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…

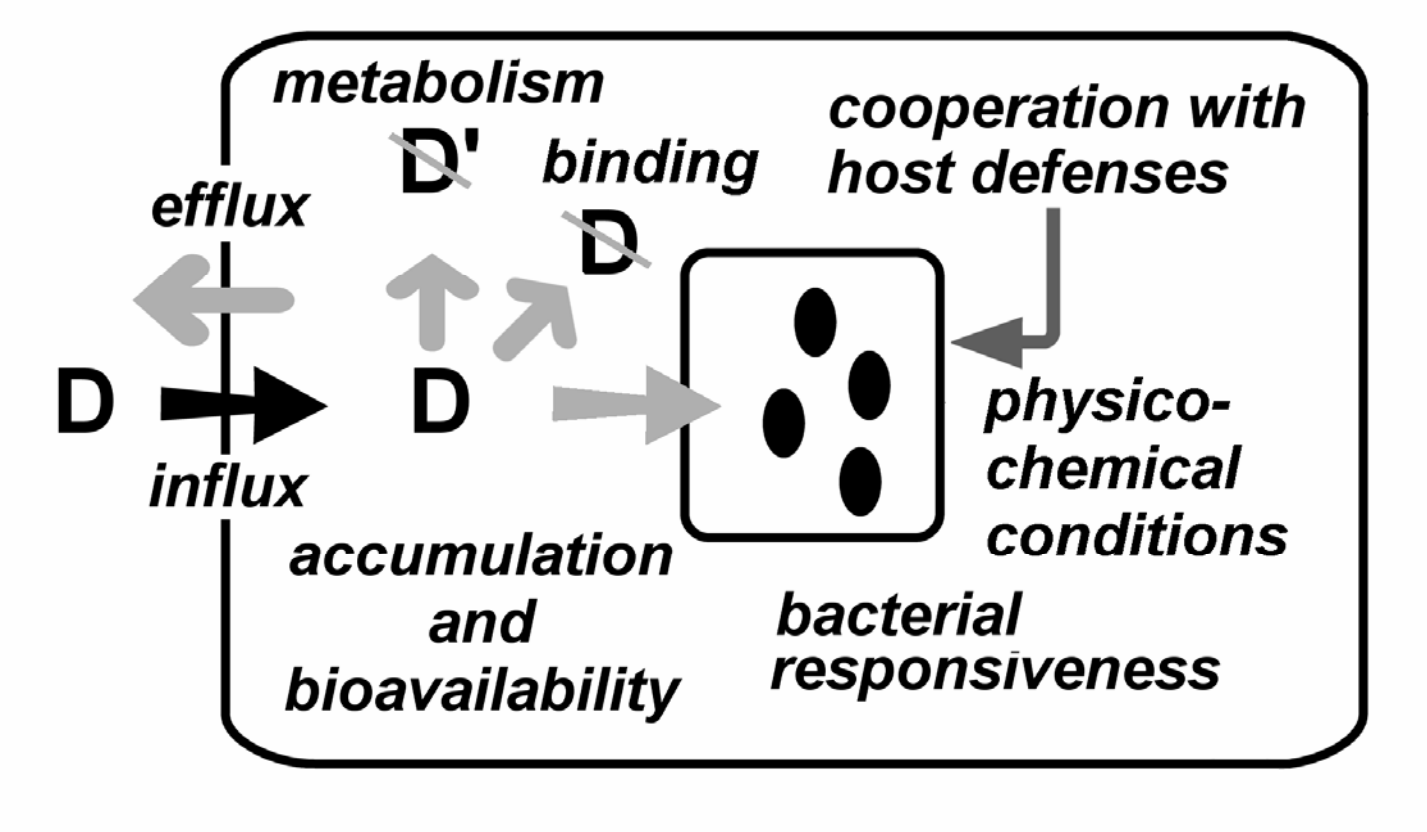
Intracellular bacterial infections

- 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 1. Uptake of azithromycin and erythromycin by various phagocytic cells

Cell type	Antibiotic[a]	Differential[b]	Antibiotic uptake	
			I/E	µg/10^7 cells
Human PMNs	Azithromycin	4.9	79	1.58
	Erythromycin		16	0.32
Murine PMNs	Azithromycin	3.9	39	0.78
	Erythromycin		10	0.20
Murine alveolar macrophages	Azithromycin	5.9	170	18.66
	Erythromycin		29	3.18
Rat alveolar macrophages	Azithromycin	5.5	60	6.58
	Erythromycin		11	1.21
Murine resident peritoneal macrophages	Azithromycin	15.5	62	6.81
	Erythromycin		4	0.43

[a] Cells were incubated for 2 h with 10 µg of the antibiotic per ml.
[b] Ratio of azithromycin uptake to erythromycin uptake. All values are statistically significant.

Gladue et al.,
AAC 33:277-82, 1989

The S. aureus problem …

J Bone Joint Surg Br. 2003 Aug;85(6):918-21.

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

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

Clin Infect Dis. 2001 Jun 1;32(11):1643-7. Epub 2001 Apr 30.

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

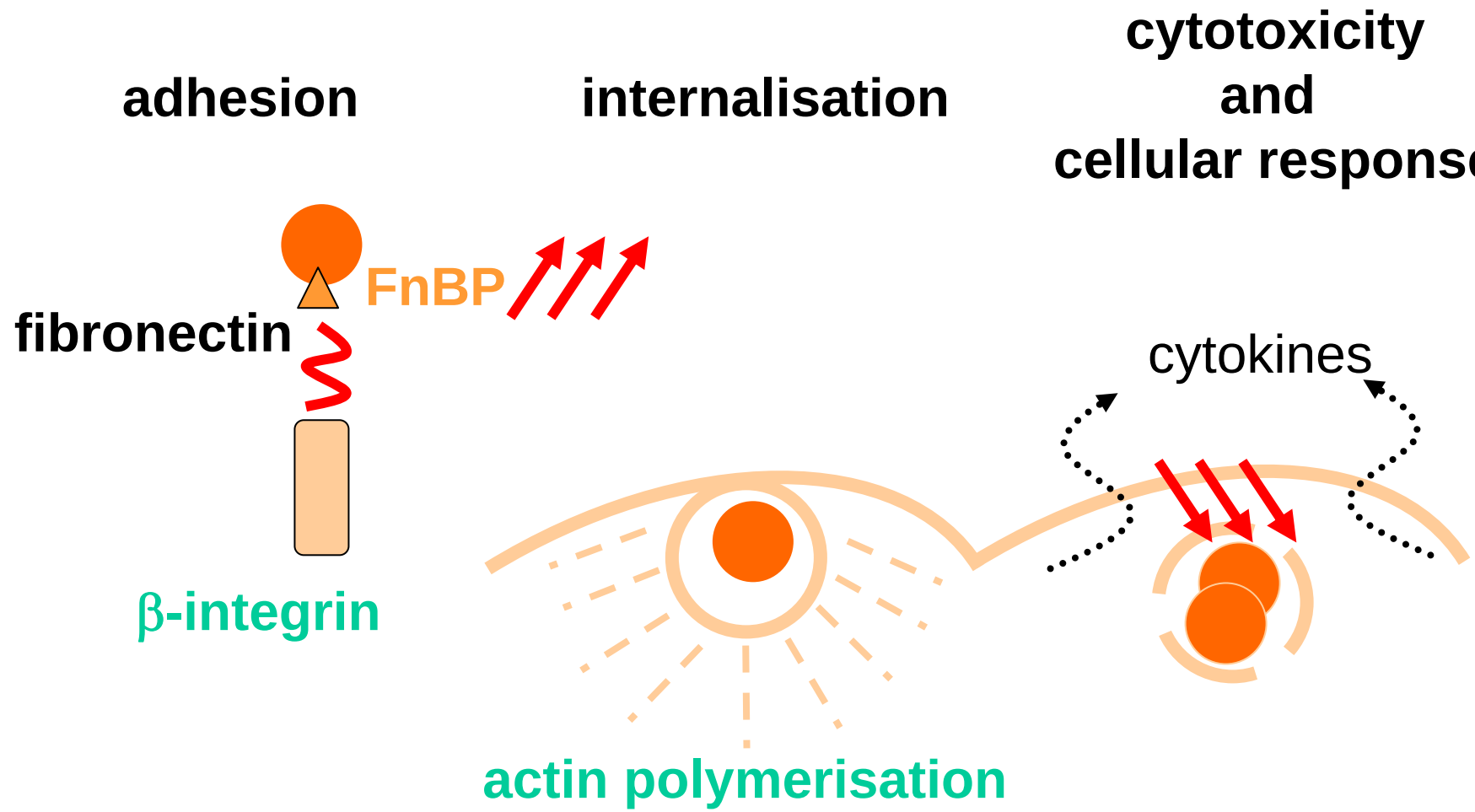
von Eiff C, Becker K, Metze D, Lubritz G, Hockmann J, Schwarz T, Peters G.

Infect Immun. 1986 Dec;54(3):833-6.

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

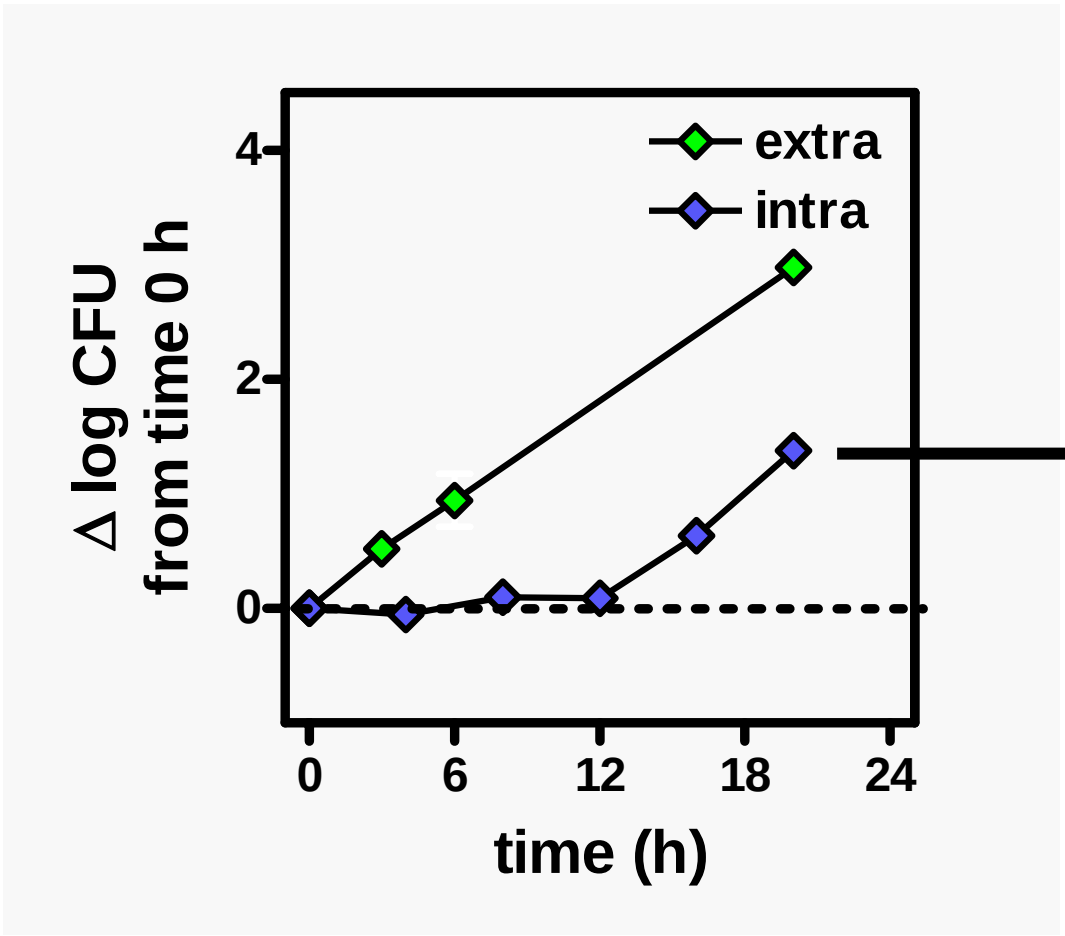
Hamill RJ, Vann JM, Proctor RA.

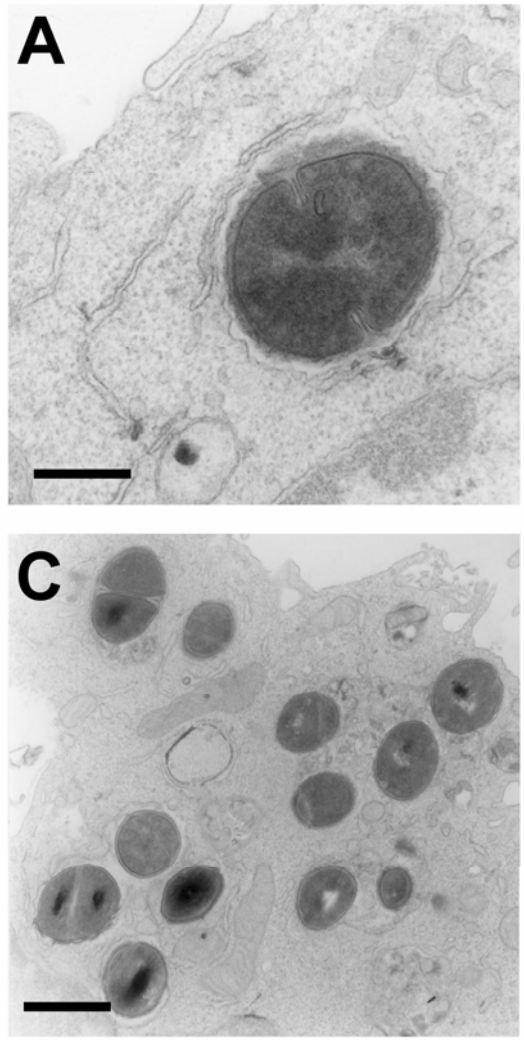
Why does S. aureus has an intracellular life, and is dangerous ?



Lowy, Trends Microbiol (2000) 8:341-342

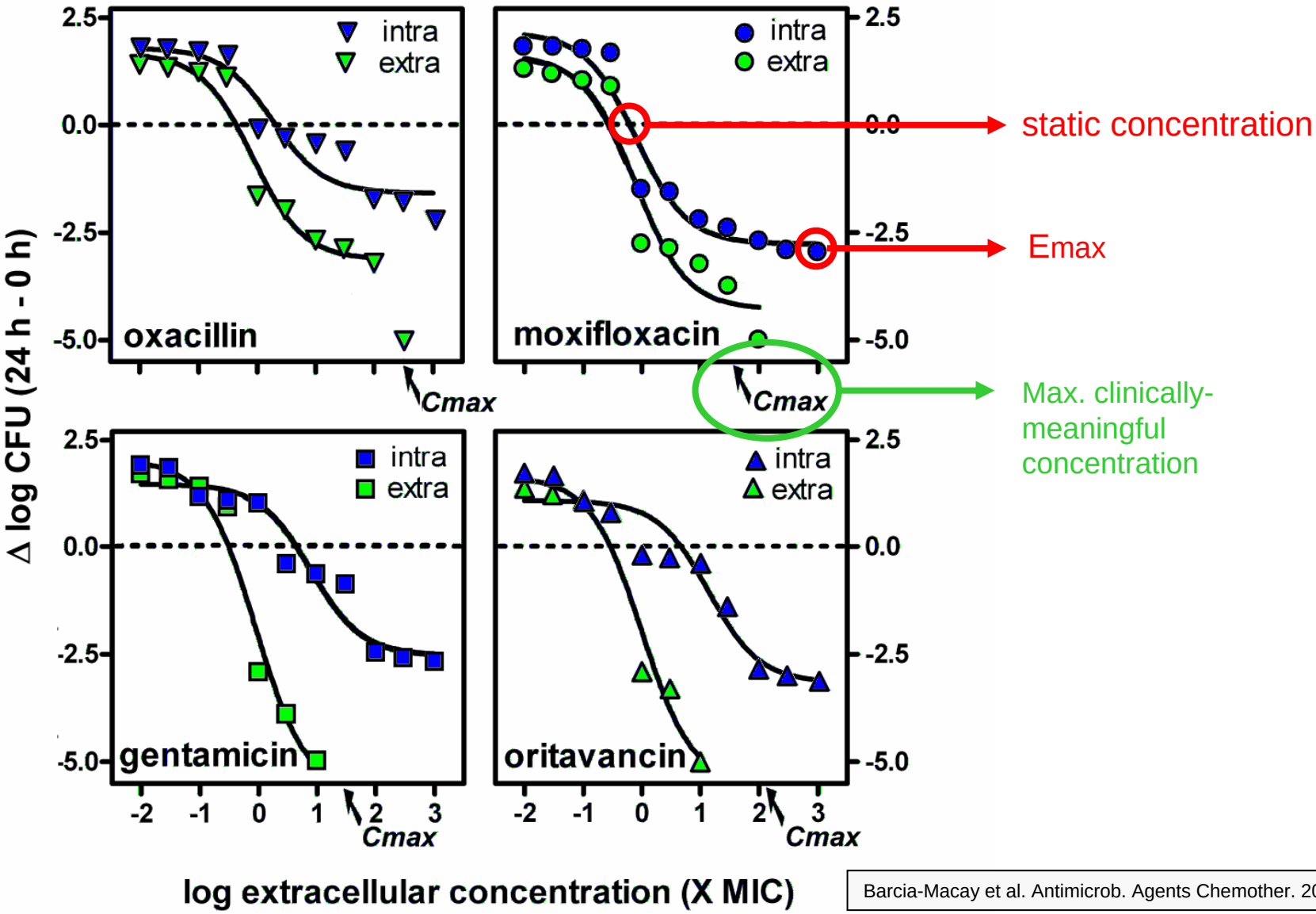
Settting up the model …





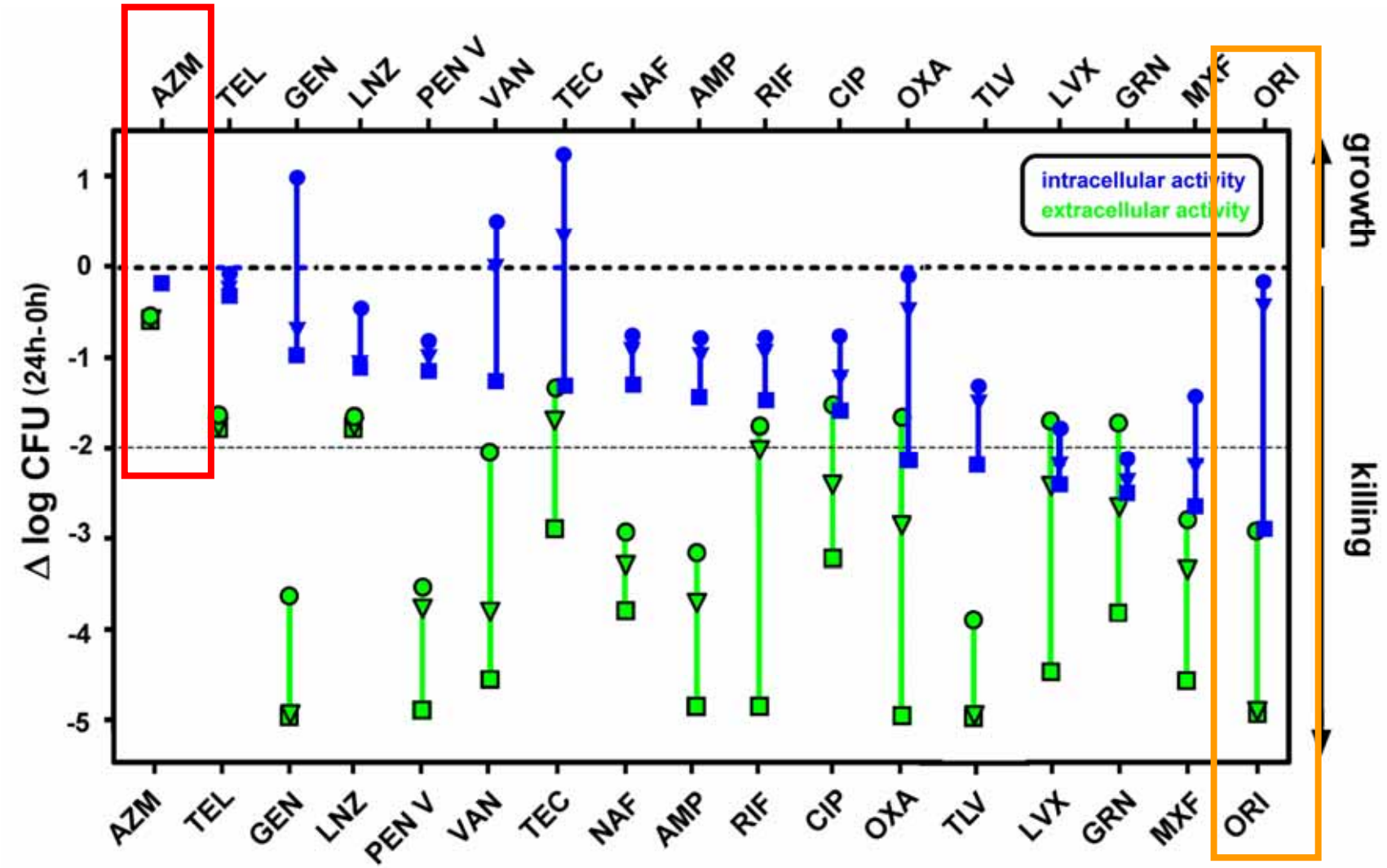
Seral *et al.* Antimicrob. Agents Chemother. 2003 47:2283-2292

Using the model…



Barcia-Macay et al. Antimicrob. Agents Chemother. 2006, in press

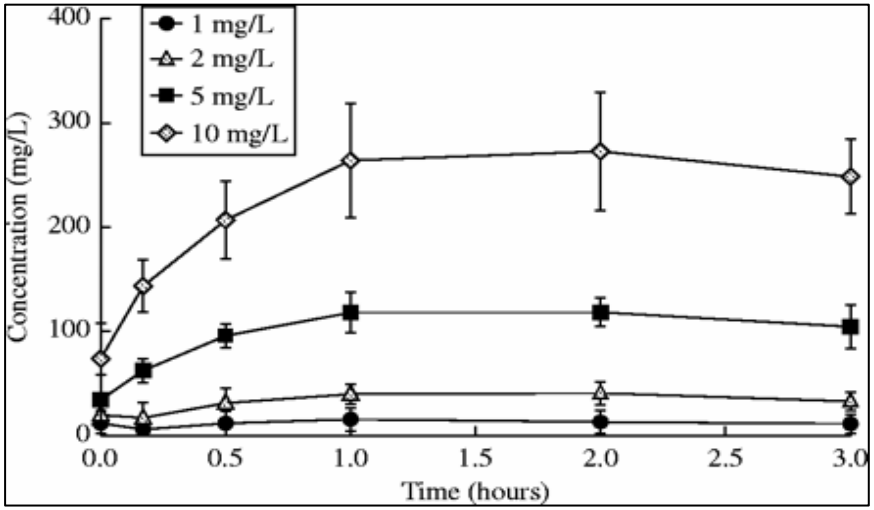
Screening available antibiotics



Barcia-Macay et al. Antimicrob. Agents Chemother. 2006, in press

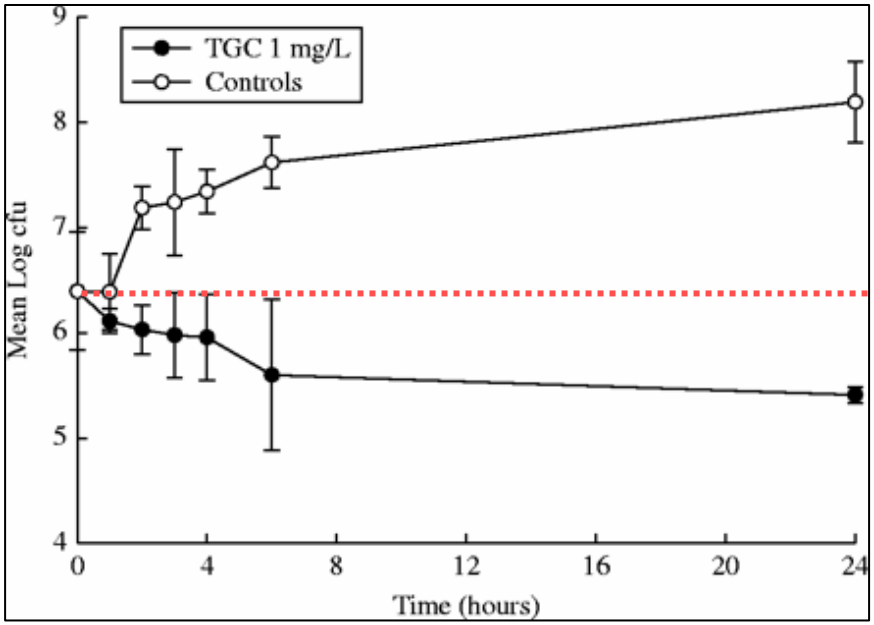
What about a new antibiotic

(tigecycline and intracellular *S. aureus*…)



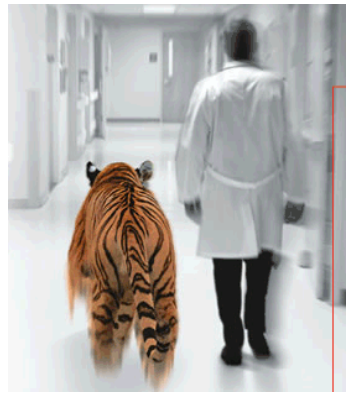
accumulation in PMN:
about 20-30 fold

Ong et al. J Antimicrob Chemother. 2005 Sep;56(3):498-501.

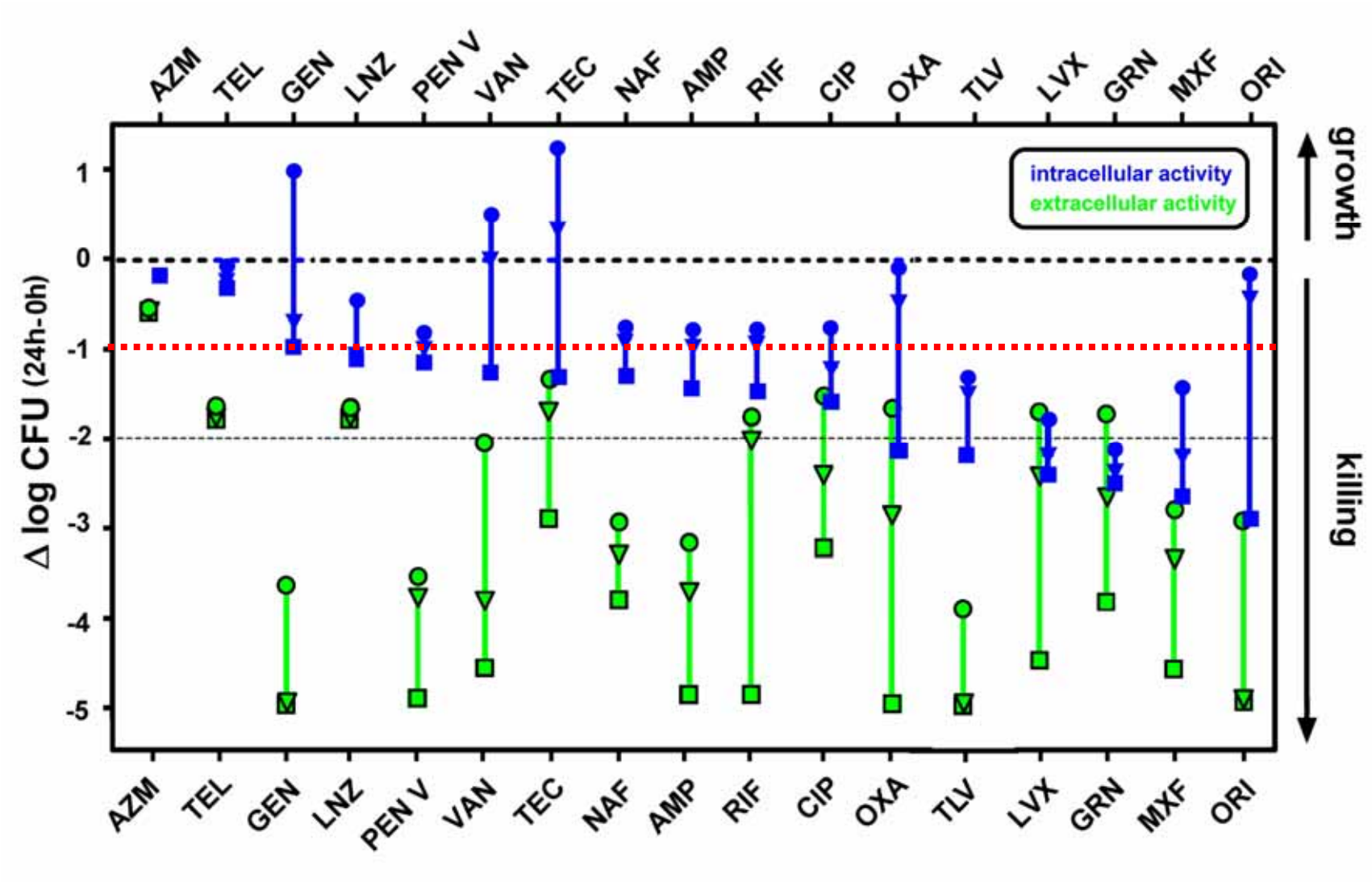


activity in PMN:
about 1 $\log_{10}$
at 1 mg/L

S. aureus ATCC 29213
(MIC = 0.25 mg/L)



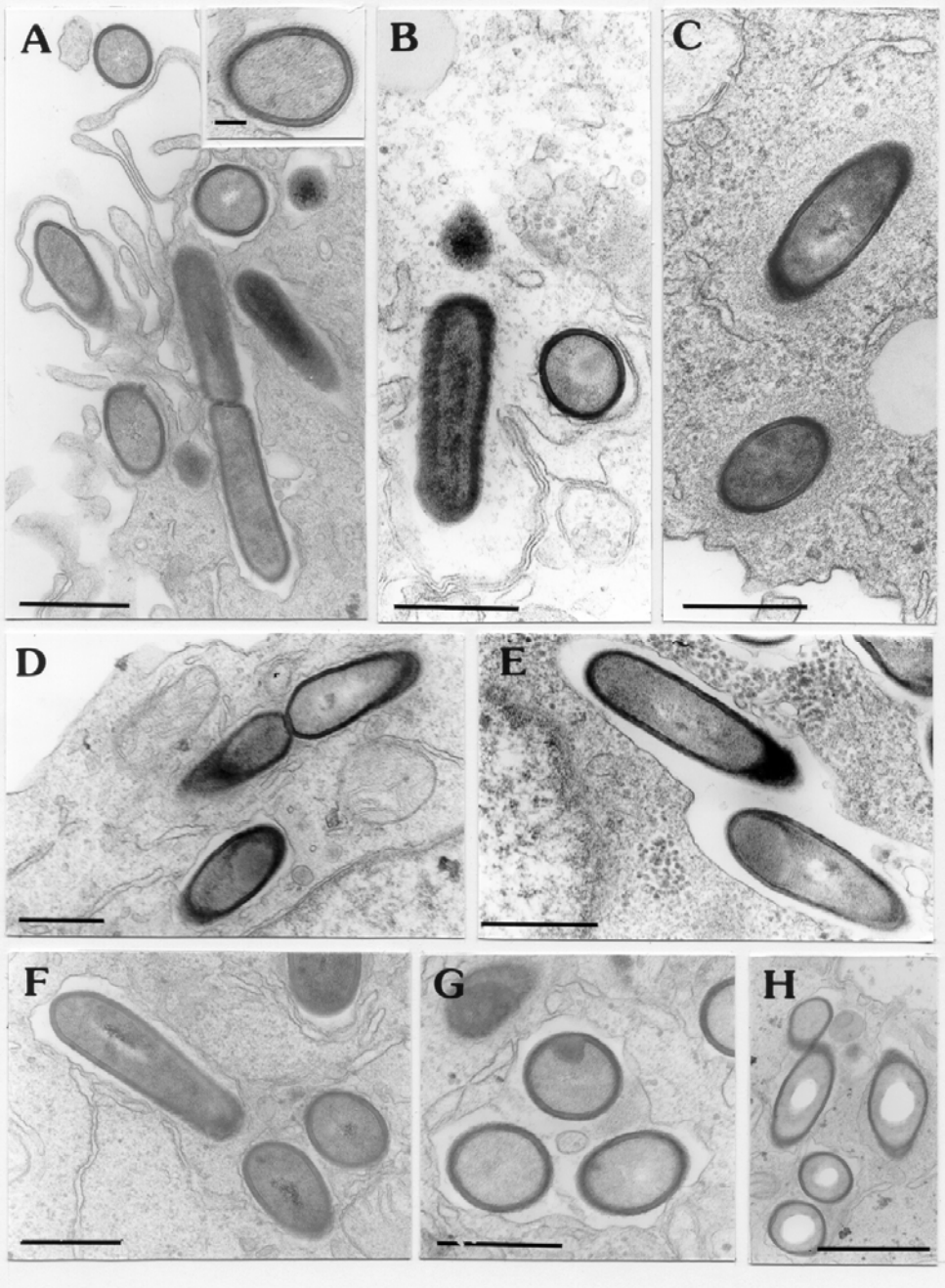
Screening available antibiotics



Cooperation with host defenses ...

The intracellular pathway
of Listeria monocytogenes ...

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

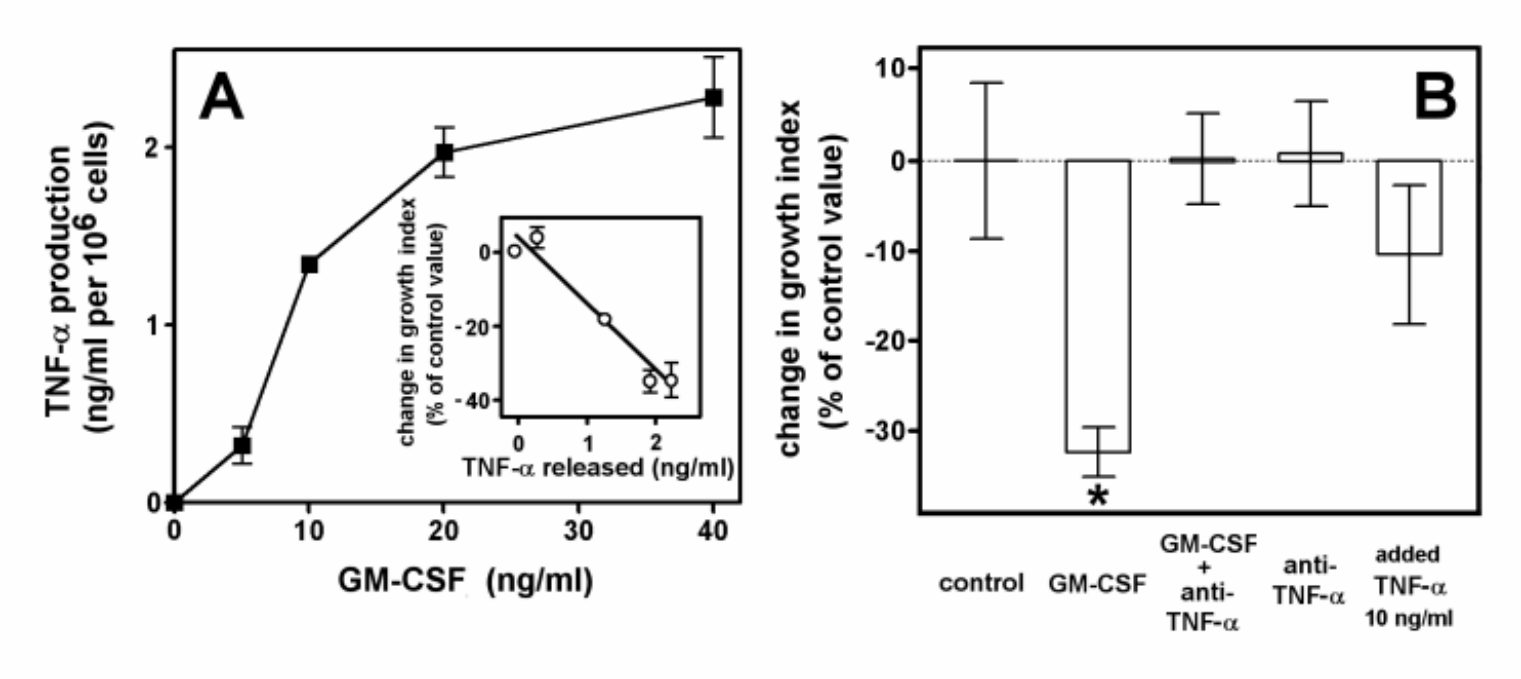


Ouadrhiri et al. Antimicrob. Agents Chemother. (1999) 43:1242-1251

Cooperation with host defenses ...

Influence of GM-CSF on

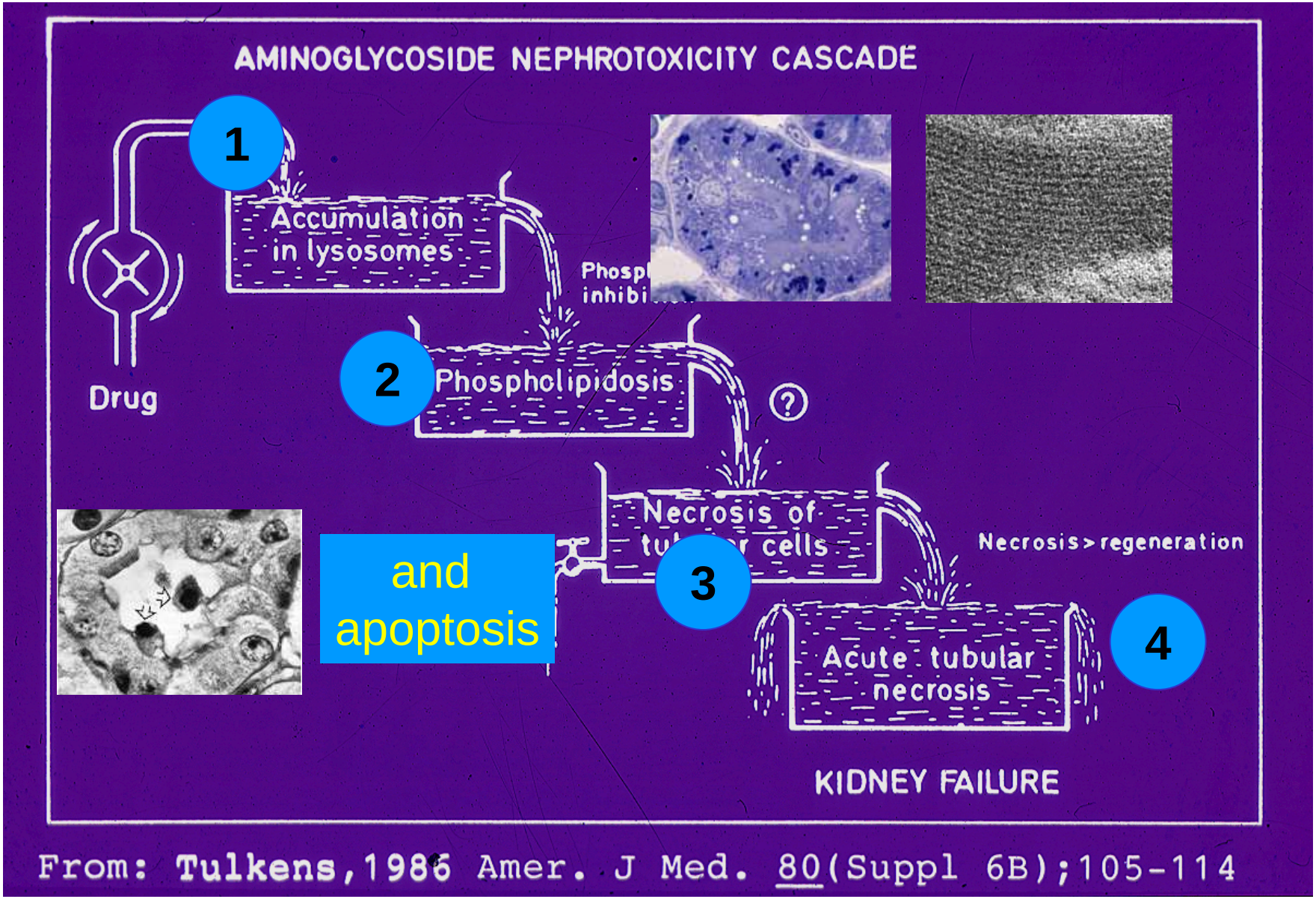
- **TNF-α production by macrophages**
- **autocrine activation of Listericidal activity**



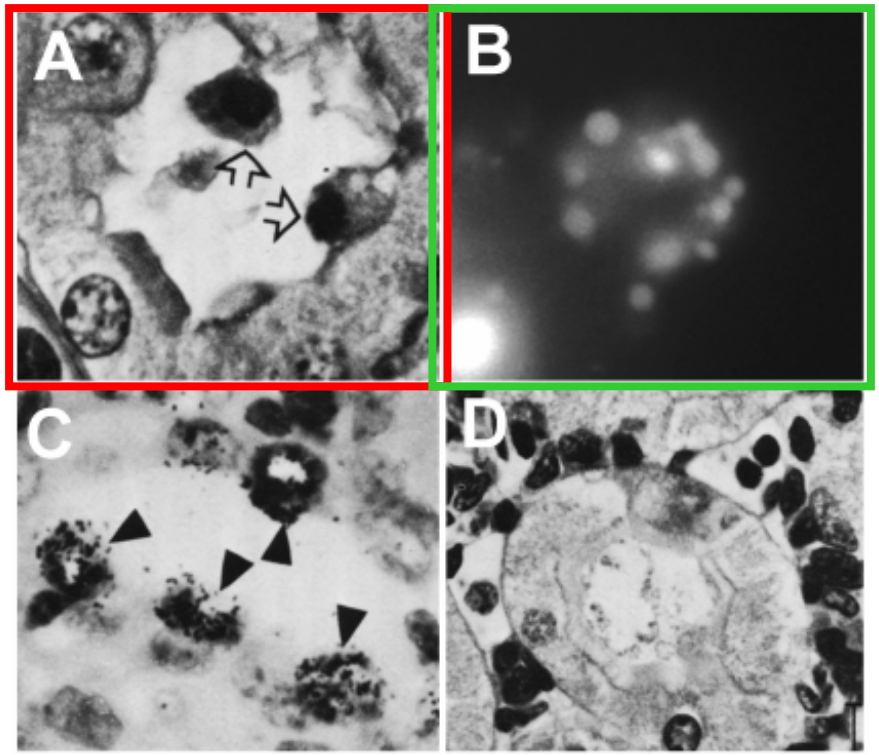
Carryn et al. J. Infect. Dis. (2004) 189:2101-2109

Toxicity …

Aminoglycoside toxicity …



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 LCC-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 ?

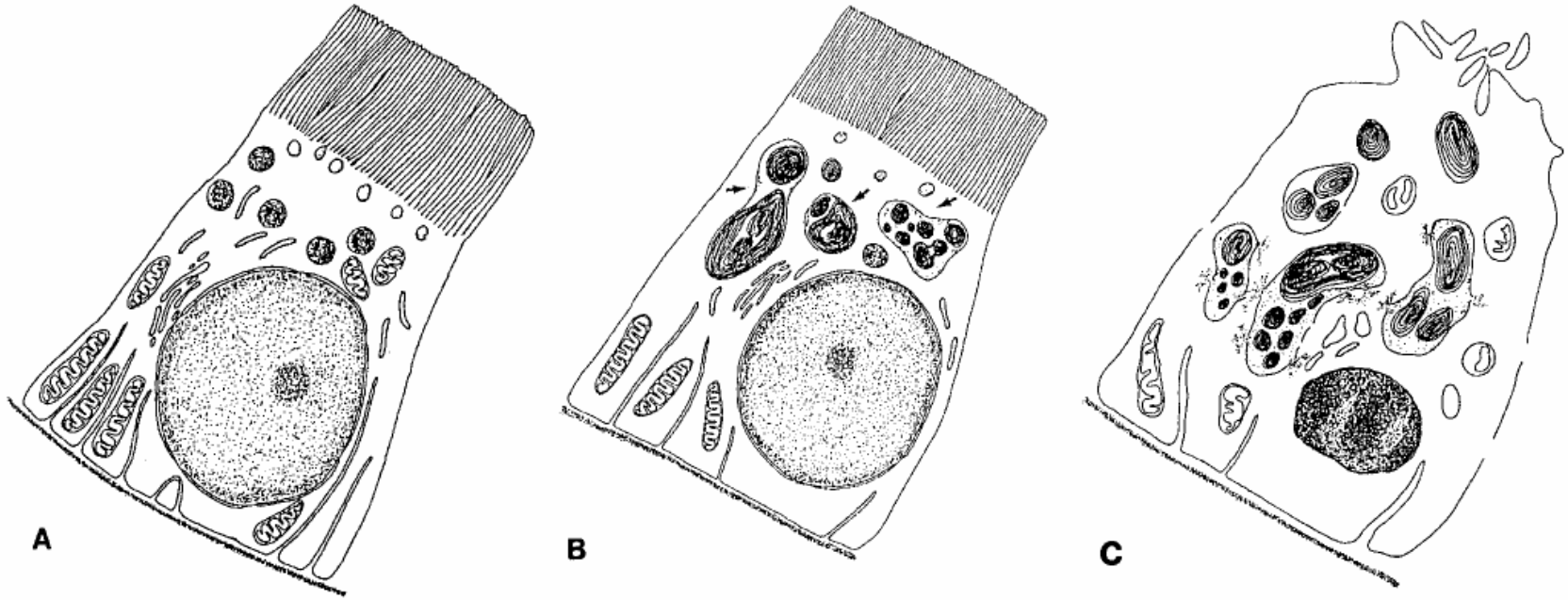


FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive deposition of polar lipids which adopt a concentric lamellar disposition (myelin-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.

Mingeot-Leclercq & Tulkens, Antimicrob. Agents Chemother. (1999) 43:1003-1012

Are lysosomes disrupted by gentamicin ?

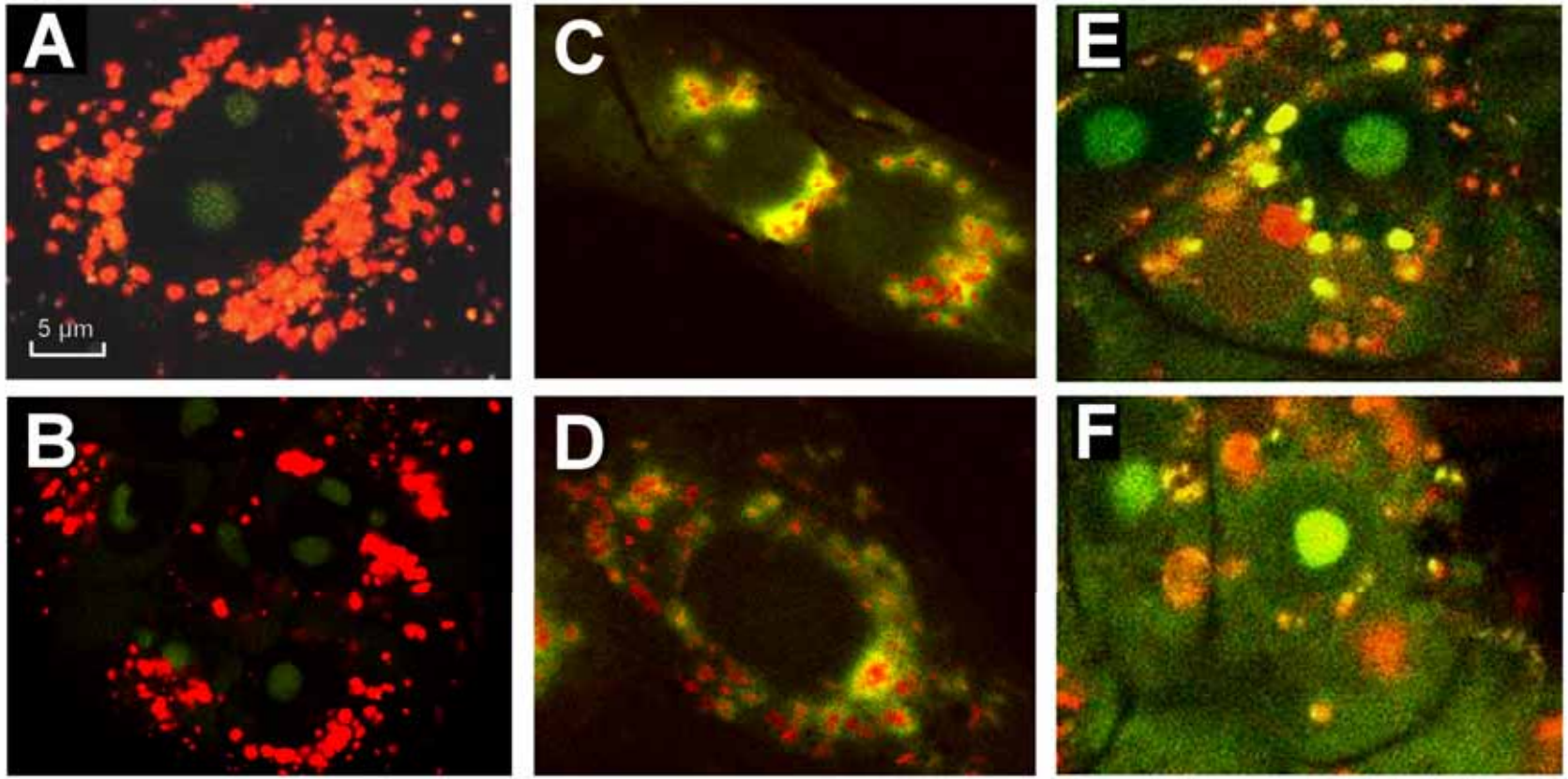


Fig. 4. Appearance of acridine orange-loaded LLC-PK1 cells in confocal microscopy. Cells were exposed to acridine orange (5 µg/ml) for 15 min and then returned to control medium for 3 h (A, B), or exposed to gentamicin (C and D, 3 mM, 3 h; E, 2 mM, 4 h) or MSDH (F, 25 µM, 3 h).

H. Servais et al. / Toxicology and Applied Pharmacology 206 (2005) 321–333

What if you by-pass lysosomes ?

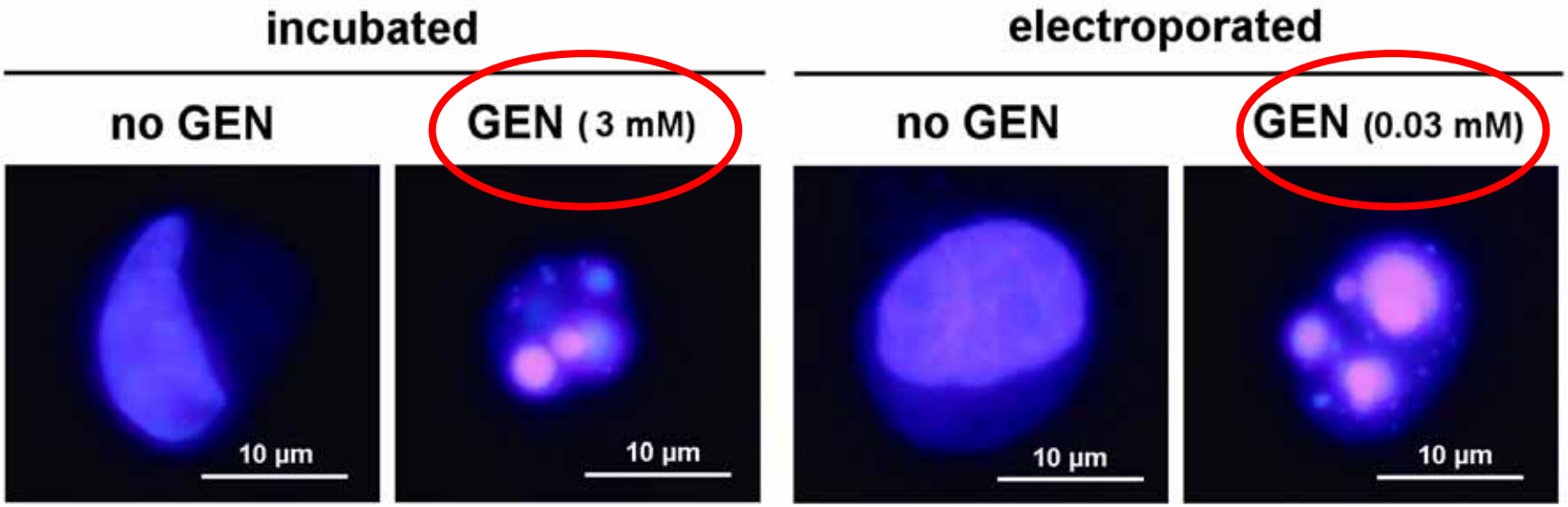
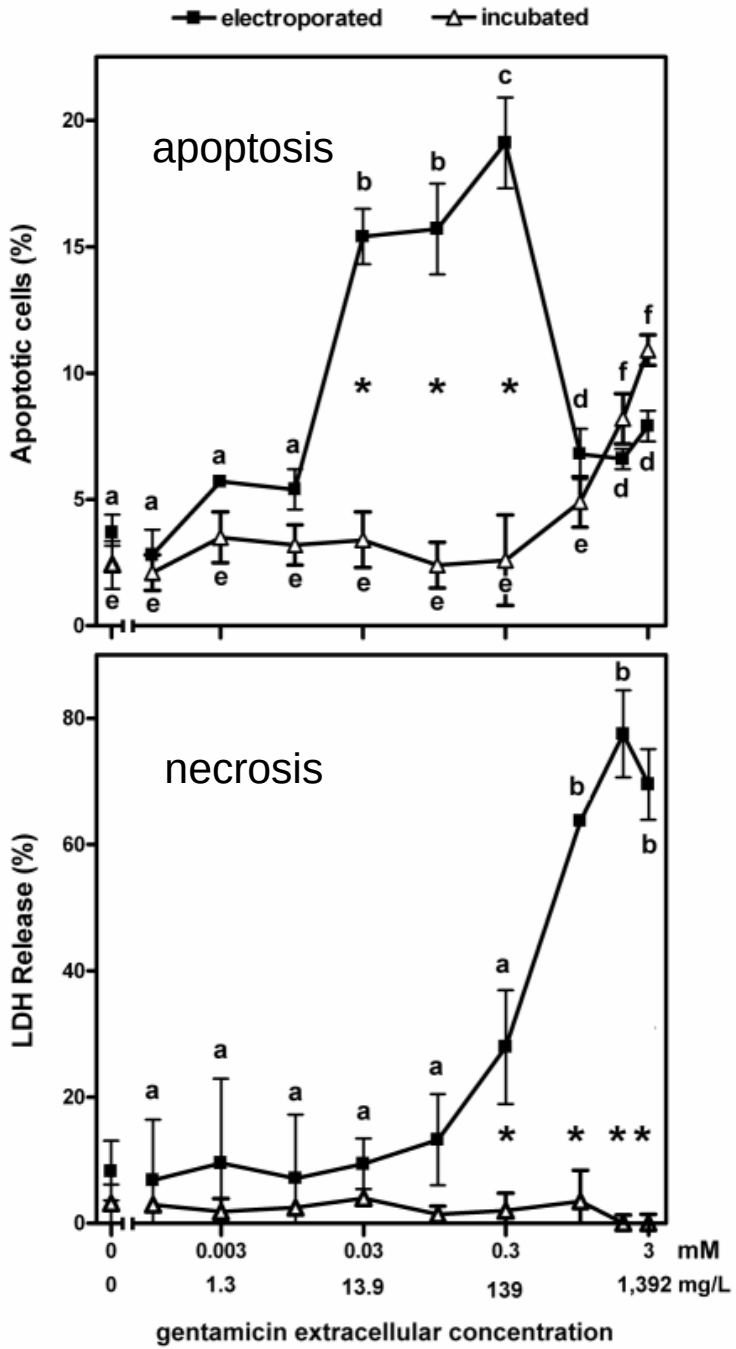


Figure 1: Staining of nuclei of LLC-PK_1 cells by 4',6'-diamidine-2'-phenylindole (DAPI). Incubated: cells were maintained for 24 h in the absence of gentamicin (no GEN) or in the presence of gentamicin (GEN) at the concentration shown (3 mM; 1.3 g/L). Electroporated: cells were electroporated in the 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.

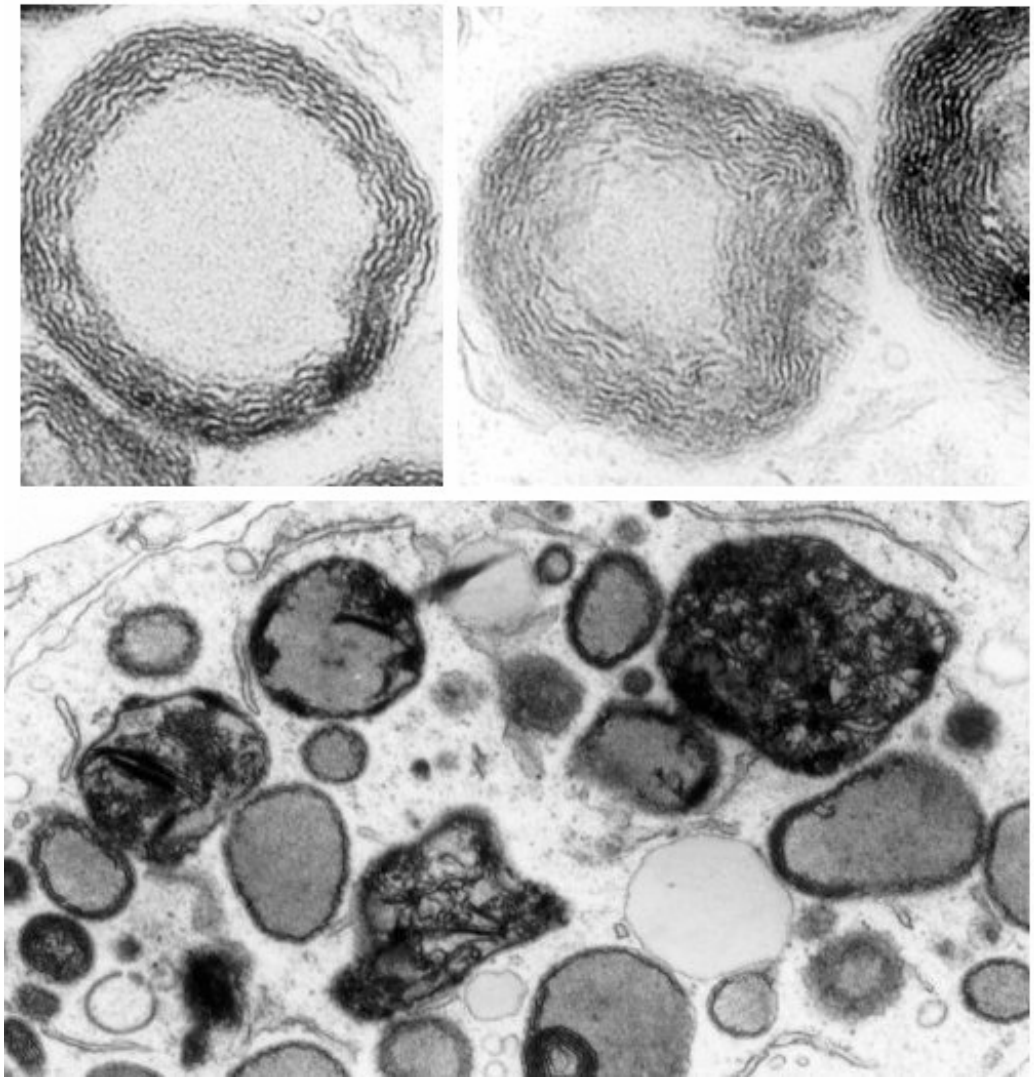
Servais et al., Antimicrob. Agents Chemother. in press

Bypassing lysosomes in cultured cells …



Servais et al., Antimicrob. Agents Chemother. in press

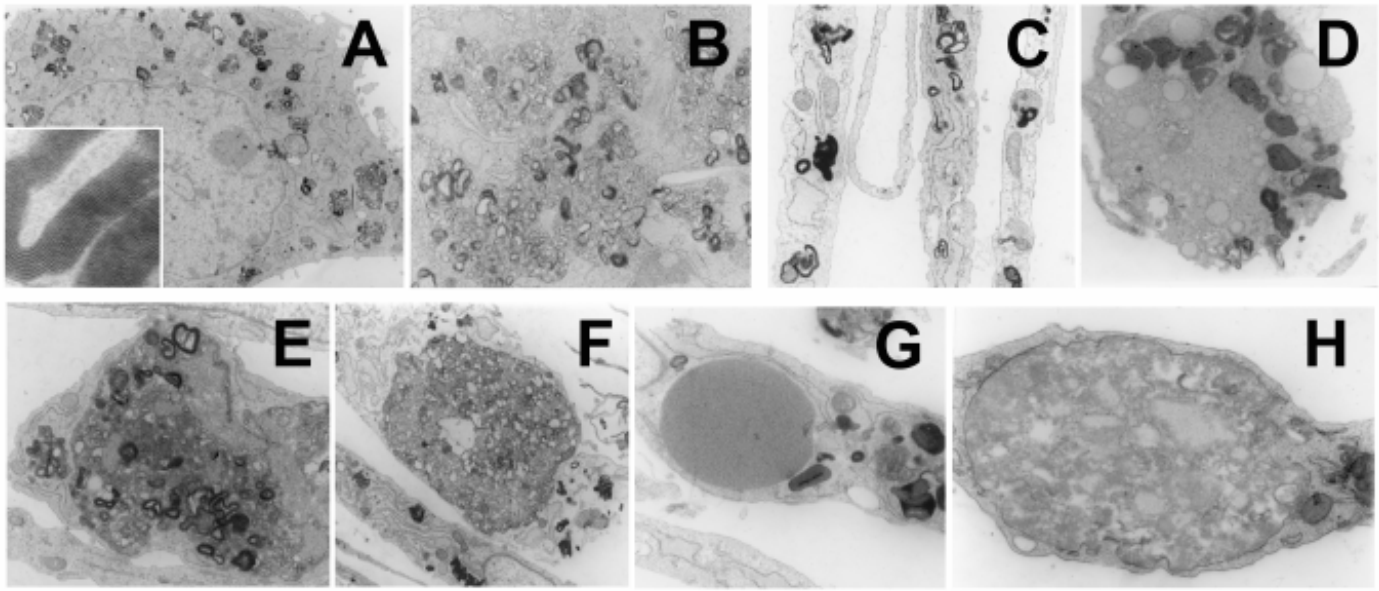
Accumulation may not be without risks: azithromycin may cause phospholipid accumulation …



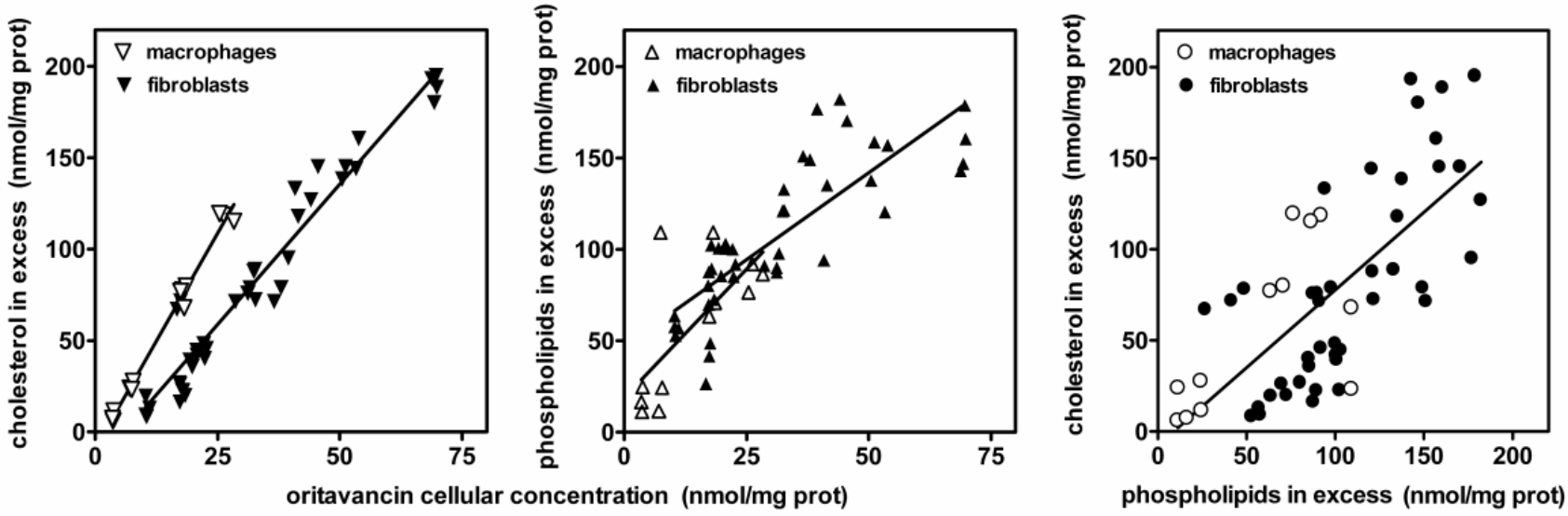
Ultrastuctural alterations observed in cultured fibroblats maintained with 0.03-0.1 mg/L of azithromycin for 7 to 16 days.

Van Bambeke et al., J. Antimicrob. Chemother. 42:761-767, 1968

Oritavancin cellular toxicity …



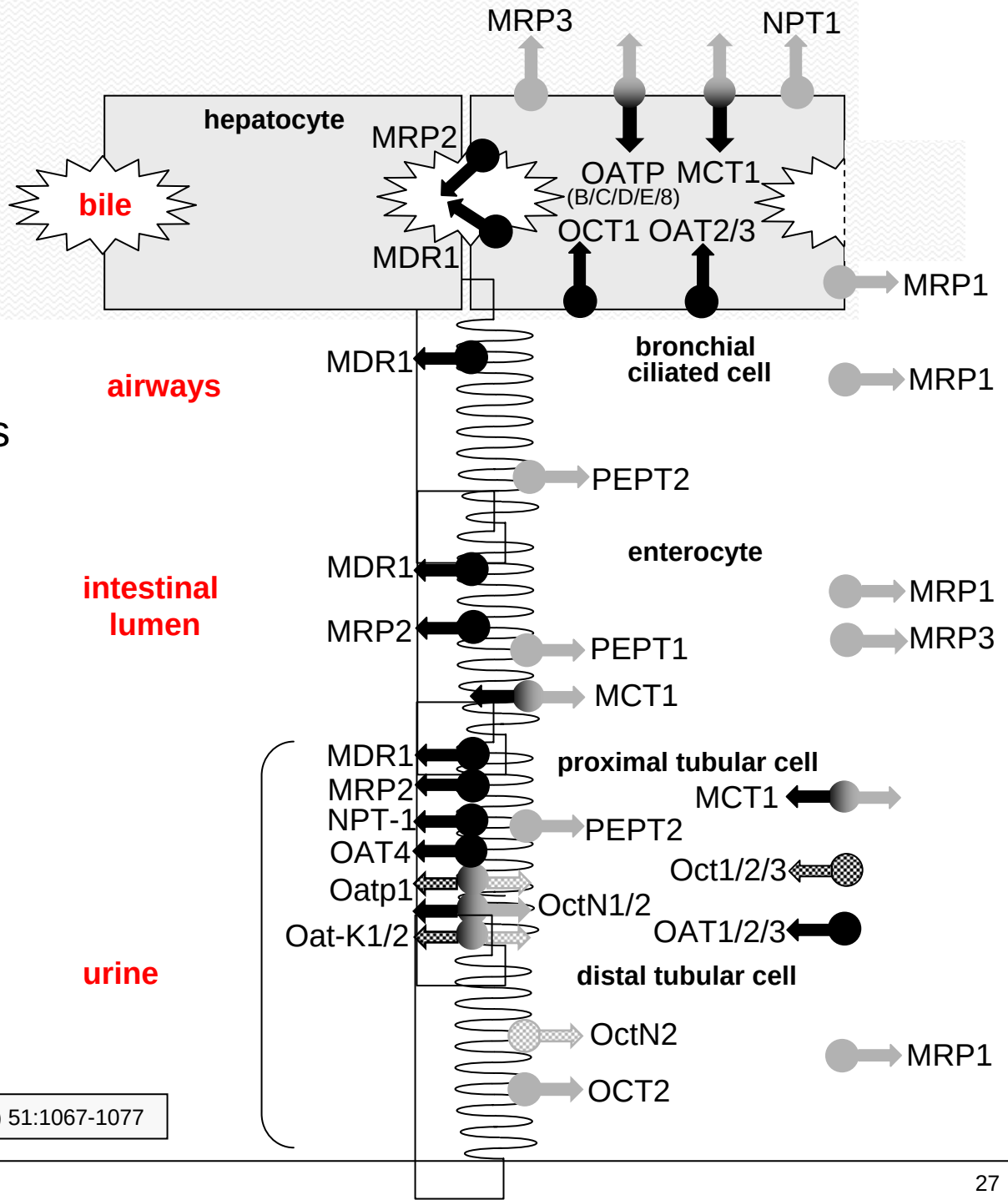
cells incubated at clinically meaningful concentrations



Van Bambeke et al. Antimicrob. Agents Chemotherapy (2005) 49:1695-1700.

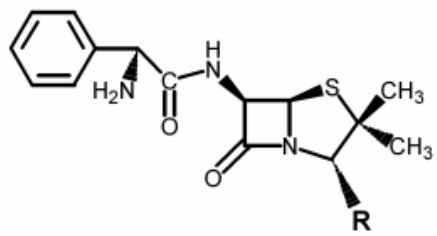
Transport …

Efflux and transport of antibiotics in eucaryotic cells I. absorbtion and excretion

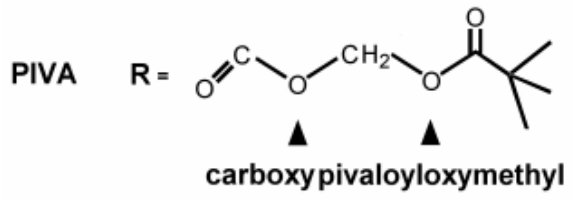


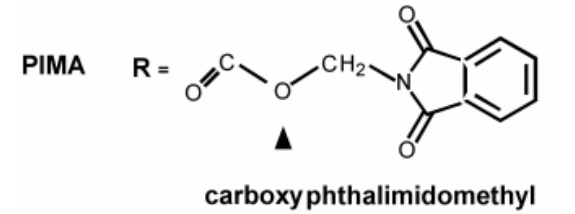
Van Bambeke et al. J. Antimicrob. Chemother. (2003) 51:1067-1077

Modeling transintestinal transport of ampicillin pro-drugs: 1) net transport



ampicillin R = C(=O)—OH





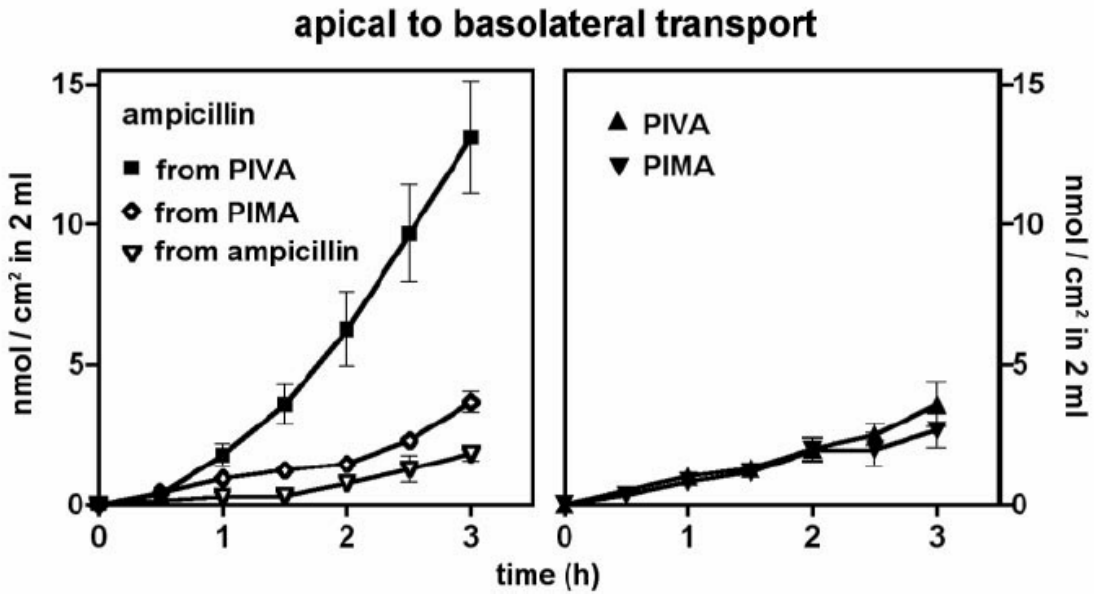
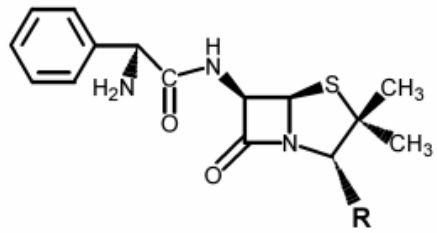


FIG. 2. Apical-to-basolateral transepithelial transport of ampicillin, PIVA, and PIMA through a Caco-2 cell monolayer at 37°C. (Left panel) Cells were incubated with PIVA, PIMA, or ampicillin (all at 0.2 mM) in the apical medium, and the appearance of ampicillin was monitored in the basolateral medium; (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.

Chanteux et al. Antimicrob. Agents Chemother. (2005) 49:1279-88.

Modeling transintestinal transport of ampicillin pro-drugs: 2) accumulation of pro-drug and conversion prodrug ➔ drug



ampicillin R = C(=O)—OH

PIVA R = carboxypivaloyloxymethyl

PIMA R = carboxyphthalimidomethyl

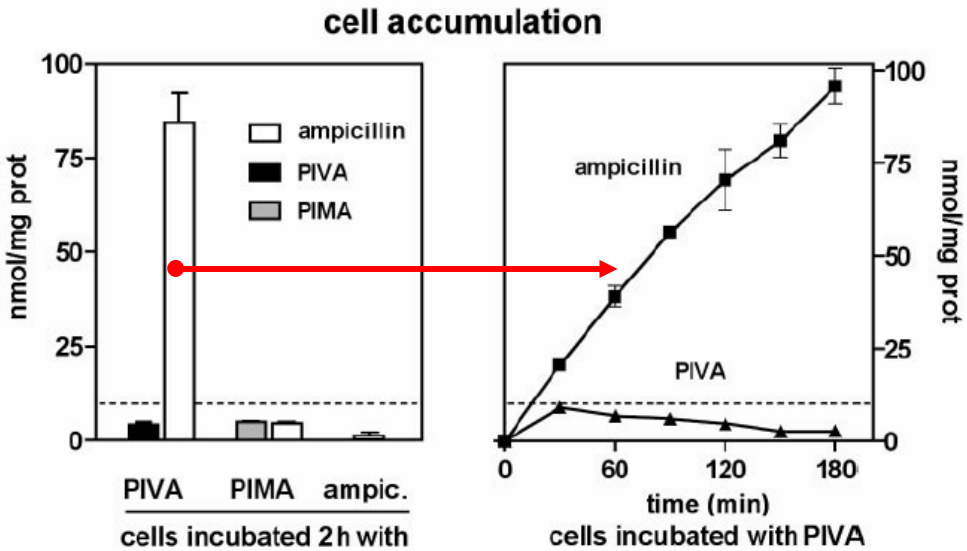
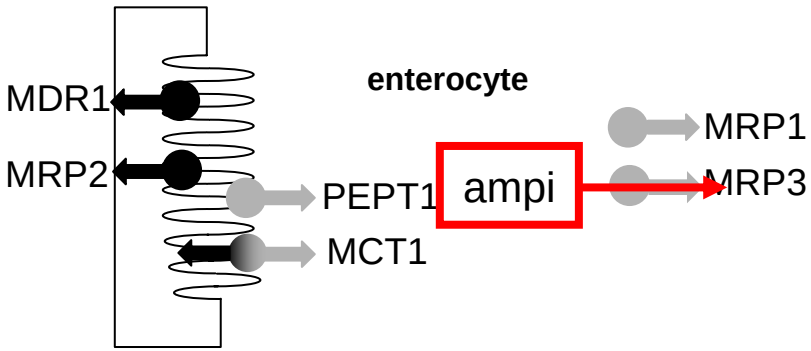


FIG. 3. Accumulation of ampicillin (ampic.), PIVA, and PIMA in cells incubated with PIVA and PIMA and with free ampicillin in Caco-2 cells. (Left panel) Cells were incubated with PIVA, PIMA, or ampicillin (abscissa) in the apical medium for 2 h, as described in the legend to Fig. 2. The ordinate shows 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.

Chanteux et al. Antimicrob. Agents Chemother. (2005) 49:1279-88.

Modeling transintestinal transport of ampicillin pro-drugs: 3) oriented efflux of intracellularly released drug



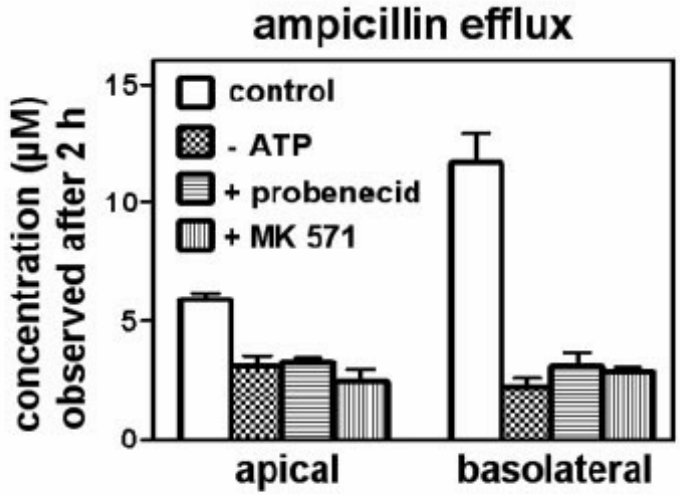
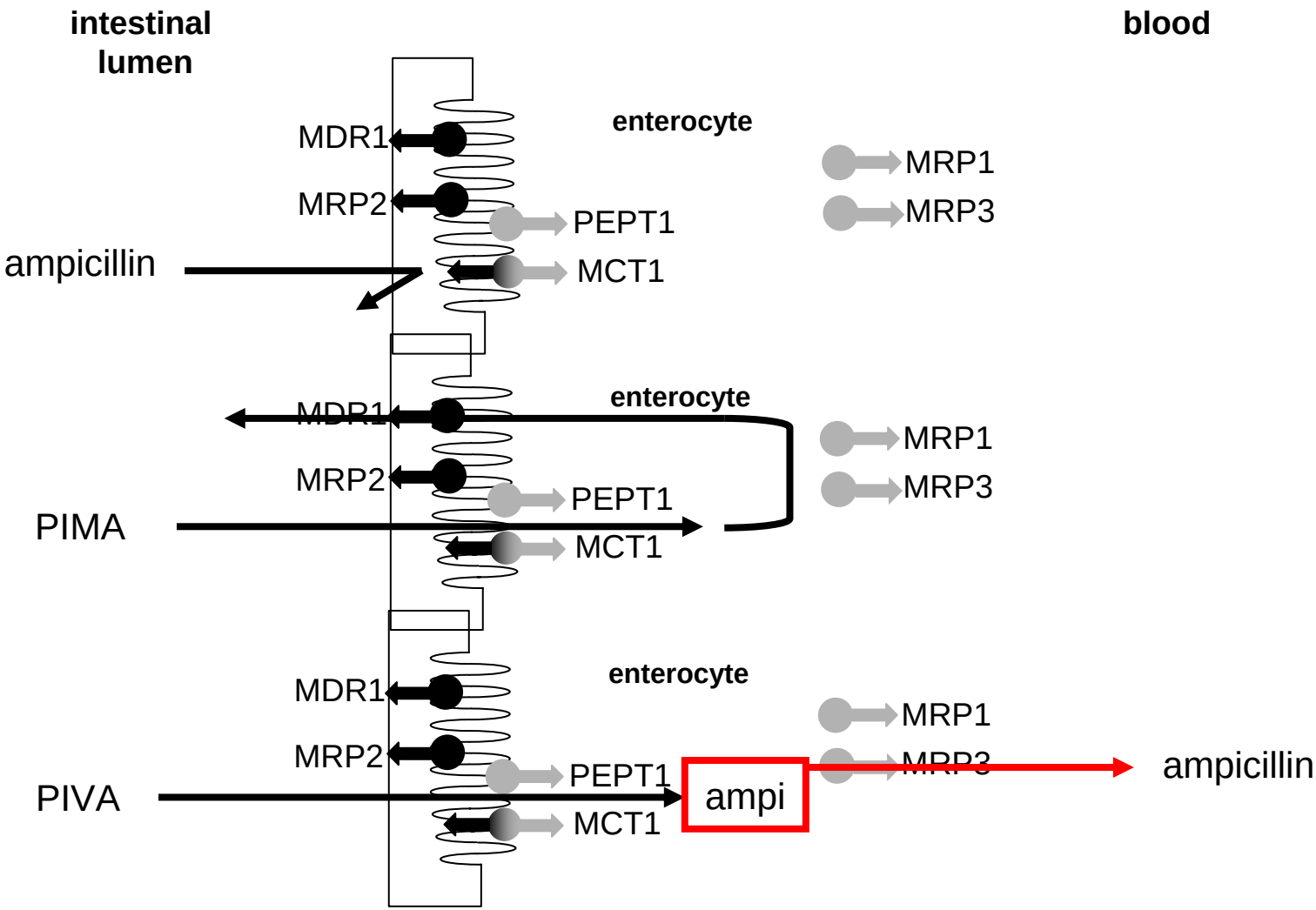


FIG. 7. Appearance of ampicillin in the apical or basolateral medium of Caco-2 cells incubated for 1 h at 37°C with 0.2 mM PIVA in the apical medium (pulse) and then transferred for 2 h in fresh medium (chase). The drug concentrations observed in the corresponding medium at the end of the chase are shown. Control, cells without other treatment; −ATP, cells preincubated for 1 h with 5 mM NaN_3 and 60 mM 2-D-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.

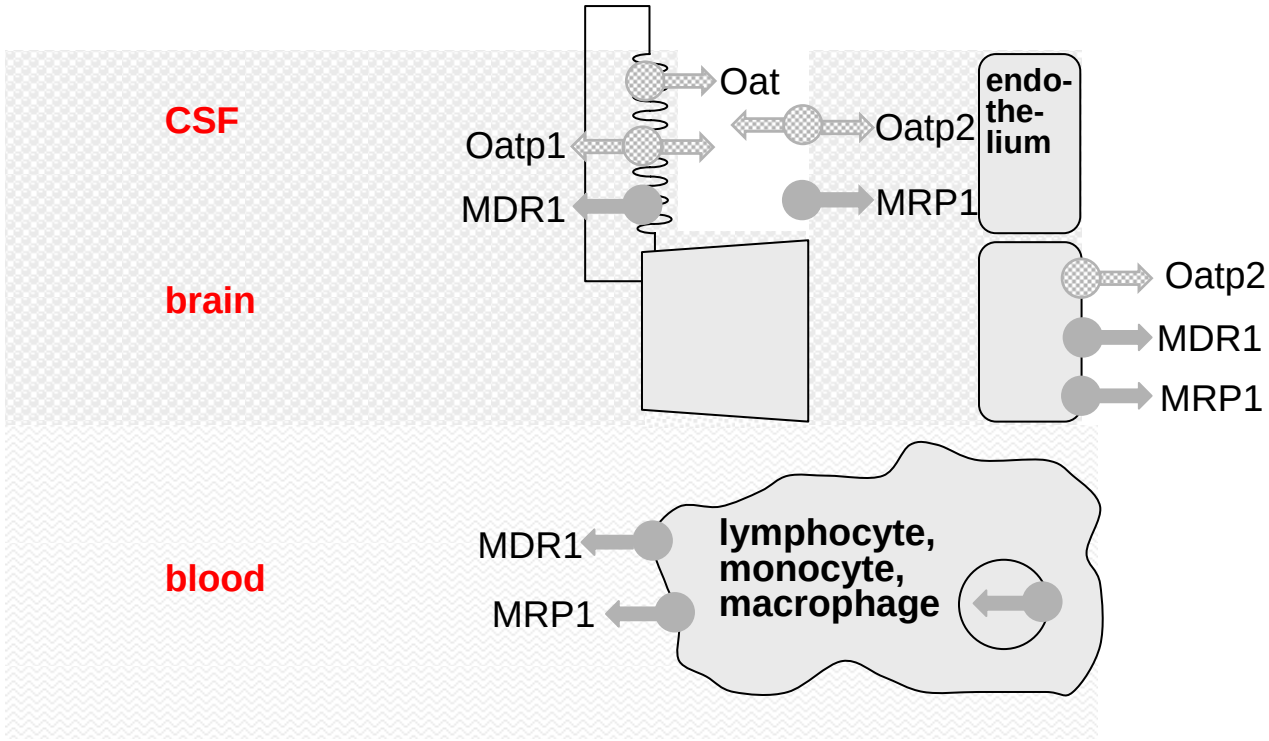
Chanteux et al. Antimicrob. Agents Chemother. (2005) 49:1279-88.

Model of trans-intestinal transport



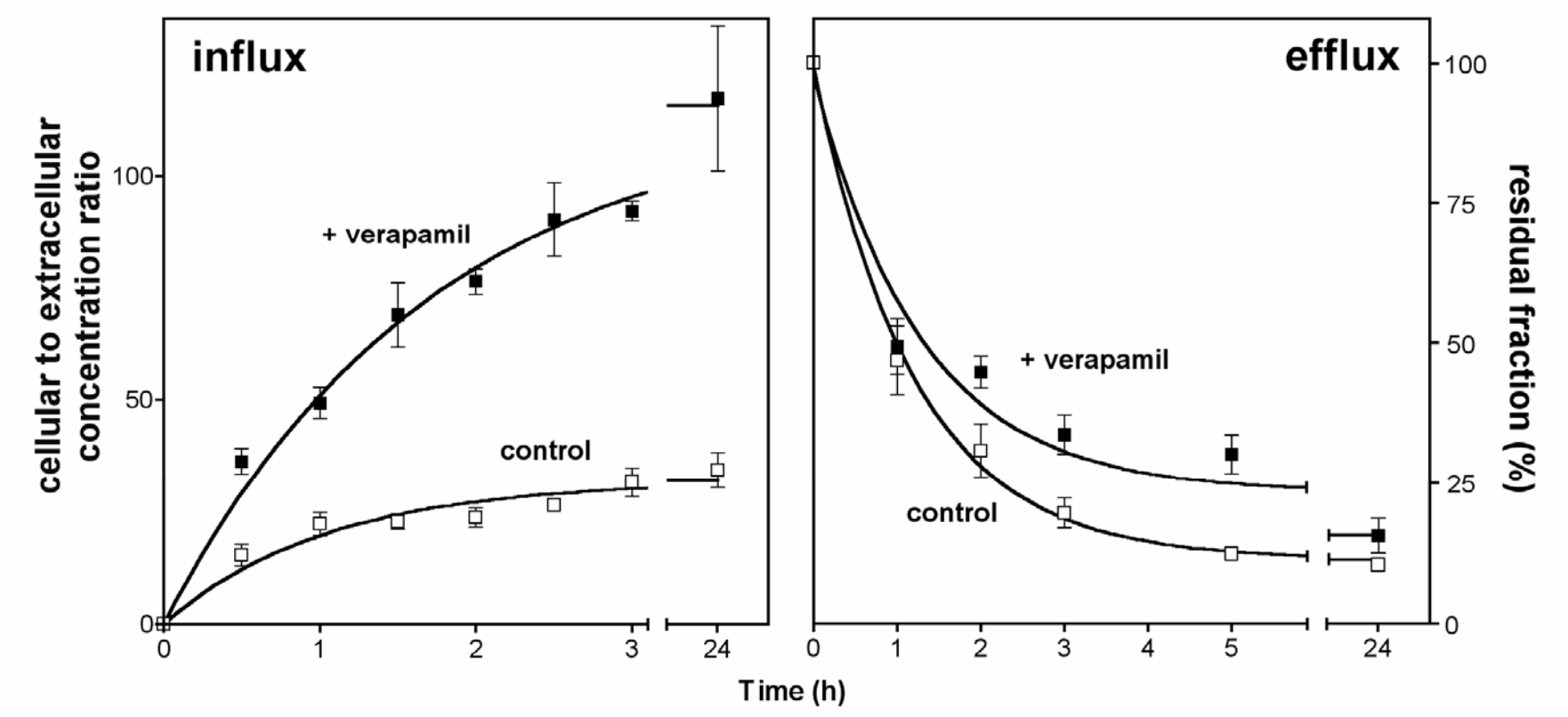
Chanteux et al. Antimicrob. Agents Chemother. (2005) 49:1279-88.

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



Van Bambeke et al. J. Antimicrob. Chemother. (2003) 51:1067-1077

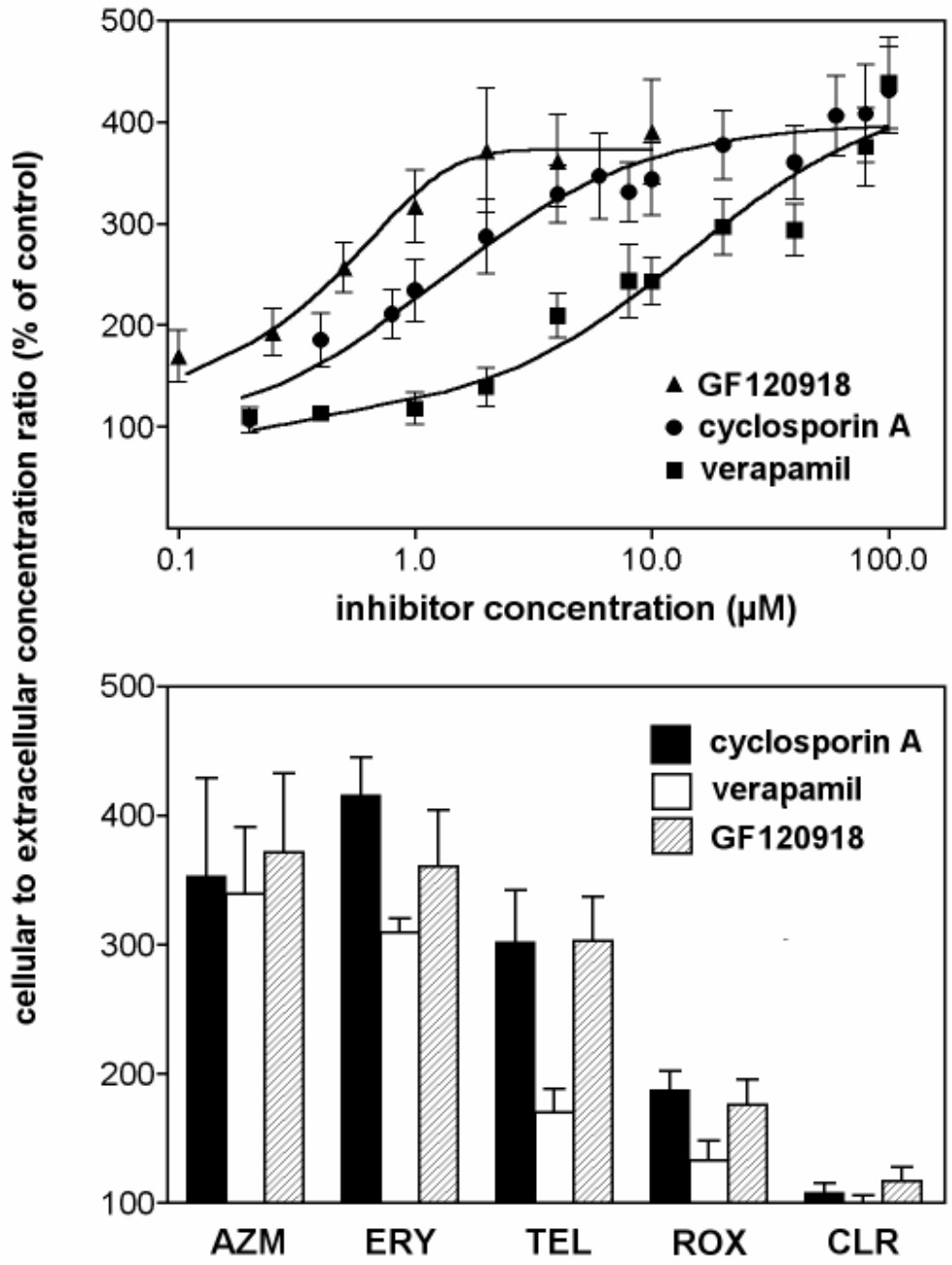
Azithromycin accumulation in macrophages is sub-optimal because of effflux through P-glycoprotein



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

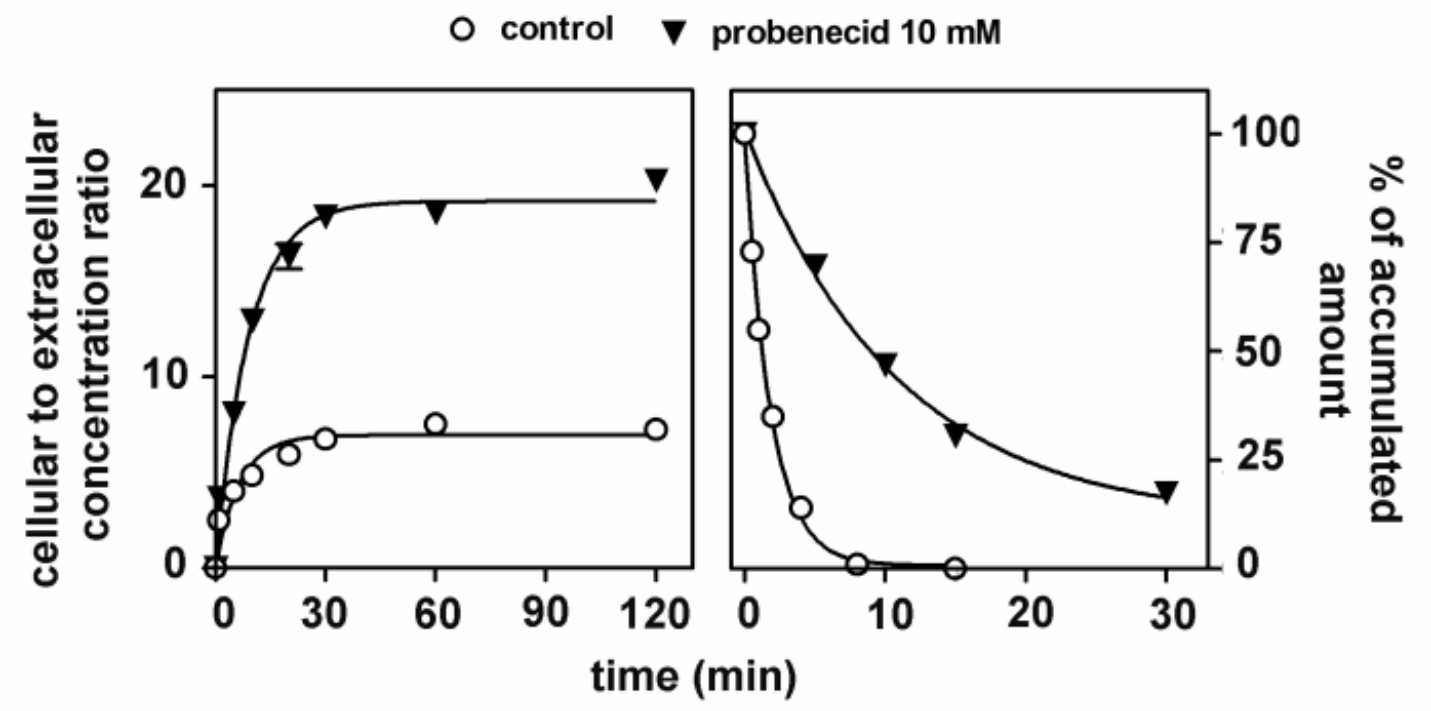
Seral et al. Antimicrob. Agents Chemother. (2003) 47:1047-1051

Characterizing P-gp-mediated efflux and ranking macrolides



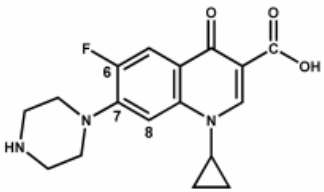
Seral et al. Antimicrob. Agents Chemother. (2003) 47:1047-1051

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

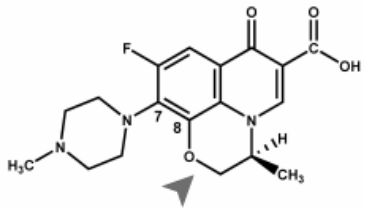


Michot et al. Antimicrob. Agents Chemother. (2004) 48:2673-2682

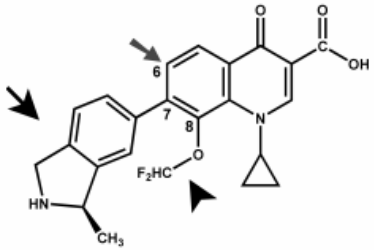
Ranking fluoroquinolones for efflux and explaining differences in accumulation



ciprofloxacin



levofloxacin



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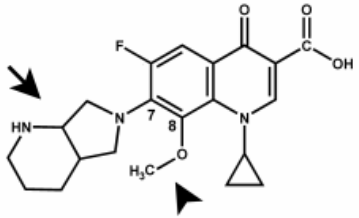

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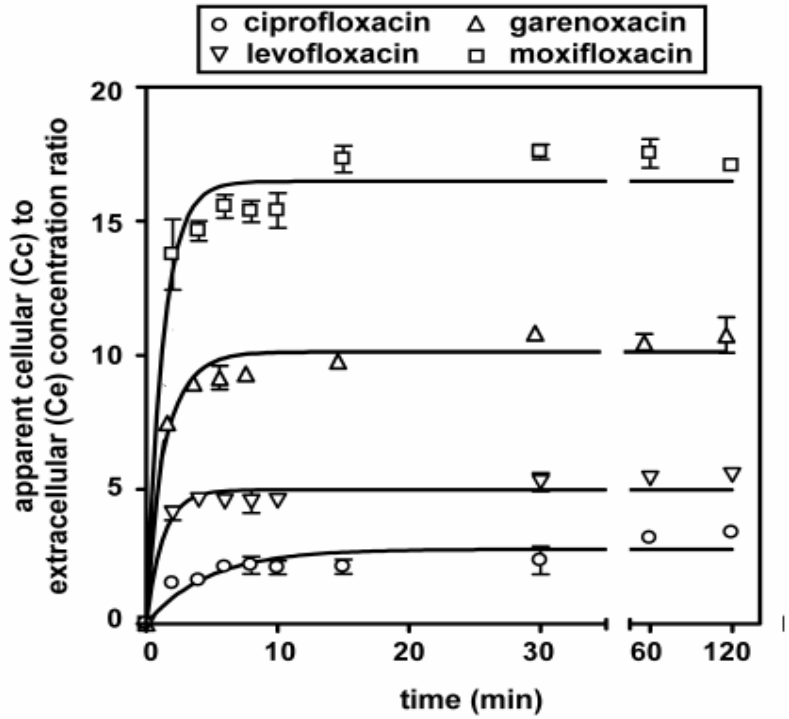
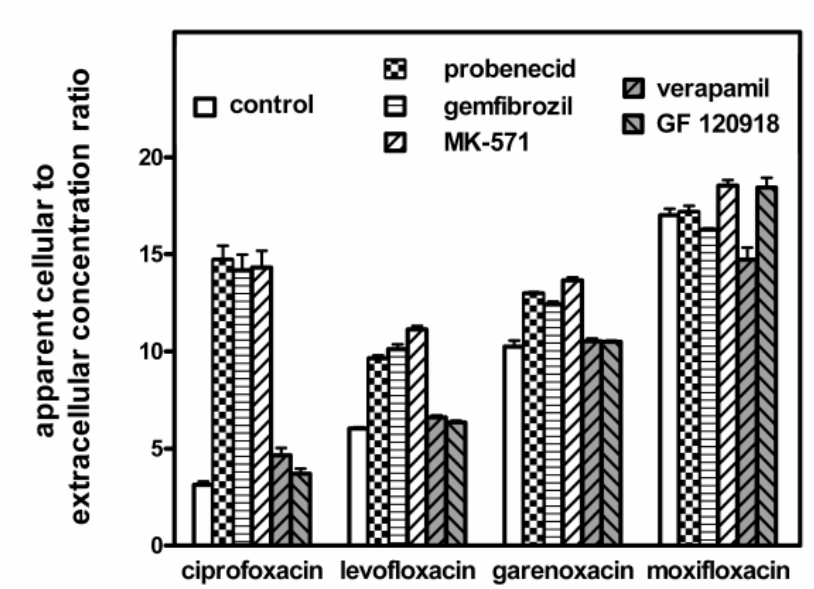
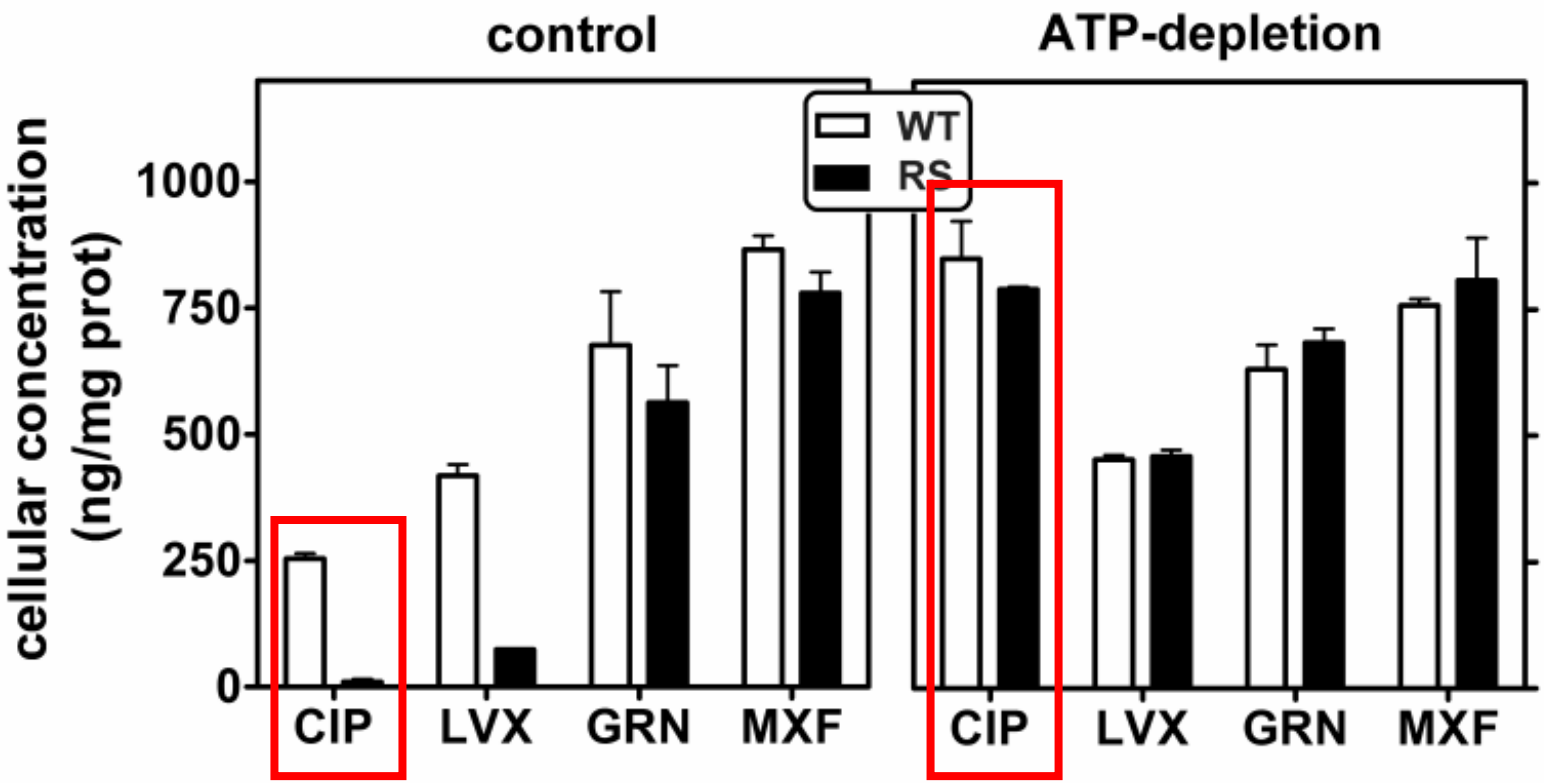




Michot et al. Antimicrob. Agents Chemother. (2005) 49:2429-2437

Making MRP-overexpressing cells

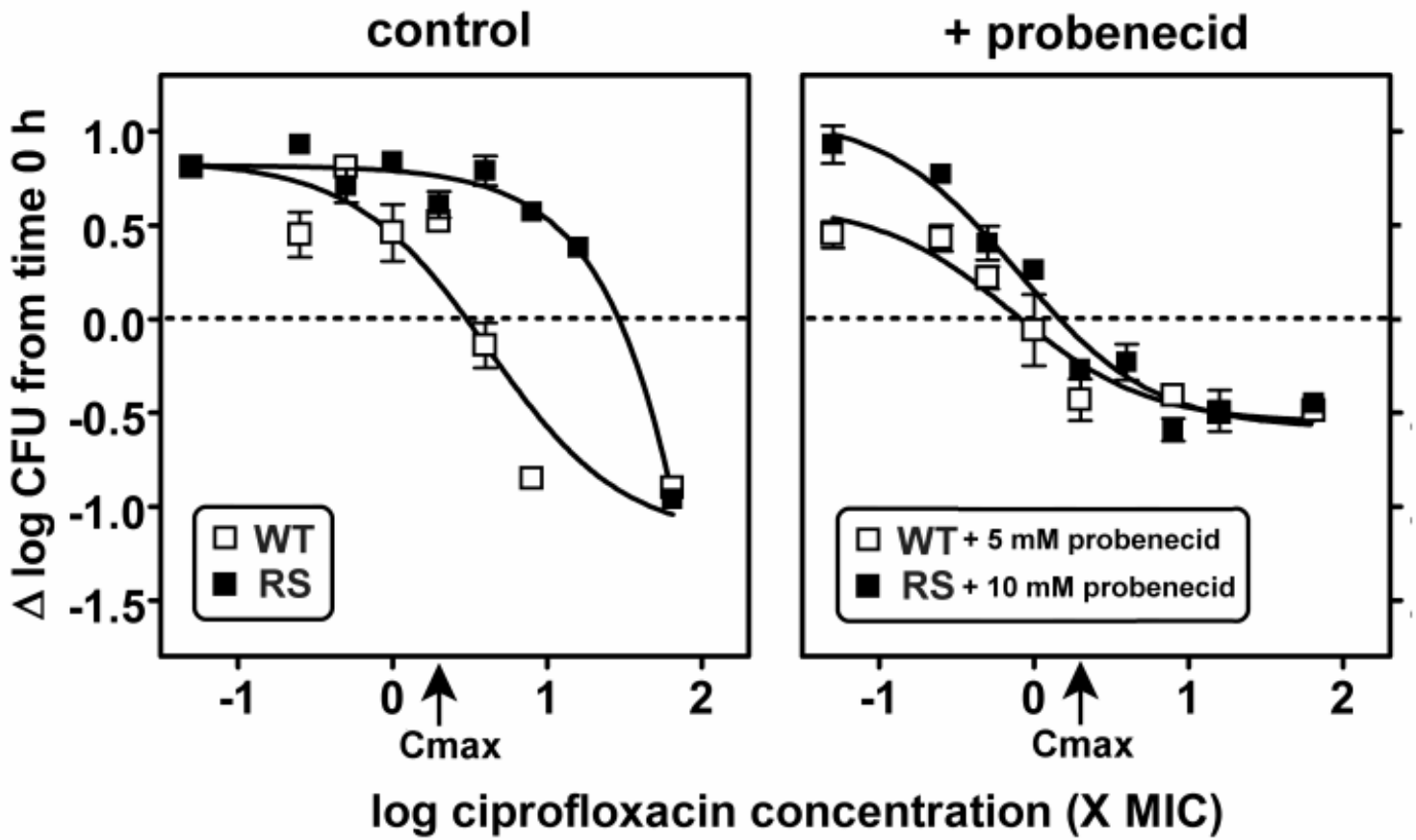
WT: wild type
RS: MRP-overexpressing cells



Michot et al. Antimicrob. Agents Chemother. 2006 (in press)

Assessing the intracellular activity of ciprofloxacin in MRP-overexpressing cells

WT: wild type
RS: MRP-overexpressing cells



Michot et al. Antimicrob. Agents Chemother. 2006 (in press)

Conclusions

Cell lines are useful to adress 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