Simulation of Membrane and Cell Culture Permeability and Transport

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Simulations Plus, Inc.

GPEN, Kansas, 2006

Outline

- Models of microscopic membrane kinetics
- Calculation of membrane entry and exit kinetics.
- Simulation of passive permeability
- Application to efflux transporter Pgp

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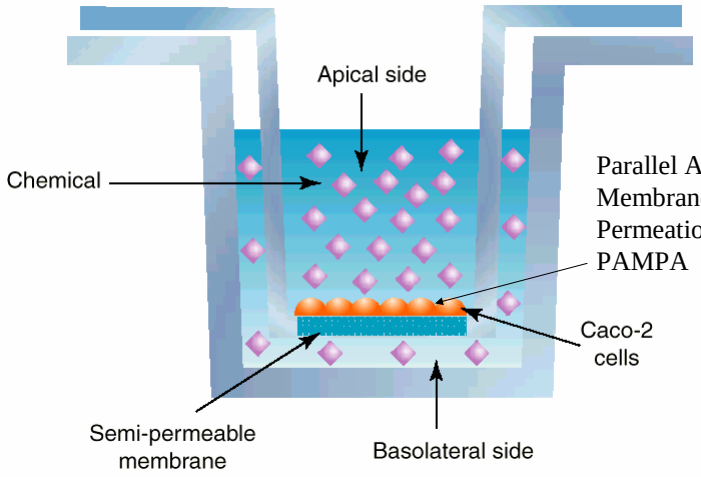
Epithelial Cell Permeability Assay



Li, A.P., DDT, 6(7):339-348

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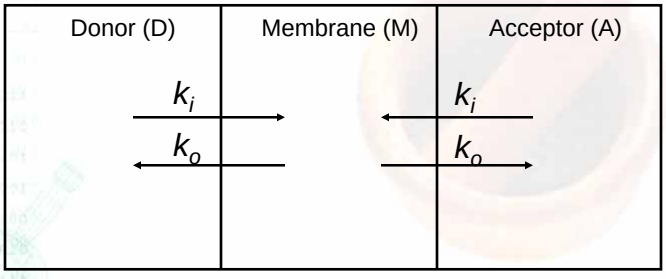
Simplest model

only the partitioning in and out of the membrane
no concentration gradients
the concentration equilibrium in each compartment reached instantaneously



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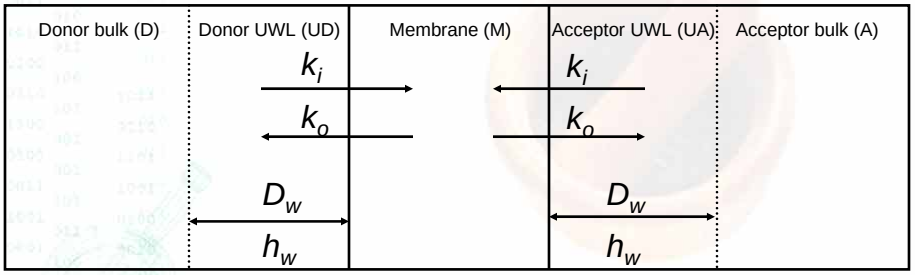
Extended model 1

Addition of diffusion through unstirred water layer (UWL) in donor and acceptor compartments concentration equilibrium in bulk aqueous phases and membrane - instantaneous



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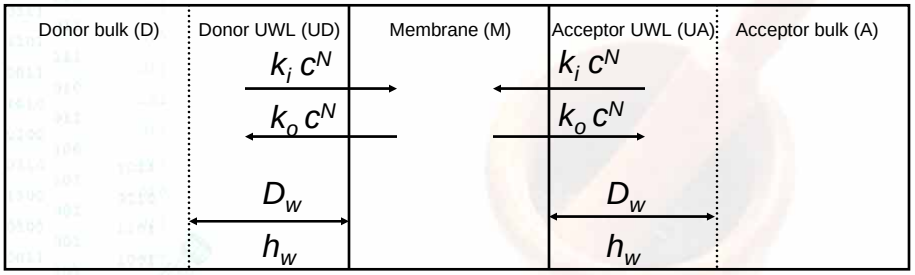
Extended model 2 - model currently in use

Addition of ionization of compounds

Assume the diffusion through the aqueous phases is the same for neutral and ionized species partitioning into the membrane - only non-ionized species.



$$c_x^N = c_{Ux} \frac{1}{1+\sum_{r=1}^{j} 10^{\sum_{s=r}^{j} pKa_s^b - (j-r+1)\times pH} + \sum_{p=1}^{i} 10^{p\times pH - \sum_{s=1}^{p} pKa_s^a}}$$

c_U-total concentration of the drug in the membrane vicinity
c^N-concentration of un-ionized species
a and *b* - acidic and basic pKa
i and *j*- number of acidic and basic pKas
x - acceptor or donor (pH in acceptor and donor compartments may be different)

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Membrane Composition

- Kansy, M. et al., J. Med. Chem. 41(7):1007 (1998)
  - 1-20% phosphatidylcholine (PC) in hexadecane
- (BAMPA) (Sugano K., et al., Int. J. Pharmaceut. 228:181 (2001)
  - biomimetic lipid composition similar to intestinal brush border membrane.
  - PC (0.8%)
  - Phosphatidylethanolamine (PE, 0.8%)
  - Phosphatidylserine (PS, 0.2%)
  - Phosphatidylinositol (PI, 0.2%)
  - Cholesterol (CHO, 1.0%)
- Collander R. Acta. Chem. Scand., 5:774 (1951)
  - $\log P_{PC}/\text{water} = \log(\alpha) + \beta \log P_{\text{octanol/water}}$
  - with parameters: α=15 and β=0.73 and $R^2 \approx 0.73$

Membrane Kinetics

Prediction of rate constants k_i and k_o for partitioning to and out of the membrane. Using the relationship and parameters (γ =0.48 and δ =0.286) from Kubinyi:

$$k_i = \frac{\gamma P}{\delta P + 1}; \quad k_o = \frac{\gamma}{\delta P + 1}$$

$\log k_1 = aN - \log(\beta' 10^{aN} + 1) + b'$ (Eq. 8)

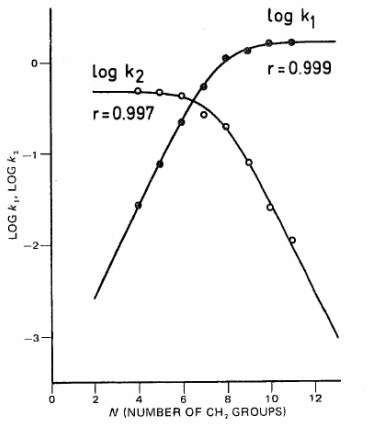
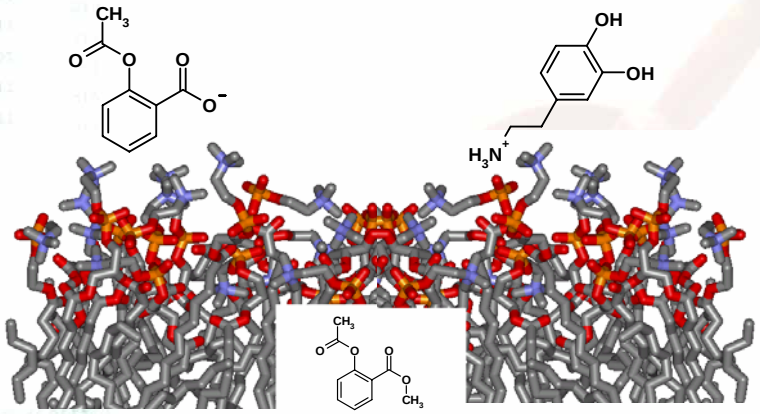


Figure 2—*Rate constants* k_1 *and* k_2 *of the partitioning of homologous quaternary alkylammonium bromides; comparison of experimental values from a three-compartment system (sodium bromide added) (2) and values calculated from Eqs. 8 and 9 (a, b, β, and c values from Table II).*

Kubinyi, H., J. Pharm. Sci. 67:262 (1978)

Passive Permeability

Transcellular



f (Hydrophobicity, H-Bonding, Ionization)

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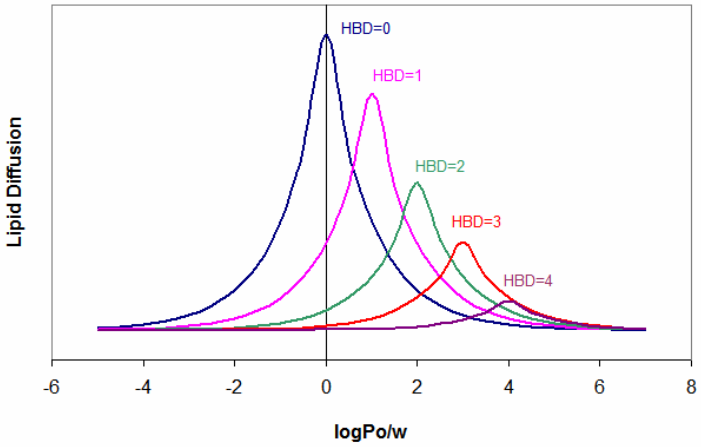
Theoretical Lipid Diffusion as a function of log P and HBDs



$LD = LD_0 + LD_{max} * \exp(-abs(HBD - logPo/w))$

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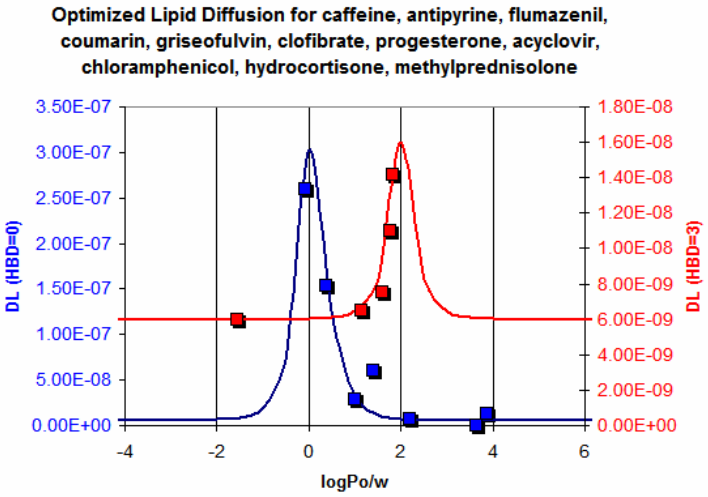
Optimized Lipid Diffusion



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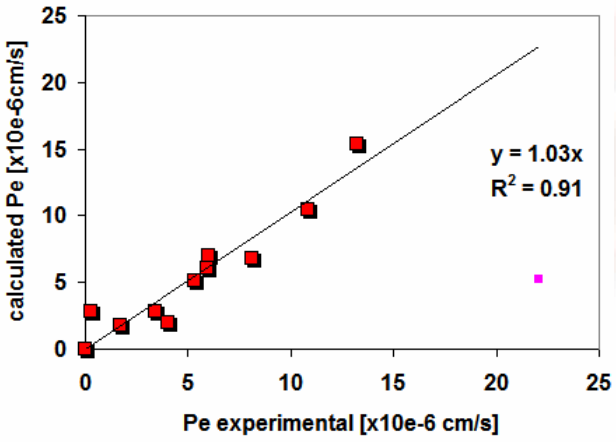
Pe calculated using DL modeled by theoretical relationship between DL and logPo/w for HBD=0 and HBD=3



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Membrane Accumulation for high log P molecules.

Dvorsky-Balaz J. Theor. Biol.185:213 (1997)

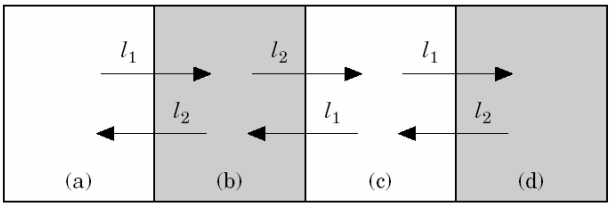

FIG. 1. The schematic outline of the system consisting of four alternating aqueous [white (a, c)] and lipoid [grey (b, d)] phases. The lipoid phases represent a membrane and either the hydrophobic core of a globular protein or further membrane. The transport rate is characterized by the rate parameters l, the subscripts indicating the direction of transport (1—from water to a lipoid phase and 2 backwards).

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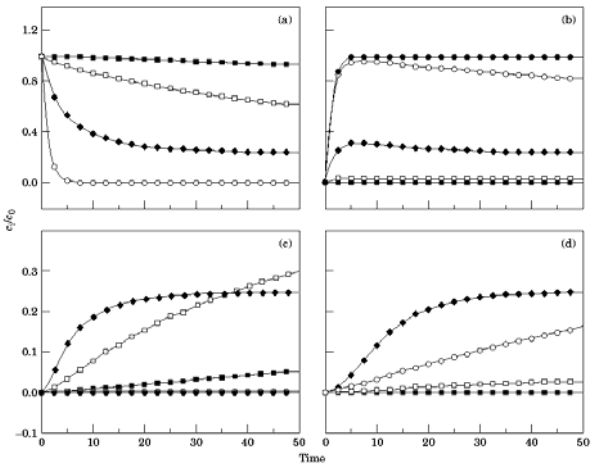
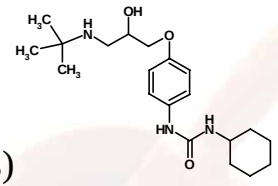


FIG. 2. The transport kinetics of the compounds differing in their partition coefficient P in individual compartments (Fig. 1) indicated by (a)–(d) in the corners of the insets. Calculated from eqn (3) using eqn (5) with the constants $\gamma = 0.245$ and $\delta = 0.286$ (Kubinyi, 1978). Individual curves hold for log $P = -2$ (■), -1 (□), 0 (◆), 2 (◇), and 4 and more (●). In (a), the last two curves are overlaid, the first and the last curve are overlaid in (d).

Talinolol



- log $D_{9.4}$ = 3.14 (Langguth)
- Basic pKa = 9.8 (QMPRPlus)
- Composite Pgp K_m = 412 μM (149 μg/mL)
  - Two sites high (72 μM) and low (1570 μM) (Langguth)
- Solubility = 1.23 mg/mL (pH = 7.4) (Gramatte)
- $Peff_{rat}$ = 0.5 x 10^{-4} cm/s (Langguth)
- $Peff_{QMPR}$ = 1.68 x 10^{-4} cm/s

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Image J Analysis of Pgp Micropig

H. Tang, AAPS Annual meeting poster, 2002

RT-PCR expression of mRNA

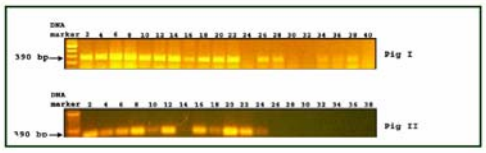
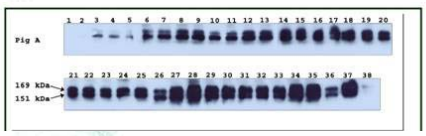
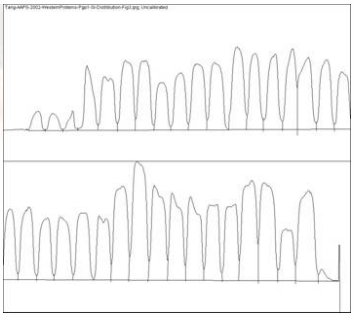


Fig 2. RT-PCR detection of *p-gp* 1 expression in two pigs. The even alternating numbers from 2 to 40 indicate segments from the proximal duodenum to the distal ileum.





Western blot of Pgp-1 from proximal to distal SI

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Mouly, S., Paine, M.F. PharmRes-20(10):1595-1598 (2003)

Pgp expression in human SI

P-gp Expression along the Human Small Intestine

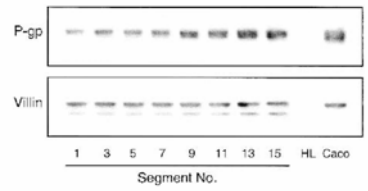

Fig. 1. Western blot showing the expression of P-glycoprotein and the control protein villin along the length of a human donor small intestine (HI-31). Segment 1 represents duodenum; segments 3, 5, and 7 represent middle to distal jejunum; and the remaining segments represent ileum. All segments measured approximately 30 cm in length. For a given intestine, the same blot was probed for both proteins, but optimal visualization of each required a different exposure time. HL, human liver homogenate. Caco, homogenate prepared from a representative Caco-2 cell monolayer treated with the CYP3A4/P-gp inducing agent $1\alpha,25\text{-}(OH)_2\text{-}D_3$. All lanes of the gel were loaded with 15 μg homogenate protein.

Table II. Relative P-gp Expression* Along the Length of Four Human Donor Small Intestines

Segment†	Donor code‡ HI-31	HI-32	HI-35	HI-40
Duodenum/proximal jejunum				
1/2	0.51	0.63	0.33	0.68
Middle to distal jejunum				
3/4	0.61	0.61	0.51	0.85
5/6	0.61	0.81	0.50	0.99
7/8	0.70	0.72	0.57	0.88
Ileum				
9/10	0.89	0.72	0.50	0.97
11/12	0.92	0.74	0.69	1.00
13/14	0.86	0.73	0.80	0.91
15/16	1.00	0.86	0.59	
17/18		1.00	1.00	
20		0.89		

* P-gp/villin IOD ratio (normalized to the maximum value).
† Each segment measured approximately 30 cm in length.
‡ HI, human intestine.

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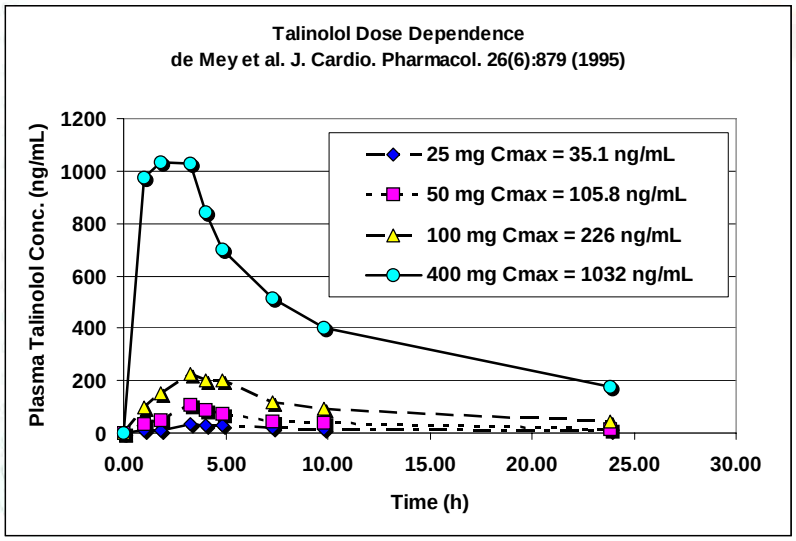
Talinolol Non-linear Dose Dependence



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Regional Window for Absorption

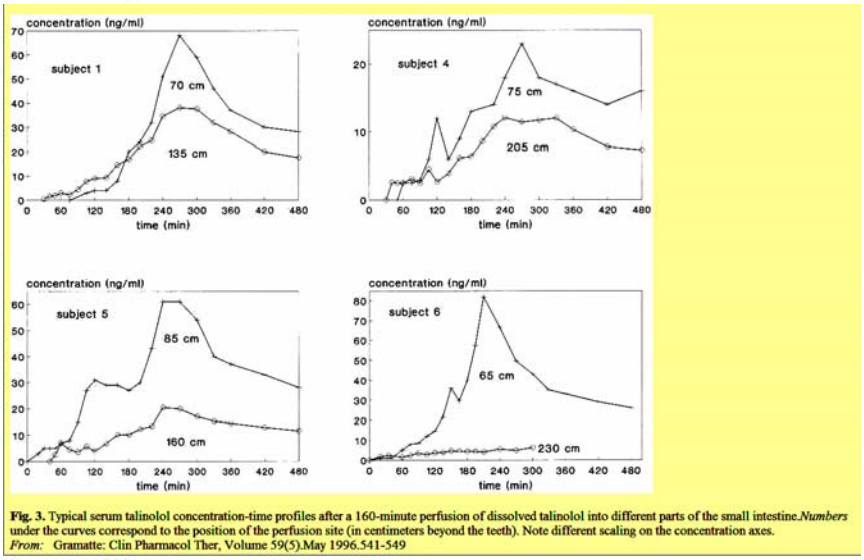

Fig. 3. Typical serum talinolol concentration-time profiles after a 160-minute perfusion of dissolved talinolol into different parts of the small intestine. *Numbers* under the curves correspond to the position of the perfusion site (in centimeters beyond the teeth). Note different scaling on the concentration axes.
From: Gramatte: Clin Pharmacol Ther, Volume 59(5).May 1996.541-549

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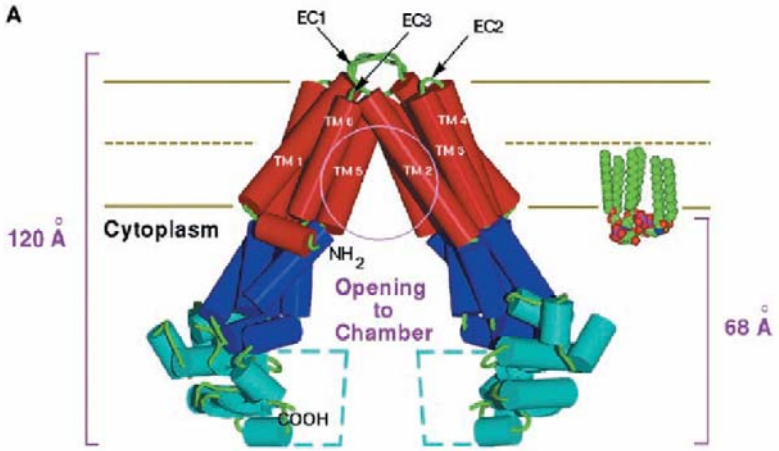
ATP-Binding Cassette Protein Msb (1JSQ) from E. Coli
Chang, Science 293:1793 (2001)



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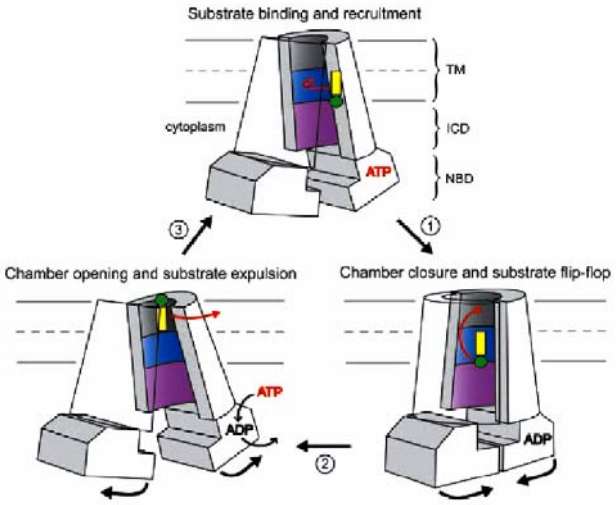

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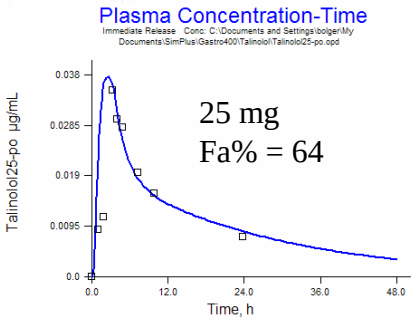
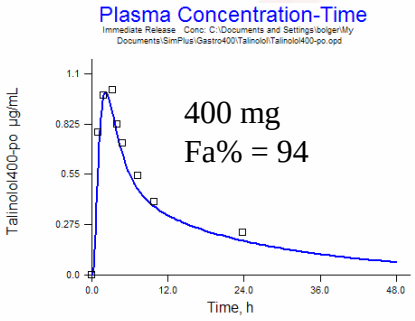
25 and 400 mg Talinolol *po*

Data from: de Mey, C., J. Cardiovasc. Pharmacol. 26(6):879 (1995)
courtesy of Peter Langguth and Daniel Wagner





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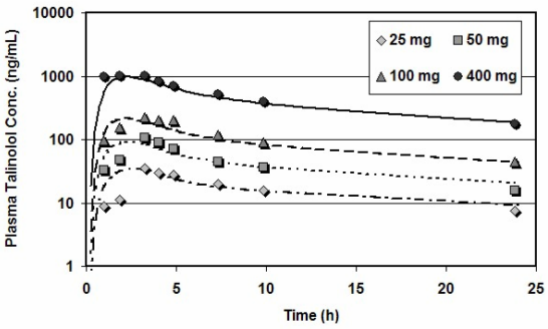
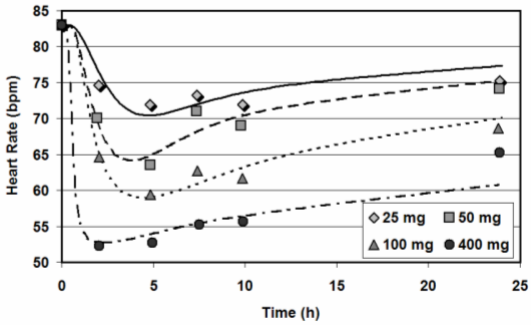


GastroPlus PK PD - Simulation of Talinolol Effect on Heart Rate

Tubic M, 2006 in press.



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Conclusions

- Membrane and cellular simulations can improve our understanding of absorption.
- Membrane concentration is important for calibration of *in vitro* transporter kinetics.
- The present state-of-the-art provides useful (not perfect) simulations.

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