Use of cell lines to study specific aspects related to bacterial infections and antibiotic transport

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The pharmacologist's and toxicologist's key question ...

- Can you model the complexity of the bacterial infection in relation to host?
- Can you model antibiotic toxicity?
- Can you model drug cellular transport?
Are these questions important?

- intracellular infection is probably a major cause of failure of antibiotic therapy in "difficult to treat" infections (suboptimal activity);
- it may also explain (partly) the emergence of resistance (exposure to pharmacologically suboptimal concentrations);
- Toxicity is often a limitation to antibiotic usage …
- If antibiotics are not transported at their site of action, they are useless…
Intracellular bacterial infections

• Obligatory or mainly intracellular:
  – respiratory infections (pneumopathies):
    • Chlamydia pneumoniae: 10% in children
    • Legionella pneumophila: frequent if immunosuppression
    • Mycobacterium spp.: frequent if immunosuppression
  – sexually transmitted diseases
    • Chlamydia trachomatis: most common pathogen in MST
  – CNS infections + other sites:
    • Listeria monocytogenes: pregnant women; immunosuppression
      (mortality: > 30 %)

• Facultative or mainly extracellular:
  – digestive tract infections
    • Salmonella spp., Shigella spp.
  – respiratory, cutaneous, etc…tract infections
    • Streptococcus spp., Staphylococcus spp.
Activity …
Antibiotic intracellular activity?

Question: which are the pharmacokinetic and pharmacodynamic parameters governing the activity of intracellular antibiotics

(Tulkens, 1991; Carryn et al., 2003)
Is accumulation in cells the solution?

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Antibiotic</th>
<th>Differential</th>
<th>Antibiotic uptake</th>
<th>Antibiotic uptake</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I/E</td>
<td>µg/10⁷ cells</td>
</tr>
<tr>
<td>Human PMNs</td>
<td>Azithromycin</td>
<td>4.9</td>
<td>79</td>
<td>1.58</td>
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<tr>
<td></td>
<td>Erythromycin</td>
<td></td>
<td>16</td>
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<tr>
<td>Murine PMNs</td>
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<td>39</td>
<td>0.78</td>
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<td></td>
<td>Erythromycin</td>
<td></td>
<td>10</td>
<td>0.20</td>
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<tr>
<td>Murine alveolar macrophages</td>
<td>Azithromycin</td>
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<td>170</td>
<td>18.66</td>
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<tr>
<td></td>
<td>Erythromycin</td>
<td></td>
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<tr>
<td>Rat alveolar macrophages</td>
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<td></td>
<td>Erythromycin</td>
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<tr>
<td>Murine resident peritoneal</td>
<td>Azithromycin</td>
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<td>6.81</td>
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<tr>
<td>macrophages</td>
<td>Erythromycin</td>
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</tbody>
</table>

* Cells were incubated for 2 h with 10 µg of the antibiotic per ml.
* Ratio of azithromycin uptake to erythromycin uptake. All values are statistically significant.
The S. aureus problem …

Intracellular Staphylococcus aureus. A mechanism for the indolence of osteomyelitis.

Ellington JK, Harris M, Webb L, Smith B, Smith T, Tan K, Hudson M.


Intracellular persistence of Staphylococcus aureus small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease.

von Eiff C, Becker K, Metze D, Lubritz G, Hockmann J, Schwarz T, Peters G.

Institut fuer Medizinische Mikrobiologie, Westfälische Wilhelms-Universität Muenster, Muenster, Germany.


Phagocytosis of Staphylococcus aureus by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections.

Hamill RJ, Vann JM, Proctor RA.
Why does *S. aureus* have an intracellular life, and is dangerous?

Setting up the model …

\[ \Delta \log \text{CFU from time 0 h} \]

Using the model...

Screening available antibiotics

What about a new antibiotic (tigecycline and intracellular *S. aureus*…)

accumulation in PMN:
about 20-30 fold

activity in PMN:
about $1 \log_{10}$
at 1 mg/L
*S. aureus* ATCC 29213
(MIC = 0.25 mg/L)

Screening available antibiotics
Cooperation with host defenses ...

The intracellular pathway of Listeria monocytogenes ...

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

Cooperation with host defenses ...

Influence of GM-CSF on
• TNF-α production by macrophages
• autocrine activation of Listericidal activity

Toxicity …
Aminoglycoside toxicity ...

From: Tulkens, 1986 Amer. J Med. 80(Suppl 6B); 105-114
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.
What is the mechanism of gentamicin–induced apoptosis and its relation to necrosis in kidney cortex?

FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive deposition of polar lipids which adopt a concentric lamellar disposition (myeloid-like structures, most commonly referred to as myeloid bodies); the other subcellular structures are usually well preserved. Later changes or changes observed with high doses (C) include the apparent rupture of lysosomes (with the release of myeloid bodies in the cytosol), extensive mitochondrial swelling and damage, dilatation of the endoplasmic reticulum cisternae, shedding of the apical brush-border villi, pericellular membrane discontinuities, and the occurrence of apoptotic nuclei. These alterations do not necessarily coexist in all cells. The figure is adapted from reference 76 and is based on the typical descriptions given in references 38, 40, 71, 76, 77, 127, and 138.
Are lysosomes disrupted by gentamicin?

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).

What if you by-pass lysosomes?

Figure 1: Staining of nuclei of LLC-PK₁ cells by 4’,6’-diamidine-2’-phenylindole (DAPI). Incubated: cells were maintained for 24 h in the absence of gentamicin (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (3 mM; 1.3 g/L). Electroporated: cells were electroporated in the absence (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (0.03 mM; 13.9 mg/L), and examined 24 h later. In the absence of gentamicin, both electroporated and incubated cells show a diffuse finely reticulated staining characteristic of euchromatin of diploid interphase animal cells. In contrast, cells electroporated or incubated in the presence of gentamicin show typical changes associated with apoptosis, consisting in the condensation and fragmentation of the nuclear material.

Servais et al., Antimicrob. Agents Chemother. in press
Bypassing lysosomes in cultured cells ...

Servais et al., Antimicrob. Agents Chemother. in press
Accumulation may not be without risks: azithromycin may cause phospholipid accumulation …

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

Oritavancin cellular toxicity …

Transport ...
Efflux and transport of antibiotics in eucaryotic cells
I. absorption and excretion
Modeling transintestinal transport of ampicillin pro-drugs: 1) net transport

![Diagram of drug structures and transport](image)

**FIG. 2.** Apical-to-basolateral transepithelial transport of ampicillin, PIVA, and PIMA through a Caco-2 cell monolayer at 37°C. (Left panel) Cells were incubated with PIVA, PIMA, or ampicillin (all at 0.2 mM) in the apical medium, and the appearance of ampicillin was monitored in the basolateral medium; (right panel) cells were incubated with PIVA or PIMA, as described for the left panel, and the appearance of the corresponding prodrug was monitored in the basolateral medium. At 3 h, the proportions of PIVA, PIMA, and mannitol present in the basolateral side corresponded to 2.1, 1.6, and 2.0% of the total amount present in the apical side, respectively. Each datum point is the mean ± standard deviation of three determinations. This experiment was repeated three times, with similar results each time.
Modeling transintestinal transport of ampicillin pro-drugs: 2) accumulation of pro-drug and conversion prodrug → drug

FIG. 3. Accumulation of ampicillin (ampic.), PIVA, and PIMA in cells incubated with PIVA and PIMA and with free ampicillin in Caco-2 cells. (Left panel) Cells were incubated with PIVA, PIMA, or ampicillin (abscissa) in the apical medium for 2 h, as described in the legend to Fig. 2. The ordinate shows the accumulation of ampicillin in each case and of PIVA and PIMA when they were incubated with the corresponding ester. (Right panel) Kinetics of accumulation of ampicillin and PIVA in cells incubated with PIVA, as in the left panel. In both panels, the dotted horizontal line indicates the cell drug content which would correspond to a 10-fold accumulation of PIVA or PIMA (compared to either the actual ampicillin concentration in the same medium [cells incubated with ampicillin] or the concentration of ampicillin that would be created in the same medium if all prodrug was converted to ampicillin [cells incubated with PIVA or PIMA]). Each datum point is the mean ± standard deviation of three determinations. This experiment was repeated three times, with similar results each time. prot, protein.
Modeling transintestinal transport of ampicillin pro-drugs: 3) oriented efflux of intracellularly released drug

FIG. 7. Appearance of ampicillin in the apical or basolateral medium of Caco-2 cells incubated for 1 h at 37°C with 0.2 mM PIVA in the apical medium (pulse) and then transferred for 2 h in fresh medium (chase). The drug concentrations observed in the corresponding medium at the end of the chase are shown. Control, cells without other treatment; −ATP, cells preincubated for 1 h with 5 mM NaN₃ and 60 mM 2-deoxyglucose to obtain ATP depletion (these conditions were maintained during the pulse and chase periods); +probenecid and +MK-571, cells incubated with these MRP inhibitors (5 mM and 100 μM, respectively) in both the apical and basolateral media only during the chase period. Each datum point is the mean ± standard deviation of three determinations. This experiment was repeated three times, with similar results each time.
Model of trans-intestinal transport

Efflux and transport of antibiotics in eucaryotic cells
II. trans-barrier passage and intracellular accumulation

Azithromycin accumulation in macrophages is sub-optimal because of efflux through P-glycoprotein

Kinetics of uptake (A) and release (B) of azithromycin in J774 murine macrophages with (open squares) or without (closed squares) 20 µM verapamil.

Characterizing P-gp-mediated efflux and ranking macrolides

Ciprofloxacin (a fluoroquinolone) is subject to MRP-mediated efflux in macrophages

Ranking fluoroquinolones for efflux and explaining differences in accumulation

Making MRP-overexpressing cells

WT: wild type
RS: MRP-overexpressing cells

Assessing the intracellular activity of ciprofloxacin in MRP-overexpressing cells

WT: wild type
RS: MRP-overexpressing cells

Conclusions

Cell lines are useful to address many questions related to antibiotic development and assessment

1. activity against intracellular pathogens...
2. cooperation with host defenses...
3. cellular toxicity ...
4. drug transport...
5. ... and probably many others if the models are chosen appropriately