Principles of Clinical Pharmacology

Module 2: Drug Metabolism and Transport

Unit 6: Concentrative and Equilibrative Drug Transport

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Objectives

- Vision, reality, and the path between.
- Methods of measuring drug transport *in vitro* and *in vivo*.
- Mechanisms of drug transport.
- Recent advances in understanding the role of membrane transport proteins.
- Clinical significance.

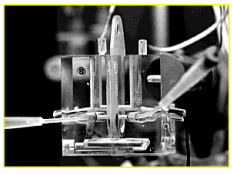
Measurement of Drug Distribution/Transport *In Vivo*

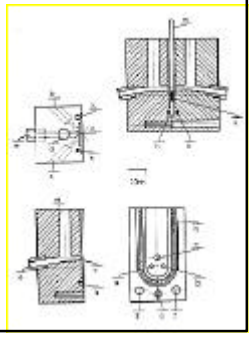
- Tissue Samples, Biopsies, and Assays
- Autoradiography
- Perfusion/Cannulation Methods
- Radiology - x-ray, PET, SPECT
- Magnetic Resonance Imaging
- Microdialysis

Measurement of Transport *In Vitro*

- Ussing chamber - transport across tissue samples - high level of control
- Everted gut sac - uptake from medium
- Uptake/Efflux by cells in culture (CHO)
- Confocal microscopy of cultured cells
  - fluorescent drugs (mitoxantrone, rhodamine)
- Monolayer cell cultures
  - Caco-2 cells, MDKII, brain microvascular endothelial

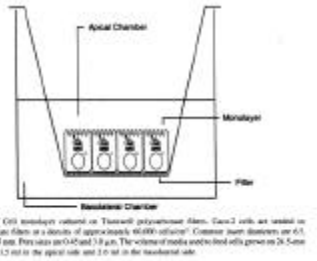
Measurement of Transport in Excised Tissue Samples





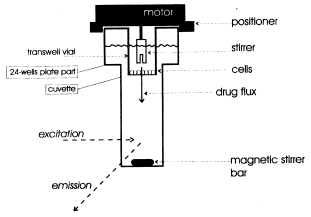
Modified Ussing-chamber allows perfusion of solutions on both sides of membrane holder, control of pressure differential, measurement of potential, conductivity, pH.
Adapted from Ref. 7.

Monolayer Epithelial Cell Culture



I.J. Hidalgo, in ref. 6, Models for Assessing Drug Absorption and Metabolism (Borchardt, et al., Eds.) Plenum Press, NY, 1996, p. 38.

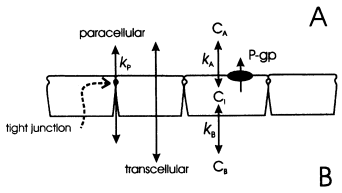
Apparatus for On-Line Fluorescence Measurement of Transport in Epithelial Cell Cultures



MDCKII ± MDR1
± SDZ PSC 833
Daunorubicin
λ_{ex} = 480, λ_{em} = 590
FITC-dextran
λ_{ex} = 480, λ_{em} = 525
Trans Epithelial Resistance (TER) = 300 - 600 Ω·mm²

Ref. 8: Wielinga, et al., J. Pharm. Sci, 88(12), 1340, 1999.

Paracellular versus Transcellular Transport

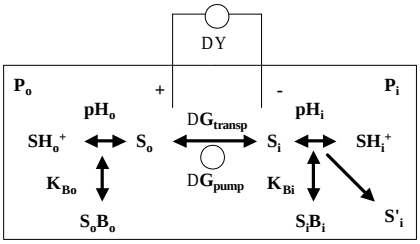


Ref. 8: Wielinga, et al., J. Pharm. Sci, 88(12), 1340, 1999.

Thermodynamics of Transport

- Transport of neutral species
- Ions & transmembrane potentials
- Ionizable species & proton gradients
- Metals and other titrants
- Macromolecular and cellular binding sites
- Coupled transport and ATP driven pumps
- Chemical conversions

Equilabrative Transport Compartment Model



Equations for Membrane Transport Thermodynamics

$$\Delta G_{transp} = 2.303RT \log[S_i]/[S_o] + nF\Delta Y + \Delta G_{pump}$$

R = 8.314 joules/mol°K = 1.987 cal/mol°K
F = 96.5 Joules/mol-mV = 23.06 cal/mol-mV

D **$[S_i]/[S_o]$ = 10x @**	**296°K (23°C)**	**310°K (37°C)**
$\Delta(\Delta G)$ =	5.67 kJ/mol	5.936 kJ/mol
=	1.35 kcal/mol	1.41 kcal/mol
$\Delta(\Delta\Psi)$ =	58.5 mV	61.5 mV

pH = pKa + log[S]/[SH]

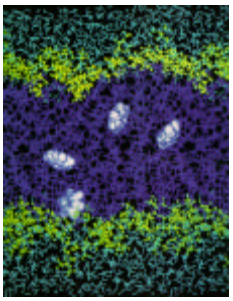
Examples Mechanism/Drug/Compartment

Diffusion	antipyrene	total body water
Ion trapping	Tc-Sestamibi	heart mitochondria
pH trapping	quinidine	renal excretion
Binding	warfarin	plasma/liver ratio
Active	captopril	GI absorption

Passive Diffusion

- Characteristics of passive diffusion
  - $k_{in} = k_{out}$, net rate = $k([S_o] - [S_i])$, non-selective
- Model Membranes
  - monolayers, bilayers, liposomes, BLMs, IAMs
- Membrane Models
  - structural, electrical, single/multiple barrier, partition adsorption/diffusive, unstirred layers
- Simulation of bilayers and transport
  - molecular dynamics
- QSAR - structure/transport correlations

Molecular Dynamics Simulation of Membrane Diffusion



From: Bassolino-Klimas, Alper and Stouch, ref. 16. See also ref. 17.

Snapshot from 10 nsec MD simulation in 100 fs steps. Showed hopping motions of 8 Å over ca 5 psec vs RMS motions of 1.5 Å. Motions differ in center and near surface, both differ from bulk organic. Rotational isomerizations (gauche/trans) gate channels between voids. Differing motions available to adamantane, nifedipine.

QSAR of Transport

- Hansch Equation
  - $\log (1/C) = -k(\log P)^2 + k'(\log P) + \rho\sigma + k''$
  - C = dose or [S] for effect (ED_{50}, IC_{50}, rate)
  - logP = partition coef or π = lipophilicity factor
  - σ = Hammett electronic substituent effects
  - k, k'', ρ = regression coefficients
- Free-Wilson Model
  - $BA = \Sigma ajXj + \mu$
  - BA = biological activity (e.g., log(1/c))
  - aj = substuent constant, Xj = substituent presence, μ = overall average activity

QSAR of Transport

Selected from: V.Austel & E. Kutter in Ref. 18.

ABSORPTION - log (%abs), log Perm, log k

Barbiturates	Gastric	log $P_{CHCL3/W}$
Sulfonamides	Gastric	log $P_{isoam\text{-}OAC/w}$
Anilines	Gastric	pKa
Xanthines	Intestine	Distribution Coef
C-glycosides	Intestine	log $P_{o/w}$, R_m

Excretion - log (%excreted), log Cl_R, log k

Penicillins	Biliary	logP, R_m
Suflathiazoles	Biliary	log$P_{o/w}$, pKa
Sufapyridines	Renal	Rm, pKa
Sulfonamides	Renal	π, pKa
Amphetamines	Renal	log$P_{h/w}$

QSAR Conclusions

- Lipophilicity (log$P_{o/w}$); CLOGP
  - GI (0.5-2.0), buccal (4-4.5), topical (>2.0)
- Water solubility
  - melting point, solvation energy
- pKa - fraction of neutral species available
- mw - $D \propto 1/\sqrt{mw}$; mw < 500 Da
- Confounding factors
  - inaccurate data, multiple mechanisms

Mediated Transport: Facilitated Diffusion and Active Transport

- Rates > passive, solute specific, high Q_{10}
- Non-symmetrical ($k_{in} \neq k_{out}$ at $[S_i] = [S_o]$)
- Saturable transport - Michaelis-Menten
- Inhibitable - competitive, non-competitive
- Regulated - inducibility & repression
- Tissue specific- differential expression
- Energy dependent - active transport
  - primary pumps - respiration, photosyn, ATPase
  - secondary transporters (coupled to H^+, Na^+ etc.)

Mechanisms of Transport and Example Drugs (in Bacteria)

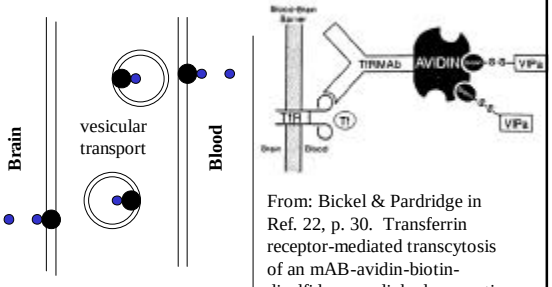
Adapted from: R.E. Hancock, p. 292, in Ref. 15.

- Passive diffusion across lipid bilayer
  - fluoroquinolones, tetracycline (hydrophobic_
- Diffusion through OM channels and porins
  - B-lactams (hydrophilic and charged)
- Facilitated diffusion
  - imipenem, catechols, albomycin, albicin
- Active Transport
  - aminoglycosides, cycloserine, phosphomycin, alaphosphin

Pinocytosis, Endocytosis, and Receptor Mediated Transcytosis

- Pinocytosis (cell sipping - non-mediated)
  - non-specific, non-saturable, bulk fluid phase uptake, large particles, polymer-conjugates, obsolete term?
- Endocytosis (receptor-mediated uptake)
  - specific, relevant to macromolecules, used to deliver small molecules as prodrugs, mediates clearance
  - insulin, growth hormone, erythropoetin, G-CSR, ILs
- Transcytosis (receptor-mediated uptake and secretion on the translateral surface)
  - useful for macromolecules and small molecule prodrugs, GI, BBB, and pulmonary epithelia.

Transcytosis Delivery of Prodrug



From: Bickel & Pardridge in Ref. 22, p. 30. Transferrin receptor-mediated transcytosis of an mAB-avidin-biotin-disulfide cross-linked vasoactive intestinal peptide.

Cell Culture and Molecular Biology Methods

- Isolation of MXR genes (Ref. 25).
  - Cells cultured from patients w/ resistant tumors.
  - mitoxantrone uptake measured microscopically
  - Cells grown under progressively selective conditions mitoxantrone, adriamycin, verapamil
  - Isolation of differentially expressed mRNA as cDNA clones and cDNA sequencing.
  - Northern analysis of mRNA expression levels.
  - Southern analysis of gene copy amplification.
  - Quantitative PCR analysis of expression levels in non-selected resistant cells.

Cell Culture and Molecular Biology Methods (Cont'd).

- Isolation of BCRP genes (Ref. 26-27)
  - cells same as from Ref. 25
  - cultured under selective conditions w/ doxorubicin and verapamil
  - RNA fingerprinting used to isolate cDNAs.
  - transfection of non-selected cells confers resistance to mitoxantrone, doxorubicin, daunorubicin
  - reduced uptake (dauno), enhance efflux (rhodamine 123)
  - Northern/Southern analysis of various cell lines

Cell Culture and Molecular Biology Methods (Cont'd II)

- Isolation of MOAT-B,C,D (Ref. 28)
  - cMOAT (cannicular multispecific organic anion transporter) = MRP previously isolated
  - MOAT-B isolated by PCR
  - Homology search against EST datase suggests MOAT-C & MOAT-D
  - EST probe isolation of cDNA from human
  - RNA blot analysis of tissue expression library
  - chromosomal location by FISH

Cell Culture and Molecular Biology Methods (Cont'd III)

- Other Cloning Methods
  - Expression cloning in oocytes
  - Homologous hybridization
  - Cloning by RT-PCR with degenerate primers
  - Cloning by functional complementation

Cell Culture and Molecular Biology Methods (Cont'd IV).

- What have you got? MXR, BCRP, MDR, MRP, ABC
  - Homology search against database - BLAST
  - Hydropathy analysis - Kyte-Dolitte
  - Sequence alignments and motifs
  - Homology modeling
  - Sequences of suggestive of transporter family

Biochemistry and Biophysics

- Functional characterization
  - Expression of Transport Activity in Vitro
  - Substrate structure/activity profiles and co-substrates (GSH, ATP, H+, Na+), uncouplers
  - Tissue distribution - EST database, RNA expression levels, antibodies, in situ methods
  - Phenotypes in Knock Out Rodents
  - Subcellular localization microscopy
  - Isolation, purification, reconstitution
  - Structural biology - EM, X-ray, NMR
  - Mechanism of substrate transport and energy coupling - enzymology, inhibition, drug design

Membrane Transporter Families

ABC Superfamily

ABC peptide transporter family

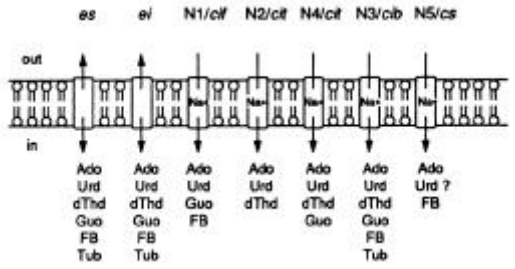
P-glycoprotein (MDR) family

- MDR1a,1b,2,3 - organic cations, lipids (PC)
- MRP1,2,3 - organic anions, GSX conjugates
- cMOAT - canalicular multispecific organic anion transporter = MRP2?
- cBAT - canalicular bile acid transport

Major Facilitator Superfamily

- POT - proton coupled oligopeptide transporter
- NT - Na+ coupled nucleotide transporter
- NTCP - N+ coupled taurocholate protein
- OATP - polyspecific organic anion transport protein
- OAT-K1 - renal methotrexate transporter
- OCT - organic cation transporter - electrogenic
- RFC - reduced folate carrier
- sGSHT - glutathione conjugate transporter

Nucleotide Transporters of Mammalian Cells



From: C.E. Cass, in Ref. 31, Fig. 3, p. 413.

Nucleoside Drug Transporters

Adapted from:C.E. Cass (Tables 1-4) in Ref 31.

Cladribine (Cl-dAdo)	Leukemia	es, ei, N1, N5
Cytarabine (araC)	Leukemia	es, ei
2-Fludarabine (F-araA)	Leukemia	es, N1, N5
Pentostatin (dCF)	Leukemia	es
Floxidine (F-dUrd)	Colon Cancer	es, ei
Didanosine (ddI)	HIV	es, NB
Zalcitabine (ddC)	HIV	es, N2
Zidovudine (AZT)	HIV	N2
Acyclovir (ACV)	HSV	NB
Gancyclovir (GCV)	HSV	es, NB
Vidarabine (araA)	HSV	es, ei, N1
Idoxuridine (IdUrd)	HSV	es
Trifluridine (F3-dThd)	HSV	ND
Ribavirin (RBV)	RNA/DNA	ND

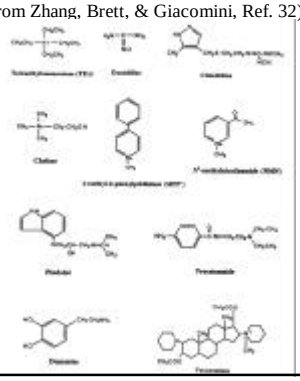
Drug Adsorption, Distribution, Tissue Uptake, and Elimination

- Gastrointestinal absorption
  - lumenal uptake into enterocyte (apical)
  - release to blood stream (basolateral)
- Hepatic clearance
  - sinusoidal uptake into hepatocyte
  - secretion of metabolites into blood
  - secretion into bile canniculi
- Renal tubular secretion (and resorption)
  - uptake from blood stream (basolateral)
  - release to lumen (apical)

- Tissue uptake to site of action
  - cell membrane uptake (and secretion)
  - uptake into subcellular compartment
- Blood Brain Barrier
  - uptake from blood stream into endothelial cell
  - secretion into brain interstitial space
- Blood Cerebrospinal Fluid Barrier - choroid plexes
  - uptake from extravascular fluid into epithelium (basolateral)
  - release to ventricle (apical)

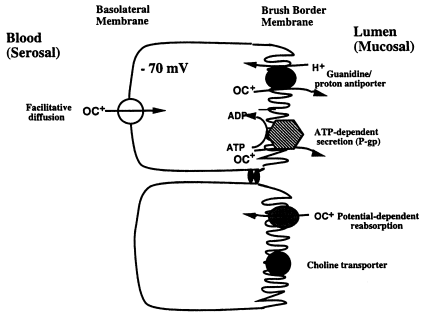
Structures of Organic Cations

(From Zhang, Brett, & Giacomini, Ref. 32)



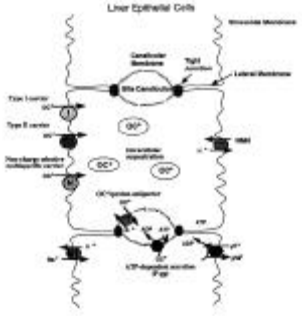
Gastrointestinal Transporters

(Modified from Zhang, Brett, & Giacomini, Ref. 32)



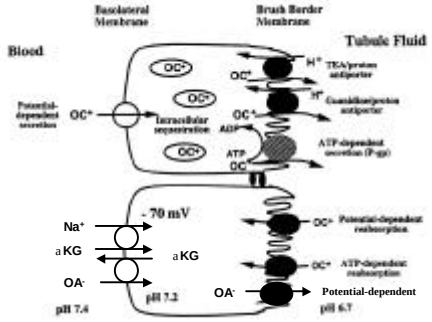
Hepatocyte Transporters

(Modified from Zhang, Brett, & Giacomini, Ref. 32)

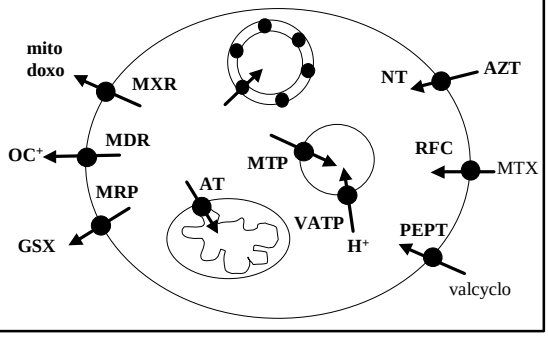


Renal Tubule Transporters

(Modified from Zhang, Brett, & Giacomini, Ref. 32)

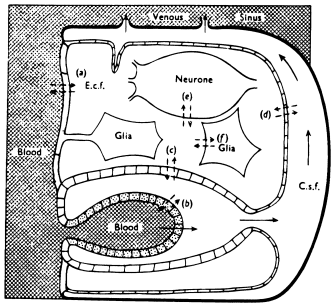


Tissue Uptake and Intracellular Drug Transport



Blood-Brain and Blood-Cerebrospinal Fluid Barriers

(From H. Dawson & M. Segal, Ref. 38).



Exploiting Nutrient Transporters to Enhance Drug Bioavailability (Ref. 43)

- Valacyclovir is an amino acid ester prodrug of the antiviral drug acyclovir.
- Oral biovailability (AUC) is increased in humans 3-5x.
- Intestinal permeability in a rat perfusion model is increased 3-10x. Effect is specific (SAR), stereospecific (L), saturable, and inhibitable by PEPT1 subsrates (cephalexin, dipeptides), and by gly-acyclovir, val-AZT.
- Competitive with ^{3}H-gly-sarc in CHO/hPEPT1 cells.
- Enhanced, saturable, inhibitable mucosal to serosal transport demonstrated in CACO-2 cells and accompanied by hydrolysis. Serosal to mucosal transport is passive.

Drug Interactions & Drug Transport

- Digoxin - cardiac glycoside, narrow therapeutic index, substrate for PgP
  - Verapamil - Ca channel blocker co-therapy for arrythmia. Increases plasma digoxin [60-90%], reduces renal clearance, apparently through inhibition of tubular PgP (44).
  - St. John's wort (Hypericum perforatum) decreased digoxin AUC by 25% after 10 days treatment through induction of PgP (45).

Drug Resistance Reversal Agents

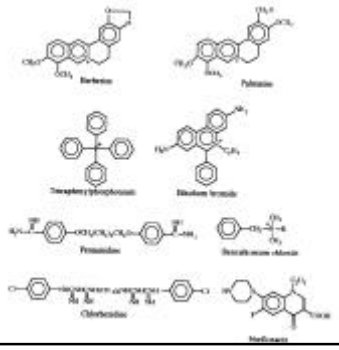
- valspodar is a PgP modulator under development by Novartis Pharma AG (46).
  - Steady state digoxin therapy was established in normal healthy volunteers (1 mg then 0.125 mg/day). Initiation of valspodar (400 mg followed by 200 mg twice per day) caused immediate and progressive increases in digoxin AUC (+211%) and decreases in total body, renal, and non-renal clearance (-67%, -73%, -58%) after 5 days.

Microbial Drug Transport and Resistance Mechanisms

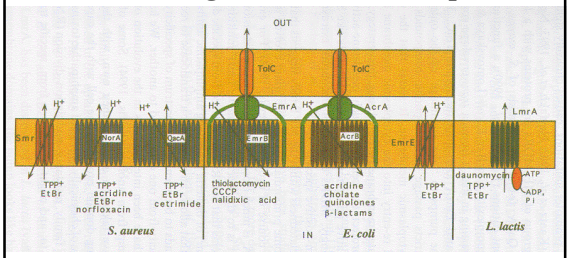
- Mechanisms of Drug Uptake in Bacteria
  - OM porins, periplasmic binders, and IM pumps
  - B-lactam channels - imipenem resistance
  - siderophore uptake is a drug delivery target
- Mechanisms of Drug Efflux in Bacteria
  - Major facilitatory (MF) family
  - RND family (AcrAB, EmrAB, TolC)
  - SMR (small multidrugresistance pumps)
  - ABC (ATP binding cassette) family

Structures of MDR substrates

(From K. Lewis, Ref. 45).



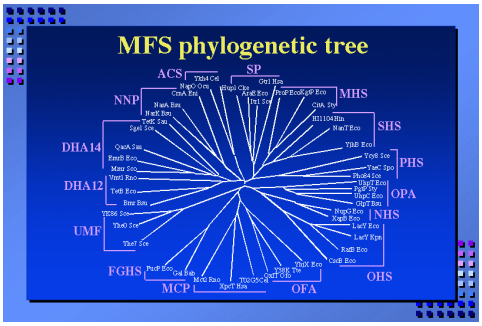
Topology Models of Microbial Multidrug Resistance Pumps



From: K. Lewis, Ref. 47, Fig. 2.

Pharmacogenomics of Transport

- Bacterial, Protozoal, and Plant Genomes
  - classification of gene transporters families
- Human Genome
  - EST databases
  - complete genome mapping and sequencing
- Bioinformatics
  - Structural Genomics
- Functional Genomics
  - High throughput screening



http://www.biology.ucsd.edu/~ipaulsen/transport/findings.html

Pharmacogenetics of Transport

- Misappropriated as term used for genetics of bacterial drug resistance.
- Polymorphism detected in p-glycoprotein in normal versus drug selected cell lines.
- RFLP predicts ivermectin neurotoxicity sensitive P-gp-deficient mice.
- hCNT1 (SPNT) from kidney and hCNT2 (small intestine) are identical except R75S.