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SYSTEMATIC STUDY OF AMINOGLYCOSIDE GROUP ANTIBIOTICS DRUG RESISTANCE

Zahraa A.H. AL-Tameemi[1*], Noor D. Almanaseer[2], Nesreen Ahmed Nasser[3]

[1]Al - Iraqia University/ Department of Quality Assurance and Academic Performance.
[2]Medlabs consultancy group -Amman- Jordan.
[3]Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

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Corresponding Author: Zahraa A.H. AL-Tameemi
Al - Iraqia University/ Department of Quality Assurance and Academic Performance.

ABSTRACT

Aminoglycosides are powerful, broad-spectrum antibiotics that can cure infections that present a serious threat to life. Upon the seminal introduction of streptomycin in 1944, an array of pivotal medications such as kanamycin, gentamicin, and tobramycin subsequently emerged. This succession of compounds incontrovertibly affirmed the utility of this category of antibiotics in managing gram-negative bacterial infections. During the 1970s, the introduction of semi-synthetic aminoglycosides, including dibekacin, amikacin, and netilmicin, demonstrated the feasibility of developing therapeutics with diverse toxicological profiles and efficacy against strains displaying resistance mechanisms to earlier generation aminoglycosides. However, since that time, the rate of novel aminoglycoside development has significantly reduced. Contrarily, after a period of intensive scientific and clinical research, we now see these medications considerably differently from how people did when they were initially presented to the clinic. Antibiotic aminoglycoside resistance has significantly impacted clinical practice. The difficulty of resistance was one of the first faced by aminoglycosides, despite their potent bactericidal efficacy. The enzymatic alteration of the antibiotic is the most frequent form of clinically significant resistance against these treatments. Consequently, an enhanced comprehension of aminoglycoside-modifying enzymes and their interactions with antibiotics is essential to promote the development of superior inhibitors and innovative semi-synthetic aminoglycosides. These novel compounds should demonstrate increased potency and efficiency while remaining unaffected by modifying enzymes. In this review, we have aimed to cover the systematic study of aminoglycoside group antibiotics drug resistance with respect to different enzymes responsible for drug modification, and genetic traits involved in drug resistance. Our objective is to present and engage in discourse regarding these advancements, not with the intention to ascertain if new molecules or feasible methods for preventing bacterial resistance and drug-induced toxicity will ultimately be incorporated into clinical practice. Instead, we aim to inspire continuous research on aminoglycosides and to keep clinicians updated on the significant progress achieved in this field.

INTRODUCTION

Streptomycin, an antibiotic with broad-spectrum activity, effectively inhibits both Gram-positive and Gram-negative bacteria.[1] Streptomycin, an antibiotic, came to light in close proximity to the introduction of penicillin into medical practice. Selman Waksman, acclaimed for this discovery and recipient of the Nobel Prize, is widely acknowledged as the primary discoverer of streptomycin. However, Albert Schatz, one of Waksman's doctoral students, was legally recognized as a co-discoverer of streptomycin and obtained a share of the drug's royalties.[2] Aminoglycosides are antibiotics produced by actinomycetes that are either natural or semisynthetic. Aminoglycosides were among the pioneers of antibiotics utilized in routine clinical practice, with several variants gaining approval for human application. During the initial phases of antimicrobial chemotherapy, aminoglycosides were widely utilized as primary therapeutic agents. Nevertheless, in the 1980s, their prominence gradually diminished as they were succeeded by cephalosporins, carbapenems, and fluoroquinolones. Aminoglycosides exhibit synergistic effects with various other antibacterial classes. The renewed interest in broad-spectrum and rapidly bactericidal antibiotics is a result of a combination of factors. These factors encompass the rising prevalence of multi-drug resistant bacteria and the potential for augmenting the safety and efficacy of this category of antibiotics through refined dosing regimens.[3] Aminoglycosides have been for decades an important part of the therapy arsenal for serious diseases. Unfortunately, the surge and spread of resistance have restricted their effectiveness. In some cases, the degrees of resistance were so high that they became practically insignificant. In clinical settings, enzymatic modification represents the predominant mechanism of resistance to aminoglycosides. Aminoglycoside-altering enzymes, encompassing nucleotidyltransferases, phosphotransferases, and acetyltransferases, are catalysts in the modification of numerous OH or NH2 groups situated within the 2-deoxystreptamine nucleus or sugar constituents.[4] Aminoglycosides, a class of antibiotics, are not commonly employed as the primary treatment modality for H. pylori infections. Instead, a combination therapy consisting of distinct antibiotics including clarithromycin, amoxicillin, or metronidazole, in conjunction with a proton pump inhibitor, is typically prescribed as the preferred course of action.[5]

The vast array of aminoglycoside-modifying enzymes identified thus far, along with the genetic characteristics where the coding genes reside, is remarkable. Furthermore, it appears that there are practically no bacteria incapable of accommodating enzymatic resistance to aminoglycosides. Presently, in addition to the pursuit of novel aminoglycosides resistant to a wide array of modifying enzymes, two primary strategies are being employed to mitigate the impact of aminoglycoside-modifying enzymes. The effective progression of these methodologies would prolong the therapeutic efficacy of established antibiotics that have demonstrated effectiveness in combatting infections. These strategies involve the formulation of inhibitors that selectively target either the enzymatic activity or the expression of modifying enzymes.[4]

Mechanism of mode of Action and pharmacology

Streptomycin, a member of the aminoglycosides, possesses antibacterial properties and interferes with ribosomal peptide/protein synthesis. Aminoglycosides exert their inhibitory effects by binding to a specific region of the 16S rRNA within the smaller 30S component of the bacterial ribosome. This binding interferes with the functioning of the ribosome and impedes the process of protein synthesis by preventing peptide bond formation. It is noteworthy that aminoglycosides exhibit hydrophilic characteristics, rendering them unable to traverse the hydrophobic barrier presented by the bacterial cell membrane. For this to occur, a mechanism involved in the cell's respiratory cycle,

specifically an electron transport process, is necessary. This explains why aminoglycosides are only effective against aerobic bacteria.[6-8]

Aminoglycosides exhibit notable effectiveness against aerobic, gram-negative bacteria while also demonstrating a synergistic impact on specific gram-positive organisms. Among the aminoglycosides, gentamicin is the most prescribed, although amikacin can be highly efficacious against resistant strains. These antibiotics find applications in the management of severe gastrointestinal and urinary tract infections, as well as in the treatment of bacteremia and endocarditis. Additionally, aminoglycosides are employed for prophylactic purposes, particularly in the prevention of endocarditis. While resistance to these antibiotics remains relatively uncommon, its occurrence is increasing. To minimize the risk of toxicity, it is advisable to avoid prolonged usage, prevent volume depletion, and exercise caution when administering potentially nephrotoxic medications concurrently.[9]

Aminoglycosides display restricted absorption when administered via the gastrointestinal tract. Upon parenteral administration, these antibiotics primarily distribute within the extracellular fluid compartment. Consequently, the presence of pathological states or iatrogenic influences that disrupt fluid homeostasis may necessitate modifications in dosage regimens. Appropriate concentrations of the drug are often attained in various body locations, including bone, synovial fluid, and peritoneal fluid following parenteral administration of aminoglycosides. However, due to their polar nature, these antibiotics exhibit limited penetration through biological membranes, resulting in typically low intracellular concentrations, except in the proximal renal tubule where higher levels can be observed. Endotracheal administration tends to yield increased concentrations in the bronchial region compared to systemic administration, although the clinical implications of these variations remain inconsistent. Moreover, concentrations below the therapeutic level are frequently observed in the cerebrospinal fluid, vitreous fluid, prostate, and brain subsequent to the parenteral administration of aminoglycosides.[11]

Bacterial resistance to aminoglycoside antibiotics:

Resistance to aminoglycosides emerges through multiple mechanisms that may coexist within a single cell. These mechanisms encompass the alteration of the target through mutation of the 16S rRNA.[12,13] Methylation of ribosomal proteins and the 16S rRNA represents an additional mechanism contributing to resistance against aminoglycosides.[14] The process is widely observed in the majority of aminoglycoside-producing organisms and clinical strains. Moreover, resistance can arise due to diminished permeability, achieved through alterations in the outer membrane's permeability or by reducing inner membrane transport[15,16], as well as through the presence of active efflux pumps[17] that assist the cell in expelling the drug molecule. Alongside these mechanisms, a significant pathway towards drug resistance involves the enzymatic deactivation of the antibiotic molecule.[4] In a majority of clinical isolates, the primary mode of resistance is the synthesis of aminoglycoside-modifying enzymes. Prominent examples of these modifying enzymes include Acetyl CoA-dependent N-acetyltransferases, ATP-dependent O-adenyl transferases, and ATP-dependent O-phosphoryl transferases. Alongside these, bifunctional enzymes also significantly contribute to aminoglycoside resistance. The modification of aminoglycosides impairs their ability to bind ribosomes in a manner that inhibits their biological function, thereby instigating drug resistance within the cells. This review emphasizes the roles various enzymes play in aminoglycoside resistance.[18]

The APH enzyme family encompasses numerous members, which can be differentiated based on three criteria: 1) substrate specificity or resistance phenotype, 2) regiospecificity of phosphoryl transfer, and 3) protein/gene sequence.[18]

APH (3') enzymes: (Aminoglycoside kinases)

Considering the frequent administration of aminoglycosides in clinical therapy, the majority of determinants associated with aminoglycoside resistance have been identified in nosocomial infections. These pathogenic strains harbor APH (3') enzymes. Over the preceding three decades, APH (3') enzymes have been discerned in both Gram-negative and Gram-positive pathogens. The rise of these enzymes has markedly curtailed the clinical applicability of aminoglycosides, encompassing kanamycin and neomycin. The resistance to other aminoglycosides, such as amikacin, isepamicin, butirosin, and lividomycin, constitutes the foundation for categorization into seven discrete classes (I-VII).[19-21] seven isomers of APH (3') enzymes are listed in table 1.

Table No.1: Isozymes of APH (3') enzymes along with substrate profile and bacterial example.

Isozymes of APH (3') enzymes	Subtype of Isozyme	Substrate	Organism	Reference
I	a-c	Kanamycin, Neomycin, Gentamycin B	*Escherichia coli* *Klebsiella pneumonia* *Acinetobacter baumannii*	[22] [30]
II	a	Kanamycin, Neomycin, Paromomycin, Gentamycin B	*E. coli*	[23]
III	a	Kanamycin, Neomycin	*Enterococcus* species *Staphylococcus* species	[24]
IV	a	Kanamycin, Neomycin, Paromomycin, Butirosin	*Bacillus cirulans*	[25]
V	a b c	Kanamycin, Neomycin, Paromomycin, Gentamycin B, Ribostamycin	*Streptomyces fradiae* *Streptomyces ribosidificus* *Streptomyces fradiae*	[26] [28]
VI	a	Kanamycin, Neomycin, Paromomycin, Butirosin, Gentamycin B	*Acinetobacter baumannii* BM2580	[27]
VII	a	Kanamycin, Neomycin, Paromomycin, Gentamycin B	*Campylobacter jejuni* and *Campylobacter coli*	[21]

Aminoglycoside N-acetyltransferases: (AACs)

Aminoglycoside acetyltransferases represent significant indicators of resistance to aminoglycoside antibiotics across most bacterial genera. These aminoglycoside-modifying enzymes participate in the covalent modification of specific amino or hydroxyl groups present in the aminoglycoside group antibiotic. This enzymatic process induces chemical modifications in the drug molecule, resulting in diminished binding affinity to ribosomes and the failure of accelerated drug uptake mediated by EDP-II. This often culminates in a significant degree of resistance. The category of aminoglycoside-modifying enzymes encompasses N-acetyltransferases (AAC), which primarily employ acetyl-coenzyme A as a donor and exert an influence on amino functionalities. Additionally, O-nucleotidyl transferases (ANT) and O-phosphotransferases (APH) are included in this group. Both ANT and APH utilize ATP as a donor and have an

impact on hydroxyl functionalities.[29] In standard aminoglycosides such as kanamycin and gentamicin derivatives, AAC affects positions 3, 2', and 6', whereas ANT influences positions 4' and 2'', and APH targets positions 3' and 2''.[30] The N-acetyltransferase (AAC)-modifying enzymes are summarized in Table 2.

Table No 2: N-acetyltransferase (AAC)-modifying enzymes.

Enzyme	Subtype of enzyme	Substrate	Organism	Reference
AAC (6′)	I (a–d,e,f–z) II	T, A, N, D, S, K, I T, G, N, D, S, K	*S. enterica,* A. *haemolyticus, E. coli S. warneri,*	**[31-34]**
AAC (3)	I (a-b) II(a-c) III (a-c) IV V	G, S, F T, G, N, D, S T, G, D, S, K, N, P, L T, S, N, D, S, A G	*S. marcesans* *E. coli* *K pneumoniae* *C jejuni* *Actinomycetes*	**[35-39]**
AAC (2′)	I(a-c)	PM,T, S, N, D, Ne	*P stuartii*	**[40]**
AAC (1)	Ia	P, L, R, AP	*E. coli*	**[41]**

The following abbreviations are used: A for amikacin, AP for apramycin, D for dibekacin, F for fortimicin, H for hygromycin, I for isepamicin, G for gentamicin, K for kanamycin, L for lividomycin, N for netilmicin, Ne for neomycin, P for paromomycin, R for ribostamycin, S for sisomicin, T for tobramycin, and PM for plazomicin.

Aminoglycoside O-phosphotransferases

Aminoglycoside antibiotics are a significant class of clinically valuable medications that are under threat from the evolution of resistant organisms. In clinical settings, aminoglycoside resistance predominantly arises due to the presence of modifying enzymes that catalyze N-acetylation, O-adenylation, or O-phosphorylation of the antibiotics. The latter group of enzymes is referred to as aminoglycoside phosphotransferases or kinases. Resistance to aminoglycoside antibiotics typically arises from the production of modifying enzymes that covalently modify the drugs through O-phosphorylation, O-adenylation, or N-acetylation. Aminoglycoside phosphotransferases (APHs) catalyze the ATP-dependent phosphorylation of these antibiotics. Notably, APH(3')-IIIa and AAC(6')-APH(2''), which are synthesized in gram-positive cocci, have been observed to phosphorylate aminoglycosides on their 3' and 2'' hydroxyl groups, respectively.[41]

Although these enzymes exhibit limited overall sequence homology, specific signature residues and sequences are shared among them. The classification of aminoglycoside phosphotransferases encompasses seven distinct classes: APH(3'), APH(2''), APH(3'off'), APH(6), APH(9), APH(4), and APH(7''). Each class comprises multiple isozymes.[30,42]

O-nucleotidyltransferase (ANT)-modifying enzymes

Nucleotidyltransferases (ANTs) play a crucial role within this category. These enzymes facilitate the transfer of an adenylyl group from the MgATP complex to multiple positions on the antibiotic molecule. ANTs comprise the smallest family among aminoglycoside-modifying enzymes (AMEs), with four crystallized structures identified: ANT(2''), ANT(3''), ANT(4'), and ANT(6').[43-46]

Bifunctional Enzyme

The enzyme being examined exhibits a bifunctional nature, characterized by the simultaneous presence of N-terminal acetyltransferase and C-terminal phosphotransferase domains. It is postulated that this configuration arose through a gene fusion event involving an AAC (acetyltransferase) and an APH (phosphotransferase). The 6′-N-aminoglycoside acetyltransferase-2"-O-aminoglycoside phosphotransferase [AAC(6′)-APH (2")] represents a bifunctional aminoglycoside-modifying enzyme. had previously been found in isolates of *Enterococcus*[47], *Staphylococcus*[48], and *Streptococcus agalactiae*.[49] Previous studies state that this bifunctional enzyme is found in *Lactobacillus* and *Pediococcus* isolates of animal origin.[50]

Strategies / Methods for overcoming the effects of aminoglycoside modifying enzymes

Considerable endeavors have been undertaken subsequent to the discovery of these modifying enzymes in order to mitigate their deleterious alterations of Aminoglycosides (AGs). Several avenues have been explored, including the development of novel AGs that remain unaffected by AMEs, the generation of AME inhibitors for co-administration with AGs, and the regulation of AME expression. As contemporary drug-discovery initiatives address the mounting challenge of antibiotic resistance, the utilization of modern high-throughput technologies, pharmacological combinations, and repurposing strategies becomes imperative.[51]

By employing a distinct regioselective catalytic oxidation methodology, we achieved enhanced efficacy of aminoglycoside antibiotics against resistant bacteria, thereby minimizing the synthesis effort necessary for acquiring novel derivatives. The process of epimerization occurring at the 3'-position of aminoglycosides holds significant promise as a fundamental basis for the development of more potent antibiotic alternatives targeting resistant bacteria. Undeniably, this groundbreaking late-stage modification of aminoglycoside antibiotics represents a revolutionary synthetic technique aimed at combating multidrug-resistant microorganisms in both academic and industrial spheres.[52]

Several methods and strategies pertaining to this approach are discussed below:

APH inhibitors

A developed ankyrin repeat (AR) protein inhibited APH(3′)-IIIa in vitro and in vivo, deviating from the typical small-molecule chemotherapeutic method.[53] CKI-7, an ATP-competitive inhibitor of casein kinase 1, has recently been investigated as an inhibitor of APH(3′)-IIIa and APH(9)-Ia.[54]

Cationic peptide inhibitors of AMEs

Considerable scientific investigation has been undertaken on numerous cationic peptides that exhibit binding properties akin to aminoglycosides, specifically targeting the negatively charged active sites of aminoglycoside-modifying enzymes (AMEs). These peptides demonstrate a notable affinity for AAC(6')-Ie, AAC(6')-Ii, APH(3')-IIIa, and AAC(6')-Ie/APH(2")-Ia. Remarkably, these peptides represent pioneering examples of broad-spectrum inhibition against AMEs.[55]

Drug combinations & repurposing

Combination therapy involving multiple antibiotics has been employed to enhance the antibacterial spectrum and achieve synergistic effects by targeting multiple bacterial elements. Such drug combinations hold potential in combating antibiotic resistance arising from the expression of aminoglycoside-modifying enzymes (AMEs). Additionally, therapeutic repurposing presents advantages as compounds already undergoing clinical trials possess

well-characterized pharmacology and toxicity profiles. The repurposing of existing compounds also proves cost-effective, reducing the time and financial resources required during the initial stages of drug research. Notably, repurposing kinase inhibitor chemical libraries developed for cancer and other conditions involving protein kinases can unveil compounds with distinct capabilities to inhibit antibiotic resistance kinases.[56]

Azithromycin, classified as a macrolide antibiotic, is widely applied in clinical settings due to its extended half-life and remarkable antibacterial effectiveness. In addressing P. aeruginosa infections, a combination approach involving aminoglycoside antibiotics (AGAs) and azithromycin is commonly adopted, which facilitates a reduction in the individual dosage of each drug. Research has suggested that gentamicin can obstruct translation and amplify the lethal influence of azithromycin on both planktonic and biofilm cells. Moreover, earlier studies have demonstrated that the combination of gentamicin or gemifloxacin with azithromycin produces significant therapeutic outcomes in the treatment of genitourinary gonorrhoea.[58]

Combination with other natural plant extracts that have antibacterial properties

Plant secondary metabolites, diverse solvent extracts, and essential oils possess antibacterial properties, exhibit synergistic antibacterial effects, and demonstrate various pharmacological activities. These natural compounds hold significant importance as sources of medicinal substances in both the present and future.

Nadaf et al. investigated the antimicrobial, antibiofilm, and antioxidant properties of H. littoralis against a range of pathogenic microorganisms, employing a combination of experimental and computational biology methodologies.[59] Subsequent to comprehensive research, it has been elucidated that the amalgamation of gentamicin and essential oils, specifically those extracted from Pelargonium graveolens and Aniba rosaeodora, demonstrates synergistic properties. This synergism significantly amplifies the antibacterial efficacy against a wide spectrum of both gram-negative and gram-positive bacterial pathogens. The combination has the potential to lower the lowest effective dose of gentamicin. Gentamicin coupled with *Acinetobacter baumannii* essential oil demonstrated a substantial synergistic antibacterial activity. Gas chromatographic analysis was used to investigate the chemical makeup of essential oils, which revealed that essential oils isolated from *Pelargonium graveolens* and *Aniba rosaeodora* included a high percentage of terpene alcohols.[60]

Combination with Antimicrobial proteins / Peptides

The rising incidence of antibiotic-resistant bacterial infections presents a considerable global challenge to both human and animal health. In response to this issue, antimicrobial peptides (AMPs) have surfaced as potential therapeutic agents, attributed to their rapid and targeted antimicrobial action against pathogens. Thus far, an extensive repertoire of over 800 antimicrobial peptides (AMPs) has been identified, originating from diverse sources such as humans, animals, plants, insects, and bacteri. [61]

In the scholarly exploration conducted by Waghmare et al., the researchers examined a unique 16 kDa antimicrobial protein synthesized by the *B. licheniformis* strain JS. The protein showcased resilience under trypsin exposure, retaining its stability even at elevated temperatures. Furthermore, the peptide, once purified, manifested the capacity to augment the effectiveness of antibiotics such as Kanamycin, Neomycin, and Streptomycin. Consequently, the isolated antimicrobial peptide holds promise for potential therapeutic advancements in the treatment of infectious diseases.[62]

CONCLUSION

In the future, it is anticipated that scientists will veer away from conventional medicinal chemistry practices and gravitate towards contemporary methodologies, including drug repurposing. This approach capitalizes on the utilization of existing medications that have undergone extensive evaluation pertaining to dosage, metabolism, and toxicology. Repurposing established compounds as inhibitors of aminoglycoside-modifying enzymes (AMEs) necessitates significantly fewer resources and time compared to the development of novel compounds from inception. However, it is crucial to acknowledge that resistance to these inhibitors will inevitably emerge, and it remains uncertain whether we will ever completely overcome antibiotic resistance.

Predominantly, the resistance to aminoglycoside antibiotics, as witnessed in clinical trials, can be attributed to the process of enzymatic modification resulting in inactivation. The advent and distribution of aminoglycoside-altering enzymes have markedly undermined the effectiveness of these antibiotics. In specific instances, this phenomenon has entirely nullified their therapeutic potential. Nucleotidyl transferases, phosphotransferases, and acetyltransferases represent the three categories of aminoglycoside-modifying enzymes that facilitate modifications at distinct OH or NH2 groups within the antibiotic molecule. The remarkable adaptability of these enzymes in utilizing new antibiotics as substrates and their efficient propagation among bacteria can be attributed to the abundance of genes encoding these enzymes, their propensity to evolve, and their prevalence within various mobile genetic elements. Consequently, nearly all medically relevant microorganisms are capable of supporting enzymatic resistance.

In order to preserve the effectiveness of aminoglycosides in combating bacterial infectious diseases, it is imperative to explore strategies that render modified aminoglycosides resistant to a wide range of modifying enzymes. Additionally, the development of inhibitors targeting aminoglycoside-modifying enzymes and inhibitors that suppress their expression through the use of antisense oligonucleotide analogs are essential. These avenues of research represent promising areas in the ongoing battle against drug resistance.

Aminoglycoside antibiotics (AGAs) have demonstrated synergistic antibacterial effects when combined with other antibacterial agents, anti-inflammatory agents, analgesics, natural plant extracts, and various compounds. This synergism enables the reduction of individual treatment dosages while maintaining favorable antibacterial activity and minimizing adverse effects. Given the observed synergistic antibacterial effects of several pharmacological combinations in laboratory settings, the utilization of such combinations holds promise in addressing the escalating challenge of bacterial antibiotic resistance. Consequently, AGAs present a promising outlook for the future in the battle against bacterial infections.

Combining antibiotics with additional medications is a key technique for combating bacterial resistance. We anticipate that the prediction of old AGAs therapeutic targets and the examination of critical bacterial growth mechanisms will set the groundwork for the discovery of novel AGAs derivatives and drugs coupled with AGAs.

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