Are intracellular drug concentrations relevant for efficacy?

A discussion about accumulation, efflux and activity …

Paul M. Tulkens, MD, PhD
Françoise Van Bambeke, PharmD, PhD

Cellular and Molecular Pharmacology
Catholic University of Louvain
Brussels, Belgium

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A simple figure ...

First statements …

• If a drug does not accumulate, it cannot be active …

Quick answer:
this is correct if you mean "it does not get in cells at all…"

More elaborate answer:
no "accumulation" does not mean that the drug is not present, and if present, it may be active if above the critical concentration for sufficient time …

Experimental evidence:
β-lactams, known for "no accumulation" are active against intraphagocytic L. monocytogenes and S. aureus if their extracellular concentration is large enough… and if you let them enough time to act…
Intraphagocytic S. aureus and β-lactams


- 24 h model
- $C_{\text{max}} = 63$ mg/L (total)
Intraphagocytic *L. monocytogenes* and β-lactams

Observation …

• The activity of $\beta$-lactams is larger than anticipated…

Quick answer:
Their concentration may simply be large enough

More elaborate answer:
The intracellular milieu may favor their activity …

Experimental evidence for a potential explanation:
• Acid pH increases the activity of $\beta$-lactams against intraphagocytic S. aureus…
• We do not have it (yet) for L.monocytogenes …
Acid pH favors the activity of β-lactams …


extracellular activity

oxacillin

And acidity compensates for poor intracellular accumulation …

What about other antibiotics …

• **Aminoglycosides** accumulate (slowly) in phagolysosomes but their activity is defeated by the acid pH for phagolysosomal organisms (*S. aureus...*) $\beta$-lactams…!

They are inactive against *L. monocytogenes* (not present in the cytosol …)

• **Macrolides** accumulate … but their activity is severely defeated by the acid pH … and they are only bacteriostatic…

• **Quinolones** accumulate modestly, but their activity is maintained at acid pH … and they have access to most intracellular compartments… Yet, their intracellular activity is at most similar to their extracellular activity …
A not so simple figure ...

Activity = accumulation x bioavailability x favorable conditions

A few words about bacterial response: the case of telavancin and VRSA...

- Lipophilic side chain
- Polar group
- Classical pharmacophore of glycopeptides
- Membrane permeability assay

Telavancin causes an increase in bacterial membrane permeability at larger concentrations... 

Televancin dual mode of action?

3 h kill curves extracellular bacteria

Barcia-Macay et al., JAC, Oct 24; [Epub ahead of print]
Intracellular activity of telavancin vs. vancomycin:
- MSSA
- MRSA

24h CFU ➔ at $C_{\text{max}}$:
- vanco: $\sim 0.5$ log
- TLV: $\sim 2$ log

Barcia-Macay et al., JAC, Oct 24; [Epub ahead of print]
Intracellular activity of telavancin vs. vancomycin:

- VISA
- VRSA

24h CFU \( \downarrow \) at \( C_{\text{max}} \):
- vanco: static
- TLV: \( \sim 1.2 \) log
The picture gets a bit more complex …

Activity = accumulation x bioavailability x favorable conditions x bacterial responsiveness
Efflux from eucaryotic cells and intracellular activity
The story of the eucaryotic ABC transporters

**cationic amphiphiles**

MDR-1 (P-glycoprotein)

**anionic amphiphiles**

MRP1-10

ATP

ADP
Antibiotics as substrates of efflux pumps

Azithromycin is cationic

Ciprofloxacin is zwitterionic
How to inhibit ABC transporters?

cationic amphiphiles

MDR-1 (P-glycoprotein)

anionic amphiphiles

MRP1-10

deoxyglucose

$\text{NaN}_3$
How to inhibit ABC transporters?

cationic amphiphiles

MDR-1 (P-glycoprotein)

verapamil

GF120918
How to inhibit ABC transporters?

- Probenecid
- Gemfibrozil
- MK571

Anionic amphiphiles

MRP1-10

ATP

ADP
Differential recognition by MDR pumps

Influence of ATP-depletion and pump inhibitors on accumulation at equilibrium

**azithromycin** & **P-glycoprotein**

**ciprofloxacin** & **MRP**

Models of intracellular infection

**L. monocytogenes**
- Cytosol

**S. aureus**
- Phagolysosomes
Influence of pump inhibitors on intracellular activity

azithromycin and *L. monocytogenes*

*Seral et al (2003) JAC 51:1167-73*
Influence of pump inhibitors on intracellular activity

azithromycin and *S. aureus*

Influence of pump inhibitors on intracellular activity

ciprofloxacin and *L. monocytogenes*

Seral *et al.* (2003) JAC 51:1167-73
Influence of pump inhibitors on intracellular activity

ciprofloxacin and S. aureus

Influence of pump inhibitors on antibiotic distribution

verapamil enhances azithromycin concentration in cytosol and vacuoles

Influence of pump inhibitors on antibiotic distribution

gemfibrozil increases ciprofloxacin cytosolic content ONLY

L. monocytogenes

S. aureus

Over-expression of efflux pumps as mechanism of « resistance »

in eucaryotic cells ...

in prokaryotic cells ...

Michot et al, AAC (2006) 50:1689-95

Godreuil et al, AAC (2003) 47:704-8
Cooperation between procaryotic and eucaryotic efflux pumps

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<tr>
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<th>wild-type bacteria</th>
<th>resistant bacteria</th>
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Cooperation between procaryotic and eucaryotic efflux pumps

ciprofloxacin

To conclude ...

\[ \text{Activity} = \frac{\text{accumulation} \times \text{bioavailability} \times \text{favorable conditions} \times \text{bacterial responsiveness}}{\text{efflux}} \]