Briefly describe the bactericidal activity of gentamicin.
Explain why its usually administered as a single daily dose
Describe the potential toxic effects of gentamicin.

Antibiotics can be
bacteriostatic – inhibit replication permitting the microbe to be killed by the host defences: macrolides, clindamycin, tetracyclines
bacteriocidal – kill organisms: beta-lactams, aminoglycosides, metronidazole, vancomycin

Minimum Inhibitory Concentration – the lowest concentration of a chemical which prevents growth of a micro-organism
Minimum Bactericidal Concentration – the lowest concentration of an antibacterial agent required to kill a particular bacterium.

Bactericidal effects:
Minimal Antibiotic Concentration: drug altering bacterial cell morphology and slowing the rate of growth
Post antibiotic effect: persistent suppression of bacterial growth
Post antibiotic leucocyte enhancement: organism more susceptible to phagocytes after antibiotic exposure.

Antibiotics are basically divided into 3 pharmacodynamic groups:

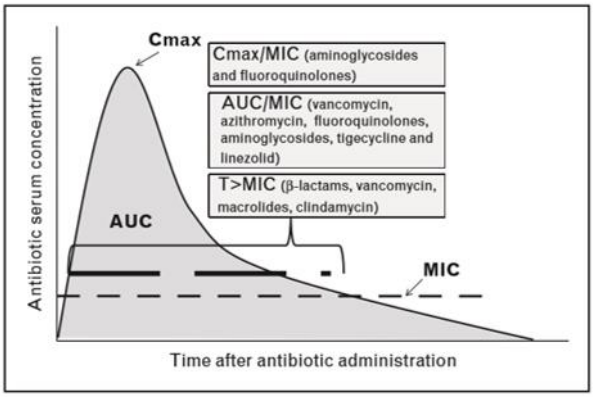


FIGURE 2. Pharmacokinetic and pharmacodynamic parameters. AUC, area under the curve; AUC/MIC, ratio of AUC to MIC (time and concentration-dependent antibiotics); C_{max}, peak antibiotic concentration; C_{max}/MIC, ratio of peak concentration to MIC (concentration-dependent antibiotics); MIC, minimum inhibitory concentration for a pathogen; T > MIC, percentage of time that the antibiotic concentration remains above MIC (time-dependent antibiotics).

1. **Concentration dependent (CMax-AUC/MIC)** – rate and duration of post-antibiotic effect are concentration dependent. The amount of drug (the total dose and its area under the curve) is more important than the dosing frequency. → aminoglycosides
2. **Time dependent (T>MIC)** – slow bactericidal action that is not improved by increasing the plasma concentration beyond maiximal killing action (4 x MIC). It is the time above the MIC that is instrumental. Variables: dosing frequency, infusion
3. **Mixed properties:** drugs that are bacteriostatic but with moderately prolonged PAEs.

Aminoglycosides are a type I (CMax + AUC) concentration dependent antibiotic. For type I drugs the rate of bactericidal activity is greatest at peak serum concentrations (CMax). As the concentration decreases the rate of bactericidal activity decreases. Higher doses not only increase the rate of reductions of bacteria but also the length of time of drug exposure to bactericidal concentrations. The higher the concentration the greater the PAE.

For aminoglycosides giving a single daily dose maximizes the CMax/AUC.
The CMax is primarily affected by the Volume of Distribution and the AUC is affected by Vd and clearance.

Aminoglycoside MOA:
Binds to the 30S subunit of the bacterial ribosome interrupting protein synthesis.
The organism is induced to produce 'false proteins'.
The post-antibiotic effect is due to synergism with leucocytes where they have enhanced phagocytosis and killing activity after exposure to aminoglycosides.

Uses:
Gram-negative bacillary infections.

Pharmaceutics
Available as parenteral, ophthalmic and topical.

Dose:
5 – 7mg/kg over 30 – 60min
Renal dysfunction
2mg/kg loading then 3 – 5mg/kg every 8 – 12hrs
BUT:
Recommended doses do not always yield desired concentrations.
Therefore dosing guided by lab after gentamicin level determined on basis of timing of last dose and patient's weight.

A
Polar cations therefore poor G.I. absorption → <1% absorbed
BUT highly absorbed from raw surfaces – wounds, burns, cutaneous ulcers

D
Polar nature precludes cellular penetration (incl. CNS) or the eye. Not very lipid soluble
Vd: approximate 25% of lean body weight and is approx ECF → 20% TBW 14L or ~200ml/kg
PB: reasonably low somewhere on the order of <10% - ~25 – 30% but seems to be influenced by cationic molecules
Zaske (1992) stated that the plasma protein binding of gentamicin (and aminoglycosides in general) is <10% and concluded that the fewer the OH groups contained in the aminoglycoside molecule, the greater the rate of protein binding. Estimation of the plasma protein binding of gentamicin in human has ranged from zero binding (Rosenkranz et al., 1978) to 20% binding (Meyers et al., 1978).
M: minimal
E: active hepatic secretion → bile concentrations 30% of plasma; freely filtered at the glomerulus
T1/2: 2 – 3hrs

Aminoglycoside Toxicity:
Adverse effects of gentamicin:
Low blood counts
Allergic responses

Neuromuscular problems
Nerve damage
Kidney damage
Ear damage

Kidney damage:'
Aminoglycosides are freely filtered at the glomerulus 5 – 10% is sequestered in the proximal tubule and can reach concentrations many times the serum.
Gentamicin accumulates in the S1 and S2 segments of the proximal tubule.
Possesses multiple amine groups which have a cationic charge at neutral pH. As a result they readily bind to anion phospholipids within the PTC in a saturable and electrostatic manner.
Here they become endocytosed and trafficked to the Golgi complex → endoplasmic reticulum → cytosol. In the cytosol they accumulate in subcellular organisms such as mitochondria and nucleus.
The aminoglycosides disrupt protein sorting and synthesis and mitochondrial function.
Dose frequency also may be important as multiple human studies suggest that giving a large dose of aminoglycoside once a day is as effective an antimicrobial regimen and less nephrotoxic than giving aminoglycosides in the conventional, divided-dose regimen

Ototoxicity:
Cochlear and vestibular toxicity.
One hypothesis is related to receptors for N-methyl-D-aspartate (NMDA), which are present at the synapse between cochlear hair cells and neural afferents. Aminoglycosides can mimic the positive modulation of polyamines at these receptors, possibly producing excitotoxic damage
Another possibility is that aminoglycosides create reactive oxygen species that damage the inner ear.
Sustained or excessive peak serum concentrations are thought to be a risk factor.

The incidence of nephrotoxicity and ototoxicity is likely related to overall exposure.

Background

a) Briefly describe the bactericidal activity of gentamicin. Explain why it is usually administered as a single daily dose.

b) Describe the potential toxic effects of gentamicin.

This question had two parts, with part a) having two sub-sections. Marks were apportioned accordingly. (I guess they meant at least 60(30:30):40

Describing the antibacterial spectrum with some examples and mentioning that bactericidal meant killing cells attracted marks.

Brief details about the bactericidal mechanism were also helpful - i.e. gentamicin binds to ribosomes causing mRNA mistranslation, creating abnormal proteins that disrupt cellular machinery and the integrity of the cell wall.

The second section of part a) asked why once daily dosing is acceptable and few candidates were able to explain this satisfactorily.

Many candidates assumed, incorrectly, that once daily dosing was due to a long half-life. The crux of the question is recognising that gentamicin has a short half-life, so should require more frequent dosing. Daily dosing with a higher dose produces an initial high (bactericidal) concentration that falls below the minimum inhibitory concentration but retains a residual bactericidal activity (the so called "post antibiotic effect").

The main benefit being accumulation and toxic effects of gentamicin are reduced.

Part b) asked for a description of the toxic effects of gentamicin and was answered better than part a). Marks were given for describing nephrotoxicity and ototoxicity, including their clinical features, frequency, contributing and exacerbating factors and the reversibility or otherwise of these effects. Including the potential for muscle weakness, interactions with muscle relaxants and mechanisms also scored marks.

Expanded answer:

Antibiotic real-world clinical pharmacology

Antibiotic effects:

The antibiotics are divided on a basic premise of:

Bactericidal: kill the organism: beta-lactams, vancomycin, the aminoglycosides, the fluoroquinolones, daptomycin, and metronidazole

Bacteriostatic: inhibit the growth of the organism: macrolides, clindamycin, the tetracyclines, the sulfonamides, linezolid, and chloramphenicol

In-vitro measures:

Minimum Inhibitory Concentration: The MIC is a measure of the potency of an antimicrobial drug. **The lowest concentration of a chemical which prevents growth of a micro-organism**. The MIC is defined as the minimal concentration of antibiotic that prevents a clear suspension of 10^5 colony-forming units (CFUs) of bacteria/mL from becoming turbid after overnight incubation; turbidity usually connotes at least a 10-fold increase in bacterial density.

Minimal Bactericidal Concentrations: The lowest concentration of an antibacterial agent required to kill a particular bacterium. It is complementary to the MIC.

For **bactericidal drugs**, the MBC is usually the same as, and generally not more than fourfold greater than, the MIC.

In contrast, the MBCs of **bacteriostatic drugs** are many-fold greater than their MICs

- ➔ Do not correspond to the density of bacteria at the site of infection,
- ➔ Do not provide information on the time course of the antimicrobial effect of fluctuating drug levels within a treated patient

Time-kill: a measure of bactericidal activity – sampling a bacterial suspension of 10^5 CFU/ml in broth at successive time intervals (e.g. 2, 4, 6, 24hrs incubation) after addition of a particular concentration of antibiotic.

In vivo:

Pharmacokinetics:

Most sites of infection are extravascular, and treatment of infections in these sites depends on movement of the antibacterial agent out of the bloodstream into interstitial and sometimes intracellular fluid.

Only an unbound drug is considered active against a micro-organism.

The ability of a drug to do so depends on tissue-related factors (such as perfusion to the tissues, the surface area of the tissue's vascular bed, and specialized vascular bed features, such as tight junctions or capillary pores) and drug-related factors (such as lipid solubility, molecular size, the drug's pKa, and plasma protein binding).

The perfusion rate is greatest for the brain, kidney, liver, and heart. It would be expected that drug concentrations would increase most rapidly in these organs. As the surface area of the capillary bed increases, the rate of diffusion also increases.

Most drugs cross biologic membranes by passive diffusion. Diffusion occurs when the drug concentrations on one side are higher than the other.

Pharmacodynamics / Phenomenology:

Bacteriostatic:

For a **bacteriostatic drug**, when drug levels are in excess of the MIC**, the bacterial count declines as a result of host defenses alone**

Bactericidal

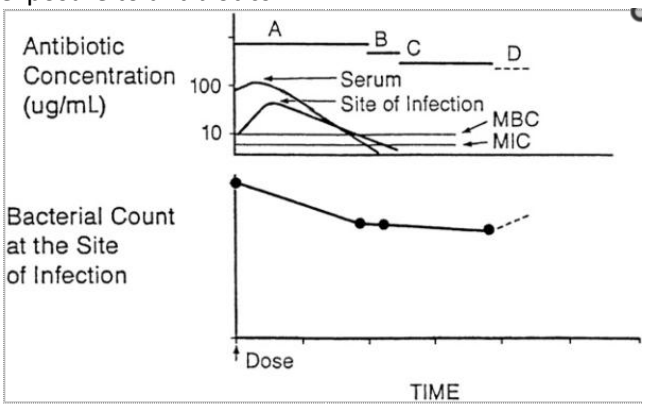
After a dose of a bactericidal drug, the bacterial count may decline in the early portion of the dosing interval, a time when levels of unbound drug exceed the MBC, as a result of both **drug effects and host defenses**. When unbound drug levels decrease to less than the MBC but still exceed the MIC, the bacterial count may remain stable or continue to decline as a result of host defenses.

When the antibiotic levels falls below the MIC then there may be persistent suppression of bacterial growth and this may be due to:

Minimal Antibacterial Concentration: drug concentration altering bacterial cell morphology and slow the rate of growth.

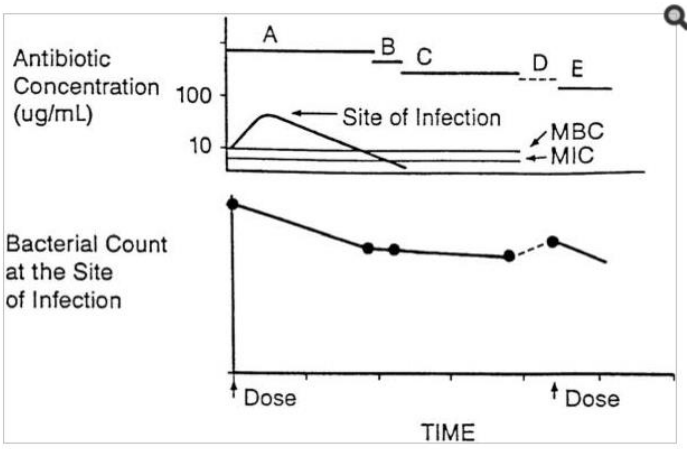
Post Antibiotic Effect: persistent suppression of bacterial growth after a brief exposure of bacteria to an antibacterial agent

Post Antibiotic Leukocyte Enhancement: organisms being more susceptible to phagocytes after exposure to antibiotics

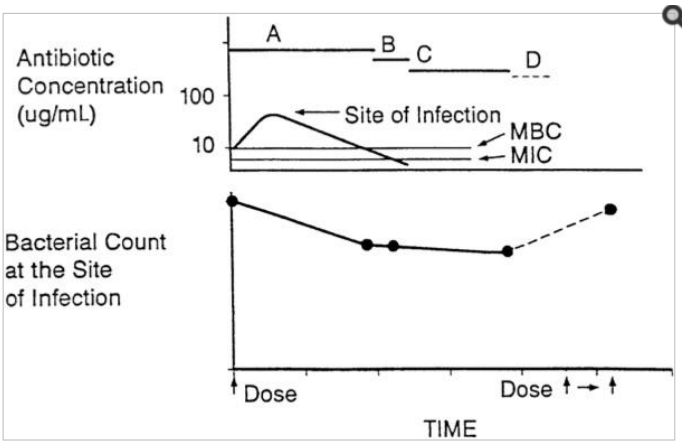


A. Duration of free drug at site of infection exceeding MBC
B. Time during which free drug levels at site of infection < MBC but >MIC
C. Persistent antimicrobial effects (PAE) but [drug] less than MIC
D. Regrowth of residual bacteria

The extent of regrowth before the next dose is given will depend in part on the inherent doubling time of the organism, on available nutrients being present in the infected tissues, and on the adequacy of host defences.
The next dose is ideally given before clinically significant regrowth occurs.



A. Duration of free drug at site of infection exceeding MBC
B. Time during which free drug levels at site of infection < MBC but >MIC
C. Persistent antimicrobial effects (PAE) but [drug] less than MIC
D. Regrowth of residual bacteria
E. Bactericidal effect of next dose



If dosing schedule is inadequate then regrowth may come to equal or even exceed the count at the beginning of the dosing interval.

In-vitro → in vivo

Usually, drug concentrations in the blood are used to determine pharmacodynamic parameters because of the relative accessibility of this body fluid and the correlation of pharmacodynamic parameters that are based on serum levels. However, the use of serum levels to determine pharmacodynamic parameters may not always be appropriate. Because infection usually occurs at extravascular sites, the use of drug concentrations in the blood will only be satisfactory if the blood levels are an adequate surrogate for levels at the site of infection. Theoretically, at equilibrium, free-drug levels in plasma and extracellular tissue fluid should be equal. However, depending on the ratio of surface area of the capillary bed to the volume of the tissue compartment, the physico-chemical characteristics of the drug, and special anatomic barriers (eg, those in the brain, eye and prostate), drug levels at the site of certain infections can be much lower than free-drug levels in plasma.
In order to relate the MIC to an invivo parameter the time/plasma concentration profile is assessed for peak concentration (Cmax) trough level (Cmin) and the Area Under the Curve (AUC).

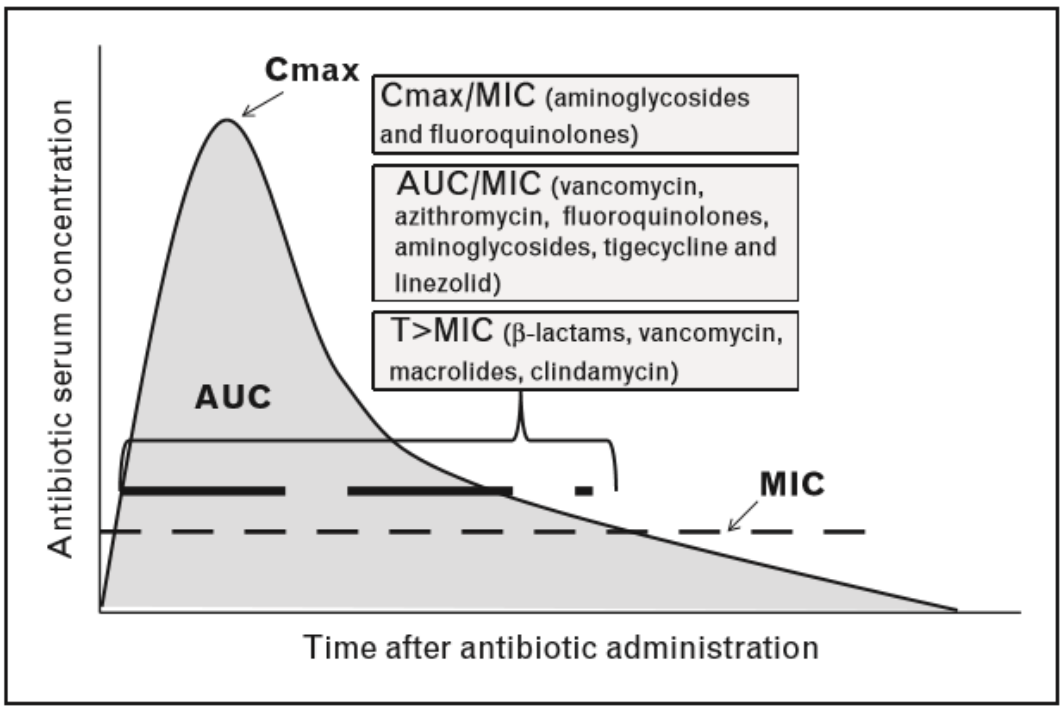


FIGURE 2. Pharmacokinetic and pharmacodynamic parameters. AUC, area under the curve; AUC/MIC, ratio of AUC to MIC (time and concentration-dependent antibiotics); C_{max}, peak antibiotic concentration; C_{max}/MIC, ratio of peak concentration to MIC (concentration-dependent antibiotics); MIC, minimum inhibitory concentration for a pathogen; T > MIC, percentage of time that the antibiotic concentration remains above MIC (time-dependent antibiotics).

Clinically useful parameters are:
The percentage of time that the antibiotic concentration remains above the MIC (T>MIC),
the ratio of the peak concentration to the MIC (CMax:MIC) and
the ratio of the Area Under the Curve concentration to MIC (AUC:MIC).

the magnitude of the pharmacodynamic parameters required to achieve a specific target (eg, bacteriostasis or various degrees of cidal action) is similar for different drugs within the same class

Dosing:
The size of the residual bacterial population at the end of each dosing interval, and ultimately the efficacy of the antimicrobial regimen, depend on the interplay of a variety of bacterial, drug, and host factors that includes
(1) the size of the initial bacterial population,
(2) the potency (MIC and MBC) and pharmacokinetic characteristics of the antimicrobial agent,
(3) the rate and extent of any bactericidal effect,
(4) the presence of a PAE,
(5) the rate of regrowth of persistent organisms, and

(6) the presence of host defenses.

Antimicrobial drugs can be divided into 3 main groups based on pharmacodynamic characteristics that affect bacterial clearance:

1. **<u>Concentration-dependent:</u> both the rate of cidal action and the duration of post-antibiotic effect for these drugs are concentration dependent over a wide range of concentrations (**CMax/MIC) this is dependent on the dose and the Vd. Consequently, the amount of drug (as quantified by Cmax and AUC relative to MIC) rather than the dosing frequency determines the efficacy for these drugs e.g. aminoglycosides and fluoroquinolones, metronidazole, daptomycin, colistin.
2. **Time-dependent:** for these agents the **bactericidal action has only a minimal relationship to drug concentrations that are greater than MIC**; these drugs have **a slow bactericidal action that is not improved by increasing the plasma concentration to more than that which elicits maximal killing action which is typically 4xMIC**; for these antibiotics **the time that the concentration remains above the MIC is of interest (T>MIC); ideally the concentration remains 2-4x the MIC of the pathogen throughout the dosing interv**al. This is achieved/optimized by increasing the frequency, or dose, or using a continuous infusion. A higher dose has been observed to cause a paradoxical decrease in bactericidal activity. Consequently there is no advantage to achieving higher concentrations. A shorter dosing frequency will increase the time that the concentrations remain >MIC e.g. B-lactams, vancomycin, macrolides, clindamycin. NB among the beta-lactams carbapenem is unique in that they have PAE against a number of gram –ve bacilli.
3. **Mixed properties: drugs that are predominantly bacteriostatic and have moderate prolonged PAEs. Because of the prolonged PAEs, their efficacy is determined less by time and more by AUC that is greater than MIC.** Here the parameter of interest is the AUC:MIC; this can be optimized by increasing the antibiotic dose. E.g. macrolides, clindamycin, tetracyclines, tigecycline, linezolid, streptogramins

Pharmacodynamic breakpoint:

A clinical susceptibility breakpoint is an interpretative criteria based on the likelihood of a favourable response of the antibiotic therapy against an organism. It is <u>the discriminating concentration</u> used in the determination of results of susceptibility testing to define isolates as susceptible, intermediate or resistant.

Bacterial strains are categorized in 3 groups based on the clinically susceptible breakpoints:

Clinically susceptible A micro-organism is defined as clinically susceptible by a level of antimicrobial susceptibility which results in an improved, or the desired, therapeutic outcome.

Clinically intermediate A micro-organism is defined as clinically intermediate by a level of antimicrobial susceptibility which results in an indeterminate therapeutic outcome.

Clinically resistant. A micro-organism is defined as clinically resistant by a level of antimicrobial susceptibility which results in a higher than expected likelihood of therapeutic failure

Clinical, pharmacological, microbiological and pharmacodynamics considerations are important in setting breakpoints. This is also very much a national context thing with different countries setting different breakpoints by way of different methodologies.

Time dependent antibiotics: Time-dependent killing refers to the time it takes for a pathogen to be killed by exposure to an antimicrobial. The goal of time-dependent killing is to optimise the duration of exposure. With time-dependent killing, postantibiotic effects (persistence of antimicrobial action after the antimicrobial is removed) are minimal. The major pharmacokinetic/pharmacodynamic parameter that correlates with clinical and bacteriologic efficacy of these drugs is the time for which the serum concentration exceeds the MIC of the pathogen. As would be expected, the required time above the MIC varies, depending on the pathogen, infection site, and drug, but is generally 40–50% of the dosing interval The major PK/PD parameter correlating with efficacy of time-dependent

antimicrobials is **the serum concentration present for 40–50% of the dosing interval, and this concentration is the susceptibility limit or breakpoint** for the dosing regimen used.

Concentration dependent: the goal of concentration dependent killing is to maximize the concentration and attain the highest possible antimicrobial concentration at the site of infection. The major pharmacodynamic parameters that correlate with clinical and bacteriologic efficacy of these drugs are the 24-h area under serum drug concentration curve (AUC) to MIC ratio, or the peak drug concentration to MIC ratio, based on free or unbound serum concentration values. So, again, the MIC remains a primary correlate of pharmacodynamic potency when correlated with the appropriate parameter. The parameters that correlate with clinical and bacteriologic efficacy are 24-h AUC/MIC ratios of ≥25–30 in immunocompetent patients, ≥100–125 in immunocompromised patients, and peak/MIC ratios of ≥10–12. **Pharmacodynamic breakpoints can be determined by the formula AUC ÷ 25 for immunocompetent patients, or AUC ÷ 125 for immunocompromised patients**.

Aminoglycosides are considered a "type I – concentration dependent" antibiotic thus the time that the plasma concentration is above the minimum inhibitory concentration is important.
For the type I drugs (aminoglycosides and fluoroquinolones) the rate of bactericidal activity is greatest at peak serum concentration (Cmax). As the drug concentration decreases, the rate of bactericidal activity decreases. Higher doses of the drug will increase not only the rate of reductions of bacteria but also the length of time of drug exposure to bactericidal concentrations. This dependence on the magnitude and the duration of exposure to bactericidal concentrations implies that concentration dependent drugs are influenced by **the Cmax and the AUC**.

After drug levels at the site of infection decrease to concentrations that are less than the MIC, there may be persistent suppression of growth that is due to a **PAE, the duration of which is also concentration-dependent for aminoglycosides** and fluoroquinolones; **the higher the drug concentration, the longer the duration of the PAE for these drugs**, and the smaller the residual bacterial population at the time of the next dose.

Indeed, **effective dosing regimens for concentration-dependent antibiotics require that either**

- **the 24-hour protein-free drug AUC/MIC value be at least 100 to 125 for aminoglycosides or fluoroquinolones against gram-negative bacilli** and from 25 to 30 for fluoroquinolones against S pneumoniae

or

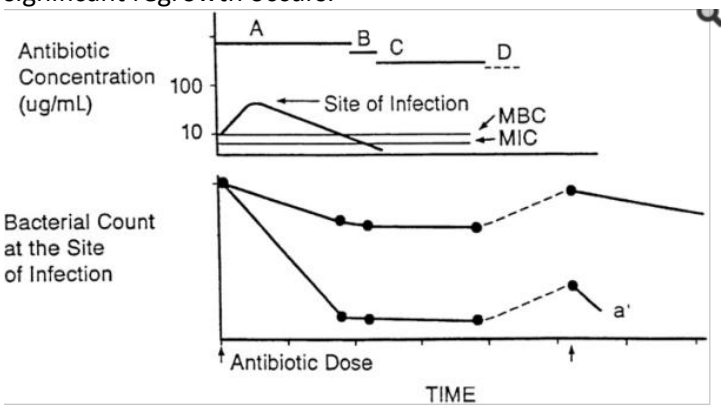
- **that the Cmax/MIC value of the causative pathogen be more than 10.**

For concentration-dependent drugs, **dosing strategies that maximize the intensity of drug exposure, such as giving the total daily dose as a single dose every 24 hours rather than giving smaller divided doses, would maximize the Cmax and possibly allow for comparable efficacy at greater convenience and lower cost.**

The AUC/MIC or the Cmax/MIC ratios also can be used to compare the effectiveness of different concentration-dependent antibiotics.
Drugs within a class having the **greater potency (ie, lower MICs) will have higher AUC/MIC or Cmax/MIC ratios and therefore can be anticipated to have greater effectiveness**. It is clear that an infection caused by **susceptible pathogens that have relatively high MICs may not be adequately treated using the standard dosage of a concentration-dependent antimicrobial agent.**
For example, **gentamicin-susceptible strains of P aeruginosa that have MICs close to the breakpoint for gentamicin of 4 µg/mL may respond suboptimally to standard dosing regimens that provide peak serum levels of gentamicin of 6 µg/mL**.
Similarly, ciprofloxacin-susceptible strains of P aeruginosa that have MICs close to the breakpoint of 2 µg/mL may respond suboptimally to standard dosing regimens that provide peak plasma levels of

ciprofloxacin of about 3 to 4 µg/mL, and levofloxacin-susceptible strains of S pneumoniae with MICs close to the breakpoint of 2 µg/mL may respond suboptimally to 500-mg dosing regimens that provide peak plasma levels of levofloxacin of about 5 to 6 µg/mL and an AUC of 55. A 750-mg dose of levofloxacin doubles the peak level and the AUC. The higher Cmax and AUC achieved using the higher dosage allow greater confidence in treating patients who may be infected with organisms for which levofloxacin MICs are high.

Higher rates of bactericidal action result in lower residual bacterial counts and longer intervals before significant regrowth occurs.



Higher doses will lead to lower residual colony counts inspit of a longer dosing interval.

Maximizing serum concentrations of drugs that exhibit concentration-dependent bactericidal activity by increasing the dose will maximize the rate and extent of bactericidal activity, if adverse effects are not also concentration-dependent.

Dose-dependent toxicity was once believed to limit the ability to administer the total daily dose of an aminoglycoside as a single dose every 24 hours, but data from animal models of infection and **human clinical trials suggest that dosing regimens that provide very high peak aminoglycoside concentrations relative to the MIC and prolonged periods of sub-inhibitory aminoglycoside concentrations have not resulted in greater nephrotoxicity than regimens that provide lower peaks but more persistent inhibitory concentrations, although the relationship between the pharmacodynamic parameters and auditory and vestibular toxicity is unclear**.

Giving the total 24-hour dose as a single dose, rather than in smaller divided doses, and using extended dosing intervals has now become the standard in most clinical settings. This strategy may be especially appropriate for treatment of many susceptible pathogens, (eg, P aeruginosa) that have MICs that are close to the breakpoint.

However, this same strategy may not be appropriate for fluoroquinolones that likely have concentration-dependent toxicity.

All aminoglycosides have similar pharmacokinetics, but there is significant variation in pharmacokinetics in normal individuals and certain patient populations. For example, volume of distribution tends to be elevated in critically ill patients, and clearance is elevated in children, in patients who have cystic fibrosis, and during pregnancy and the early postpartum period, and it is depressed in cases of renal insufficiency. **The Cmax is primarily affected by the volume of distribution, and the AUC by the volume of distribution and clearance.** Consequently, measurement of aminoglycoside levels is especially important early in the course of treatment, and doses should be adjusted to achieve therapeutic levels.

Gentamicin

Antibiotic class: aminoglycoside

The aminoglycosides induce the production of ‘false proteins’ and are bactericidal. They are active against gram negative orgamisms. Gentamicin is not active against anaerobes because oxygen is needed for its uptake into the bacterial cell.

Post antibiotic effect

The post-antibiotic effect is defined as the time required for an organism to demonstrate viable regrowth following the removal of an antibiotic.

The higher the aminoglycoside dosage, the greater the post-antibiotic effect, up to a certain maximal response. In vivo, the post-antibiotic effect for aminoglycosides is prolonged by the synergistic effect of host leukocyte activity. It is believed that leukocytes have enhanced phagocytosis and killing activity after exposure to aminoglycosides