MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

November 14, 1974

AMINOGLYCOSIDE ANTIBIOTICS

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INTRODUCTION

The term "aminoglycoside antibiotics" is a convenient but imprecise designation for a large group of chemically related antibiotics including streptomycin, neomycin, kanamycin, and gentamicin. Unlike penicillin, which was discovered accidentally, each of the aminoglycoside antibiotics was discovered as the result of a deliberate search. Recognizing the need for effective means of treating infections due to gram negative bacteria, Waksman and associates in 1939 began a program to screen microorganisms for production of antibiotics active against them. Between 1939 and 1943, these studies led to the isolation of many antimicrobial products including actinomycin and streptothrycin, but none of these agents had sufficient antibacterial activity and little enough toxicity to be clinically useful as an antibiotic. After screening more than 10,000 different microbes, a strain of *Streptomyces griseus* was found that produced a new antibiotic, now called streptomycin, that had a broad spectrum of activity against many bacteria including gram negative bacilli, staphylococci and the tubercle bacillus (1). The rapid progress in the study of streptomycin following its discovery has been summarized as follows by Weinstein: "In less than two years extensive bacteriological, chemical, and pharmacological investigations of streptomycin had been carried out, and its clinical usefulness was established. Controlled studies of the therapeutic efficacy of the drug in man were supervised by the National Research Council and supported by large contributions from privately owned pharmaceutical and chemical companies; this constituted the first privately financed, nationally coordinated, clinical drug evaluation in history. By early 1947, these investigations were completed and streptomycin was released by the National Research Council for general clinical study and use" (2). In 1947, dihydrostreptomycin was produced by chemical reduction of streptomycin. Neomycin was isolated from a strain of *Streptomyces fradae* by Waksman and Lechevalier in 1949 (3). Umezawa and his coworkers in Japan isolated kanamycin in 1957 from a strain of *Streptomyces kanamyceticus* (4). Paromomycin was isolated in 1959 by Coffey and coworkers from an isolate of *Streptomyces rimosus* from a sample of soil in Colombia (5). Early reports on the characterization of gentamicin, an antibiotic produced by a strain of *Micromonospora purpurea*, appeared in 1963 (6), and during the last 11 years gentamicin has become one of the most commonly used antibiotics in hospitalized patients. Many aminoglycosides have been tested and discarded because of insufficient

activity or excessive toxicity, while newer ones are continuously being discovered and tested for possible clinical usefulness. Among the promising new aminoglycosides that are not yet available for general clinical use are tobramycin, sisomycin, amikacin (BB-K8), and butirosin. Several important symposia about kanamycin and gentamicin have been published and provide a wealth of clinical and bacteriological data about these important antibiotics (7-10). References 11 and 12 also provide useful reference materials concerning the molecular biology and the clinical use of antibiotics.

STRUCTURE AND CLASSIFICATION

The comparative chemistry of the aminoglycoside antibiotics has been reviewed by Rinehart (13). In chemical terms an aminoglycoside is a compound containing an amino sugar glycosidically linked to some other molecule. Many different groups of antibiotics contain amino sugars linked in this manner, and the term aminoglycoside antibiotics, though useful, is not specific enough to characterize the antibiotics considered here. Other structural components that are present in all of the antibiotics commonly called aminoglycosides are derivatives of inisotol called aminocyclitols (Fig. 1).

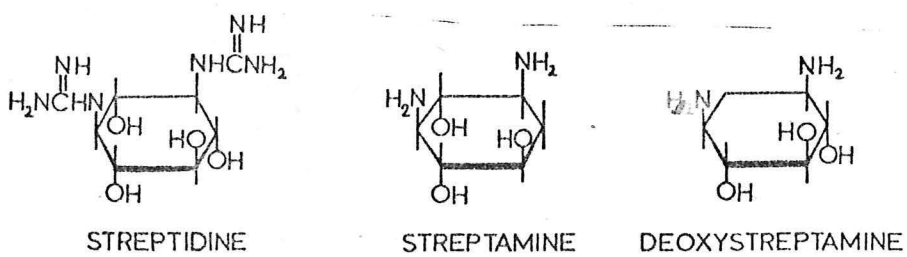
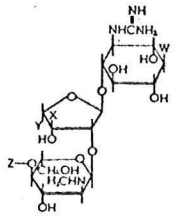


FIGURE 1
Characteristic aminocyclitols (Haworth projections)
(Ref. 13)

All of the aminoglycoside antibiotics in clinical use today can be characterized as aminoglycosidic aminocyclitols. Streptomycin is a derivative of streptidine, while other familiar aminoglycosides such as the neomycins, kanamycins, and gentamicins are all derivatives of deoxystreptamine. The deoxystreptamine containing aminoglycosides can be separated into two large subgroups which have substitutions at different positions on the deoxystreptamine molecule. The "1,2-substituted" deoxystreptamine antibiotics, including the neomycins, hybramycins, and paromomycins, have substituents attached to adjacent hydroxyl groups on the deoxystreptamine ring. In contrast, the "1,3-substituted" group of aminoglycosides, including the kanamycins, gentamicins, and tobramycin, contain substituents on hydroxyl groups that are not adjacent in the deoxystreptamine molecule. Structural formulas of the more important aminoglycoside antibiotics are given in Figures 2 through 6.

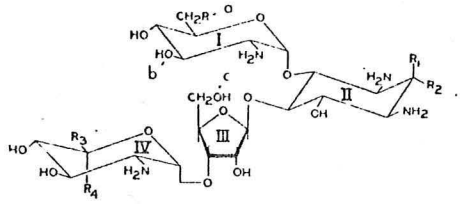


	W	X	Y	Z
STREPTOMYCIN	$-NHC(=NH)NH_2$	-CHO	$-CH_3$	-H
DIHYDROSTREPTOMYCIN	"	$-CH_2OH$	"	"
MANNOSIDOSTREPTOMYCIN	"	-CHO	"	*
HYDROXYSTREPTOMYCIN	"	"	$-CH_2OH$	-H
BLUENSOMYCIN	$-OCONH_2$	"	$-CH_3$	"

* ∝-D-MANNOPYRANOSIDO-

FIGURE 2

Streptidine-bluensidine antibiotics (Haworth projections) (Ref. 13)



	R	R_1	R_2	R_3	R_4
NEOMYCIN B	NH_2	H	H	H	CH_2NH_2
NEOMYCIN C	NH_2	H	H	CH_2NH_2	H
HYBRIMYCIN A1	NH_2	OH	H	H	CH_2NH_2
HYBRIMYCIN A2	NH_2	OH	H	CH_2NH_2	H
HYBRIMYCIN B1	NH_2	H	OH	H	CH_2NH_2
HYBRIMYCIN B2	NH_2	H	OH	CH_2NH_2	H
PAROMOMYCIN	OH	H	H	CH_2NH_2 or H	H or CH_2NH_2

FIGURE 3

Structure of the neomycins, butirosin, ribostamycin, and paromomycin. Neamine (or paromamine) consists of rings 1 + 2, neobiosaminide of rings 3 + 4, and ribostamycin of 1 + 2 + 3. The hybrimycins contain a streptamine ring (hybrimycin A_1 and A_2) or epistreptamine ring (B_1 and B_2) instead of 2-deoxystreptamine. Butirosin (1 + 2 + 3) has a 4-amino-2-hydroxybutyryl substituent on N-1 of 2-deoxystreptamine. Arrows indicate where these antibiotics are N-acetylated by kanamycin acetyltransferase to yield 6'-N-acetyl antibiotics (a), and o-phosphorylated by neomycin-kanamycin phosphotransferase (b) and by lividomycin phosphotransferase (c).

(Ref. 27)

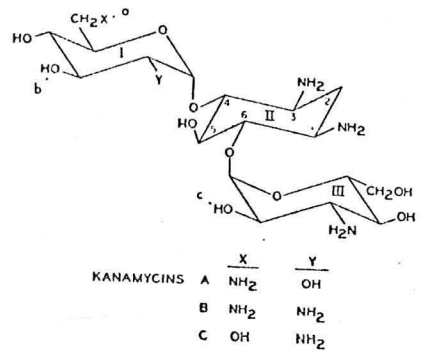


FIGURE 4

Structure of kanamycins A, B, and C. Arrows indicate the sites of N-acetylation by kanamycin acetyltransferase to yield 6'-N-acetyl antibiotics (a), the site of o-phosphorylation by neomycin-kanamycin phosphotransferase (b), and the site of o-adenylylation by gentamicin adenylyltransferase (c).

(Ref. 27)

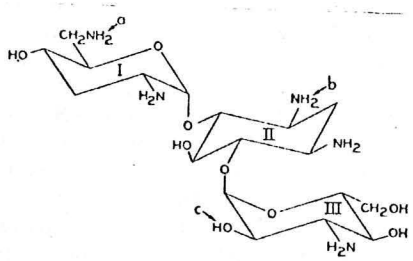


FIGURE 6

Structure of tobramycin (nebramycin factor 6). The arrow indicates where this antibiotic can be enzymatically N-acetylated by kanamycin acetyltransferase to yield 6'-N-acetyl tobramycin (a), by gentamicin acetyltransferase I to yield 3-N-acetyl tobramycin (b), and o-adenylylated by gentamicin adenylyltransferase (c).

(Ref. 27)

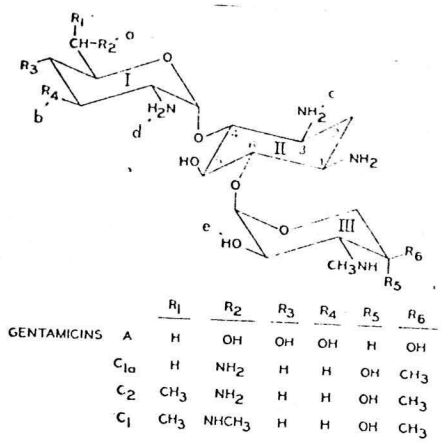


FIGURE 5

Structure of the gentamicins. Ring I is purpurosamine, II is 2-deoxystreptamine, and III is gentosamine (gentamicin A) or garosamine (gentamicin C's). Sisomycin is 4,5-dehydrogentamicin C_{1a} (the purpurosamine ring is reduced). In dihydrosisomicin, the carbon #6 group of that ring is inverted (L-sugar). Arrows indicate where this group of antibiotics can be N-acetylated by kanamycin acetyltransferase to yield 6'-N-acetyl antibiotics (a), by gentamicin acetyltransferase II to yield 2'-N-acetyl antibiotic (d), by gentamicin acetyltransferase I to yield 3-N-acetyl antibiotic (c), o-phosphorylated by neomycin-kanamycin phosphotransferase (gentamicin A only) (b); and o-adenylylated by gentamicin adenylyltransferase (e).

(Ref. 27)

Some of the aminoglycoside antibiotics used clinically are in fact mixtures of closely related compounds. For example, kanamycin is almost entirely composed of kanamycin A, but traces of kanamycin B are present; and gentamicin is a mixture of gentamicins C1, C1a, and C2. Among the newer aminoglycosides undergoing testing, amikacin (BB-K8) is a semisynthetic derivative of kanamycin, and sisomycin is 4,5-dehydrogentamicin C1a.

All of the aminoglycoside antibiotics are basic compounds that are readily soluble in water. They are most commonly available as sulfate salts, and they are stable in solution.

ANTIBACTERIAL ACTIVITY

The clinically useful aminoglycoside antibiotics have similar spectra of activity against most species of bacteria. Aminoglycosides are bactericidal against most susceptible bacteria and have optimal activity at slightly alkaline pH. A summary of recent data on the susceptibility to the aminoglycoside antibiotics streptomycin, kanamycin, gentamicin, and tobramycin of gram negative bacilli and gram positive cocci isolated at Parkland Memorial Hospital between February 1, 1974, and April 15, 1974, is presented in Table 1. These unpublished data were provided by Dr. Paul Southern and Ms. Earline Kutscher.

Most aminoglycosides have a broad spectrum of activity against gram negative bacilli and against *Staphylococcus aureus*. Aminoglycosides also have significant activity against *Mycobacterium tuberculosis*, but only streptomycin has been widely used as a first line drug for the therapy of tuberculosis. During the past 30 years, resistance to streptomycin has become common among clinical isolates of gram negative bacilli. At the present time gentamicin has a slightly broader spectrum of activity than kanamycin against most gram negative bacilli. Most strains of *Pseudomonas aeruginosa* are resistant to kanamycin but susceptible to gentamicin. In addition, most strains of *Proteus morganii* isolated at Parkland Memorial Hospital are resistant to kanamycin but susceptible to gentamicin. The incidence of gentamicin resistant strains of *Pseudomonas aeruginosa* appears to be increasing at Parkland Memorial Hospital. In a study of *Pseudomonas aeruginosa* strains isolated two years ago, 91% were inhibited by 5 µg/ml of gentamicin (14). In contrast, at the present time only 72% are inhibited by 5 µg/ml of gentamicin. Strains of *Pseudomonas aeruginosa* resistant to more than 100 µg of gentamicin/ml have also been isolated at Parkland Memorial Hospital, particularly from the adult and pediatric intensive care burn units (15). Most of the gentamicin resistant strains of *Pseudomonas aeruginosa* isolated locally have been susceptible to tobramycin but resistant to amikacin and butirosin. The antibacterial activity of newer aminoglycosides against gram negative bacteria has been evaluated in studies from various institutions (16-24). One of the more striking observations from these *in vitro* studies is the greater activity of tobramycin than gentamicin against *P. aeruginosa*, but whether this will be clinically significant remains to be established. No single pattern of cross resistance exists between gentamicin resistance and resistance to the newer aminoglycoside antibiotics. Therefore, routine testing of gram negative bacilli for susceptibility to several aminoglycosides remains desirable.

All of the aminoglycoside antibiotics are believed to have similar mechanisms of action. Among them, the antibacterial action of streptomycin has been

TABLE 1

SUSCEPTIBILITY TO AMINOGLYCOSIDES OF BACTERIAL STRAINS ISOLATED AT PARKLAND MEMORIAL HOSPITAL BETWEEN FEBRUARY 1, 1974 AND APRIL 15, 1974

Species	Number Isolated	*Percent of Strains Inhibited by* [a]			
		Streptomycin 20 µg/ml	Kanamycin 20 µg/ml	Gentamicin 5 µg/ml	Tobramycin 5 µg/ml
Gram Negative Bacilli					
Escherichia coli	736	77	90	96	96
Enterobacter cloacae	108	74	69	88	76
Enterobacter aerogenes	65	83	91	97	92
Enterobacter agglomerans	10	100	90	100	100
Klebsiella pneumoniae	284	80	82	96	86
Proteus mirabilis	206	97	98	100	N.T.
Proteus morganii	25	79	4	96	N.T.
Pseudomonas aeruginosa	263	31	5	72	94
Pseudomonas maltophilia	16	13	19	19	19
Acinetobacter (Herellea)	45	60	87	64	71
trobacter diversus	32	81	75	100	72
Citrobacter freundii	12	83	92	83	83
Serratia species	47	75	76	100	89
Providencia stuartii	31	39	26	32	39
Gram Positive Cocci					
Staphylococcus aureus (coag. +)	365	96	98	N.T.	N.T.
Staphylococcus epidermidis (coag. -)	94	74	81	N.T.	N.T.
Enterococcus	208	9	9	N.T.	N.T.
Viridans streptococci	15	87	93	N.T.	N.T.
Streptococcus pneumoniae	15	60	13	N.T.	N.T.
Bacillus species	18	56	89	N.T.	N.T.

a) Minimal inhibitory concentrations for all bacteria except Proteus species were determined by agar dilution susceptibility tests. Susceptibilities of Proteus species were determined by disk diffusion tests and data given are percent of strains reported to be susceptible. These data were compiled from unpublished studies by Dr. Paul Southern and Earline Kutscher.

studied in the greatest detail (reviewed in references 11, 25). The aminoglycoside antibiotics interact with bacterial ribosomes and interfere with synthesis of protein in bacterial cells. The biochemical experiments which led to the discovery of this mode of action for streptomycin are closely related to experiments on the genetics of resistance to streptomycin in bacteria. In *Escherichia coli*, single step mutations that confer resistance to very high concentrations of streptomycin occur at low frequency. Other mutants of *E. coli* can be isolated that grow only in the presence of streptomycin, and such strains are designated streptomycin dependent. Genetic studies have shown that the determinants of streptomycin susceptibility, resistance, and dependence are allelic, suggesting that mutational changes in a single protein can produce each of the three phenotypes. When hybrid bacterial strains are constructed that carry genes determining both streptomycin resistance and streptomycin susceptibility within a single cell, susceptibility is found to be dominant over resistance.

With the development of systems for studying protein synthesis *in vitro*, the action of streptomycin has been studied at the biochemical level. Protein synthesizing systems can be prepared by mixing ribosomes and soluble fractions prepared from streptomycin susceptible and streptomycin resistant strains of *E. coli*, and protein synthesis can be measured in the presence or in the absence of streptomycin. Such studies have shown that streptomycin susceptibility, resistance, or dependence is a property controlled by the bacterial ribosomes and not by soluble factors. Similar experiments with 30 and 50S ribosomal subunits have shown that the smaller 30S ribosomal subunit controls susceptibility or resistance to that drug. Ribosomes from susceptible cells bind streptomycin efficiently, but ribosomes from resistant cells do not. Analysis of the individual proteins derived from the 30S ribosomal subunit of *E. coli* has shown that a single protein designated P10 (or S12) is altered in streptomycin resistant or streptomycin dependent strains (26).

When *E. coli* is treated with streptomycin, protein synthesis is inhibited, abnormal proteins are formed as the result of misreading of the genetic message, and the bacterial cells die. Inhibition of protein synthesis and misreading of the genetic code occur in the presence of all of the aminoglycoside antibiotics that have been tested. However, neither of these effects is sufficient to explain the bactericidal action of streptomycin or the dominance of streptomycin susceptibility over streptomycin resistance. Studies of protein synthesis *in vitro* using natural messenger RNA have clarified this problem. In the presence of appropriate cofactors, streptomycin poisoned ribosomes are capable of initiating protein synthesis, but elongation of polypeptide chains does not occur. Polysomes are present in the cell and turnover of messenger RNA occurs, but effective protein synthesis is blocked. The initiation complexes formed with streptomycin poisoned ribosomes are unstable, and the streptomycin-ribosome complexes are released from initiation complexes and can reassociate with new molecules of messenger RNA. The dominance of streptomycin susceptibility is thus believed to result from a cyclic blockade of polysomes by streptomycin poisoned ribosomes in the cell.

Benveniste and Davies have recently reported studies on the relationship between structure and antibiotic activity among the deoxystreptamine containing aminoglycosides (27). They have shown that the antibacterial activity of these aminoglycosides is strongly affected by the positions and by the number of amino groups in the amino sugars attached to the deoxystreptamine molecule. At least one amino group on each of the sugars is required for antibacterial activity, and activity is increased by increasing the number of amino groups. Modification of

the amino groups by acetylation appears to neutralize their contributions to antibacterial activity. The aminoglycoside antibiotics containing 1,2-substituted deoxystreptamine are more potent on a weight basis than those containing 1,3-substituted deoxystreptamine. Benveniste and Davies have pointed out the current need for a simple and direct assay which would relate structural requirements for antibacterial activity to those responsible for the various toxic effects of aminoglycoside antibiotics. Such an assay would be very helpful in providing a rational basis for designing more effective and less toxic aminoglycosides.

BACTERIAL RESISTANCE

There are several important differences between the mechanisms of resistance to aminoglycosides that are observed in bacterial mutants selected *in vitro* and in resistant bacteria isolated directly from clinical specimens. The occurrence of single step mutations that confer resistance to high levels of streptomycin has already been mentioned, and resistance of this type is due to alterations in a specific ribosomal protein responsible for the binding of streptomycin to ribosomes. Resistance determined by alterations in ribosomal structure has not been observed with kanamycin, gentamicin, and other related aminoglycosides (27). In clinical isolates of aminoglycoside resistant bacteria, antibiotic resistance is usually determined by drug resistance factors (R factors) and not by alterations in ribosomal structure (28). In addition, some strains of aminoglycoside resistant bacteria have a reduced ability to take up these antibiotics (29).

R factors are important in clinical medicine because they can determine bacterial resistance to multiple antibiotics and can sometimes be transferable from one bacterial strain to another (28,29). R factors are a specific example of a group of genetic elements in bacteria called plasmids, and the term resistance factor is therefore equivalent to resistance plasmid. Plasmids are extrachromosomal genetic elements carrying information that is not essential to the survival of their bacterial host under normal conditions. Within cells, plasmids exist as circular molecules of double stranded DNA that are separate from the bacterial chromosome and are capable of replication. As shown in Figure 7, there are two general classes of plasmids.

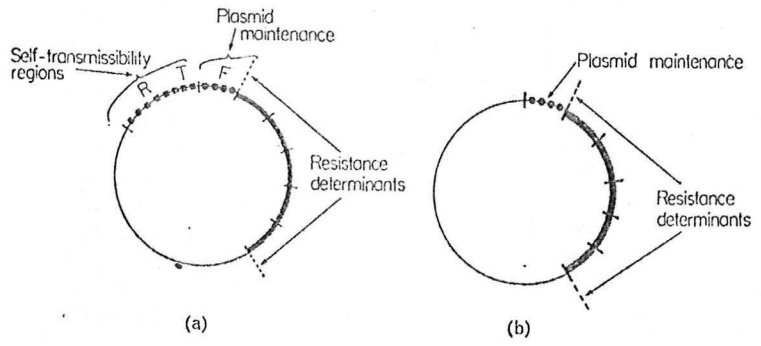


FIGURE 7

Diagrammatic representations of the two main types of resistance plasmid. (a) self-transmissible, (b) phage-transmissible. In example (a), the RTF region is made up of a maintenance region (●●●●) coupled to a region specifying sexual characters (■■■■). In (b), only the maintenance region is found. The distribution of the resistance determinants round the plasmid is diagrammatic. In most cases mapping data are lacking. (Ref. 11)

All plasmids have certain features in common. To ensure their survival within cells, plasmids must have genes that enable them to replicate and persist within bacterial cells. In Figures 7A and 7B this section of the plasmid is designated *plasmid maintenance* functions. One class of plasmids, represented diagramatically in Figure 7A, is designated self transmissible. This term indicates that such plasmids can be transferred from one bacterium to another by conjugation, and that the plasmid itself contains the genetic information necessary for these transfer functions. Self transmissible plasmids direct synthesis of new antigens associated with surface structures called pili. These pili are important in establishing contact between bacterial cells, and during conjugation they serve as the intracellular bridges through which genetic material (DNA) is transferred from the donor to the recipient cells. In Figure 7A, the genes that determine these functions are designated as the *self transmissibility regions* of the plasmids. Other plasmids, represented diagramatically in Figure 7B, cannot promote their own transfer from cell to cell. They can be transferred, however, if they are present in a cell that also contains a self transmissible plasmid. They can also be transferred by transduction, a process in which a bacterial virus serves as a vector for transportation of the plasmid DNA from a donor to a recipient cell.

The genes involved in plasmid maintenance and self transmissibility are characteristic of plasmids in general. However, plasmids can carry additional and potentially unique genetic information that is not required either for their own replication and maintenance or for the viability of their host cells. Such additional genes may determine resistance to antibiotics, resistance to heavy metals, resistance to environmental agents such as ultraviolet irradiation, the production of toxins, etc. (Table 2).

TABLE 2

Naturally Occurring Resistance to Antibacterial Agents

Aminoglycosides:	Sulfonamide
Kanamycin	Erythromycin
Neomycin	Lincomycin
Gentamicin	Trimethoprim
Lividomycin	Heavy metals:
Tobramycin	Nickel
Streptomycin	Cobalt
Spectinomycin	Mercury
β-Lactam antibiotics:	Lead
Penicillins	Bacteriophages
Cephalosporins	Ultraviolet light
Chloramphenicol	Colicins
Tetracycline	

(Ref. 28)

Resistance factors or R factors were first discovered because they contained genes determining resistance to antibiotics and because they could be transferred rapidly from cell to cell. R factors are common in bacteria isolated from clinical specimens in all parts of the world. Clinically significant resistance to aminoglycoside antibiotics is most commonly determined by genes that are present in resistance factors (28,29). Many studies have demonstrated that

aminoglycoside resistant bacteria carrying R factors can inactivate specific aminoglycoside antibiotics. It has now been established that the resistance determinants associated with aminoglycoside resistance direct the synthesis of enzymes that can modify aminoglycosides and lead to their inactivation. These reactions involve modifications of the aminoglycosides by acetylation, by phosphorylation, or by adenylylation. Benveniste and Davies have summarized the structural properties of aminoglycosides necessary for them to be substrates for these enzymes, and have tabulated the available information concerning the different types of aminoglycoside modifying enzymes discovered to date (28). Some of their data are reproduced in Tables 3 and 4.

Some of these principles can be illustrated with data from aminoglycoside resistant bacteria isolated at Parkland Memorial Hospital. Strains of *Pseudomonas aeruginosa* resistant to high levels of gentamicin (100 µg/ml or greater) were found to contain acetyltransferase activities and to inactivate gentamicin by acetylation (15). Some of these strains also contain a kanamycin phosphorylating enzyme. Among the gentamicin resistant strains, most are susceptible to tobramycin but a few are resistant to tobramycin. The acetyltransferase activities from tobramycin susceptible and resistant strains can be distinguished *in vitro*, and the differences in susceptibility of the bacteria to tobramycin can be explained by differences in the ability of the acetylating enzymes to utilize tobramycin as a substrate. Self transmissibile plasmids determining gentamicin resistance in our strains of *Pseudomonas aeruginosa* were not demonstrated. Tobramycin resistant strains of *Enterobacter cloacae* and of *Klebsiella pneumoniae* have also been isolated from the burn units at Parkland Memorial Hospital (30). Tobramycin resistance in these strains is associated with an aminoglycoside acetylating activity that resembles kanamycin acetyltransferase, and tobramycin resistance can be readily transferred by conjugation from these strains to an *E. coli* recipient. These observations illustrate two other points. First, strains of *Pseudomonas aeruginosa* resistant to high levels of gentamicin are present in Parkland Memorial Hospital, particularly in the burn units, and the possibility that they could become disseminated throughout the hospital is real and must be constantly monitored. Second, although tobramycin is an experimental drug which has only been used to a limited extent here at Parkland Hospital, a reservoir of tobramycin resistant gram negative bacteria containing self transmissible R factors for tobramycin resistance is present in the hospital environment. These observations reinforce the basic principle that a tenuous balance exists between the development of effective new antibiotics and the evolution and dissemination of bacteria resistant to them. Such a balance is subject to change with time.

One clinically useful fringe benefit has resulted from the discovery of bacterial enzymes that modify aminoglycoside antibiotics. These enzymes have been used to develop enzymatic assays for gentamicin and for other aminoglycoside antibiotics, and such assays provide a rapid and accurate method to measure the concentrations of aminoglycosides in serum or other body fluids (31-34). Assays for aminoglycosides can also be performed by microbiological methods (35-37) or by radioimmunoassays (38,39). Such methods have provided an important tool for studies on the clinical pharmacology of the aminoglycoside antibiotics.

PHARMACOLOGY

Although there are minor differences in pharmacologic properties of the various clinically useful aminoglycosides, the similarities between these drugs are much more striking than the differences (7-10,12,40). When aminoglycosides

TABLE 3
(Ref. 28)

Aminoglycoside Modifying Enzymes

Enzyme	Bacterial source	Modification
Kanamycin acetyltransferase (KAcT)	R+ *E. coli* *P. aeruginosa*	6-amino group of an amino hexose is acetylated
Gentamicin acetyltransferase I (GAcT I)	*P. aeruginosa* *K. pneumoniae* *E. coli*	3-amino group of 2-deoxystreptamine is acetylated
Gentamicin acetyltransferase II (GAcT II)	*Providencia*	2-amino group of an amino hexose is acetylated
Streptomycin-spectinomycin adenylyltransferase (SAdt)	R+ *E. coli*	hydroxyl group of a D-*threo* methylamino alcohol moiety is adenylylated
Gentamicin adenylyltransferase (GAdt)	R+ *E. coli* R+ *K. pneumoniae*	2-hydroxyl group of an amino hexose is adenylylated
Streptomycin phosphotransferase (SPT)	R+ *E. coli* *S. aureus* *P. aeruginosa*	3-hydroxy group of N-methyl L-glucosamine is phosphorylated
Neomycin-kanamycin I phosphotransferase (NPT) II	R+ *E. coli* *P. aeruginosa* *S. aureus*	3-hydroxyl group of an amino hexose is phosphorylated
Lividomycin phosphotransferase (LvPT)	*P. aeruginosa* R+ *E. coli*	5-hydroxyl group of D-ribose is phosphorylated

TABLE 4
(Ref. 28)

Enzymatic Inactivation of Aminoglycoside Antibiotics

Antibiotic	Inactivating enzyme[a]								
	KAcT	GAcT I	GAcT II	SAdT	GAdT	SPT	NPT I	NPT II	LvPT
kanamycin A	+	–	–	–	+	–	+	+	–
B	(+)	(+)[b]	–	–	+	–	+	+	–
C	–	–	–	–	+	–	+	+	–
neomycin B or C	(+)	–	–	–	–	–	+	+	+
paromomycin	–	–	–	–	–	–	+	+	+
lividomycin B	–	0	0	–	0	–	+	+	+
butirosin	(+)	–	–	–	–	–	–	+	0
vistamycin	+	–	–	–	–	–	+	+	+
gentamicin C_{1a}	(+)	+	+	–	+	–	–	–	–
C_2	(+)	+	+	–	+	–	–	–	–
C_1	–	+	+	–	+	–	–	–	–
A	–	–	(+)[b]	–	+	–	+	+	–
sisomicin	(+)	+	+	–	+	–	–	–	–
tobramycin	(+)	+	(+)[b]	–	+	–	–	–	–
nebramycin factor 2	–	–	–	–	–	–	–	–	–
streptomycin	–	–	–	+	–	+	–	–	–
spectinomycin	–	–	–	+	–	–	–	–	–
BBK-8[c]	+	–	–	–	–	–	–	–	–

[a] The abbreviations for the enzymes are explained in Table 2. + means an enzymatic modification inactivates the antibiotic, (+) that it is only partially inactivated, and – that it is not a substrate. Zeros denote reactions that have not been tested.

[b] These three antibiotics are modified by the enzyme, but are poor substrates and strains are essentially sensitive to them.

[c] BBK-8 is a new kanamycin derivative (224).

are administered orally, only traces of the drugs are absorbed from the gastrointestinal tract. The drugs remain biologically active within the lumen of the gut and exert their antibacterial effect on the normal flora of the bowel. Significant blood levels of these drugs are not observed after oral administration except in the presence of renal failure, when they may accumulate and reach toxic levels. Absorption of aminoglycosides is also poor after topical application to the skin. Neomycin has been used extensively for oral administration to patients with hepatic coma, and neomycin is also a component of many creams and ointments designed for topical application. The use of gentamicin applied topically to patients with severe burns has been associated with the emergence of gentamicin resistant bacteria including *Pseudomonas aeruginosa* (41), and topical use of aminoglycosides other than neomycin is discouraged at the present time.

For systemic therapy, the aminoglycosides must therefore be injected parenterally, and they are usually administered by the intramuscular or intravenous route. At the present time, streptomycin is used primarily in the therapy of tuberculosis, but it is also the drug of choice for certain uncommon bacterial infections such as plague and tularemia. Kanamycin and gentamicin are widely used for the treatment of serious gram negative bacillary infections caused by organisms that are known to be resistant to other less toxic antibiotics. In addition, kanamycin and gentamicin have been used extensively to provide broad coverage against gram negative bacilli during initial therapy in patients with sepsis of undetermined etiology. Because of the similarities between aminoglycosides and the voluminous literature about these drugs, studies with gentamicin will be cited in the following paragraphs to illustrate the more important points about pharmacology of aminoglycosides. Selected data concerning the pharmacology of aminoglycosides are also summarized in Table 5.

The aminoglycoside antibiotics are rapidly absorbed after intramuscular injection and become distributed throughout the extracellular space of the body (42,43). Maximum concentrations in serum are observed within one hour after intramuscular injection. Aminoglycosides do not enter erythrocytes efficiently, and almost all of the aminoglycoside in blood is normally present in the plasma. With the exception of streptomycin, none of the aminoglycosides is significantly bound to plasma proteins (44). Binding of streptomycin is weak and probably does not exceed 35%. The half life of these antibiotics in plasma in normal adults varies somewhat from individual to individual, but is usually between 2 and 3 hours (45). None of the aminoglycosides is metabolized within the body. Aminoglycosides do not cross the blood-brain barrier efficiently, and maximal concentrations in cerebrospinal fluid are a small fraction of peak blood levels (46, 47). The concentrations of aminoglycosides obtained in peritoneal fluid, pleural fluid, pericardial fluid, and thoracic duct lymph represent significant fractions of blood levels (46,47).

Elimination of aminoglycosides from the body occurs primarily by glomerular filtration, and aminoglycosides are neither secreted nor reabsorbed by the renal tubule (42,43). Therefore, the clearance of aminoglycosides that are not bound to plasma proteins approximates the glomerular filtration rate, while the clearance of streptomycin is somewhat less. Small quantities of aminoglycosides are excreted in bile, and in patients without obstruction of the cystic duct mean gentamicin concentrations are approximately one third of simultaneous serum levels (48). The concentrations of gentamicin and of tobramycin in bronchial secretions have been measured in a canine model (49,50) and peak concentrations of gentamicin in bronchial secretions were 26% and 52% of simultaneous blood levels at 1 and 2 hours after injection.

TABLE 5

Summary of Pharmacology of Aminoglycosides in Adults With Normal Renal Function

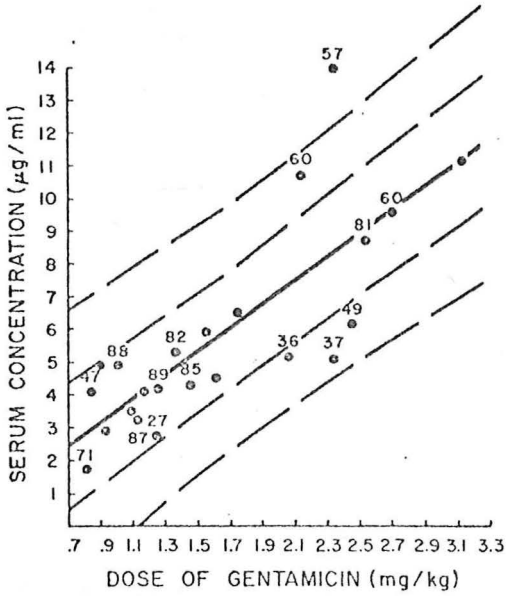
Drug	*Normal Dosage or Dose Studied*	*Serum Half Life (hours)*	*Dosage Studied*	*Peak Concentration Average or Range*		*% of Dose Excreted in Urine*
				Serum (μg/ml)	*Urine (μg/ml)*	
Streptomycin	0.5-2 g/day	2-3	500 mg IM	6-42	300-400	50- 60
			1000 mg IM	25-50	1000	
Kanamycin	15 mg/kg/day divided q12h	3	500 mg IM	14-29		
			1000 mg IM	18-40	140-250	50 in 4 hr
Gentamicin	5 mg/kg/day divided q8h	1.6-3.6	1.25 mg/kg IM	5- 7	5-100	30-100
Tobramycin	3-4 mg/kg/day divided q8h	1.6-3	80 mg IM	3.7 ± 0.6	20- 83	60
			40 mg IM	2.4 ± 0.3		60
Amikacin	150 mg/M^2 q6h	2.2-2.8	250 mg IM	11.9	1000	75 in 6 hr
			500 mg IM	20.6		

The recommended daily dosages are similar for streptomycin, kanamycin, and amikacin; recommended total daily doses for these three aminoglycosides usually do not exceed 1 gm in normal adults (Table 5). Following equivalent doses, the mean peak blood levels of streptomycin, kanamycin, and amikacin are similar, and mean peak concentrations after intramuscular injection of 500 mg doses of these drugs approximate 20 µg/ml. Very high concentrations of active drug are found in the urine during the first few hours after an intramuscular injection, and 50 to 100% of an injected dose is recovered in the urine in 24 hours. The recommended dosages of gentamicin and tobramycin on a weight basis are significantly less than for the other aminoglycosides. Following intramuscular injection of approximately 80 mg of gentamicin or tobramycin, mean peak concentrations of these aminoglycosides in serum are approximately 4 or 5 µg/ml.

These generalizations concerning the pharmacokinetic responses to various dosages of aminoglycoside antibiotics represent average values obtained from studies with large numbers of patients. With each of the aminoglycosides, however, considerable variation is observed in peak blood levels after a standardized dose (47, 51-53). In addition, serum half lives of aminoglycoside antibiotics vary considerably from individual to individual, even among patients with normal creatinine clearances (45,52). Although the reasons for these variations are poorly understood, the phenomenon has been well demonstrated for several aminoglycosides. Representative data for gentamicin are presented in Figures 8 and 9. The data in Figure 8 represent serum gentamicin concentrations observed one hour after single intramuscular injections of gentamicin at various dosages. The data in Figure 9 represent increments in serum gentamicin concentration (ΔG) after various doses of gentamicin in patients receiving multiple injections during treatment of bacterial infections. Both sets of data show a statistically significant correlation between increasing serum concentration and increasing dose of gentamicin, and the mean peak blood level or mean increase in blood level after standard dosages of gentamicin observed in these studies are comparable to other reports in the literature (43,47,51). Nevertheless, when individual measurements are considered, there is significant scatter in the data. Observations such as these suggest that accurate prediction of peak serum levels of aminoglycoside antibiotics in individual patients treated with standard dosages of these drugs is very difficult and may be impossible. The observed range of variability in the serum half life of aminoglycosides in patients with normal renal function further complicates the prediction of gentamicin blood levels during a course of therapy.

Because aminoglycosides are excreted primarily by glomerular filtration, major modifications of dosage must be made in the presence of renal insufficiency. The basic principles to be considered in modifying the dosage for renal insufficiency are similar for all aminoglycosides. Data obtained in studies of gentamicin will be used to illustrate these principles. After a single intramuscular dose of gentamicin, the peak serum level obtained is independent of the degree of renal insufficiency, but the serum half life increases progressively as glomerular filtration rate decreases. Based on these observations, Gingell and Waterworth suggested that usual doses of gentamicin could be given to patients in renal failure if the interval between doses is increased to compensate for reductions in glomerular filtration rate (54). They also predicted that more sustained blood levels could be obtained by administering a normal loading dose of gentamicin followed by smaller doses at more frequent intervals.

Subsequent investigators have attempted to provide simple rules or nomograms for calculating dosages of gentamicin in the presence of renal failure. McHenry ,



The concentration of gentamicin in serum ır after im injection is plotted against the dose of gentamicin (mg/kg of body weight) in 23 patients. The endogenous creatinine clearances (Ccr) are indicated on the points that represent patients with Ccr <90 ml/min. The best straight line (the solid line) calculated by the method of least squares was $y = 3.5x + 0.09$. The dashed lines enclosed the 68% and 95% confidence intervals (±1 and 2 SD) for prediction of concentrations in serum calculated from the formula $S_Y = S_{y.x} \sqrt{1 + \frac{1}{n} + \frac{x^2}{\Sigma x^2}}$, where $S_{y.x} = \pm 1.8$.

FIGURE 8
(Ref. 52)

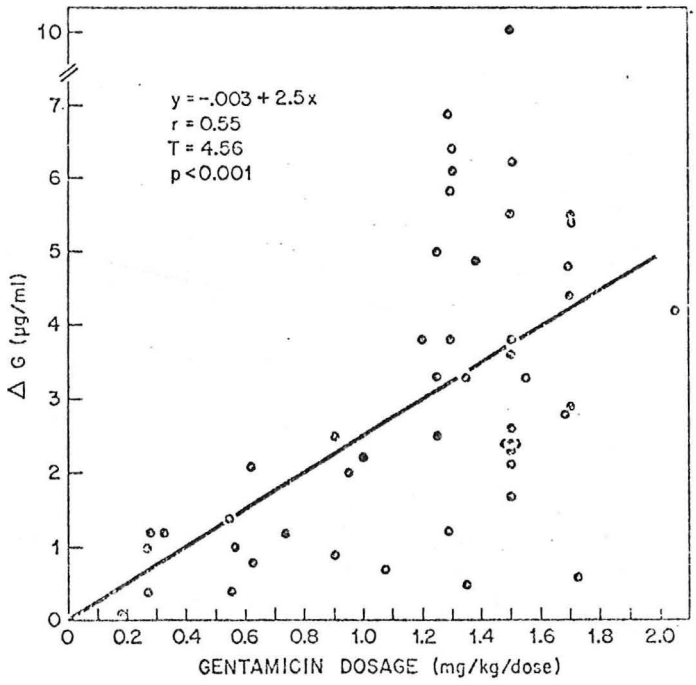


FIGURE 9
(Ref. 79)

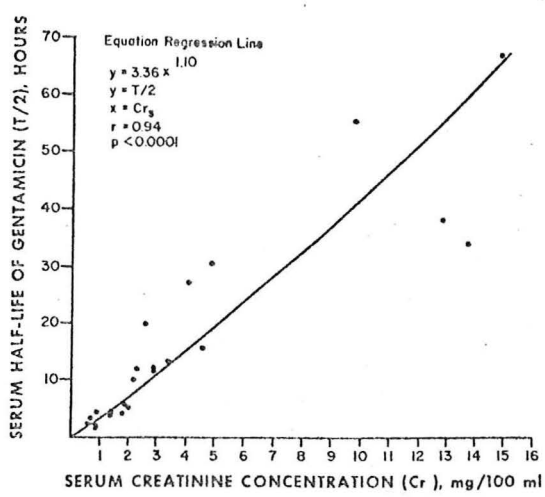


FIGURE 10

Relation of serum half life of gentamicin (T/2) to serum creatinine concentration in 24 patients (Ref. 45)

Cutler, and their coworkers (45,55) noted that the serum half life of gentamicin increased almost linearly with increasing serum creatinine concentration, as shown in Figure 10. Based on this empirical relationship, the serum half life of gentamicin in hours can be estimated by multiplying the serum creatinine concentration (in mg%) by 4. If standard doses of gentamicin (approximately 1.5 mg/kg) are given after every second half life, excessive accumulation of gentamicin in the body will not occur. This has led to the practice of estimating the appropriate interval between doses (in hours) in patients with renal failure by multiplying the serum creatinine concentration by 8. When this regimen is used, the interval between doses of gentamicin may be as long as three days. For this reason, low blood levels of gentamicin may be present for relatively long periods preceding each dose of gentamicin. Chan, Benner, and Hoeprich, citing the theoretical desirability of maintaining effective therapeutic levels of gentamicin in the serum at all times, have recommended that gentamicin be given in smaller doses at more frequent intervals to patients with renal insufficiency (56). Based on an approximately linear correlation between the elimination constant for gentamicin and creatinine clearance, they have devised a nomogram to estimate appropriate maintenance doses of gentamicin at various creatinine clearances (Fig. 11). In their system, a loading dose of 1.7 mg/kg is administered to all patients, and appropriately reduced doses are given subsequently at intervals of 8 hours.

Although these regimens for use of gentamicin in patients with renal insufficiency are based on logical principles, several practical problems may occur when they are used. As mentioned above, measurements of serum gentamicin concentrations have shown that the accuracy of predicting blood levels of gentamicin based on standardized regimens is limited. In addition, accurate data correlating blood levels of gentamicin with effectiveness of therapy in specific bacterial

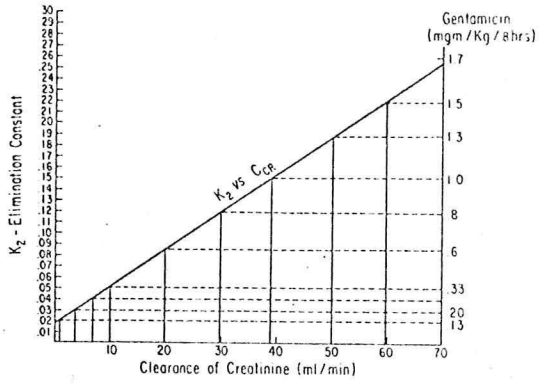


FIGURE 11

Dosage Nomogram for Patients With Renal Failure (Ref. 56)

infections are limited (56), and clinical data comparing the results of therapy with variable dosage and variable frequency regimens for administration of gentamicin in patients with renal failure are completely lacking.

When aminoglycoside antibiotics are used in patients with severe renal failure, the effects of peritoneal dialysis and hemodialysis on dosage regimens must also be considered. Again, data concerning gentamicin are used to illustrate the principles involved. The clearance of gentamicin has been measured both during peritoneal dialysis and during hemodialysis in man. When a single dose of gentamicin was given before peritoneal dialysis, the serum half life of gentamicin during dialysis was 12.5 hours (58). Peritoneal clearance of gentamicin during dialysis is in the range of 7 to 20 ml/min (59,60). Available data suggest that approximately 25 to 40% of an administered dose can be eliminated during a 24 hour period of peritoneal dialysis. In patients with peritonitis undergoing peritoneal dialysis who receive intramuscular gentamicin, concentrations of gentamicin observed in dialysis effluent were much less than simultaneous blood levels (61). Since gentamicin is not bound to serum protein and can equilibrate between peritoneal fluid and blood by diffusion, it is possible to maintain stable concentrations of gentamicin in serum and peritoneal fluid during dialysis by incorporating gentamicin into the dialysis fluid at the desired concentration (usually about 5 mg/liter) (61,62). Clearance of tobramycin by peritoneal dialysis appears comparable to the experience with gentamicin (63). During hemodialysis, the dialysance values for gentamicin may vary as much as two to three fold with different artificial kidneys, and dialysance values varied from 28 to 71 ml/min in four different artificial kidneys tested (64). These should be compared to renal gentamicin clearances of 90-130 ml/min in adults with normal kidneys. Thus, substantial removal of gentamicin during hemodialysis can be expected. In functionally anephric patients undergoing hemodialysis at intervals of 2 to 3 days at Parkland Memorial Hospital, we have usually begun gentamicin therapy with a dose of 1.5 to 2 mg/kg and have repeated the dose at the completion of each hemodialysis.

TOXICITY

It is well known that aminoglycosides are potentially toxic drugs, and among the serious side effects nephrotoxicity and ototoxicity are prominent (12). Toxicity can occur with doses of aminoglycosides comparable to or slightly above those optimal for therapy, so there is a narrow margin of safety for these drugs. In addition to ototoxicity and nephrotoxicity, many other toxic effects occur and can be separated into three general groups (65,66). Reactions occurring immediately after administration of the first dose of an aminoglycoside can be related either to direct pharmacological activities or to allergic reactions from prior sensitization to these drugs. Reactions occuring during the course of therapy may be due to sensitization or to other poorly defined mechanisms. Because aminoglycosides have a broad spectrum of antibacterial activity, suppression of normal bacterial flora may result in complications that are secondary to the antibacterial activity of the drugs. Pseudomembranous enterocolitis associated with oral administration of aminoglycosides is a good example of this problem (67).

TABLE 6
(Ref. 65)

Summary of Adverse Reactions to Kanamycin (FDA Files)

Total Drug-Related Reactions		43 Cases
1. Nephrotoxicity		11 cases
a. Includes 2 deaths		
b. Includes 3 cases with VIII n. damage		
2. Ototoxicity		14 cases
4 with complete hearing loss		
3. Respiratory Death (I.P. admin.)		1 case
4. CNS		6 cases
a. Bulging fontanelle	1 case	
b. Acute brain syndrome, hysteria	1 case	
c. Blurred vision	4 cases	
5. Anaphylactic Shock		2 cases
6. Hypersensitivity Skin Reactions		9 cases

A summary of adverse reactions to kanamycin is presented in Table 6. Although the incidence of specific adverse reactions may vary with different aminoglycosides, the types of reactions listed here are typical. Several antibiotics including aminoglycosides are weak neuromuscular blocking agents and have a curare like activity. If d-tubocuranine is assigned a relative neuromuscular blocking activity of 1,000, then the activity of polymyxin B would be 5, neomycin 2.5, streptomycin 0.7, dihydrostreptomycin 0.6, and kanamycin 0.5 (68). Fatal respiratory paralysis may occur due to this neuromuscular blockade, and has been observed most frequently when kanamycin or neomycin is administered intraperitoneally in excessive doses to surgical patients who have received anesthetics and muscle relaxants such as succinylcholine (12,65). Administration of aminoglycosides to patients with myasthenia gravis may aggravate muscle weakness (69). Peripheral neuropathy has been reported after administration of 500 mg of kanamycin sulfate into the vertebral canal at the time of a lumbar disc operation

(70). An anecdotal report of neuromuscular blockade following administration of gentamicin to two patients who had received prior courses of therapy with streptomycin or kanamycin has been made (71). Fortunately, this complication is extremely rare, and the neuromuscular blockade following administration of aminoglycosides is reversible and responds to infusion of calcium salts or administration of cholinesterase inhibitors. Anaphylactic shock and hypersensitivity skin reactions occur infrequently after treatment with aminoglycosides (65). Other adverse reactions that have been reported during therapy with gentamicin include nausea, vomiting, rash, urticaria, decreased hematocrit, and depression of granulocytes (66). These are rare and have all been reversible.

TABLE 7
(Ref. 66)
Comparison of Ototoxicity of Aminoglycosides

Antibiotic	Source of Data	Number of Cases	Ototoxicity
Kanamycin ..	Finegold *et alii* (1958)	106	5·7%
	Bristol Laboratory medical files	1,815	4·9%
Neomycin ..	Waisbren and Spink (1950)	64	8·0%
	Carr *et alii* (1950)	6	67·0%
Streptomycin ..	Keefer and Hewitt (1948)	1,957	3·6%
Gentamicin ..	Schering medical files	1,327	2·3%

Good epidemiological data are available concerning the incidence and types of ototoxic manifestations associated with various aminoglycoside antibiotics. Incidence data for kanamycin, neomycin, streptomycin, and gentamicin are summarized in Table 7. Significant ototoxicity occurs in 2 to 6% of patients treated with these antibiotics (66). Hearing loss, vestibular dysfunction, or both may occur. The histological lesion associated with ototoxicity is loss of hair cells in the cochlea or semicircular canals (66,72). Streptomycin and gentamicin cause vestibular damage more frequently than deafness, while neomycin and kanamycin are more likely to cause deafness. When audiometry is performed, high frequency hearing loss occurs more frequently and may precede the development of symptomatic hearing loss. Finegold analyzed variables associated with kanamycin ototoxicity and found that patients with ototoxicity had received larger average daily doses, longer courses of therapy, and larger total doses of kanamycin than patients without ototoxicity (Table 8). Arcieri (66), Jackson (73) and their collaborators have reviewed ototoxicity associated with gentamicin (Table 9). Prior therapy with ototoxic drugs, including any of the aminoglycoside, and age above 50 years were significant risk factors. Renal functional impairment prior to therapy was present in 20 of 33 patients who developed ototoxicity, and gentamicin serum levels above 10 µg/ml were detected in 8 of 13 patients in whom serum levels were obtained. The widely quoted recommendation that peak serum levels of gentamicin should not exceed 10 µg/ml is based primarily on these observations of risk factors for ototoxicity with gentamicin. There are no satisfactory data available to correlate peak serum levels of gentamicin with other forms of toxicity.

TABLE 8
(Ref. 65)

Relation of Age and Dosage to Kanamycin Ototoxicity

Category	Average Total Dose (g)	Average Daily Dose mg/kg wt	Average Number Days Therapy	Average Age
Total Group (106 patients)	28.2	27.6	15.6	52.9
Patients with Ototoxicity (22 patients)	46.7	36.0	20.3	50.8
Patients without Ototoxicity (84 patients)	23.3	25.2	14.6	53.4

TABLE 9
(Ref. 66)

Factors Possibly Related to Ototoxicity (31 Cases)

Characteristics	*Number of Patients*
Renal functional impairment	20
Prior ototoxic drug therapy	16
Age about 50 years	13
Elevated serum gentamicin level*	8 (10 µg/ml to 40 µg/ml)

* *Serum levels were available in only 13 of the 31 cases*

The study of gentamicin nephrotoxicity is more elusive than ototoxicity. Based on the retrospective study of patients with acute renal failure, prior therapy with gentamicin is a significant risk factor for the development of renal failure. Schultze and collaborators concluded that gentamicin was the major factor in the development of acute renal failure in 4 of 22 patients and was a contributing factor in 2 additional patients (74). Among patients treated with kanamycin or gentamicin, elevations of BUN or creatinine during therapy are common (65,75). In a review of 117 patients treated with kanamycin at Parkland Memorial Hospital, Sanford reported that 4 patients with normal renal function at the start of therapy developed acute renal failure with maximal values of BUN exceeding 100 mg% (76). Early experience with gentamicin is shown in Table 10. Among 131 patients treated with gentamicin in whom pretreatment values of BUN were available, 68 had some increase in BUN, and 31 of these had increases of BUN from normal to abnormal or from abnormal to markedly elevated values (75). Wilfert reviewed the records of 100 consecutive patients treated with gentamicin and identified 5 in whom gentamicin was felt to be the major cause of deteriorating renal function (77). Hewitt has reviewed 1,450 cases treated with gentamicin, among whom 70, or 4.8%, had evidence of decreasing renal function during therapy (78). The majority of these patients had pre-existing renal disease which became

TABLE 10
(Ref. 75)

*Changes from Pretreatment Values of BUN During or After Therapy With Gentamicin Sulfate in 131 Patients**

Changes in BUN	Number of patients
No increase:	
Values within normal range (5-22 mg/100 ml)	33
Initial values abnormally high (>22 mg/100 ml)	11
"No change," values not given by investigator	19
Total	63
Increase during or after treatment:	
Increase within normal range	37
Increase from normal to abnormally high	19
Increase from abnormally high to higher values	12
Total	68

* From file of cases reported to Schering Corp., Bloomfield, N. J.

worse during therapy with gentamicin. Among 7 patients with gentamicin nephrotoxicity whose renal function was apparently normal at the start of therapy, 4 had received either cephalothin or cephaloridine in addition to gentamicin. Other investigators have also suggested that simultaneous administration of gentamicin with cephalosporins may increase the risk of nephrotoxicity (79,80). Acute renal failure associated with gentamicin therapy has usually been reversible. Prospective studies are urgently needed to define clearly the factors associated with high risk for the development of gentamicin nephrotoxicity.

Several techniques are now available to permit the measurement of concentrations of gentamicin or other aminoglycosides in serum or other body fluids (31-39). Several investigators have recommended monitoring gentamicin therapy with blood levels, but specific indications for performing such assays are not clearly defined. In a prospective study of 100 patients treated with gentamicin, Dahlgren and Hewitt measured both peak and trough concentrations of gentamicin in serum (81). They made the interesting observation that rising values of creatinine concentration during therapy occurred in approximately one third of patients with trough levels above 2 µg/ml but did not occur in patients with trough levels less than 2 µg/ml. Goodman and collaborators have studied patients at Parkland Memorial Hospital and have confirmed a correlation between gentamicin trough levels above 2 µg/ml and rising serum creatinine concentrations during therapy with gentamicin (53). It is not yet established whether trough levels, peak levels, total dose of gentamicin, or some combination of these variables has primary importance for the development of nephrotoxicity in man. Therapy with gentamicin should be reserved for seriously ill patients with presumed sepsis or with infections by bacteria that are resistant to less toxic antibiotics. For this reason, most patients who have developed renal failure during therapy with gentamicin were ill patients receiving multiple drugs who also had other physiological derangements that could contribute to renal failure. Well designed prospective studies with large numbers of patients will be necessary to sort out these variables and to define the mechanisms associated with aminoglycoside nephrotoxicity in man.

MONITORING BLOOD LEVELS OF GENTAMICIN

At the present time, the following indications for measuring serum concentrations of gentamicin should be considered. Measurements of peak levels are useful to document that a "therapeutic" concentration of the antibiotic is achieved during therapy. Peak levels should probably be determined in infections with gentamicin susceptible bacteria that do not respond promptly to therapy and in infections with relatively resistant organisms when maximal therapeutic blood levels may be essential. In practice, however, lack of clinical response has been an uncommon reason for determining serum levels of aminoglycosides. Measurements of trough levels are most helpful in patients who are likely to accumulate the drug and who might be subject to an increased risk of the toxic side effects of gentamicin. Measurement of trough levels of gentamicin seems indicated in patients with deteriorating renal function, in patients receiving prolonged courses of therapy, and in patients with stable renal insufficiency treated by variable dosage regimens in order to maintain persistently high serum concentrations of the drug.

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