

In Vivo techniques
  (BUI, intravenous injection, in situ perfusion)

Specialized in vivo techniques
  (microdialysis, transgenic animals)
Strategies to Improve the CNS Delivery of Drugs

1) improve physiochemical properties
2) use existing influx transport systems
3) reduce affinity for efflux transporters
   problem: multidrug resistance
4) temporary breakdown of the BBB
   hyperosmotic (mannitol)
   inflammatory mediators (RMP-7)
5) drug delivery systems
   implants/direct delivery
   polymeric carriers
   nanoparticles
   peptide vectors
   immunodirected vectors
   efflux inhibitors
CNS Drug Distribution in the Future

The role of various drug efflux proteins in the blood-brain and blood-CSF barriers in CNS targeting can be examined using a variety of complimentary experimental approaches. These include:

- biochemical and molecular biological methods
  (expression of functional protein and RNA message)
- in vitro models using transfected cell cultures
- in vitro models using cell cultures of CNS barriers
- in vivo models using novel sampling techniques
- in vivo models that have been genetically engineered

These approaches will allow a specific quantitative analysis of the biological determinants of drug action in the CNS that are related to drug delivery across the cellular barriers in the CNS.
Acknowledgements

Haiying Sun
Hua Yang
Qin Wang
Michele Fontaine
Tim Spitzenberger
Christine Brandquist
Haiqing Dai
Ying Chen
Dr. Mini Kurumboor
Naveed Shaik
Dr. Paul Stemmer
Dr. Donald Miller

Dr. Alexander Kabanov
Dr. Elena Batrakova
Tanya Bronich

Center for Neurovirology
Dr. Howard Gendelman
Dr. Yuri Persidsky

Lilly Cancer Research
Dr. Anne Dantzig
Dr. James Starling

Yale University
Dr. Alan Sartorelli
Dr. Rick Finch

Novartis Pharma
Dr. Michel Lemaire
Dr. Peter Marbach

Alfred Schinkel

Funding
National Institutes of Health
Lilly Cancer Research Laboratories
Novartis Pharma
UNMC Graduate Fellowship