Combination of Drugs: An Effective Approach for Enhancing the Efficacy of Antibiotics to Combat Drug Resistance

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Abstract

Currently available antibiotics have been effective in treating infectious diseases; however, the development of resistance to these drugs has led to the emergence of new and the re-emergence of old, infectious diseases. Therefore, newer antibiotic approaches with mechanistic differences are needed to combat antimicrobial resistance. Combining antibiotics is an encouraging strategy for increasing treatment efficacy and for controlling resistance evolution. This approach may include the combination of one antibiotic with another antibiotic and the development of adjuvants that either directly target resistance mechanisms, like inhibition of β-lactamase enzymes, or indirectly target resistance by interrupting the bacterial signaling pathways, such as two-component systems. Other natural products, like essential oils, plant extracts, and nanoparticles, can also be combined synergistically with antibiotics. The aim of this chapter is to highlight the strategy of treating infections with arrays of drugs rather than discrete drugs. We have addressed here three categories of approaches being used in combination therapy: the inhibition of targets in different pathways, the inhibition of distinct nodes in the same pathway, and the inhibition of the same target in different ways. Here, we have described the most recent developments toward combination therapies for the treatment of infectious diseases caused by multidrug-resistant bacteria.

Keywords

Antibiotic · Combination therapy · Drug resistance · Synergy

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1 Introduction

In recent years, bacterial infections have become a global health challenge due to the emergence of multidrug resistance in pathogenic strains. The indiscriminate use of antibiotics has led to an alarming increase in resistance among microorganisms, and also opened the door for re-emergence of old infectious diseases (Fair and Tor 2014). Majority of infectious diseases are caused by biofilm-forming strains that are several 1000-fold tolerant to antibiotics (Hoiby et al. 2010). As a result, the existing antibacterial drugs are becoming less effective and it has forced the investigators to develop newer varieties (Khameneh et al. 2016; Zaman et al. 2017). Various approaches have been developed and employed by researchers to eliminate antibiotic resistance, though understanding the underlying mechanisms. These include the removal of antibiotics from the bacterial cell through efflux pumps, enzymatic modification or degradation of the antibiotics, and modification of the antibiotic targets (Kalan and Wright 2011). Thus, overcoming resistance requires the use of various approaches, like inhibiting the enzymes that degrade or modify the antibiotic to a non-active form, hindering antibiotic efflux, enhancing antibiotic entry into the cell, and changing the physiology of the cells to render them more sensitive to antibiotic killing (Kalan and Wright 2011; Zaman et al. 2017).

However, the higher prevalence of resistant strains is making the solution as extremely difficult and necessitates newer approaches. Therefore, novel antimicrobial discovery and drug combinations are being explored in order to combat the multidrug-resistant (MDR) phenotype. The toxic effects of antibiotics are lowered in drug combinations and the potency of antimicrobial compounds also get increased against resistant strains (Khameneh et al. 2016). Application of synergistic activity between antibiotics and non-antibiotics is also exploited. Such efforts include the combination of an antibiotic with a non-antibiotic adjuvant compound to directly target resistance mechanisms or by interfering with the bacterial signaling pathways (Worthington and Melander 2013a). One such strategy is the coupling of β-lactam antibiotics with β-lactamase inhibitors (Worthington and Melander 2013b). Several plant extracts, essential oils, phytocompounds, and nanoparticles have also exhibited synergistic interactions with various classes of antibiotics against microorganisms, including drug-resistant strains (Hemaiswarya et al. 2008; Allahverdiyev et al. 2011; Khan and Ahmad 2011; Khan et al. 2012). In clinical settings, two or more antimicrobial drugs are often combined to treat MDR infections (Worthington and Melander 2013a), including those caused by bacteria and fungi. For example, a combination of four drugs is being used for the treatment of *Mycobacterium tuberculosis* infections (Mitchison and Davies 2012). The emergence of many new MDR pathogens has indicated that monotherapy is no longer satisfactory to treat these infections, and instead, combination therapy should be utilized (Tamma et al. 2012).

The success of combination therapy against microbial infections depends on its ability to combat the infection, avoid resistance, minimize host toxicity, and leave the natural microflora intact. To further boost the efficacy of combination therapy while minimizing drug concentrations, local drug delivery is also necessary. Overall, the key features of a combination treatment include (i) enhancement of antibiotic

activity by synergistic effect, (ii) prevention of resistance emergence, (iii) possession of anti-biofilm activity, (iv) improvement of antibiotic penetration to cell and tissues, and (v) inhibition of virulence factors, such as toxin or enzyme production in pathogens (Hagihara et al. 2012). When drugs are combined, their individual effects on cells may be augmented or weakened, resulting in either synergistic, antagonistic, or no interactions (Khameneh et al. 2016). High-throughput studies have resulted in the identification of drugs based on their interactions with established antibiotics, thereby enabling the prediction of drug interactions (Bollenbach 2015). It has also been found that the mechanism of action of drugs in combination therapy can significantly differ from that of the single drugs. Therefore, the selection of proper combinations is critical, and necessitates an understanding of the potential interactions between the antimicrobial compounds (Yeh et al. 2009; Hamoud et al. 2014). This approach is not only restricted to the use of biologically active compounds; the use of smart controlled delivery strategies could also be considered. Overall, the conceptual and technical establishment for the rational design of effective drug combinations is quickly developing (Bollenbach 2015; Khameneh et al. 2016).

In this chapter, we aimed to summarize recent approaches used in combining antibiotics based on their mechanisms of action. We have briefly considered examples of combination therapies that pair antibiotics with other naturally occurring antibacterial agents, such as plant products and nanoparticles, to formulate new prospects for future studies. We have also addressed the opportunities and challenges in making influential use of drug combinations.

2 Combination Approaches of Antibiotics

The combination approach can be divided into three categories based on the drug target: (1) combining antibiotics that target different pathways (e.g., treatment of *Mycobacterium tuberculosis* infections with a combination of isoniazid, rifampicin, ethambutol, and pyrazinamide), (2) combining antibiotics that target different parts of the same pathway (e.g., sulfamethoxazole and trimethoprim), and (3) combining antibiotics that attack the same target by multiple mechanisms (e.g., streptogramins and virginamycin) (Fischbach 2011; Worthington and Melander 2013b; Hamoud et al. 2014).

2.1 Combination Approaches that Target Different Pathways

Utilizing drugs that target multiple pathways is one of the most successful approaches to combat antibiotic resistance. A prime example of this is DOTS chemotherapy, used in treating *Mycobacterium tuberculosis*, which employs a combination of four drugs: isoniazid, an inhibitor of the enoylreductase subunit of fatty acid synthase; rifampicin, an RNA polymerase inhibitor; ethambutol, an inhibitor of arabinosyl transferases involved in cell wall biosynthesis; and pyrazinamide, whose mechanism of action is not well understood (Fischbach 2011). Thus with this therapy, at least three pathways are inhibited at once, meaning that even if a strain of *M.*

tuberculosis manages to protect one of the pathways, other crucial pathways will be obstructed.

The incredible improvements in survival for HIV-infected patients have been made possible because of combination approaches (Richman 2001). Treatment for such patients includes the combination of two nucleoside reverse transcriptase inhibitors, emtricitabine and tenofivir. One of them adds raltegravir, an integrase inhibitor; the second adds the non-nucleoside reverse transcriptase inhibitor, efavirenz; and a third adds a mixture of ritonavir and darunavir, both protease inhibitors (Lennox et al. 2009). With this therapy, HIV is not completely eradicated, but instead becomes a manageable chronic illness.

It should be noted that many effective combinations of drugs targeting different pathways are not only limited to antibiotics, but also include pairings with non-antibiotic adjuvants as well (Smith et al. 2013; Hamoud et al. 2014).

2.1.1 Combinations with Non-antibiotic Adjuvants

One of the prominent strategies for the treatment of MDR bacterial infections is to combine an antibiotic with a compound that is non-antimicrobial alone, but that assists in the enhancement of drug activity. For example, the compound may act by blocking the mechanism of resistance to the antibiotic. Such an approach is particularly attractive as resistance development is minimized (Worthington and Melander 2013a). Three common adjuvant types with clinical achievements are antiseptics, inhibitors, and biological (bacteriophage) or natural (phyto-compounds, nanoparticles) compounds. Additionally, several other known pharmaceutical compounds, such as antihistamines, antihypertensives, antispasmodics, anti-inflammatory drugs, and tranquilizers, are now being discovered as antibiotic adjuvants (Ejim et al. 2011; Smith et al. 2013).

2.1.1.1 Antiseptic Adjuvants

Antiseptics or biocides are the most commonly used adjuvants. For example, chlorhexidine, a bisbiguanide, is used to either kill or inhibit the growth of pathogens and is reported to show multiple sites of targets (Muller and Kramer 2008). Their ability to permeate and disrupt the membrane or inactivate ATPase has made them a very effective choice in combination (McDonnell and Russell 1999). For example, the coating of catheters with an antibiotic/antiseptic combination has shown significant efficacy against a variety of pathogens (Wu and Grainger 2006). It should be noted that despite their success, the development of resistance has been reported for in vitro combinations of chlorhexidine or silver sulfadiazine (antiseptics) and minocycline or rifampicin (antibiotics) (Lewis 2005).

2.1.1.2 Inhibitor Adjuvants

Inhibitor adjuvants augment the bactericidal treatments by targeting the applicable mechanisms of resistance (Roemer et al. 2013; Drawz et al. 2014). Multiple adjuvants are used to counter enzymatic degradation of antibiotics. Augmentin, for example, is a combination of β-lactam antibiotic (amoxicillin) and β-lactamase inhibitor (clavulanic acid). In this combination, the in vivo β-lactamase production

in bacteria is inhibited by clavulanic acid, facilitating inhibition of cell wall biosynthesis by amoxicillin. In this case, the addition of the adjuvant has allowed for the continued use of amoxicillin to treat infections caused by pathogens that may develop resistance to β-lactam antibiotics (Ball 2007).

Compounds inhibiting efflux pumps have also been exploited in several antibiotic combinations in order to reduce the prevalence of a resistant phenotype. For example, reserpine, a well-known mammalian MDR pump inhibitor, when used in combination with ciprofloxacin, has resulted in suppression of resistance in *Staphylococcus aureus* and *Streptococcus pneumoniae* strains (Lomovskaya et al. 2001). Similarly, celecoxib, a nonsteroidal anti-inflammatory drug (NSAID) that inhibits the MDR1 efflux pump, when combined with antibiotics like ampicillin, kanamycin, chloramphenicol, and ciprofloxacin, results in improved sensitivity of *S. aureus* to these antibiotics (Kalle and Rizvi 2011). The use of inhibitor as adjuvant is advantageous compared to antiseptic adjuvants for two reasons: (i) the antiseptic adjuvants are antimicrobial in nature, whereas inhibitor adjuvants being non-antimicrobial, can avoid the evolution of resistance against them (Worthington and Melander 2013a), and (ii) the complementary act of an inhibitor adjuvant toward the action of its antimicrobial counterpart does not promote the development of new mechanisms of resistance (Hamoud et al. 2014).

2.1.1.3 Biological and Natural Adjuvants

Use of natural and biological adjuvants with antibiotics is a very encouraging approach and has shown its extensive application. Many investigators have demonstrated that the combination of an antibiotic with a bacteriophage adjuvant can lead to a more effective therapy than either agent alone (Petty et al. 2007; Ghannad and Mohammadi 2012). In Georgia, for example, a company named PhagoBioDerm is using a combination of a lytic phage and ciprofloxacin in a biodegradable polymer matrix (Markoishvili et al. 2002). Biological adjuvants in drug combinations naturally enhance antimicrobial efficacy as they target multiple sites of action in pathogens that will not allow to develop resistance easily. In a study by Barekzi et al. (2002), IgG antibodies were used as adjuvants to promote a host immune response, while also suppressing any additional development of resistance, as the bacteria do not experience any direct selective pressure against them.

There are many reports on the use of natural compounds as synergistic adjuvants, especially plant-derived essential oils, extracts, and phytocompounds, such as eugenol, cinnamaldehyde, geraniol, and thymol, in combination with antifungals, like azoles, and antibiotics, like vancomycin (Khan and Ahmad 2011; Hamoud et al. 2014). The use of biosurfactants, such as sophorolipid, has also shown synergistic interaction with many antibiotics. Importantly, the use of phytocompounds and biosurfactants is considered safe, and has been approved by the FDA for use in pharmaceuticals and food (Navare and Prabhune 2013).

Due to their possession of antimicrobial activities, metallic nanoparticles, such as silver, zinc, and gold, represent an effective class of agents for overcoming bacterial resistance. Unfortunately, metallic nanoparticles are considered toxic at pharmacological doses, which cause restrictions in their use. However, studies have

revealed that the combination of various nanoparticles with antibiotics lessens the toxicity of both agents toward human cells by reducing the dosage required while at the same time increasing their bactericidal efficacy (Allahverdiyev et al. 2011).

2.1.1.4 Screening of Previously Approved Drugs as Adjuvants

Discovery of newer antibiotic adjuvants could be achieved by screening the drugs approved previously for other medications. It is an interesting approach, as these drugs are well known for their toxicology and pharmacology profiles. Systematic screenings of approved non-antibiotic compounds for antimicrobial potential have uncovered various compounds from many drug classes, including antihistamines, antihypertensives, antispasmodics, anti-inflammatory drugs, and tranquilizers. These drugs display activity against a broad spectrum of Gram-positive and Gram-negative bacteria (Worthington and Melander 2013a).

2.2 Combination Approaches that Target the Same Pathway

Combining antimicrobial compounds with different targets in the same pathway is a more specified strategy than targeting different pathways. If the proper pathway is chosen, this could result in a very effective strategy. There are two points of consideration for selecting this approach: (i) The targeted pathway must be essential to the survival of the pathogen, such as a requirement for folate to synthesize dTMP, or a precursor for DNA synthesis. (ii) The pathway chosen should not be non-meaningful, as it may lead to resistance (Fischbach 2011). Targeting two steps in the same pathway offers a more perilous strategy than attacking two or more separate pathways, as it may lead to an increase in antibiotic resistance. Despite this, in most cases it is still more effective than monotherapies, which are comparatively less potent at inhibiting a single pathway (Payne et al. 2007; Read and Huijben 2009; Pena-Miller et al. 2013).

2.3 Combination Approaches that Act on the Same Target

If the drugs in combination have the same target, then the approach becomes very less diversified, such as is the case for a combination of antibiotics that act on the bacterial ribosome. However, in a study by Fischbach (2011), synercid, which is a semisynthetic combination of two drugs, it was shown that both of the components bind to adjacent regions in the 50S ribosomal subunits, resulting in 10–100-fold more efficacy than either drug alone. As the target was of a critical and conserved nature, the authors were able to achieve enhanced antimicrobial efficacy. Therefore, the selection of an appropriate, vital target is a critical prerequisite for this strategy and may help counter the inherent risk, in terms of resistance generation, of attacking a single target.

3 Combination Approaches in Combating Polymicrobial Infections

In addition to being useful in treating single species, combination approaches are quite successful and crucial for the treatment of polymicrobial infections (Ahmed et al. 2013). The majority of infectious diseases are associated with medical devices, which often harbor more than one pathogen, resulting in more antimicrobial tolerant, mixed species infections (Moran et al. 2007; Marculescu and Cantey 2008; Aggarwal et al. 2013). Therefore, the ability of combinational drugs to target multiple pathogens, including multispecies biofilm communities, is emerging as a valuable tool in fighting infections. For example, combinations of three antibiotics, such as a β-lactam, a glycopeptide, and an aminoglycoside, have demonstrated highly improved activity against multidrug-resistant *S. aureus* (MRSA) strains when compared to two antibiotic combinations (Wood et al. 2012). Furthermore, combinations of antibiotics showing a varied range of mechanisms of action are very effective in suppressing the development of resistance. Due to diverse modes of action, antibiotics in combinations, such as protein synthesis inhibitors (macrolides, aminoglycosides, tetracyclines, lincosamides, and chloramphenicol), DNA synthesis inhibitors (fluoroquinolones and quinolones), folic acid synthesis inhibitors (sulfonamides and diaminopyrimidines), and cell wall synthesis inhibitors (polypeptide antibiotics, preservatives, and analgesics), is very effective in combating polymicrobial infections (Wood et al. 2012; Ahmed et al. 2013).

4 Consequences of Drug Combinations

Initially, it appears that the use of drug combinations would address multiple resistance development, but in fact, it may actually promote the evolution of drug resistance (Hegreness et al. 2008; Yeh et al. 2009; Pena-Miller et al. 2013). As indicated through in vitro studies, resistance to aminoglycosides can lead to increased sensitivity to other antimicrobials (Lazar et al. 2013). The use of many drugs in combination carries with it the danger of evolving "super-pathogens" due to the co-evolution of multidrug-resistant variants and desensitization to other antibiotics of the same class (Ahmed et al. 2013). In order to overcome this challenge, combination therapies should be designed to reduce the emergence of multidrug-resistant bacteria while increasing the efficacy of the treatment.

The drug combinations must also be crafted while considering the effect of drug–drug interactions, drug metabolism, compound ratios, the doses required for drug adsorption, and also the rate of excretion for each drug in the treatment (Kalan and Wright 2011; Goldberg et al. 2012; Roemer et al. 2013). The administration of two or more drugs in synergy may alter the pharmacokinetics of drug delivery, and could be toxic to the host cells and valuable natural microflora. Novel synergistic drug therapies may cope with some of these common challenges via multiple methods, including the use of a hybrid single antibiotic with two distinct functions, such as lantibiotic and nisin (Walsh 2000; Hasper et al. 2006). Remarkably, some

combinations of antimicrobial agents can actually alleviate the toxicity of single agents alone, e.g., when nanoparticles are combined with antibiotics. This happens due to a decrease in amount required for activity in combination when compared to individual use (Allahverdiyev et al. 2011). Also, the mechanisms of action of the individual drugs must be considered to avoid any antagonistic interactions, such as with the combination of certain DNA synthesis inhibitors with protein synthesis inhibitors (Bollenbach et al. 2009). Finally, combinations of drugs should be done only after understanding the mechanism of action in combination to obtain innovative combinations, as with the combination of an antibiotic with non-antibiotic adjuvant or inhibitor of quorum sensing.

5 Use of Antibiotics in Combination with Plant Products and Nanoparticles

Another promising approach in managing antibiotic resistance is the use of natural antimicrobial substances, such as plant extracts, essential oils, or their active compounds. These products possess high antimicrobial activity and have also demonstrated antioxidant, anti-inflammatory, immune modulatory, regenerative, and other beneficial properties (Chao et al. 2008; Sadlon and Lamson 2010; Miguel 2010; Silva and Fernandes Jr 2010). The drug synergism between antimicrobial agents and bioactive plant products is a new concept, and in order to control a particular disease, in vitro experimentation should be carried out with various antibiotics in combination with plant products. This way, a proper combination may be administered to the patient for early and safe recovery from a specific ailment. In general, plant products are safer and cheaper, and their use can reduce the administration doses of antibiotics. A few examples of such combinations are summarized in Table 1.

Further, it has been found that when certain nanoparticles are combined with antibiotics, the bactericidal activity of drug is restored against resistant strains (Li et al. 2005; Fayaz et al. 2009). Also, when antibiotics are tagged with nanoparticles that can also act as efficient drug delivery agents (Chaloupka et al. 2010), the concentration of antibiotics at the site of drug-bacterial interaction is increased. This facilitates the binding of antibiotics to bacteria, resulting in increased efficacy, and the overcoming of bacterial resistance to antibiotics, such as vancomycin (Gu et al. 2003; Allahverdiyev et al. 2011). Because they possess substantial antibacterial properties, the nanoparticles of copper, gold, iron, silver, titanium, and zinc are being investigated in combination with other antibiotics. Some of the key studies are summarized in Table 1.

5.1 Promising Combinations: Essential Oils and Nanoparticles

Some investigators have also studied the interactions of essential oil components with polymeric nanoparticles for delivering oil-active compounds into the site of microbe-host interaction. Chen et al. (2009) prepared nanoparticles by grafting two

Table 1 Examples of synergistic combinations of various antibiotics with plant products or nanoparticles

Plant products	Antibiotics	Strains	References
Methanol extract of *Euphorbia hirta* leaves	Erythromycin	*S. aureus*	Adikwu et al. (2010)
Ethanol extract of *Mangifera indica* L. peel	Tetracycline and erythromycin	*S. aureus*	Souto de Oliveira et al. (2011)
Spices and herbs like *Coriandrum sativum*, *Cumium cyminum*, *Mentha piperita*, *Micromeria fruticosa* L., and *Rosmarinus officinalis*	Cephalothin, ceftriaxone, gentamicin, and nystatin	Gram-positive and gram-negative bacteria	Toroglu (2011)
Ethanolic extracts of *Rhus coriaria* (seed)	Oxytetracycline HCl, penicillin G, cephalexin, sulfadimethoxine, and enrofloxacin	Multidrug-resistant *Pseudomonas aeruginosa*	Adwan et al. (2010)
Ethanol extracts from the leaf and stem of *Salvadora persica*	Tetracycline	*S. aureus*	Ahmed et al. (2009)
Essential oils of *Cinnamomum verum*, *Cymbopogon citratus*, *Thymus vulgaris*, and *Syzygium aromaticum* and their active compounds, including cinnamaldehyde, eugenol, thymol, geraniol, and citratus	Azole drugs	*Candida albicans* and filamentous fungi	Khan and Ahmad (2011), Khan et al. (2012), and Khan and Ahmad (2013)
Nanoparticles			
Silver nanoparticles	Amoxicillin, ampicillin, erythromycin, kanamycin, and chloramphenicol	*E. coli*, *S. aureus*, *Micrococcus luteus*, *E. coli*, and *Salmonella typhi*	Li et al. (2005) and Fayaz et al. (2009)
TiO_2 nanoparticles	Penicillins, cephalosporins, and aminoglycosides	MRSA	Roy et al. (2010)
ZnO nanoparticles	Aminoglycosides, cephalosporins, glycopeptides, lincosamides, macrolides, penicillins, and tetracyclines	*S. aureus*	Thati et al. (2010)
Chitosan-capped gold nanoparticles	Ampicillin	Gram-positive and gram-negative bacteria	Chamundeeswari et al. (2010)

active compounds of essential oils, namely eugenol and carvacrol, on chitosan nanoparticles. The authors found stronger antibacterial activity of grafted eugenol and carvacrol against *E. coli* and *S. aureus* compared to the original chitosan nanoparticles. Similarly, Hu et al. (2009) synthesized chitosan nanoparticles embedded with thymol, and studied their antibacterial activity against *S. aureus*, *Bacillus subtilis*, and some species of fungi. They observed that this combination was more potent than thymol alone. However, it should be taken into consideration that the combination of nanoparticles and essential oils could not be applicable in coatings of medical devices, due to the volatile characteristics of essential oils. However, such combinations could be exploited for the treatment of topical infections, due to the presence of antimicrobial properties in both agents, as well as the various healing aptitudes of essential oils (Allahverdiyev et al. 2011).

6 Conclusion

The emergence of multidrug resistance among microbial pathogens is a global problem that requires improvements in the present methods of, and novel discovery of, antimicrobial strategies. The use of single drugs is no longer a satisfactory approach to combatting this problem. Though the "one drug–one target" approach is no longer optimal, drugs administered in combination provide multiple targets, resulting in greater efficacy and a reduction in resistance. Treatment of many life-threatening diseases, such as cancers, HIV, and tuberculosis is dependent on combination therapy, and similarly, drug couplings, such as antibiotic/adjuvant combinations, for the treatment of infectious diseases caused by MDR pathogens has attracted considerable attention. Furthermore, the use of high-throughput screening of drug compounds that have been previously approved for other applications has led to the discovery of many potential adjuvants. In addition, the combination of nanoparticles or phyto-products with antibiotics results in reduced toxicity of drugs toward human cells. Due to this combinatorial approach, the efficacy of many previously effective antibiotics is restored and can once again be utilized to combat emerging resistance. However, the pitfall of drugs in combination should be taken under consideration. There have been reports on issues with drug–drug interactions, optimized drug ratios, and dosing amounts for pharmacokinetics properties of each compound, and thus the combination therapies with specific dosages and synergistically active drugs are of utmost importance for their ability to increase the efficacy of antibiotics and decrease resistance generation.

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