Combinational Synergy of Antibiotics and Antimicrobial Peptides

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Abstract: Combination of existing antibiotics have been introduced to increase the efficacy for a long time. Synergistic combinations of antibiotics are most desirable due to reduction of doses, toxicity and side effects of the individual antibiotic as well as prolonging in evolution of antibiotic resistance. Recently, antimicrobial peptides (AMPs) are gaining attention as viable alternatives to antibiotics due to the broad spectrum of activities and abundantly present in multicellular organisms that act against invading microbes. The combination of AMPs and conventional antibiotics enables to reduce the overall cost and widen the power of synergistic effects. Synergistic combinations are also reported among AMPs. Despite enormous advantages of synergistic combinations and several reports and uses in clinical medicine, only few underlying mechanisms are revealed. In this review, we overviewed the mechanisms of antimicrobial agents' synergistic combinations and introduced the proteome chip approach for predicting new synergistic combinations and deciphering the synergy mechanisms.

Keywords: Synergistic, Combination, Antibiotic, Antimicrobial peptide, Mechanism, Target analysis.

INTRODUCTION

Discovery and development of different antibiotics in past has revolutionized clinical medicine. Soon the magic bullets were followed by the emergence of resistance microbes. The condition is getting worst day-by-day due to rapid evolution of multidrug-resistant microbes, causing serious impact on human morbidity. Moreover, antibiotics cause collateral damage to commensal microbes and activate immune response leading to increase atopic and autoimmune disease [1].

Several solutions are currently investigated to address above problems. One approach is through combination strategy for exploiting the full potential of existing antibiotics. Another is to search for the potential natural antimicrobial agents like antimicrobial peptides(AMPs) that can boost existing antibiotic power or directly have lethal action against pathogenic microbes. AMPs are usually cationic peptides of organisms acting against invading microbes (primary defense). In this review, we will explore the potential of synergistic combinations between antibiotic-antibiotic, antibiotic-AMP and AMP-AMP. Moreover, we have focused on the mechanism of actions of synergistic combinations.

ANTIBIOTIC-ANTIBIOTIC COMBINATIONS

Combinational therapy is a multi-beneficial approaches supported by clinical successes in past few decades [2-4]. Drug combinations can be additive, synergistic or antagonistic depending on their combined effect being equal to, greater than or less than that of the expected value based on their individual effects [5,6]. The notable advantage achieved through the combination of antibiotics is the synergistic effects which reduce doses, toxicity and side effects of the individual antibiotic as well as delay in evolution of antibiotic resistance [7-9]. Due to these features, research has been intensively focused on finding synergetic drug combinations. Table **1** summarize the synergistic antibiotic pairs and their mechanism of action against specific microbes.

Although many synergistic combinations have been reported, only a few of their mechanisms are known. Understanding the synergistic mechanisms of combinational antimicrobial therapy can increase the therapeutic efficacy and reduce its unwanted use. In principle, synergistic mechanism behind some antimicrobial combination is relatively simple through physicochemical effects: where one drug facilitates the bioavailability of other drug [10]. For example, streptomycin and penicillin combination [11], gentamicin and vancomycin combination [12] and, erythromycin and penicillin combination [13], in which penicillin and vancomycin targets the cell envelope to increase the permeability for the entry of streptomycin, gentamicin and erythromycin inside the cell. Whereas streptomycin, gentamicin and erythromycin target on bacterial ribosome, causing misreading of the genetic code and inhibits translocation to disrupt protein synthesis. Figure **1** depicts the mechanisms of

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Table 1: Synergistic Combinations and their Mechanisms of Action Against Microbes

Synergistic Antibiotic Combination	Mechanism of Action	Coverage Against
Penicillin and Streptomycin	facilitate uptake of Streptomycin	Streptococcus viridans
Vancomycin and Gentamicin	facilitate uptake of Gentamicin	penicillin-resistant pneumococci
Penicillin and Erythromycin	facilitate uptake of Erythromycin	-
Trimethoprim and Sulfamethoxazole	both target on same pathway (Folic acid pathway)	*E.coli, Shigella, Yersinia enterocolitics, Pneumocystis carinii*
Ampicillin and Dicloxacillin	both target on same protein (Penicillin binding protein)	Gram –negative infection
Quinupristin and Dalfopristin	both target on same protein (Exit channel in 50s ribosomal unit)	*Straphylococci, Vancomycin resistance Enterococcus faecium*
Tetracycline and Streptomycin	Unknown	*Brucella, Yersinia pestis, Pseudomonas mallei*
Tetracycline and Chloramphenical	Unknown	*Pseudomonas paeudomallei*
Isoniazid and Rifampin/Ethambutol	Unknown	*Mycobacterium tuberculosis*
Dapsone and Rifampin	Unknown	*Mycobacterium leprae*
Sulfonamide and Tetracycline	Unknown	*Chlamydia trachomatis*
Erythromycin and Doxycycline	Unknown	-
Gentamicin and Ciprofloxacin	Unknown	-

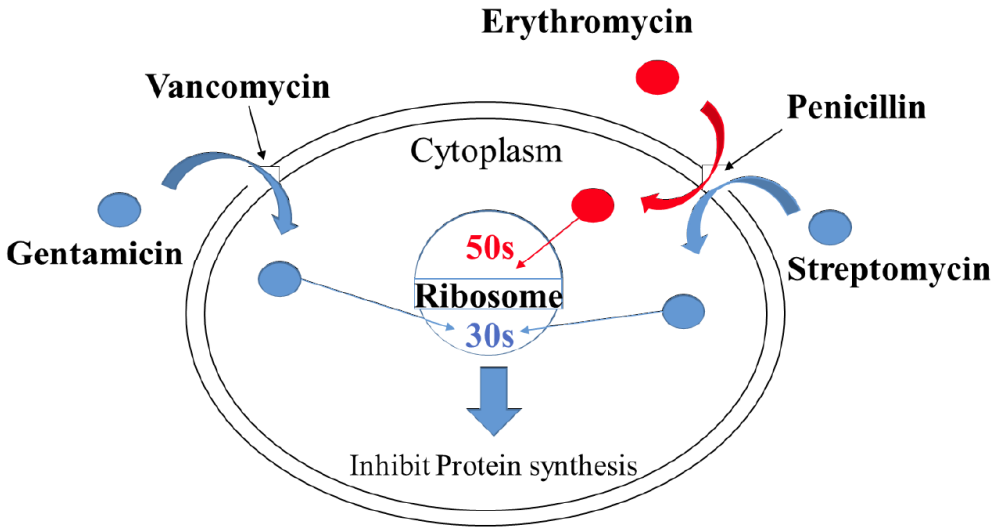

Figure 1: Schematic diagram showing the mechanism of action of synergistic antibiotic combination. Most common mechanism is that one antibiotic facilitates the entry of other antibiotics. After the entry inside the cell, the other antibiotic act on intracellular target and kill the cell. Penicillin and Vancomycin targets on bacterial cell wall and facilitate the entry of Erythromycin, Streptomycin and Gentamicin. After entry inside the cell, Erythromycin target on the 50s subunit of ribosomes whereas Streptomycin and Gentamicin targets on the 30s subunits of ribosomes.

synergistic combination, in which one antibiotic facilitates the entry of other antibiotic inside the cell.

Another interesting synergistic mechanism drew our attention: dual inhibition on the same pathway. Trimethoprim and sulfonamides attacks FolA and FolP in folic acid biosynthesis pathway, respectively [14]. This dual inhibition on the same pathway causes great synergistic effect. Similarly, if the combinations of the multiple antimicrobial treatments target the proteins with the same function (redundant proteins) or pathways with the same function (related pathways), it

would also cause a synergistic effect because both main and alternative proteins or pathways would be disabled.

AMP-ANTIBIOTIC AND AMP-AMP COMBINATIONS

To further expand the scope of treatment, along with antibiotics combination, combination with natural antimicrobial peptides (AMPs) have been tried [15-19]. AMPs are usually cationic and amphiphilic. AMPs have been isolated from bacteria, fungi, plants and animal including human. AMPs are mostly gene encoded peptides initially synthesized as pre-pro-peptides which after cleavage of per- and pro-segment from N-terminal become fully functional [20]. These short chain peptides are active against Gram-positive and Gram-negative bacteria, fungi, protozoa, parasites, cancer cells and also viruses [21, 22].

AMPs exert multiple modes of action which can be categorized into two types: membrane lysis and no-membrane lysis (intracellular targets) [23]. Usually, dual mechanisms are reported for several AMPs and are concentration dependent. AMPs cause membrane lysis at high concentration and no-membrane lysis at low concentration [24]. Moreover, wide spectrum, selective target, lower toxicity and diverse mechanism of action make AMPs an ideal candidate as future medicine [25].

Recently, AMPs were reported to boost the activities of antibiotics with a great synergistic effect [16, 18, 19]. Thus, a new hope has emerged for fighting the battle by combining conventional antibiotics with AMPs. Table **2** summarize the synergistic combinations between AMP-AMP and AMP-antibiotic.

Table 2: Summary Table of Reported AMPs and Antibiotics Combinations

AMPs and Antibiotics Combination	References
Lactoferricin B + PR-39	Ho *et al* (2016)
Lactoferricin B + Bac7	Ho *et al* (2016)
Tachyplesin I + Magainin	Kobayashi *et al* (2001)
apidaecin + Magainin II	Fieck *et al* (2010)
Indolicidin + Protegrin 1	Yan and Hancock (2001)
Human neutrophil defensin-1 + Vancomycin	Yeaman *et al* (1992)
Buforin II + Minocycline	Giacometti *et al* (2000)
Tritrpticin + Ceftazidime	Cirioni *et al* (2006)
Cryptin-2 + Amplicillin	Rishi *et al* (2011)
Nisin Z + Ampicillin; Streptomycin	Naghmouchi *et al* (2012)
Nisin Z + Streptomycin	Naghmouchi *et al* (2012)

MECHANISM BASED ON TARGET ANALYSIS

Target analysis would be a good approach to explore the synergistic mechanisms in antimicrobial combinations. By analyzing the targets of the antibiotics or AMPs, it will allow us not only to decipher synergistic mechanisms of the known combinations but also to predict synergistic combinations. In past, exploring the underlying synergistic combinations was labor-intensive, time consuming trial-and-error experiments. Target analysis will provide an effective

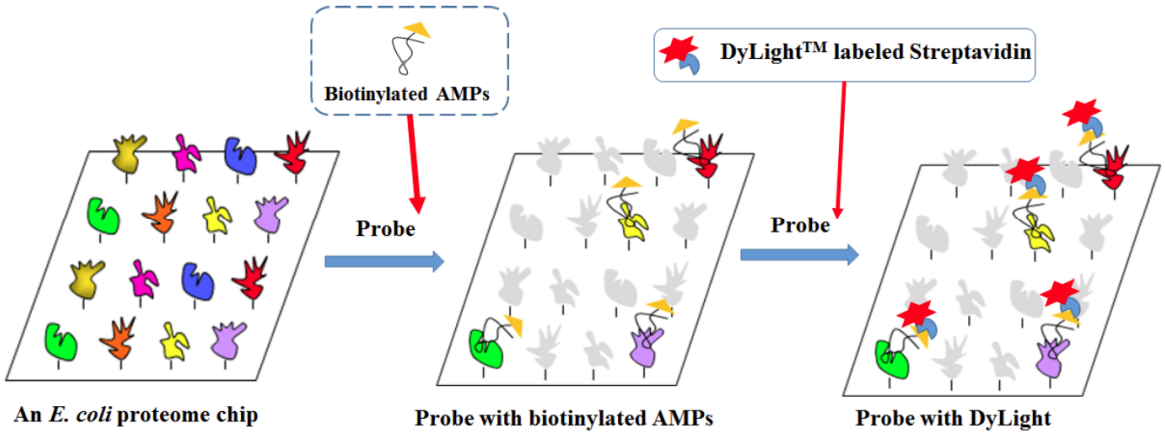

Figure 2: Schematic diagram of the identification of protein targets of AMPs by using *E. coli* proteome microarray assay. To identify the proteins targets AMPs, *E. coli* proteome chips were incubated with biotinylated AMPs. DyLight labeled streptavidin was then probed with chips to identify the targets of individual AMPs. DyLight labeled anti-His antibody was used to detect the relative amount of the proteins.

design of synergistic combinations. Xu [26] also reported that the targets of drugs are important elements for the development of effective drug combinations. However, the targets of AMPs and antibiotics were not comprehensively investigated and analyzed. Usually, only one or two targets are known for an antibiotic, which is not enough to analyze or predict their synergistic mechanism.

In our Lab, we have developed a method for the systematical and comprehensive identification of all the targets of AMPs and antibiotics through proteome microarrays. Proteome microarray is an excellent tool which provides a high-throughput and rapid platform for the identifications of protein targets. Figure **2** depicts the schematic diagram of probing biotinylated AMPs on *E. coli* proteome microarray, comprise of ~42,000 proteins, purified and probed individually on the glass slide coated with aldehyde. DyLight labeled streptavidin and DyLight labeled anti-His antibody were probed to identify the targets of individual biotinylated AMPs and detect the relative amount of the proteins on the *E. coli* proteome microarray, respectively. In our previous work, *E. coli* proteome microarrays were applied to identify the targets of Lactoferricin B (Lfcin B), found in human gut from the digestion of bovine milk. Our study suggested that Lfcin B influenced the metabolic process via multiple protein targets [27].

The proteome targets of AMPs: bactenecin 7 (Bac 7), a hybrid of Pleurocidin and Dermaseptin (P-Der) and a proline-arginine-rich antibacterial peptide (PR-39) were also identified by using *E. coli* proteome microarrays [28]. The comprehensive analysis of the protein targets of four AMPs (LfcinB, Bac7, P-Der and PR-39) showed common enrichment in the same pathway. Purine metabolism was enriched in both Lfcin B and Bac7. Lipopolysaccharide (LPS) biosynthesis was enriched in both Lfcin B and PR-39. This result showed the possibility of synergy between Lfcin B and Bac7 and also Lfcin B and PR-39. To validate this prediction, the synergistic combination between Lfcin B and Bac7 as well as Lfcin B and PR-39 were experimentally tested *in vivo*. Figure **3** (**A** and **B**) shows the growth inhibition by individual and combined AMPs on *E. coli* MG1655 at 7.33 hours. Significant differences in cell growth were observed between expected and experimental combination of AMPs. The

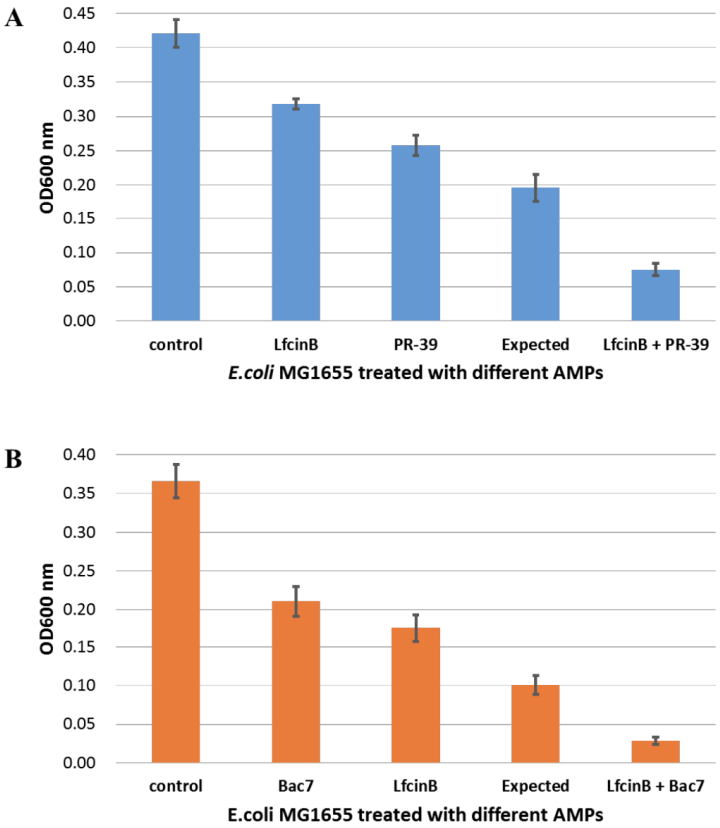

Figure 3: Treatment of different AMPs on *E. coli* MG1655 and the growth recorded after 7.33 hr. A. the growth inhibition by individual and combined Lfcin B and PR-39. **B**. the growth inhibition by individual and combined Bac7 and Lfcin B. Expected value is calculated by the inhibition of one AMP upon the other AMP individually.

expected value was calculated by the individual inhibitory effect of two AMPs in turns. The experimental result of combined Lfcin B and PR-39 shows significantly greater inhibitory effect than the expected value calculated by the Lfcin Bupon PR-39 individual inhibitory effect. This result indicates a synergy between Lfcin B and PR-39 (Figure **3A**). Similarly, Bac 7 and Lfcin B is a synergistic pair due to the much higher effect of combined treatment of Bac 7 and Lfcin B than the expected value obtained from the individual Bac 7 and Lfcin B inhibitory effect (Figure **3B**). Thus, the growth inhibition assay successfully validated the predictions of the new synergistic combination pairs and also deciphered the mechanism of synergistic combination based on the focused damage of most proteins on a pathway [28]. These results indicated the importance to identify all the targets of individual antimicrobial agents comprehensively and thoroughly for the investigation of synergistic effect.

CONCLUSION

In summary, synergistic combinations have proved to be the powerful therapy which can overcome the disadvantage of individual antimicrobial reagent and provide new options against pathogens. More researches should be carried out to discover new synergistic combination. To use the combinational therapy more specifically, we also need to explore the mechanisms involve in synergistic combinations. The mechanisms of synergistic combinations are much more complicated than it was previously explained with a single target molecule of an antimicrobial reagent. Our proteome microarray approach has provided a platform for systematical and comprehensive identification of AMP targets. Target analysis enables precisely prediction of a new synergistic combination and explains the mechanism of synergistic combinations. In future, we will keep analyzing more AMPs and antibiotics to predict more combinations and decipher the mechanism of synergistic combinations. The new synergistic combinations with fully explained mechanism will be powerful weapons in the war of the mankind against pathogens.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

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