Examining the interaction of drugs and membranes to improve selectivity

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The pharmacologist's and toxicologist's key question …

• What do we know exactly about membranes (vs. proteins)
  – composition and lipid-distribution heterogeneity
  – structure and micro/macrostructure – influence from and on proteins
  – fusion and rupture

• Drug disposition: membranes and penetration/efflux
  – the case of two fluoroquinolones

• Drug toxicity toxicity
  – the case of aminoglycosides, azithromycin, and lipoglycopeptides

• Drug activity activity
  – the case of new anti Gram (+) antibiotics (daptomycin, telavancin, … and lantibiotics) and a few words on rafts as drug targets
Heterogeneity of lipid distribution among organelles: the case of cholesterol macro-distribution

Fig. 1. PEG-Chol Labels the Golgi Apparatus in Human skin Fibroblasts

Cells were fixed, permeabilized and triply labeled with iPEG-Chol (green), anti-TGN46 (Golgi, red), and TOPRO-3 (nucleus, blue). The lower right figure shows the merge. Bar, 20 μm. Golgi labeling by PEG-Chol indicates the accumulation of cholesterol in the organelle in human skin fibroblast. Photograph courtesy of Kumiko Ishii.

Membrane microheterogeneity: role of P-glycoprotein and impact on cholesterol trafficking

**Figure 5.** Functional scheme of P-gp involvement in cellular cholesterol trafficking. In cells, according to an oversimplified view, the cholesterol amount is regulated between endogenous cholesterol biosynthesis and esterification in the ER, an exogenous cholesterol import from LDL by endocytosis and a cholesterol export to HDL [133, 134]. The active cholesterol flux mediated by P-gp from the cytosolic to the exoplasmic leaflet of the plasma membrane supports a role for P-gp in cholesterol enrichment of rafts and caveolae, leading to increased integration of caveolin-1 in plasma membrane, and possibly to upregulation of other steps in intracellular cholesterol trafficking. (ER, endoplasmic reticulum; TGN, trans-Golgi network.)
How lipids influence proteins structure and organization...

Possible consequences of hydrophobic mismatch for protein structure and organization. The green cylinder represents the hydrophobic part of a membrane protein. (a) Positive mismatch by itself would lead to exposure of hydrophobic groups to a hydrophilic environment at the interface. Possible adaptations are (b) tilting of transmembrane segments to reduce their effective length, (c) self-association, (d) changes in backbone conformation or (e) changes in the orientation of the sidechains. (f) In multicomponent systems, transmembrane segments that are too long might partition into thicker domains.

*Current Opinion in Structural Biology* 2006, 16:473-479

www.sciencedirect.com
How protein influence membrane micro-organization

Possible mismatch-induced effects of proteins on lipids are (a) stretching of lipids under conditions of positive mismatch, (b) disordering of the lipid acyl chains and formation of non-lamellar structures under conditions of negative mismatch, and (c) sorting of lipids by recruitment of lipids with the best-matching length from mixtures of lipids.
Lipid rafts are defined as specialized, dynamic microdomains that can be found in plasma membrane, and they are enriched with cholesterol and sphingolipids.

What are rafts and how are they formed?

Figure 3: Schematic representation of biomembrane rafts emphasizing the asymmetry of the rafts in the two monolayers, which have different sizes (and probably different lifetimes). Both monolayers have liquid-ordered phases with cholesterol but the phospholipids interacting with cholesterol are different and hence induce slightly different L_o phases. This figure suggests that superposition of rafts in two monolayers requires a coupling via a transmembrane protein. The association can be also fortuitous and is likely to be temporary.

How proteins may create lipid microdomains (rafts)

**Figure 1: Lipid rafts.** Proteins with a high affinity for selected lipids are suggested to form lipid shells (9). It remains to be determined whether shells can already represent a functional unit or whether larger (raft) structures are required to generate functional domains. Protein–protein interactions between shell proteins can create larger functional units called lipid rafts (A). Dual-acylated proteins (including GPI-anchored proteins) can associate with pre-existing lipid rafts based on their low affinity for raft lipids of the acyl moiety. This affinity can be enhanced by lipid-protein and/or protein–protein interactions (B). By oligomerization of low-affinity raft proteins, enough low affinity lipid-interacting moieties may be combined to stabilize a functional raft domain (C). Under these conditions the oligomerization process may create and stabilize raft domains.

*Traffic* 2004; 5: 247–254
Various views and hypotheses for rafts

specific associations with "raft" proteins

mosaic of domains
(with cholesterol-driven partition)

oscillations from "monomeric" to "assembled" structures

actively maintained structures

pre-existing organization

induced 'rafts'

Mayor & Rao Traffic. 2004 Apr;5(4):231-40
Rafts and membrane movements

Figure 2: Multiple pathways of endocytosis. Different types of invaginations occur at the plasma membrane to mediate membrane endocytosis. Many surface receptors are internalized into coated pits by a clathrin-dependent pathway. Clathrin-independent, raft-dependent internalization pathways are distinct in caveolae and noncoated invaginations, but both are dynamin-independent and require different small GTPases. All pathways lead to the endosomal compartment, while caveolae fuse with another sorting compartment known as the caveosome. The relationship (if any) between the early endosomal compartment and the caveosome is not yet understood.

Rafts and virus budding

FIG. 3. Model of HIV-1 assembly and budding through membrane rafts. gp160 trimerizes within the ER and, on reaching the TGN, associates with rafts because of its affinity for lipid rafts. It then migrates to the plasma membrane. Pr55\(^{\text{gag}}\) and Pr160\(^{\text{gag-pol}}\) oligomerize around two genomic RNAs and associate simultaneously with plasma membrane rafts due to the anchoring myristate and intrinsic properties of the MA domain. This allows the binding of MA to the cytoplasmic tail of glycoproteins. The cytoplasmic Nef protein, after palmitoylation, associates with the inner leaflet of the plasma membrane raft. The raft coalescence results in Nef incorporation into HIV-1 particles and in the enrichment of the envelope in lipid rafts. Then HIV-1 matures (cleavage of Gag precursors in MA, CA, NC, p6, and enzymes) and buds from the plasma membrane rafts. Nef protein is initially bound to membrane rafts. When encapsidated into HIV-1 particles, Nef is partly cleaved off by the viral protease into a soluble domain, which is thought to bind to the RNP. Membrane rafts are represented as shaded grey regions within the lipid bilayer.
Membrane fusion: the role of lipids

Membrane fusion: mechanisms

Separated Vesicles  Aggregated Vesicles  Initiation of Fusion  Fusion

proteins play a key role in nearing membranes (viz. viruses)

but lipids do the job …

role of gp41 helical regions (HRs) to near viral and host cell membranes.

Cervia & Smith, Clinical Infectious Diseases 2003;37:1102-1106
Ultrastucture of cell secretion ...

Fig. 2  AFM images (a and b) show dilation of the porosome to allow expulsion of vesicular contents. Section analysis through one of the porosomes in (a) and (b) show enlargement of the porosome following stimulation of secretion. (c) Exposure of live pancreatic cells to gold conjugated-amylase antibody, results in specific localization of gold to the edge of the porosome. (d) AFM micrograph of a stimulated, and fixed, pancreatic cell showing a pit (yellow arrowhead) with immunogold localization of amylase specific antibody (blue arrowhead) associated with the porosome [19]. AFM images courtesy of Dr. Bhanu P. Jena.
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• **Drug disposition: membranes and penetration/efflux**
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• **Drug toxicity toxicity**
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Antibiotic acting intracellularly...

Question: what is the importance of influx and efflux in overall activity
(modified from Tulkens, 1991; Carrayn et al., 2003)
Example of intracellular bacteria

The intracellular pathway of *Listeria monocytogenes* ...

A-C: control
D-E: with gamma-interferon

"In and out" of closely related fluoroquinolones in macrophages

"In and out" of closely related fluoroquinolones in macrophages


Drugs and membranes Munich, Germany - 7 Feb 2006
Fluoroquinolone model of penetration and efflux in macrophages
What if you diffuse faster
(and are not recognized by efflux transporters?)

• You get a higher cellular accumulation
  – ↙ activity of moxifloxacin against intracellular *Listeria*, *S. aureus*, etc...

• Bacterial and eucaryotic efflux share many similarities
  – ↙ activity of moxifloxacin against pneumococci, *Listeria*, and all organisms with efflux-mediated resistance

• Combination of both ...
Cooperation between procaryotic and eucaryotic efflux pumps

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<th>wild-type bacteria</th>
<th>resistant bacteria</th>
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Lismond et al. ICAAC 2006 – poster A1108
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Aminoglycoside toxicity...

From: Tulkens, 1986 Amer. J Med. 80(Suppl 6B);105-114
Aminoglycoside intracellular pathway ...

Sandoval & Molitoris, Am J Physiol Renal Physiol 286: F617-F624, 2004
Aminoglycoside bind to lipid bilayers …

Perturbation of intracellular traffic by aminoglycoside binding to bilayers ...

Control, 37,500X

+ Gentamicin, 37,500X

+ Gentamicin, 85,500X


Intralysosomal gentamicin disrupts lysosomes...

Fig. 4. Appearance of acridine orange-loaded LLC-PK1 cells in confocal microscopy. Cells were exposed to acridine orange (5 μg/ml) for 15 min and then returned to control medium for 3 h (A, B), or exposed to gentamicin (C and D, 3 mM, 3 h; E, 2 mM, 4 h) or MSDH (F, 25 μM, 3 h).

Is the lysosomal membrane specifically sensitive to gentamicin-induced disruption?

Calcein-release experiments

liposome composition and pH conditions mimicking the
- ■ lysosomal membrane
- □ outer mitochondrial membr.
- △ inner mitochondrial membr.

% of calcein released

hours

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Aminoglycoside-induced lysosomal destabilization and perturbation of traffic causes apoptosis in kidney and renal cells …

Morphological changes in rat renal cortex (A,C,D) upon treatment with gentamicin at low doses (10 mg/kg; 10 days) and in cultured LLC-PK1 renal cells (B) upon incubation with gentamicin (under conditions causing a drug accumulation similar to that observed in rat renal cortex of the animals treated as indicated in A, B, and C [approx. 10 µg/g;]

Servais et al. In: Toxicology of the Kidney (Target Organ Toxicology Series), 2004, chap. 16, pp 635-685.
Aminoglycoside-induced apoptosis ...

Brutal release of gentamicin in the cytosol

Azithromycin-induced phospholipidosis

Ultrasound alterations observed in cultured fibroblasts maintained with 0.03-0.1 mg/L of azithromycin for 7 to 16 days.

Biochemical studies show a predominant accumulation of phosphatidylcoline (no marked excess in cholesterol)

Azithromycin-induced modulation of membrane fluidity

Use of giant liposomes

Micropipet experiments:

$k_c = \text{bending modulu (energy necessary to bend the bilayer)}$

Relative increase of the apparent surface

- in azithromycin solution
- without azithromycin

Fa et al. Biophysical Society 49th Annual Meeting, 2005
Azithromycin-induced modulation of membrane microstructure

Use of atomic force microscopy to detect changes in membrane surface

AFM on DOPC:DPPC 1:1 bilayers:

DPPC gel domains (white) in DOPC fluid matrix (dark); height difference: 1.10±0.05 nm

Addition of azithromycin + 60 mn: only one uniform fluid phase visible

Current interpretation:

Azithromycin inserts in bilayers and increases lipid mixing

Fa et al. Biophysical Society 49th Annual Meeting, 2005
Oritavancin-induced lipid storage

cells incubated at clinically meaningful concentrations
Oritavancin-induced lipid storage

c-co-accumulation of phospholipids and cholesterol

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Daptomycin...
Daptomycin...

- Step 1: Calcium-dependent PG binding/insertion
- Step 2: Oligomerization (micelle formation)
- Step 3: Membrane distortion and ion leakage, depolarization
- Step 4: Lethal downstream events

PG = phosphatidylglycerol negatively-charged and abundant in procaryotic cells

J. Silverman, 45th ICAAC, 2005
Daptomycin: model 1

Interaction via the acyl chain and binding to PG

Integration in membrane facilitated by Ca^{++}

Aggregation

Pore formation and bacterial death

Daptomycin: alternate model

a) oligomerization in presence of Ca^{++}
b) insertion in membrane
c) drug-induced change in membrane curvature
d) leakage and bacterial death

e) Strauss & Hancock, Biochim. Biophys. Acta 2006; 1758:1215-1223
Telavancin: a membrane destabilizing derivative of vancomycin

Telavancin is a membrane destabilizing derivative of vancomycin. It contains a lipophilic side chain and a polar group. This causes an increase in bacterial membrane permeability. The classical pharmacophore of glycopeptides is highlighted. A membrane permeability assay was performed, showing that telavancin has a higher permeability effect than vancomycin.

Televancin dual mode of action?

Classical mode of action

Membrane destabilizing mode of action

3 h kill curves extracellular bacteria

Why is telavancin specific of bacteria?

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Why is telavancin specific of bacteria?

Cytoplasm    Membrane    Cell surface

D-Ala + D-Ala
Ligase ATP
D-Ala - D-Ala
D-Ala - D-Ala
ATP
UDP-L-Ala-D-Glu-L-Lys-L-Ala-D-Ala
MurF ATP
UDP-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala
pentapeptide

Binding site for the "vancomycin" pharmacophore

Binding site for the "membrane destabilizing" pharmacophore

Lipid II

UDP - L-Ala - D-Glu - L-Lys - D-Ala - D-Ala
UDP - L-Ala - D-Glu - L-Lys - D-Ala - D-Ala
UDP - L-Ala - D-Glu - L-Lys - D-Ala - D-Ala
UDP - L-Ala - D-Glu - L-Lys - D-Ala - D-Ala
UDP - L-Ala - D-Glu - L-Lys - D-Ala - D-Ala
UDP - L-Ala - D-Glu - L-Lys - D-Ala - D-Ala
D-Ala - D-Ala
Ligase ATP
D-Ala - D-Ala
D-Ala + D-Ala

Drugs and membranes Munich, Germany - 7 Feb 2006
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**Fig. 1.** Lipid II segregation caused by lantibiotics visualized by fluorescence microscopy. (A) Nisin in action. After the addition of 2 to 4 μl of a 2 mM nisin solution, the peptide started to diffuse into the field of view from the bottom right corner. This image is a snapshot of three vesicles that have either not yet encountered nisin (vesicle a), just encountered nisin (vesicle b), or already been exposed to nisin for some time (vesicle c). Scale bar, 60 μm. (B) GUV doped with NBD-labeled lipid II before treatment with mutacin 1140. (C) Mutacin 1140–induced segregation of NBD-labeled lipid II. (D) Snapshot of a GUV treated with mutacin 1140 just at the onset of the lipid II segregation; the interior remains black. The green fluorescence of the NBD-labeled lipid II and the red fluorescence of Texas Red were sequentially detected with the use of two lasers.
Lantibiotics...

**Fig. 2.** Nisin segregates lipid II into nonphysiological domains in vivo. (A) GUV containing 0.5 mole percent wild-type lipid II 15 min after the addition of fluorescently labeled vancomycin. (B) *B. megaterium* cells that were incubated for 10 min with labeled vancomycin (2 μg/ml). The arrows point at newly formed division sites or older exemplars. (C) *B. subtilis* stained with fluorescent vancomycin (4 μg/ml). (D) *B. megaterium* cells after incubation for 10 min with fluorescein-labeled nisin (0.5 μg/ml). The arrow marks where the bacterium has already divided. (E) *B. subtilis* cells after incubation with fluorescein-labeled nisin (4 μg/ml). The bottom image in (E) and the insets in (B) to (D) show Nomarski images.
One step ahead: targeting lipid A

Eritoran

- LPS inhibitory activity manifested via down-regulation of the intracellular generation of pro-inflammatory cytokines IL-6 and TNF-alpha in human monocytes. 

- inhibition of TLR4 with eritoran in an in situ murine model significantly reduces MI/R injury and markers of an inflammatory response.
  (Shimamoto et al. Circulation. 2006 Jul 4;114(1 Suppl):I270-4)
Another step ahead: targeting rafts

- Toxins, bacterial-, and viral-pathogens exploit cholesterol and/or lipid rafts to gain a foot hold in their target hosts…

- Statins cause lipid raft disruption by impairing cholesterol synthesis. Since lipid rafts have been implicated both in antigen internalization, antigen processing and presentation may be a selective target of statins. (Ghittoni et al Eur J Immunol. 2006 Nov;36(11):2885-93).

- Inhibition of sphingolipids biosynthesis may be a new approach to treatment of hepatitis C (since the virus replication complex resides in in rafts) (Sakamoto et al. Nat Chem Biol. 2005 Nov;1(6):333-7.)

- Association of the serotonin transporter with lipid rafts may represent a mechanism for regulating serotonergic signaling in the central nervous system, through the modulation of the cholesterol content in the cell membrane… (Magani et al., J. Biol. Chem. 2004 Sep 10;279(37):38770-8)
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- Modulation of rafts to which P-glycoprotein is associated may also modulate resistance to P-gp-effluxed drugs
Conclusions

Lipids are potential drug targets …

1. for modulation of transport…

2. for toxicity …

3. for activity …

Considerably more systematic research is warranted…