

Pharmacodynamic concepts and their definitions

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Pharmacodynamic parameters

- **MIC/MBC**
- **Time-killing curves**
- **Concentration-dependent vs. non-dependent killing**
- **Tolerance**
- **Paradoxical effect**

Pharmacodynamic parameters

- **PAE; Postantibiotic effect**

In vitro

In vivo

- **PA SME; Postantibiotic sub-MIC effect**

- **ERT, CERT**

Pharmacodynamic parameters

- **PME; post- MIC effect**
- **PALe; postantibiotic leucocyte enhancement**
- **SME; sub-MIC effect**

Minimal inhibitory concentration; MIC

- **The amount of antibiotic which has to be added to a variety of different microorganisms in order to produce a publication in a scientific journal**

Differences between MIC determinations in vitro and in vivo

In vitro

- **Static concentrations**
- **Physiologic pH, maximal growth conditions**
- **Protein-free medium**
- **Read after 24 hours**
- **No immunsystem**
- **Constant (often low) inoculum**

In vivo

- Variable concentrations**
- Variable conditions**
- Albumin, glycoproteins**
- Can not be determined**
- Leucocytes, macrophages**
- Variable inoculum**

MIC methods

- **Broth macrodilution in tubes**
- **Broth dilution in microtiter plates**
- **Plate dilution method**
- **E-test (disc diffusion)**

Correlation between MIC and in vivo efficacy

- **Correlation between 24h static dose and MIC in mouse thigh model**
- **Correlation between log ED50 and log MIC in a mouse peritonitis model**
- **Some correlation between high MICs and clinical failures**

Time-killing curves

- **In vitro killing curves correlate rather well with in vivo killing curves**

Concentration dependent vs. non-dependent killing

In general

- **β -lactam and glycopeptide antibiotics; non concentration-dependent killing**
- **Quinolones and aminoglycosides; concentration-dependent killing**

Concentration dependent vs. non-dependent killing

- **Macrolides are mainly bacteriostatic. Newer macrolides acts more bactericidal.**
- **Streptogramins and ketolides are slowly bactericidal**

Tolerance

- **Genotypic tolerance**

$$\text{MBC/MIC} \geq 16$$

- **Phenotypic tolerance**

Most antibiotics do not kill non-growing bacteria with the exception of carbapenems

Paradoxical effect "Eagle effect"

Definition

- **An antibiotic concentration above the maximally effective concentration at which the killing rate of the bacteria is paradoxical reduced**

Paradoxical effect "Eagle effect"

- **Have been described in vitro for**
- **Penicillins; staphylococci
streptococci gr. B
H. influenzae**
- **Quinolones; E. coli**
- **Aminoglycosides; gramnegatives**
- **In vivo phenomenon??**

Control related effective regrowth time; CERT

- **Defintion**
- **$CERT = T - C$**
- **T= the time required for resumption of logarithmic growth and increase of one log₁₀ to occur over the preexposed inoculum in the test tube**
- **C= corresponding time for the control culture**

Conclusions

- **The postantibiotic effect in vitro can in most cases predict the in vivo PAE**
- **The PA SME and PME reflect the in vivo situation better than the PAE**
- **Antibiotic/bacterial combinations that exhibit a PAE also have a PA SME**
- **The PMEs are shorter in comparison with the PA SMEs**

Conclusions

- ***β -lactam and glycopeptide antibiotics***
- **Slow bactericidal effect**
- **Non-concentration dependent killing**
- **Paradoxial effect common in vitro**
- **Carbapenems can kill phenotypic tolerant bacteria**
- **PAE and PA SME only against grampositive bacteria**
- **Effect is correlated to time > MIC**

Conclusions

- **Quinolones and aminoglycosides**
- **Fast bactericidal effect**
- **Concentration dependent killing**
- **Paradoxical effect described in vitro**
- **PAE and PA SME against both grampositive gramnegative bacteria**
- **Effect is correlated to AUC/MIC**

Conclusions

- **Macrolides**
- **Mostly bacteriostatic**
- **Non-concentration dependent killing**
- **Have the longest PAEs and PA SMEs**
- **Effect is correlated to time > MIC**

Conclusions

- **Pharmacodynamic parameters in relation to pharmacokinetics are important to define optimal dosing regimens**

The postantibiotic and sub-MIC effects in vitro and in vivo

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Postantibiotic effect; PAE in vitro

Definition:

- Suppression of bacterial growth after short exposure of organisms to antibiotics

$$PAE = T - C$$

T= The time required for the exposed culture to increase one \log_{10} above the count observed immediately after drug removal

C= The corresponding time for the unexposed control

Postantibiotic effect in vitro

The PAE is dependent on:

- Type of antibiotic
- Type of bacterial species
- Concentration of the antibiotic
- Duration of exposure
- Size of the inoculum
- Growth phase of the organism

Postantibiotic effect in vitro

PAE against Grampositive bacteria

<u>Antibiotics</u>	<u>hours</u>
• Penicillins	1-2
• Cephalosporins	1-2
• Carbapenems	1-2
• Quinolones	1-3
• Proteinsynthesis inhibitors	3-5

Postantibiotic effect in vitro

PAE against Gramnegative bacteria

<i>Antibiotics</i>	<i>hours</i>
• Penicillins	0
• Cephalosporins	0
• Carbapenems	(1)
• Quinolones	1-3
• Proteinsynthesis inhibitors	3-8
• Aminoglycosides	2-4

Postantibiotic effect in vitro

PAE against P. aeruginosa

<u>Antibiotics</u>	<u>hours</u>
• Penicillins	0
• Cephalosporins	0
• Carbapenems	1-2
• Quinolones	1-2
• Aminoglycosides	2-3

PAE in vitro Methods

1. Viable counts

Methodological pitfalls

- **may overestimate killing**
- **negative PAEs are common with β -lactams**
- **and gram-negatives due to forming of filaments**
- **similar inocula of the control and the pre-exposed culture are desirable**

PAE in vitro Methods

2. Optical density

Methodological pitfalls

- killing cannot be measured due to a detection limit of 10^6 cfu/ml
- control curves at different inocula and viable counts after drug removal are necessary to be performed to ensure that PAE culture and control are at the same inoculum

PAE in vitro Methods

3. ATP measurement

Methodological pitfalls

- bactericidal activity is underestimated due to dead but intact (not lysed) bacteria still containing intracellular ATP
- PAE is overestimated due to falsely elevated ATP content

PAE in vitro Methods

4. Morphology

- Phase contrast microscopy
 - the time it takes for the bacteria to revert to 90% bacilli
- **Ultrastructural changes**
 - the changes in structure correlates well with the PAE measured with viable counting

5. ³H-thymidine incorporation

- correlates well with the PAE measured with viable counting

Postantibiotic effect in vivo

Definition

$$PAE = T - C - M$$

- T= the time required for the counts of cfu in thighs of treated mice to increase one log₁₀ above the count closest to but not less than the time M
- C= the time required for the counts of cfu in thighs of untreated mice to increase one log₁₀ above the count at time zero
- M= the time serum concentration exceeds the MIC

PAE in vivo

- Observed in several animal models
- In vitro data are predictive of in vivo results except that in vivo PAE are usually longer due to the effect of sub-MICs and/or the effect of neutrophils
- The major unexplained discordant results are for β -lactams and streptococci

PAE in vivo

Animal models

- Thigh infections in mice
- Pneumonia model in mice
- Infected treads in mice
- Infected tissue cages in rabbits
- Meningitis model in rabbits
- Endocarditis model in rats

Mechanisms of PAE

- **β -lactam antibiotics.**

At least for *S. pyogenes* and penicillin it has been shown that PAE stands for the time it takes for the bacteria to resynthesize new PBPs

Mechanisms of PAE

- **Erythromycin and clarithromycin:**

50S ribosomal subunits were reduced during 90 min and protein synthesis during 4 h (PAE) due to prolonged binding of the antibiotics to 50S.

Mechanisms of PAE

- **Aminoglycosides:**

Binding of sublethal amounts of drug enough to disrupte DNA, RNA and protein synthesis. The time it takes to resynthesize these proteins.

With a half-life of >2.5 h, the PAE disappears, reflecting a sufficient time for the repair mechanism to be restored.

Postantibiotic sub-MIC effect; PA SME

Definition

- The effect of subinhibitory antibiotic concentrations on bacteria previously exposed to suprainhibitory concentrations

$$\text{PA SME} = T_{\text{PA}} - C$$

- T_{PA} = the time it takes for the cultures previously exposed to antibiotics and thereafter to sub-MICs to increase by one log₁₀ above the counts observed immediately after washing.
- C = corresponding time for the unexposed control

Post-MIC effect; PME

Definition

- The effect of sub-MICs on bacteria previously exposed to a constant decreasing antibiotic concentration

$$\text{PME} = \text{Tpme} - \text{C}$$

- Tpme = The time for the counts in cfu of the exposed culture to increase one \log_{10} above the count observed at the MIC level
- C = the time for an unexposed control to increase one \log_{10}

Mechanism of PA SME?

- The PAE of β -lactam antibiotics seems to represent the time necessary to synthesize new PBPs. When bacteria in the PA-phase are exposed to sub-MICs, most PBPs are still inactivated and only a small amount of the drug is needed to prolong the inhibition of cell multiplication until a critical number of free PBPs are once more available

Postantibiotic leucocyte enhancement; PALE

- Bacteria pretreated with antibiotics for a brief period of time show increased susceptibility to intracellular killing and phagocytosis
- In general, antibiotics that produce the longest PAEs exhibit maximal PALEs

Sub-MIC effects; SME

Definition

- The effect of subinhibitory antibiotic concentrations on bacteria not previously exposed to suprainhibitory concentrations

$$\text{SME} = T_s - C$$

- T_s = the time it takes for the cultures exposed to sub-MICs to increase by one log₁₀ above the counts observed immediately after washing
- C = corresponding time for the unexposed control

Sub-MIC effects

- The minimum antibiotic concentrations that produces a structural change in bacteria seen by light or electrom microscopy
- The minimum antibiotic concentration that produces a one log₁₀ decrease in the bacterial population compared to the control
- Loss or change of bacterial toxins

Sub-MIC effects

- Loss of surface antigens resulting in decreased adhesion
- Increased rates of phagocytic ingestion and killing
- Increased chemotaxis and opsonization

Mechanism of sub-MIC effects

- SME probably tests the distribution of antibiotic susceptibility in the bacterial population, in which there are subpopulations that are inhibited by concentrations less than the MIC. The SME would therefore represent the time it takes for the population with the higher MIC to become dominant