In vitro pharmacodynamic evaluation of intracellular activity of antibiotics alone or in combination against a small colony variant (SCV) of *Staphylococcus aureus*

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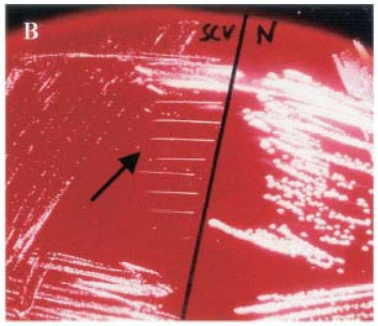
Ehrlich II

Small Colony Variant (SCV): a phenotype that facilitates persistent and recurrent infections

- SCVs show a particular phenotype
  - Growth: tiny, non-pigmented, non-hemolytic colonies
  - Reduced production of α-toxin
  - Auxotrophic for haemin, thymidine, menadione…
  - Can revert to their normal phenotype

Growth of SCV in comparison to normal isogenic phenotype on Columbia blood agar

Kahl BC et al. J. Clin. Microbiol. 2003;41: 410-3



- SCVs have been isolated in patients suffering from chronic, relapsing and difficult-to-treat infections
  - CF *Vergison et al. J. Antimicrob. Chemother. 2007; 59: 893-9*
  - Infection of skin (Darier's disease), bone (osteomyelitis), device-associated infections …

Proctor RA et al. Clin. Infect. Dis. 1995; 20: 95-102.
von Eiff C et al. Clin. Infect. Dis 2001; 32: 1643-1647.
von Eiff C et al. Injury 2006; 37 (Suppl 2): S26-33.

SCVs are difficult to eradicate

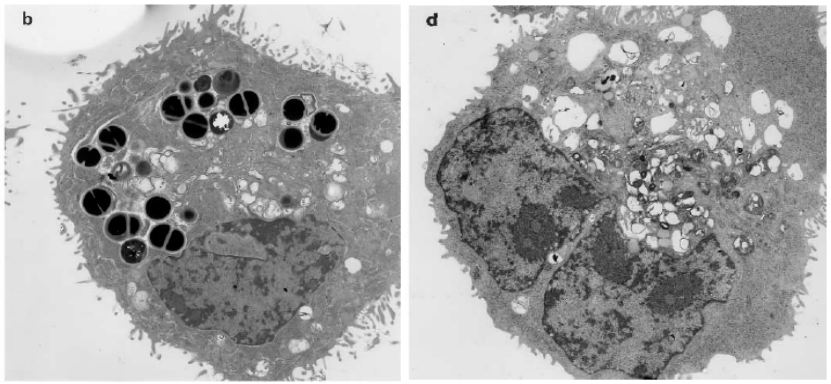
- Reduced susceptibility to antibiotics
  - Slow growth
  - Metabolic defects

Chuard C et al. J. Antimicrob. Chemother. 1997; 39: 603-608
Proctor RA and von Humboldt A. Drug Resist. Updat.1998; 1: 227-325

- More persistent within intracellular milieu ⇒ evasion host defenses and antibiotic actions

Intracellular persistence assay within human keratinocyte with SCV and NP

Von Eiff et al. Clin. Iinfect. Dis. 2001; 32: 1643-7.



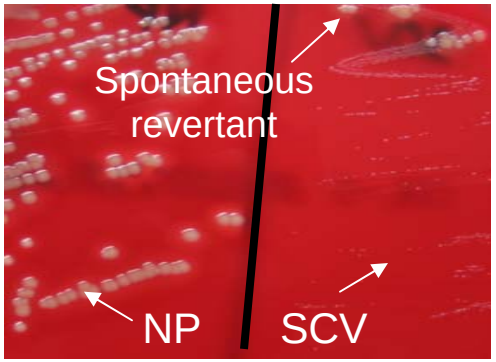
SCV, 48 h NP, 48 h

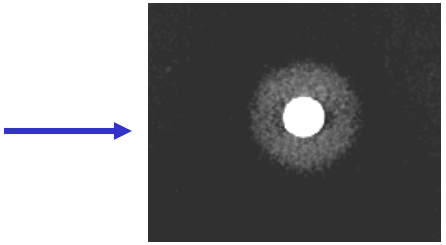
How to target intracellular SCVs ?

- AB pharmacokinetic properties:
  - Capacity to penetrate within host cells and to rejoin the infected compartment.
- AB pharmacodynamic properties:
  - Capacity to exert optimal bactericidal activity
    - in the intracellular environment
    - even against slowly growing bacteria

Aims of the study

- To compare the intracellular activity of a series of ATBs
  - against a stable mecA-negative, thymidine-dependent SCV variant, isolated from a CF patient, its isogenic normal phenotype (NP) and revertant counterparts.
  - In a model of THP-1 human macrophages





Thymidine dependency

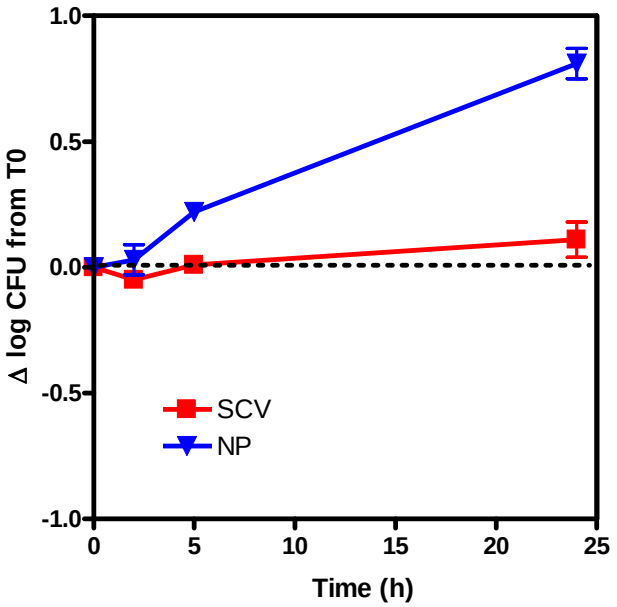
Selected antistaphylococcal agents:

- First line antistaphyloccal agents: OXA, GEN, VAN, MXF, CLI, RIF.
- New alternatives for resistant *S. aureus*: LZD, TGC, DAP, Q-D.
- Investigational compounds: ORI, TLV.

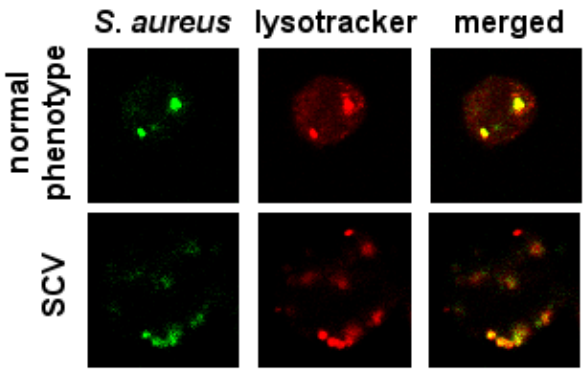
- To examine the potential interest of combining ATBs against intracellular SCVs

Description of the model

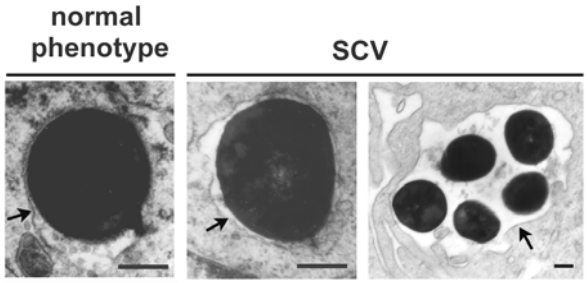
Intracellular growth of SCV versus its NP counterpart



Subcellular localization

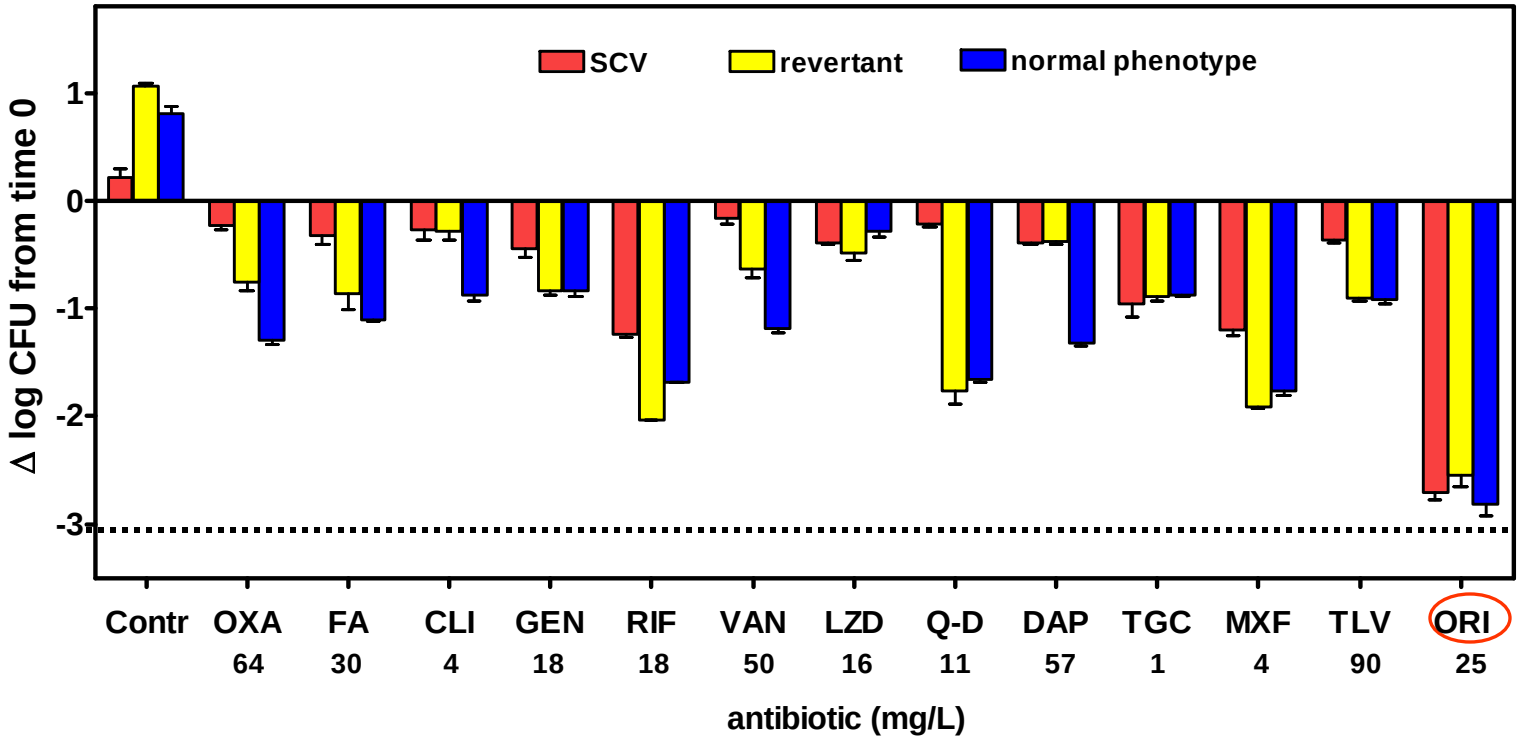


SCV and NP localized in acidic intracellular compartments



Intracellular activity : SCV versus isogenic normal phenotype and revertant

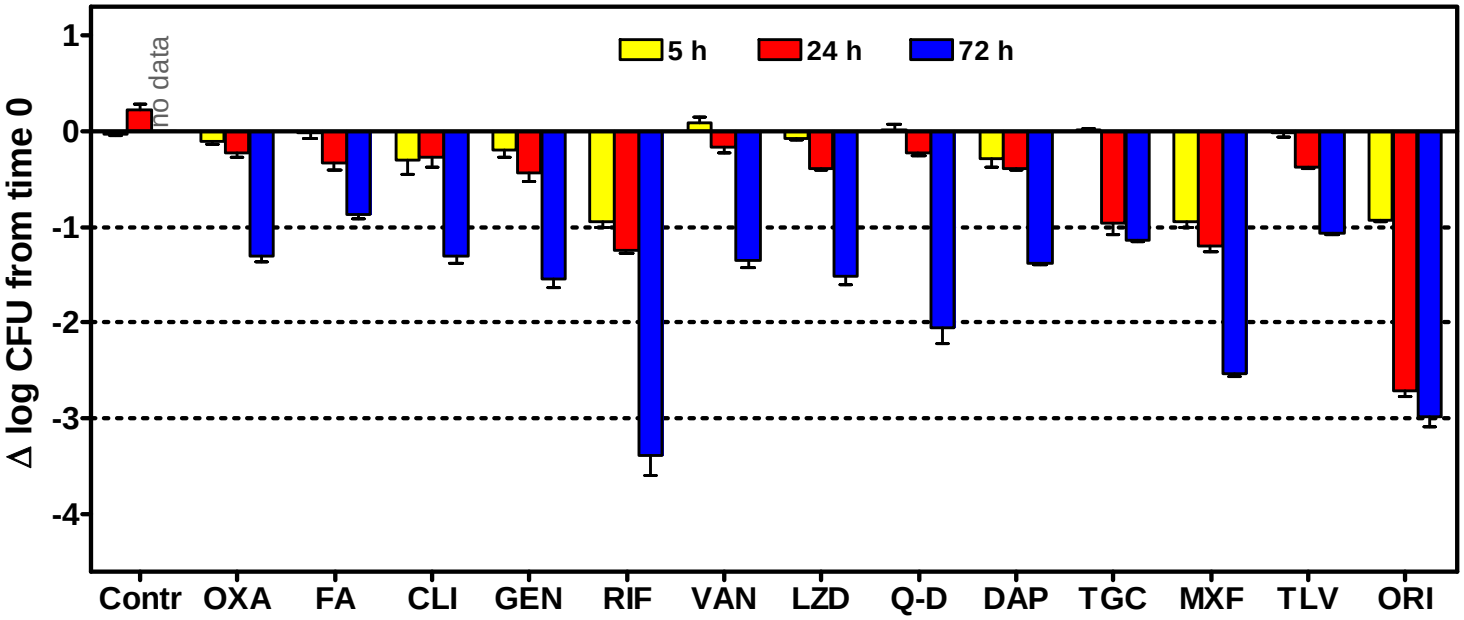
THP-1; 24 h, antibiotics at Cmax



- Intracellular growth of NP and revertant is markedly increased
- Activity is higher against NP and revertant for all but LZD, ORI, TGC
- Enhancement is more pronounced for membrane-acting ATB: OXA, VAN, DAP, TLV
- ORI is the most active drug, with no difference against SCV or NP

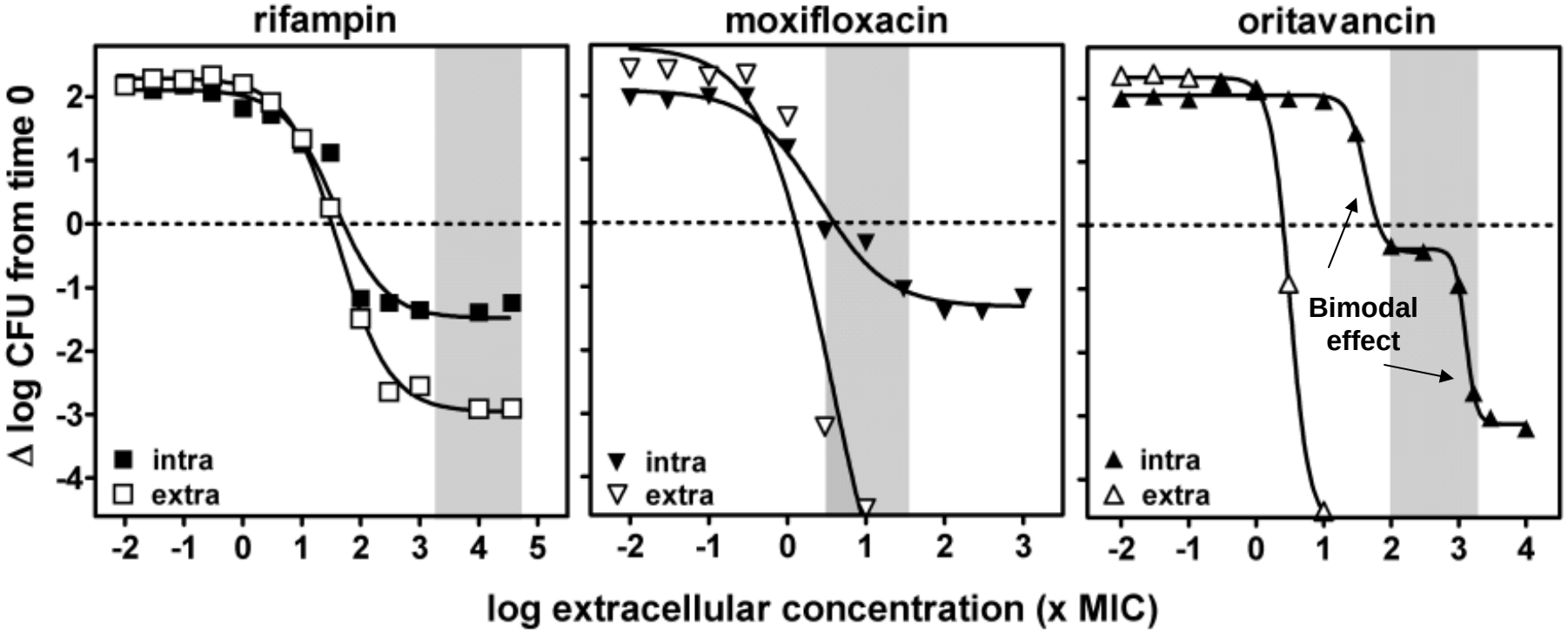
Intracellular activity against SCV : time effect

THP-1; SCV, antibiotics at Cmax for up to 3 days



- **At 5 h,** no marked change compared to initial inoculum
- **At 24 h,**
  - most antibiotics caused only a modest decrease in bacterial counts (< 1 log decrease)
  - an approx. 1 log decrease was reached for RIF, TGC, MXF
  - ORI achieved more than 2 log decrease
- **At 72 h,**
  - the activity of most ATBs (but TGC) increased (0.5-1 log)
  - ORI remained one of the most active drugs

Dose-response curves of the 3 most active antibiotics against extra- and intra-cellular SCV (24 h of exposure)



Gray zones: clinically-relevant range of concentrations

- Extracellular activity:
  - Highly bactericidal, concentration-dependent effects
- Intracellular activity:
  - RIF and MXF show markedly reduced activity
  - ORI shows a bimodal effect with maximal activity ≈ 3 log

Activity against extra- and intra-cellular SCV: dose-effect response

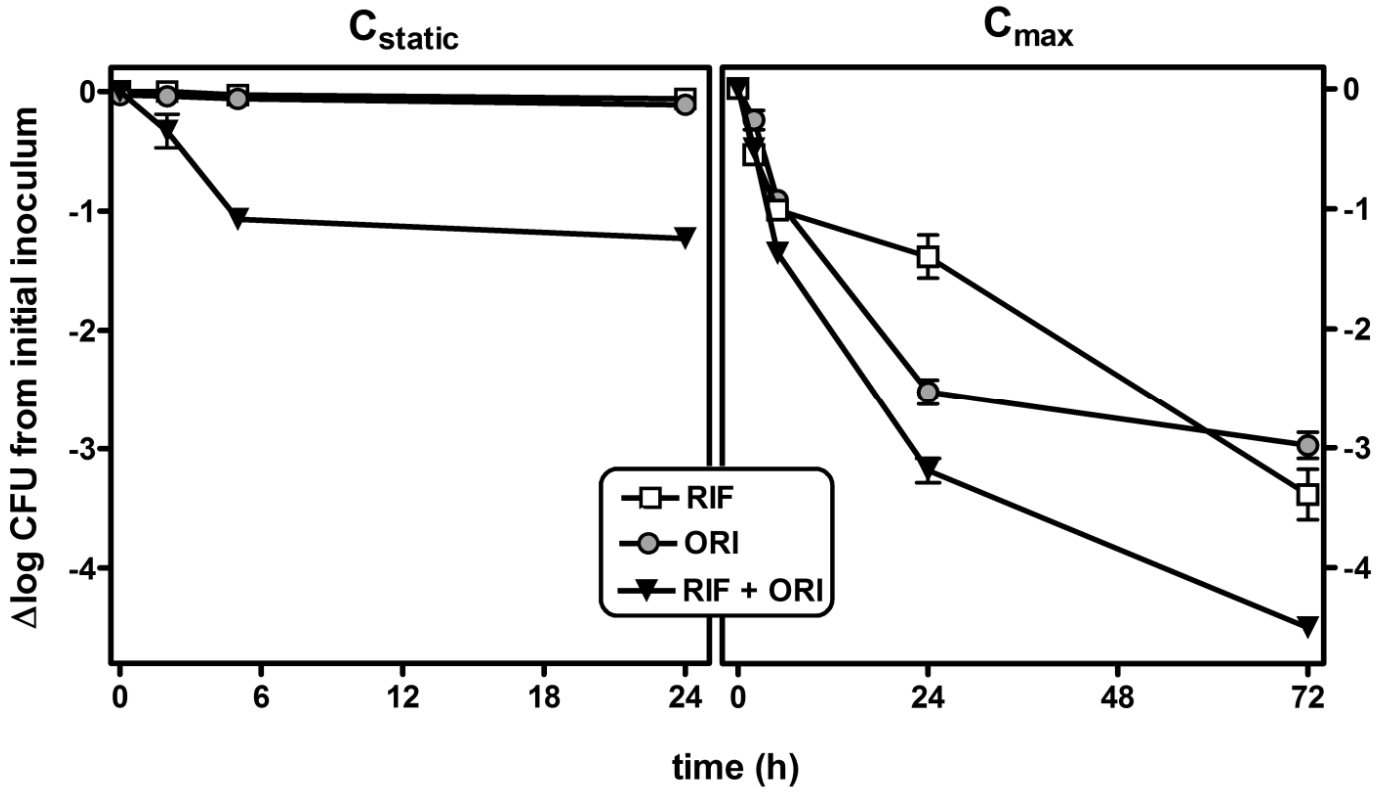
Curve analyses for all drugs

antibiotic	Intracellular [b]				Extracellular [c]			
	E_{max} [d] (CI)	EC_{50} [e] (CI)	C_{static} [f]	R^2	E_{max} [d] (CI)	EC_{50} [e] (CI)	C_{static} [f]	R^2
OXA	-0.39 (-0.52 to –0.27) A,a	0.83 (0.58 to 1.17) A,a	2.03	0.966	-3.91 (-4.07 to –3.76) A,b	5.50 (4.71 to 6.43) A,b	1.66	0.993
FA	-0.36(-0.52 to –0.20) A,a	10.70 (7.77 to 14.72) B,a	6.29	0.970	-1.43 (-1.52 to –1.34) B,b	4.78 (4.15 to 5.51) B,b	2.45	0.994
CLI	-0.45 (-0.51 to –0.39) C,a	0.47(0.39 to 0.57) A,a	1.47	0.990	-2.59 (-2.85 to –2.23) C,b	2.52 (1.79 to 3.55) C,b	1.47	0.967
GEN	-0.58 (-0.76 to –0.40) A,a	0.63 (0.41 to 0.97) A,a	1.62	0.947	< -4.5 D,b	2.33 (1.75 to 3.11) C,b	1.04	0.986
RIF	-1.72 (-2.04 to –1.40) B,a	42.60 (29.42 to 61.66) C,a	5.69	0.960	-3.02 (-3;19 to –2.85) E,b	43.35 (37.66 to 49.90) D,b	4.57	0.994
VAN	-0.36 (-0.62 to –0.10) A,a	6.61 (3.77 to 11.61) D,a	5.18	0.915	-4.25 (-4.42 to –4.00) F,b	4.99 (4.27 to 5;84) E,b	1.58	0.992
LZD	-0.54 (-0.84 to –0.23) A,a	0.61 (0.38 to 0.97) A,a	1.87	0.948	-3.24 (-3.49 to –3.00) E,b	2.72 (2.21 to 3.35) C,b	1.43	0.989
Q-D	-0.60 (-0.76 to –0.44) A,a	0.25 (0.15 to 0.40) A,a	1.02	0.934	-3.50 (-3.70 to –3.30) G,b	0.75 (0.58 to 0.97) G,b	0.80	0.981
DAP	-0.50 (-0.64 to –0.35) A,a	4.17 (2.91 to 5.96) E,a	3.45	0.964	-3.78 (-4.03 to –3.53) A,b	4.54(3.58 to 5.76) H,b	1.68	0.983
TGC	-1.11 (-1.26 to 0.95) C,a	0.80 (0.50 to 1.17) A,a	1.23	0.960	-1.75 (-1.85 to –1.64) H,b	1.31(1;08 to 1.59) I,b	1.27	0.989
MXF	-1.32 (-1.45 to –1.19) C,a	2.49 (1.92 to 3.23) F,a	1.85	0.980	< -4.5 D,b	3.01 (1.45 to 6.26) J,b	1.12	0.923
TLV	-0.35 (-0.53 to –0.17) A,a	0.52 (0.31 to 0.85) A,a	3.89	0.931	< -4.5 D,b	0.88(0.64 to 1.21) G,b	0.52	0.971
ORI [g]	-0.43 (-0.56 to 0.30) na	41.61 (35.79 to 48.38) na	65	0.990	< -4.5 D,n	3.15 (3.05 to 3.24) na	1.50	0.976
	-3.13 (-3.22 to –3.00) na	1272 (1212 to 1334) na	NA	0.994				

Reduced killing activities against intracellular SCV: lower E_{max}

Activity of antibiotic combination against intracellular SCV

At fixed concentrations with RIF or ORI (over the time)



- At C_{static}: 1.2 log after 24 h.
- At C_{max}: reaching the limit of detection (sterilize the infected macrophages)

Activity of combination RIF-ORI against intracellular SCV

Fractional maximal effect (FME) approach

- Analyse the nonlinear pharmacodynamics exhibited by antibiotics
- Examine the effect of combination for calculated and not arbitrarily chosen concentrations

Effect (E): decrease of inoculum after 24 h. Sigmoid E_{max} model $\Rightarrow$ E_{max}, EC_{50}

$$E = \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n}$$

FME is defined by the ratio observed effect/theoretical effect

ATBs (A et B) are combined to a FME=1.

5 pairs: 0.1 FME_A + 0.9 FME_B, 0.3 FME_A + 0.7 FME_B, 0.5 FME_A + 0.5 FME_B, 0.7 FME_A + 0.3 FME_B, 0.9 FME_A + 0.1 FME_B

Correspoding concentrations to be tested alone and in combination:

$$C_{xp} = \frac{FME \; x \, EC_{50}}{1 - FME}$$

FME (for ATB alone or in combination) is then plotted versus concentrations

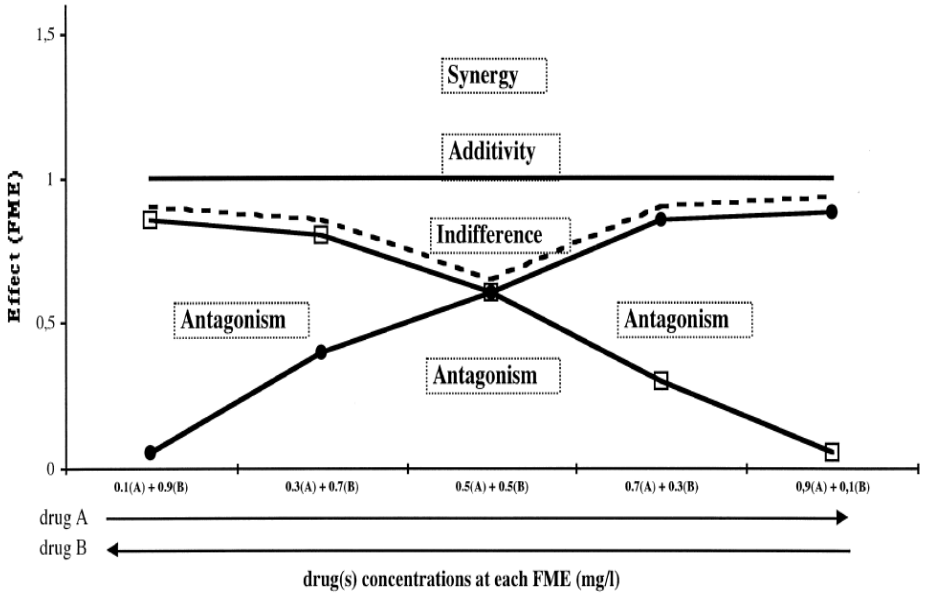
Interaction evaluation:

Additivity: effect equal to the theoretical sum of the effects of each ATB tested alone, FME=1

Synergy (FME > 1),

Antagonism: FME < best FME of ATB used alone

Indifference: FME between antagonism and additivity



Li RC et al. AAC 1993; 37: 523-531; Desbiolles N et al. AAC 2001; 45:3328-3333.

Activity of combination RIF-ORI against intracellular SCV

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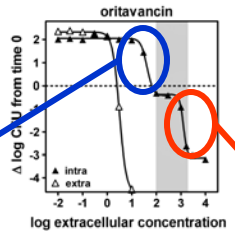
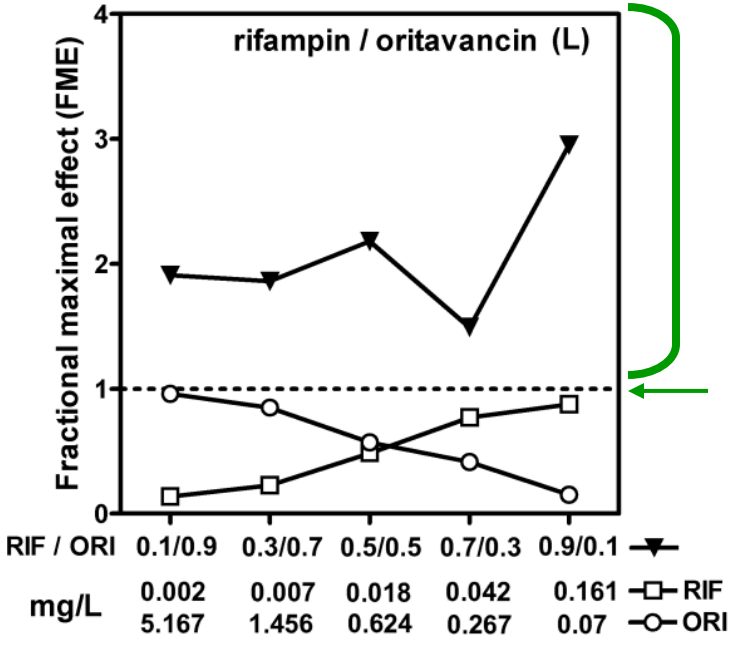
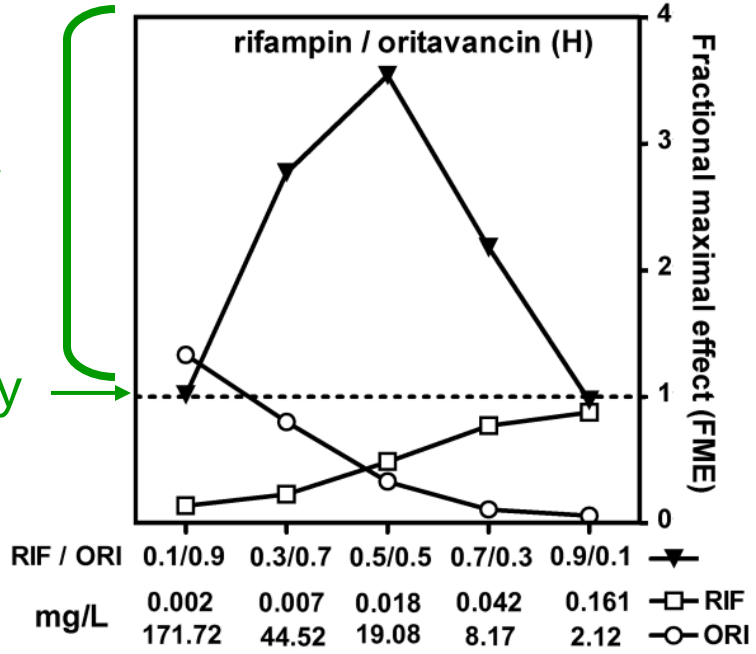




synergy

additivity



Combination RIF-ORI is highly synergistic over a wide range of concentration ratios

Conclusions

- Most currently available anti-staphylococcal agents act only poorly on intracellular SCVs compared to its normal phenotype and revertant counterparts, unless upon prolonged exposure.

- None of the tested antibiotics is able to sterilize the infected macrophages, which may explain the difficulty of eradicating these organisms in CF patients.

- Among clinically-available antibiotics, RIF and MXF are the most active. Only ORI proves bactericidal activity towards both intracellular SCV and NP.

- Combination RIF-ORI is highly synergistic over a wide range of concentration ratios, reaching the limit of detection when used both at C_{max}.

- Our results suggest the interest of evaluating ATBs against intracellular SCV to select most appropriate therapies.

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- FACM team