Review Article

Analysis of Aminoglycosides

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ABSTRACT

Aminoglycosides are potent antibiotic and have narrow (low risk-benefit ratio) therapeutic ranges. For gentamicin, tobramycin and netilmicin, the risk of ototoxicity and nephrotoxicity increases if peak levels are consistently maintained above 12 to 14 µg/ml or trough levels consistently exceed 2µg/ml. For amikacin, peak levels above 32 to 34 µg/ml or trough levels greater than 10µg/ml have been associated with a higher risk of ototoxicity and nephrotoxicity. Serum trough concentration above 2µg/mL is predictive of toxicities. Concentrations are taken anywhere from 6 h post-dose to trough concentrations to ensure reasonable clearance. Because of aminoglycoside's spectrum of antimicrobial susceptibility, nephrotoxicity ototoxicity with higher doses both of which are associated with elevated trough levels and sustained elevated peak levels, therapeutic drug serum concentration levels monitoring for aminoglycosides is necessary to obtain the correct dose, ensure optimal treatment and prevent toxicity. Hydration reduces risk of ototoxicity. Kidney function and levels of aminoglycoside in patient's blood may be performed periodically to monitor their progress or check for side effects. Once-daily gentamicin aminoglycoside has been used along with ampicillin or amoxicillin in streptococcal infections for synergistic effects in endocarditis or acute pyelonephritis. Antimicrobial resistance, a global problem because of their use in inappropriate ways, is particularly pressing in developing countries, where the infectious disease burden is high and cost constrains the replacement of older antibiotics with newer, more expensive ones. Amikacin may be effective against some gentamicin resistant bacteria and so is more suitable for hospital settings where gentamicin resistance rates are high.

Keywords: Aminoglycoside, Dose, Resistance; Therapeutic drug monitoring, Nomogam, Toxicity.

INTRODUCTION

Aminoglycoside is a medicinal and bacteriologic category of traditional Gram-negative antibacterial therapeutic agents that inhibit protein synthesis and contain as a portion of the molecule an amino-modified glycoside (sugar). Usually, once cultures of the causal organism are grown and their susceptibilities tested, amino-glycosides are discontinued in favour of less toxic antibiotics. Aminoglycoside antibiotics display bactericidal activity against gram-negative aerobes (pseudomonas, acinetobacter, and enterobacter) and some anaerobic bacilli (bacteria that cannot grow in the presence of oxygen) where resistance has not yet arisen, but generally not against Gram-positive and anaerobic Gram-negative bacteria.[1] They include the first-in-class aminoglycoside antibiotic streptomycin derived from *Streptomyces griseus*, the earliest modern agent used against tuberculosis, and an example that lacks the common 2-deoxystreptamine moiety present in many other class members. Other examples include the deoxystreptamine-containing agents: kanamycin, tobramycin, gentamicin, and neomycin. Amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin, and tobramycin have the same basic chemical structure. To highlight their specific biological origins, gentamicin and other related antibiotics produced by this genus (verdamicin, mutamicin, sisomicin, netilmicin, retymicin) generally have their spellings ending in ~micin and not in ~mycin. Gentamicin is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system. It is available as a generic medication. For injection Gentamicin, a type of aminoglycoside is synthesized by the fermentation of bacteria *Micromonospora purpurea* a genus of Gram-positive bacteria widely present in the environment (water and soil) in 1963. Gentamicin, sold under brand names Garamycin among others, is an antibiotic used to treat nosocomial respiratory tract infections, soft tissues infections, bone infections, endocarditis, pelvic inflammatory disease, pneumonia, urinary tract infections, and empiric therapy for serious infections such as sepsis of Gram-negative bacteria including *Pseudomonas aeruginosa*, Proteus, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, Serratia, the Gram-positive Staphylococcus susceptible bacteria and complicated intra-abdominal infections.[2] Gentamicin is also useful against *Yersinia pestis*, its relatives, and *Francisella tularensis* (the organism responsible for Tularemia seen often in hunters and/or trappers). It can be given intravenously, by injection into a muscle, or topically. Topical formulations may be used in burns or for infections of the outside of the eye. In the developed world it is often only used for two days until bacterial cultures determine what antibiotics the infection is sensitive to. The wholesale cost of Gentamicin is between 0.05 and 0.58 USD per day and less expensive.

Receiving an aminoglycoside antibiotic can often mean the difference between life and death for some patients because of a significant variability in the relationship

between the dose administered and the resultant plasma level in blood. The mortality rate from Gram-negative bloodstream infection in children (43.5 %) was more double that of malaria.[3] Aminoglycosides especially Gentamicin do retain activity against the majority of Gram-negative clinical bacterial isolates resistant to other aminoglycoside in many parts of the world.[4] It appears to be safe for use during breastfeeding. It works by stopping the bacteria from making protein which typically kills the bacteria. Aminoglycosides do not pass into breast milk to any great extent, so nursing mothers may be prescribed aminoglycosides without injuring their infants. Their potential benefits may warrant use of the drug in pregnant women despite potential risks (damage to their infants' hearing, kidneys, or sense of balance). This revived interest in the use of aminoglycosides has brought back to light the debate on the two major issues related to these compounds, namely the spectrum of antimicrobial susceptibility and toxicity to the nephrons (nephrotoxic) and the eighth cranial nerve which is responsible for hearing and balance (ototoxic) - tinnitus (a ringing sound in the ears), dizziness or loss of balance, hearing loss, especially high frequency sounds. The recent emergence of infections as meningitis, tuberculosis, and plague due to Gram-negative bacterial strains with advanced patterns of antimicrobial resistance has prompted physicians to reevaluate the use of these antibacterial agents in adults and children including neonates.[5]

Antimicrobial Resistance

Antimicrobial resistance a global problem because of their use in inappropriate ways, is particularly pressing in developing countries including India, where the infectious disease burden is high and cost constrains the replacement of older antibiotics with newer, more expensive ones.[6] Enterococci are characteristically resistant to a wide variety of antimicrobial agents making single-drug therapy often ineffective. Some Enterobacteriaceae, Pseudomonas spp., Enterococci, Staphylococcus aureus and other Staphylococci are resistant to gentamicin sulfate to varying degrees. Bacterial resistance to gentamicin is generally developed slowly. Aminoglycoside resistance includes the deactivation of aminoglycosides by aminoglycoside-modifying enzymes which act on specific sites of the aminoglycosides causing acetylation via aminoglycoside acetyltransferase (AAC), adenylation via aminoglycoside nucleotdyltransferase (ANT) or phosphorylation via aminoglycoside phosphotransferase,[7,8] this is a major mechanism by which clinical isolates of gram-negative and gram positive bacteria cause an enzymatic modification of the amino or hydroxyl group's aminoglycoside, causing them to bind poorly to ribosome and thus fail to trigger energy-dependent phase II, which allows bacteria to survive.[9] Other mechanisms include the reduction of the intracellular concentration of aminoglycosides by changes in the outer membrane permeability which is usually a nonspecific resistance

mechanism, inner membrane transport, active efflux or drug trapping, the alteration of the 30S ribosomal subunit target by mutation, and finally methylation of the aminoglycoside binding site. Many antibiotics are often prescribed for duration of 5-7 days. Nevertheless it is reasonable to discontinue therapy even after a shorter period if the patient's symptoms have resolved. There are however certain infections where prolonged treatment is necessary (Table 1).

In some conditions e.g. uncomplicated cystitis in women and gonococcal urethritis in males, single dose regimens have been shown to be effective. Despite their short half-life, single injection of aminoglycoside total daily dose may be preferred because they exert long and concentration dependent post-antibiotic effect. Once daily gentamicin aminoglycoside has been used in conjunction with ampicillin or amoxicillin beta-lactam antibiotic in streptococcal infections for their synergistic effects, in particular in endocarditis and acute pyelonephritis respectively.[10] Severe sepsis, septic shock and systemic enterococcal infections, such as endocarditis in neutropenic patients are usually treated with a combination of two antimicrobial agents: one specific action against the cell wall, such as a beta-lactam or a glycopeptide (i.e., penicillin, ampicillin or vancomycin) and an aminoglycoside, which inhibits bacterial protein synthesis (i.e., gentamicin or streptomycin). For preventing bacterial resistance, these agents act synergistically to enhance broad spectrum coverage, killing of the bacteria, since the aminoglycoside has increased uptake into the cell, after cell wall damage by the beta-lactam agent.

In-Vitro Diagnosis of Resistance

Gentamicin and streptomycin high-level aminoglycoside resistance (HLAR) differentiation disks are used to detect high-level aminoglycoside resistance in *Enterococcus faecalis* and *E. faecium*. These disks are designated for use for HLAR testing, in accordance with the NCCLS performance standards for susceptibility testing.

Formula

Each HLAR Differentiation Disks contains the following specified concentrations of the appropriate antibiotics on high quality 6mm diameter filter paper disks:

Gentamicin   120µg   50 disks/cartridge

Streptomycin   300µg   50 disks/cartridge

Components

Gentamicin is composed of a number of related gentamicin components and fractions which have varying degrees of antimicrobial potency. Gentamicin Sulfate (40 mg in 1 mL), active ingredient Sodium metabisulfite (2.9 mg in 1 mL), edetate disodium anhydrous (0.1 mg in 1 mL), methylparaben, propylparaben, sulfuric acid and water, inactive Ingredients are present in composition of Gentamicin. The main components of gentamicin include

members of the gentamicin C complex: gentamicin C1, gentamicin C1a, and gentamicin C2 which compose approximately 80% of gentamicin and have been found to have the highest antibacterial activity. Gentamicin A, B, X, and a few others make up the remaining 20% of gentamicin and have lower antibiotic activity than the gentamicin C complex.

Storage and Shelf Life

Upon receipt it should be stored at -20°C. away from direct light. A small supply of disks for use within one week can be stored at 4 degrees C. The disks should not be used if there are any signs of deterioration, discolouration, or if the expiration date has passed. It should be protected from light, excessive heat, and moisture.

Precaution

This product is for *in-vitro* diagnostic use only and is to be used only by adequately trained and qualified laboratory personnel. Approved biohazard precautions and aseptic techniques should be observed. All laboratory specimens should be considered infectious and handled according to "standard precautions". For additional information regarding specific precautions, Clinical and Laboratory Standards Institute document M29 should be referred.

Specimen Collection

It should be used only with cultures of isolated organism. These are high-concentration disks; standard susceptibility disks for HLAR testing should not be used.

Procedure

Disks are allowed to equilibrate to room temperature. A suspension (equivalent to a McFarland 0.5 opacity standard) of the organism to be tested is prepared using a pure 18-24 hour culture. A sterile non-toxic cotton swab (Cat. no. 258061WC) is dipped into the organism suspension. The swab is rotated several times, pressing firmly on the inside wall of the tube above the fluid level. This will remove excess inoculums from the swab.

The dried surface of Mueller Hinton agar plate (Cat. no. H11 or G45) is evenly inoculated by streaking the swab over the entire surface of the plate in three directions, as for a routine disk diffusion test. One Gentamicin disk (120µg) and one Streptomycin disk (300µg) were aseptically placed on the media surface, far enough away from each other to leave room for zones of inhibition (greater than 30mm apart). With sterile forceps, each disk is gently tapped to the media surface to ensure uniform diffusion of the antibiotic into the medium. Plates are inverted and incubated aerobically at 35 degrees C for 18-24 hours. Plates are examined for confluent or almost confluent growth, and zones of inhibition for each disk are measured (If growth is unacceptable, the test cannot be interpreted). Re-incubation of the plate for an additional 24 hours may be done to verify susceptibility of the strain to streptomycin. HLAR Differentiation Disks are 6mm (in diameter) filter paper disks, and should appear white in colour. Signs of deterioration should be checked. If product has passed the expiration date, it should not be used. For frequency of quality control testing, Clinical and Laboratory Standards Institute should be referred. The Acceptable Quality Limit (AQL) is a method widely used to measure a production order sample to find whether or not the entire product order has met the client's specifications. For the diffusion technique using the 10µg gentamicin disk the criteria provided in Table 2 should be achieved.

Susceptibility Test Methods

The three most commonly used methods for HLAR detection are agar dilution, broth micro-dilution, and disk diffusion using high-concentration disks.[11] Susceptibility profile nosocomial and community-acquired pathogens should aid the physician in selecting an antimicrobial drug for treatment.

Dilution Technique

Quantitative methods are used to determine antimicrobial minimal inhibitory concentrations (MICs). These MICs provide estimates of the susceptibility of bacteria to antimicrobial compounds.

MICs should be determined using a standardized test method 1, 3. Standardized procedures are based on a dilution method (borth or agar) or equivalent with standardized inoculums concentrations and standardized concentrations of gentamicin powder. The MIC values should be interpreted according to criteria provided in Table 3.

Table 1: Minimum Duration Conditions of Treatment.

Infection	Minimum duration of treatment	Infection	Minimum duration of treatment
Tuberculosis	4 -6 months	Atypical pneumonia	2 - 3 weeks
Empyema and lung abscess	4 - 6 weeks	Pneumococcal meningitis	7 days
Endocarditis	4 weeks	Pneumococcal pneumonia	5 days
Osteomyelitis	4 weeks	-	-

Table 2: Acceptable Quality Control Ranges for Gentamicin.

Quality Control Organism	Minimum Inhibitory Concentrations (mcg/mL)	Zone Diameter (mm)
Escherichia coli ATCC 25922	0.25 to 1	19 to 26
Pseudomonas aeruginosa ATCC 27853	0.5 to 2	17 to 23
Staphylococcus aureus ATCC 25923	Not Applicable	19 to 27

Table 3: Susceptibility Interpretive Criteria Against Streptomycin

Pathogen	Susceptibility Interpretive Criteria					
	Minimal inhibitory concentration(mcg/mL)			Zone Diameter		
	S	I	R	S	I	R
Enterobacteriaceae[a]	≤ 4	8	≥16	≥ 15	13 to 14	≤ 12
Pseudomonas aeruginosa	≤ 4	8	≥16	≥ 15	13 to 14	≤ 12
Staphylococcus species[b]	≤ 4	8	≥16	≥ 15	13 to 14	≤ 12

S= Susceptible, **I** = Intermediate, **R**: resistant; a For *Salmonella* and *Shigella* spp., aminoglycosides may appear active *in-vitro* but are not effective clinically; the results should not be reported as susceptible.; b For staphylococci that test susceptible, aminoglycosides are used only in combination with other active agents that test susceptible.

Table 4: Pharmacokinetic Monitoring of Aminoglycosides

Drug range (mg/L) Aminoglycoside	Time to steady state	Sampling time	Therapeutic range (mg/L)
Amikacin Kanamycin Gentamicin Netilmicin Tobramicin	Adults (< 30 y): ~ 2.5-15 h (> 30 y): ~ 7.5-75 h Children: ~ 2.5-12.5 h Neonate: ~ 10-45 h	Peak 0.5-1 h after IV infusion (1 h after IM)	Peak 15-25, Trough< 5
Streptomycin	10-15 h	Peak 1-2 h after IM Trough < 5	Peak 15-40

Peak = (MD / t_{inf} x Vd x Kel) x (1 - $e^{-Kel\,x\,tinf}$ / 1 - $e^{-Kel\,x\,tau}$) Volume of distribution (Vd) = 0.27 L/kg x DW

Dosing weight (DW) = LBW + [(ABW - LBW) x CF]; Correction factor (CF) should be made for Amikacin (38%), Gentamicin (43%), Kanamycin (no correction), Netilmicin (50%) and Tobramycin (58%) respectively.

Elemeination rate (Kel) = 0.01 + (CrCl x 0.0024); Ideal maintenance dose (IMD) = Kel x Vd x Cpt_{max} x (1- $e^{-Kel\,x\,tau}$ 1-$e^{-Kel\,x\,tinf}$)

Where, Trough = Peak * $e^{-Kel\,x\,(tau - tinf)}$; tinf = length of infusion (Extended interval method - Initial dose).

Table 5: Initial Dosing of Aminoglycosides

A.   **Gentamicin & Tobramycin Initial Dosing**

CrCL (mL/min)	High-Dose Extended-Interval* (Gentamicin/Tobramycin)	Conventional / Traditional (Gentamicin/Tobramycin)	Synergy** (Gentamicin/Tobramycin)
> 60	7 mg/kg Q24H	1.7 mg/kg Q8H	1 mg/kg Q8H
40-59	4 – 7 mg/kg Q36H	1.7 mg/kg Q12H	1 mg/kg Q12H
30-39	4 – 7 mg/kg Q48H	1.7 mg/kg Q24H	1 mg/kg Q24H
20-29	Not recommended	1.7 mg/kg Q24H	1 mg/kg Q24H
<20	Not recommended	2 mg/kg load, then dose by level	1 mg/kg load, then dose by level
Hemodialysis	Not recommended	2 mg/kg load, then 1.5 mg/kg post-HD	1 mg/kg q48-72H; consider redosing for pre-HD or post-HD Cp < 1mg/L
CRRT	Not recommended	1.5 – 2.5 mg/kg Q24-48H	1 mg/kg Q24H, then by level

*See Hartford nomogram for monitoring of once-daily dosing regimens
**Alternative for synergy: 3mg/kg Q24H for Streptococci and *Streptococcus bovis* endocarditis

B.   **Amikacin Initial Dosing**

CrCL (mL/min)	High-Dose Extended-Interval* (Amikacin)	Conventional / Traditional (Amikacin)
> 60	15 – 20 mg/kg Q24H	5 – 7.5 mg/kg Q8H
40-59	15 mg/kg Q36H	5 – 7.5 mg/kg Q12H
30-39	15 mg/kg Q48H	5 – 7.5 mg/kg Q24H
20-29	Not recommended	5 – 7.5 mg/kg Q24H
<20	Not recommended	5 mg/kg load, then dose by level
Hemodialysis	Not recommended	5 – 7.5 mg/kg post-HD
CRRT	Not recommended	10 mg/kg load, then 7.5 mg/kg Q24-48H

See Hartford nomogram for monitoring of once-daily dosing regimens– divide level by half then plot on graph

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Table 6: Dosage schedule guide for adults with normal renal function (dosage at eight-hour intervals) 40 mg/mL.

| Obese Patient's Weight		Usual Dose for Serious Infections 1 mg/kg q8h		Dose for Life-Threatening Infections 1.7 mg/kg q8h**	
Kg	(lb)	(3 mg/kg/day) q8h	mL/dose	(5 mg/kg/day)	mL/dose
40	(88)	40	1	66	1.6
45	(99)	45	1.1	75	1.9
50	(110)	50	1.25	83	2.1
55	(121)	55	1.4	91	2.25
60	(132)	60	1.5	100	2.5
65	(143)	65	1.6	108	2.7
70	(154)	70	1.75	116	2.9
75	(165)	75	1.9	125	3.1
80	(176)	80	2	133	3.3
85	(187)	85	2.1	141	3.5
90	(198)	90	2.25	150	3.75
95	(209)	95	2.4	158	4
100	(220)	100	2.5	166	4.2
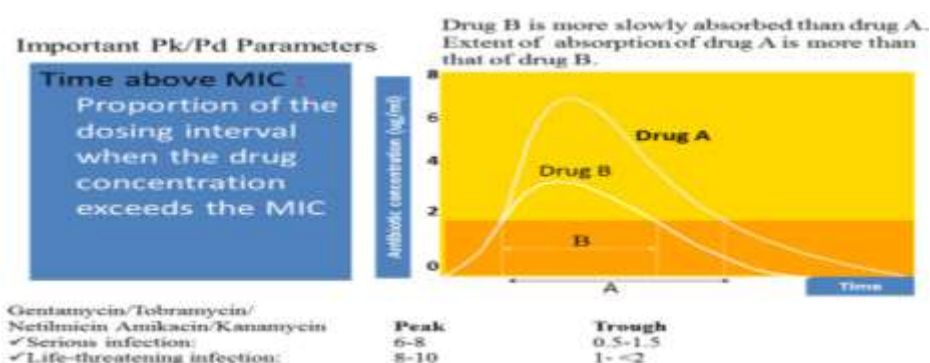
Figure 1: Applied pharmacokinetics and principles of drug dosing

Table 7: Loading Dose

Site of infection or indication	Desired peak concentration	Loading dose (mg/kg)
Uncomplicated lower urinary tract infection, gram positive endocarditis, synergy with beta-lactams for serious gram positive infections	2-4 µg/mL	0.6 to 1.2
Gram-negative sepsis or other serious gram-negative infections	6-8 µg/mL	2.5
Gram-negative pneumonia or acute life-threatening gram negative infection in a critically ill patient.	7-9 µg/mL	3.0

Table 8: Comparative Information on Toxicity of Aminoglycoside

Aminoglycoside	Vestibular	Cochlear	Nephro	Neuromuscular
Amikacin	+	++	++	+
Gentamicin	++	++	++	+
Streptomycin	+++	+	+	++
Tobramycin	++	++	++	+

Quantitative methods that require measurement of zone diameters also provide reproducible estimates of the susceptibility of bacteria to antimicrobial compounds. The zone size should be determined using a standardized test method. The standardized procedures require the use of standardized inoculum concentrations and paper disks

impregnated with 10µg of gentamicin 2, 3. When performing the disk diffusion test to determine high-level resistance to aminoglycosides, it is important to remember that standard gentamicin and streptomycin disks (10µg each) that are used for routine disk diffusion testing cannot be used. Only high-concentration disks can be used to determine aminoglycoside resistance. The disk diffusion values should be interpreted according to criteria provided in Table 3.

Diffusion Technique

Interpretation of Results

Sensitive

Zone size is greater than or equal to 10mm. A report of susceptible (S) indicates that the antimicrobial is likely to inhibit growth of the Enterococcus pathogen if the antimicrobial compound reaches the concentration usually achievable at the injection site necessary to inhibit growth of the pathogen.

Intermediate

Zone sizes of 7 to 9 mm are considered inconclusive, and should be retested by an alternative method (i.e., standard agar screen or broth micro-dilution methods). A report of intermediate indicates that the result should be considered equivocal, and the microorganism is not fully susceptible to alternative feasible drugs, the test should be repeated. This category implies possible clinically applicability in body sites where the drug is physiologically concentrated or in situations where the high dosage of the drug can be used. This category also provides a buffer zone that prevents small uncontrolled technical factors from causing major discrepancies in interpretation.

Resistant

Resistant no zone of inhibition (6mm): A report of resistant (R) indicates that the antimicrobial is not likely to inhibit growth of the HLAR Enterococcus pathogen if the antimicrobial compound reaches the concentration usually achievable at the injection site, other therapy should be selected.

Toxicities

Side effects of gentamicin can range from nausea and vomiting to numbness/tingling, muscle twitching or weakness, seizure, low blood counts, allergic response, neuromuscular problems nerve damage, (nephrotoxicity), inner ear problems (ototoxicity). A disadvantage of the aminoglycosides (gentamicin, tobramycin and netilmicin) is their association with 10-25% reversible nephrotoxicity (nonoliguric renal failure) [12] and 11% ototoxicity (inner ear problems),[13] both of which are associated with elevated trough levels (above 2µg/ml) and sustained elevated peak levels (above 12 to 14 µg/ml) of creatinine and a hypoosmolar urinary output developing after several days of therapy. For amikacin, peak levels above 32 to 34 µg/ml or trough levels greater than 10µg/ml

have been associated with a higher risk of ototoxicity and nephrotoxicity. The common symptoms of inner ear damage are: tinnitus, hearing loss, vertigo, trouble with coordination and dizziness. Chronic use of gentamicin progressively accumulates in endolymph and perilymph of inner ear and that the half life in these fluids is 5 to 6 times greater than that of plasma half-life and can affect two areas of the ears. First, damage of the inner ear hair cells can result in irreversible hearing loss. Second, damage to inner ear vestibular apparatus can lead to balance problems. Aminoglycosides can cause inner ear problems: permanent vestibular (problems with balance) and/or auditory (problems with hearing) ototoxicity.[14] Vestibulotoxicity is difficult to diagnose and there is no reliable monitoring process. A genetic predisposition to aminoglycoside auditory ototoxicity is due to a mutation of mitochondrial DNA.[15] Toxicity of Gentamicin is the most common single known cause of bilateral vestibulopathy, accounting for 15-50% of all cases. For reducing the risk of ototoxicity it is recommended to stay hydrated.

This medication may rarely cause a severe intestinal condition (*Clostridium difficile*-associated diarrhea) due to a type of resistant bacteria and during treatment or a week to months after treatment has stopped. Still, the relatively frequent occurrence of nephrotoxicity and ototoxicity during aminoglycoside treatment makes physicians reluctant to use these compounds in everyday practice.

Mechanism of Action of Aminoglycosides

Gentamicin is a bactericidal antibiotic that works by irreversibly binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis. Aminoglycosides display concentration-dependent bactericidal activity against "most gram-negative aerobic and facultative anaerobic bacilli" apart from some bacilli and methicillin-resistant staphylococci, but not against gram-negative anaerobes and most gram-positive bacteria. They require only short contact time, and are most effective against susceptible bacterial populations that are rapidly multiplying. The inhibition of protein synthesis is mediated through aminoglycosides' energy-dependent, sometimes irreversible binding, to the cytosolic, membrane-associated bacterial ribosome. (Aminoglycosides first cross bacterial cell walls—lipopolysaccharide in gram-negative bacteria)—and cell membranes, where they are actively transported. The aminoglycosides primarily act by binding to the aminoacyl site of 16S ribosomal RNA within the 30S ribosomal subunit, leading to misreading of the genetic code and inhibition of translocation. The initial steps required for peptide synthesis, such as binding of mRNA and the association of the 50S ribosomal subunit, are uninterrupted, but elongation fails to occur due to disruption of the mechanisms for ensuring translational accuracy. The subset of aberrant proteins that are incorporated into the bacterial cell membrane may then

lead to changes in its permeability and then to "further stimulation of aminoglycoside transport". The 2-deoxystreptamine, amino-sugar portion in kanamycins, gentamicins and tobramycin are implicated in the association of the small molecule with ribosomal structures that lead to the infidelities in translation (ibid.). Inhibition of ribosomal translocation—i.e., movement of the peptidyl-tRNA from the A- to the P-site—has also been suggested. Spectinomycin, a related but distinct chemical structure class often discussed with aminoglycosides, does not induce mRNA misreading and is generally not bactericidal. Finally, a further "cell-membrane effect" also occurs with aminoglycosides; "functional integrity of the bacterial cell membrane" can be lost, later in time courses of aminoglycoside exposure and transport. The ensuing antimicrobial activity is usually bactericidal against susceptible aerobic gram-negative bacilli. This mechanism of action is similar to other aminoglycosides.

Clinical Pharmacokinetics

Clinical pharmacokinetics is the process of using pharmacokinetic principles and the pharmacodynamic criteria to assist in the selection of appropriate drug dosage regimens for individual patients. The ultimate aim of drug therapy is to achieve efficacy without toxicity. This involves achieving a plasma concentration (Cp) within the 'therapeutic window', i.e. above the minimal effective concentration (MEC), but below the minimal toxic concentration. Monitoring of drug serum concentration levels for toxicity is recommended to avoid both excessive and sub-therapeutic concentration thereby preventing toxicity and ensuring efficacy.[16] Clinical pharmacokinetics is about all the factors that determine variability in the Cp and its time-course. Peak indicates efficacy in serious infections at 6-8 µg/mL, life-threatening at 8-10 µg/mL, UTI at 4-6 µg/ml and Synergy at 3-5 µg/mL. Trough concentrations are measured within 30 minutes of the next dose. Trough indicates toxicity such as serious infection at 0.5-1 µg/mL; life-threatening at 1-2 µg/mL and hospital acquired pneumonia: <1 µg/ml). When monitoring trough concentration (just prior to next dose), dosage should be adjusted so that levels above 2 µg/mL are avoided. Post antibiotic effect is noted at 15 mg/kg, a single dose of amikacin whereas 7.5 mg/Kg two doses exert its concentration dependent bactericidal action.[17]

Predictor of Efficacy

If the Pharmacokinetic (Pk)/ Pharmacodynamic (Pd) index for the unbound drug in the subject is above the PK\PD index obtained from pre-clinic: Higher Peak/MIC concentration (8-10 times), the more extensive and the faster is the degree of bactericide (Figure 1).

Drug doses should usually be reduced in renal disease in proportion to predicted reduction in clearance of the active moiety. Pharmacokinetic monitoring is to individualize a patient's dose of the drug by analyzing the patient's drug plasmatic concentrations. Because of Aminoglycoside's spectrum of antimicrobial susceptibility and toxicity, therapeutic drug monitoring (TDM) is necessary to minimize toxicities and obtain the correct dose by current immunoassay methods.[18] TDM is used mainly for monitoring drugs with narrow therapeutic ranges, drugs with marketed pharmacokinetic variability, medications for which target concentrations are difficult to monitor and drugs known to cause therapeutic and adverse effects increasingly the dosing regimens and TDM targets are specified to maximize efficacy. Sampling time is after three to five half-lives of the drug, the individual drug dosage regimen for optimal patient-benefit is for the clinical condition such as patient's characteristics as age, weight, organ function and concomitant drug therapy by maintaining plasma or drug concentrations within the targeted therapeutic range or window. Information regarding sampling time in relation to the dose, the dosage history, patient's response and the desired clinical targets can be used to identify the most appropriate dosage regimen to achieve the optimal response with minimal toxicity. It can help to identify problems with medication compliance among noncompliant patient cases.

Applied Pharmacokinetics and Principles of Drug Dosing

When given by IV infusion over 30 minutes, amino-glycosides follow a three compartment pharmacokinetic model; alpha (distribution), ß (elimination) and gamma (tissue release). When infused over one hour, the distribution phase is usually not observed. The gamma phase begins approximately sixteen hours post infusion, drug that was tissue bound to various organs is released. The amount released from tissue is very small, but does accumulate over time, contributing to AG toxicity. The peak concentration at 30 to 60 min after intramuscular injection is expected to be in range of 4 to 6 µg/mL. When monitoring peak concentrations after intramuscular or intravenous administration dosage should be adjusted so that prolonged levels above 12 µg/mL are avoided (Table 4).

Aminoglycoside level monitoring can be done by several methods-trough level only, peak and trough level measurement, using nomograms, area under curve methods or Bayesian systems. Renal function should be monitored at the start of treatment and every 2-3 days thereafter (more frequently if unstable) If aminoglycoside treatment is expected to continue >72 hours, patients should be informed of potential ototoxicity, and they should be assessed ideally at the start of treatment and then every week (in extreme cases every 2-3 days with a view to stopping treatment if toxicity develops). For patients with altered volume distribution or creatinine clearance, monitoring frequency should be individualized as appropriate and expert help sought. Although AG's do not distribute into adipose tissue, they do enter the extracellular fluid contained therein. Therefore, obese

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patients require a correction in the weight used for V_d calculation: LBW + 40% of weight above LBW.

Smaller the V_d lower the tissue binding for drug. Low V_d indicates mainly vascular distribution and haemodialysis is effective to eliminate drugs with low V_d e.g. apparent volume of distribution for Streptomycin and gentamycin is 0.25 L/kg Vd; for Cystic fibrosis: 0.35 L/kg V_d due to increase in extracellular fluid brought about by the disease process. If large the V_d drug is tightly bound. Higher V_d indicates concentrated / accumulation in tissues. Hemo-dialysis is not effective in drugs with high V_d, loading dose is required. Patients with ascites have additional extracellular fluid because of accumulation of ascitic fluid, which increases the V_d to approximately 0.32 L/kg. ICU patients may have a V_d 25-50% above normal.

Pharmacokinetics and Once-daily Dosing

The use of aminoglycosides as a single daily dose diminishes its nephrotoxicity[19] providing at least the same effectiveness as a similar dose divided in two or three applications per day.[20] The rationale for this is due to two pharmacodynamic characteristics of the drug: the post-antibiotic effect and the bactericide power dependent on concentration. The post-antibiotic effect is the persistent inhibitory effect after drug removal or metabolization and elimination observed with many gram-negative organisms.[21] These agents exhibit a post-antibiotic effect in which there is no or very little drug level detectable in blood, but there still seems to be inhibition of bacterial re-growth. This is due to strong, irreversible binding to the ribosome, and remains intracellular long after plasma levels drop, and allows a prolonged dosage interval. Depending on their plasma level of the drug concentration, they act as bacteriostatic or bactericidal agents.[22]

Aminoglycosides are small, hydrophilic molecules with a volume of distribution similar to extracellular fluid volume and clearance proportional to glomerular filtration rate.[23] Aminoglycosides are 20 times more active in alkaline than in acidic medium, highly polar compounds that do not enter cells readily and lipid insoluble and have very poor gastrointestinal absorption. They are largely excluded from the central nervous system and the eye. In the presence of active inflammation, cerebrospinal fluid level of aminoglycosides reaches 20% of their plasma level and in neonatal meningitis the level may be higher. In patients with extensive burns, altered pharmacokinetics may result in reduced serum concentration of aminoglycosides. In such patients treated with gentamicin, measurement of serum concentrations is recommended as basis for dosage adjustment. Burns patients, may develop augmented renal clearances and enhanced aminoglycoside clearances, which may suggest the need for an incrementally shortened dosing frequency.[24] Alterations in volume of distribution can be very large in sepsis of burn injuries leading to unstable or unknown fluid balances, resulting in a reduced peak

concentration if the dose is unchanged.[25] Monitoring of C_{max} would only be necessary where the patient has a volume of distribution that is significantly different from 'normal' patients. Therefore, C_{max} monitoring of critically ill, obese and burns patients might be reasonable. The widespread use of once-daily dosing of aminoglycosides means that monitoring of C_{max} has become redundant because most once-daily doses will achieve therapeutic targets. To confirm that a larger dose does achieve the optimal target, TDM can be performed by sampling 30 min after the end of the intravenous infusion.[26] For once-daily dosing, trough concentration monitoring may not be useful in some patients, who may have undetectable concentrations. For dosing every 36 or 48 h in renal dysfunction, trough concentration monitoring is suggested to ensure that re-dosing does not risk toxicities. With concentration-dependent antimicrobials, an increased volume of distribution will reduce the ability of a prescribed dose to achieve a target C_{max}. A C_{max}/MIC ratio of 8–10 should be targeted, with the precise C_{max} guided by known MIC data or by local antibiogram data. Sawchuk and Zaske, MacGowan and Reeves, Begg and Nicolau nomograms have been developed to aid dosing.[27-30] The relative merits of these approaches are discussed by Begg and Barclay.[31] However, it is noted that some patients are under-dosed with the Begg and the Australian therapeutic guidelines nomogram[32] and others potentially overdosed (Nicolau nomogram).[30] As a result of this, and the fact that computer facilities are available at many institutions, use of freely available Bayesian adaptive feedback software (http://www.tciworks.info) has been recommended where skilled clinical pharmacists or pharmacologists are available.[33] Such software can facilitate achievement of C_{max}/MIC ratios of 8–10 and AUC_{0-24} targets of 70–120 mg h l^{-1}.[34,35] Bayesian software includes a population pharmacokinetic model describing the covariates descriptive of altered pharmacokinetic parameters in patients. As TDM data from the individual patient are included, the software is able to confirm the likely parameters in the individual patient and therefore provide accurate dosing recommendations that can achieve therapeutic PK/PD targets.

The impact of aminoglycoside TDM, however, is best noted in its improvement in health outcomes. The 'active' TDM strategy used PK dosage optimization at the start of treatment, subsequent Bayesian adaptive control and ongoing patient follow-up.

The 'standard' TDM strategy used attending physician dosing and utilized TDM on request only. The 'active' TDM strategy resulted in shorter hospitalization and reduced nephrotoxicity.[36] Many un-well patients have impaired renal function.

If the dose is not adjusted, reduced aminoglycoside clearance will predispose to nephrotoxicity or ototoxicity. In such cases, an extension of the dosing frequency is suggested. In any event, the dosing interval should seek to maximize use of the aminoglycoside post-antimicrobial

effect.[28] To ensure that reasonable clearance is occurring, it has been suggested that concentrations are taken anywhere from 6 h post-dose to trough concentrations.

Dosing

Aminoglycoside (AGs) is cleared by the kidneys unchanged, and excretion is directly proportional to creatinine clearance in adults.

Patients with normal renal function show 125 ml/min creatinine clearance (CrCL). Functional status of kidneys can be determined by Creatinine Clearance. For determining the aminoglycoside dose, creatinine clearance (CrCL) is calculated with the Cockcroft-Gault equation.

$$Creatinine\ Clearance\ (for\ Males)$$
$$= \frac{(140 - Age) \times Wt\ (Kg)}{Plasma\ Creatinine\ Conc. \frac{(SrCr)mg}{dl} \times 72}$$

For Females, creatinine clearance (CrCL) is calculated by multiplying result of Creatinine Clearance (for man) by 0.85. Traditional dosing method should be used for less than 30 ml/min CrCl. Initial interval is 24 hrs for 30 to 39 ml/min, 60 ml/min and above and 36 hrs for 40 to 59 ml/min based on estimated creatinine clearance (Table 5).

The dosage of aminoglycosides in obese patients should be based on an estimate of the lean body mass. It is desirable to limit the duration of treatment with aminoglycoside to short term. The recommended dosage of gentamicin sulfate for patients with serious infection and normal renal function is 3mg/kg/day, administered in three doses every eight hours. Intensive care unit patients are often hyper metabolic and therefore eliminate AG's more rapidly. Cystic fibrosis patients show a 50% increase in elimination rate of aminoglycoside. Aminoglycoside dosing should be 5mg/kg/24hrs, 5mg/kg/36hrs and 5mg/kg/48hrs at CrCl $\geq$ 50ml/min, CrCl 30-49ml/min and CrCl 20-29ml/min respectively in cystic fibrosis patients. For patients with life-threatening infections, dosage up to 5mg/kg/day may be administered in three or four equal doses every eight hours. The dosage should be reduced to 3mg/kg/day as indicated clinically (Table 6).

Loading Dose is a single or few quickly repeated doses given in the beginning to attain target concentration rapidly (Table 7).

If large the V_d drug is tightly bound. Higher V_d indicates concentrated / accumulation of drug in tissues. Hemo-dialysis is not effective in drugs with high V_d. loading dose is required. Loading dose increases with apparent volume of distribution (aV_d) into which drug immediately and uniformly distributed and with half-life volume. It is used in emergency to hasten achieving therapeutic concentration. It may be calculated as

$$Loading\ Dose$$
$$= \frac{Target\ Css \times V_d}{F\ (a\ fraction\ of\ the\ dose\ reaches\ systemic\ circulation\ in\ the\ active\ form)}$$

Where C_{ss} : Steady state concentration; F : a fraction of the dose reaches systemic circulation in the active form. It increases with a V_d and $t^{1/2}$.

Thus loading dose is governed only by V_d and not by CL. 2mg/kg DW is loading dose for Gentamicin, Tobramycin and Netilmicin whereas 7.5 mg/kg DW is loading dose for Amikacin and Kanamycin.

Maintenance Dose

This dose is one that is to be repeated at specified intervals after the attainment of target Cpss so as to maintain the same by balancing elimination. The dose rate-Cpss relationship is linear only in case of drugs eliminated by first order kinetics. It is governed by CL or half-life ($t^{1/2}$) of the drug. If facilities for measurement of drug concentration are available, attainment of target level in a patient can be verified subsequently and the dose rate adjusted if required. The maintenance dose rate is computed by the following equation:

$$Maintenance\ dose\ rate\ = \frac{Target\ Cpss \times CL}{F}$$

Maintenance dose (Dosing Rate IV) = Css X Clearance (CL)

Maintenance dose (MD) = DW X QD dose

QD dose: 15 mg/kg for Amikacin, kanamycin; 5 mg/kg for Gentamicin, Tobramycin, Netilmicin.

Dosing (for obese patient) = 0.4 X (ABW- IBW) + IBW + Weight Obese Patient has 20% more body weight than ideal body weight in kg.

Where, ABW= actual body weight; AGE= age in years; IBW= ideal body weight in kg; IBW in men = 50 kg + 2.3 kg per inch of height over 60 inches; IBW in women= 45.5 kg + 2.3 kg per inch of height over 60 inches; SrCr =Serum creatinine in mg/dl.

Pharmacokinetic Formulas for Dose Calculation

Nomo grams and formulae related to serum creatinin levels have been constructed to modulate in treatment-regimens.[37] Due to reduced muscle mass and impaired metabolism of creatine to creatinine in a number of patients with severe liver disease, estimations of creatinine clearance based on serum creatinine measurements (e.g., Cockroft-Gault method) in these patients are often inaccurate. Cockroft-Gault method will not work for muscle wasting (patient's CrCl will be overestimated), oedematous patients, ascites and acute renal failure.

This may represent non-steady state serum creatinine levels and may underestimate the level of renal impairment.

A major body burn increases the basal metabolic rate resulting in a marked increase in AG elimination.

Contraindications

Aminoglycosides can exacerbate weakness in patients with myasthenia gravis, and their use is therefore contraindicated in patients with mitochondrial diseases as it may result in impaired mtDNA translation, which can lead to hearing loss, cardiac toxicity, and renal toxicity.

Gentamicin should not be used if a person has a history of hypersensitivity such as anaphylaxis shock or other serious toxic reaction to gentamicin or any other Aminoglycosides. Intramuscular injection of gentamicin in mothers can cause muscle weakness in the newborn.

Gentamicin is not recommended in pregnancy unless the benefits outweigh the risks for the mother. Gentamicin can cross the placenta and several reports of irreversible bilateral congenital deafness in children have been seen[38].

Geriatrics

Older adults may be more sensitive to the effects of this drug, especially kidney damage. Renal function should be assessed before beginning and during therapy in elder persons due to a decline in glomerular filtration rate. This population can have longer than usual gentamicin levels in the body. CrCl can overestimate renal function in the elderly, 20% reduction of calculated CrCl should be considered for patients >80 years of age. It should be used cautiously in persons with renal, auditory, vestibular or neuromuscular dysfunction.

Pediatrics

Gentamicin may not be appropriate to use in children, including newborns and infants. Hypocalcemia, hypokalemia and muscle weakness have been reported after gentamicin injection. Higher serum levels and a longer half-life are noted in pediatrics. The renal function should be checked periodically during therapy. Amikacin, tobramycin, amphotericin B, cidofovir, cisplatin, polymyxin B, cephalosporins such as cephaloridine, ibuprofen, nonsteroidal anti-inflammatory drug may affect the kidneys or hearing may increase the risk of kidney damage or hearing loss if taken with gentamicin.

Overdose

If overdose is suspected, one should contact a poison control center or emergency room right away. In the event of over dosage or toxic reactions, hemodialysis may aid in the removal of gentamicin from blood and is especially important if renal function is, or becomes compromised. The rate of removal of gentamicin is considerable less by peritoneal dialysis than its hemodialysis. Rapidly changing renal function, which may occur with acute renal failure in the patient with septic shock, must be anticipated to avoid overdose.

Missed Dose

For the best possible benefit, it is important to receive each scheduled dose of this medication as directed. If one misses a dose, one should contact doctor or pharmacist right away to establish a new dosing schedule. The dose should not be doubled to catch up.

Precautions

Before using gentamicin, one should tell doctor or pharmacist if one is allergic to it; or to other aminoglycoside antibiotics (such as tobramycin, amikacin); or if one has any other allergies. This product may contain inactive ingredients (such as sulfites), which can cause allergic reactions or other problems. Before using this medication, one should tell doctor or pharmacist for medical history, especially of: cystic fibrosis, hearing problems (including deafness, decreased hearing), kidney problems, low blood minerals (including potassium, magnesium and calcium), myasthenia gravis, Parkinson's disease. Gentamicin may cause live bacterial vaccines (such as typhoid vaccine) not to work as well. Therefore, one should not have any immunizations/vaccinations while using this medication without the consent of doctor. Before having surgery, one should tell doctor or dentist about all the products one uses (including prescription drugs, nonprescription drugs, and herbal products). Anti-diarrhea products or narcotic pain medications should not be used if one has any of the following symptoms because these products may make him worse. One should tell doctor right away if one develops persistent diarrhea, abdominal or stomachs pain or cramping, blood or mucus in own stool.

CONCLUSION

All *enterococci* naturally have low-level resistance to aminoglycosides, which invalidates use of the disk test with usual concentrations of antimicrobial agents. HLAR is only meaningful for a testing method. When an enterococcal strain has high-level resistance to the aminoglycoside, there is no synergism and combination therapy with a beta-lactam drug which would not have the desired bactericidal effect. Therefore, it is important to detect the presence of high-level resistance in order to predict aminoglycoside synergy. Strains that show HLAR to gentamicin, the most commonly used and best aminoglycosides against enterococci, possess one or more aminoglycoside-modifying enzymes. These enzymes may make them resistant to one or more of a variety of other aminoglycosides, including tobramycin, netilmicin, and amikacin, but not streptomycin by adenylylation, acetylation, or phosphorylation, impaired entry of aminoglycoside into the cell and deletion or alteration of receptor protein on the 30S ribosomal subunit. Other HLAR enzymes are active against streptomycin, but not gentamicin. Thus, testing gentamicin and streptomycin is preferred to provide information on the two most active of the aminoglycosides that do not show cross-resistance to each other. If a strain has high-level resistance to both gentamicin and streptomycin, tobramycin will not be effective. There are some strains that have emerged that will still be amikacin or netilmicin susceptible, however, and testing for high-level resistance in these agents may

be indicated. Many isolates of *E. faecalis* and *E. faecium* have acquired high-level resistance to one or more of the aminoglycosides, while other enterococci and viridans streptococci have not yet acquired the genes for resistance. Performing *in-vitro* susceptibility testing of clinical isolates of *E. faecalis* and *E. faecium* from systemic infections is critical for determining which combination of agents may be effective therapy. More hearing loss than vestibular disturbance is experienced with amikacin. Auditory damage, results in tinnitus and high-frequency hearing loss initially, or as permanent vestibular damage, evident by vertigo, ataxia, and loss of balance with streptomycin and gentamycin. Nephrotoxicity results in rising serum creatinine levels or reduced creatinine clearance. Gentamicin is one of the most nephrotoxic of the class. Streptomycin has the lowest nephrototoxicity among aminoglycosides. In neonates, infants and children, where possible, serum levels should be measured and the dose should be adjusted to provide the desired serum level. Toberamycine is least likely to produce neuromuscular blockade. Risk of toxic reactions is low in patients with normal renal function who do not receive gentamicin sulfate at higher doses or for longer periods of time than recommended. Only one aminoglycoside, paromomycin, is used against parasitic infection. Their correct dosage can considerably reduce or attenuate toxicity. Hence, it is essential to understand which risk factors are related to nephrotoxicity and to correct them whenever possible. Recent advances in the understanding of the effect of various dosage schedules of aminoglycosides on toxicity have provided a partial solution to this problem, although more research still needs to be done in order to overcome this problem entirely.

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