INTERACTIONS OF BIOACTIVE COMPOUNDS FROM PLANTS AND ANTIBIOTICS: FOCUS ON ANTIBACTERIAL COMBINATION EFFECTS

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Abstract: The rapid evolution of bacterial drug resistance and the alarming slowdown in development of new antibiotics is spurring attention towards multidrug treatments. Multiple drug resistance that continually challenges human health have resulted from the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. A vicious cycle is generated as multidrug resistant pathogens force us to depend on additional broad-spectrum antibiotics to treat these infections, leading to yet more resistance. Whole-genome sequencing studies have been able to elucidate that bacteria evolve so rapidly with dozens of de novo drug-resistance mutations occurring at high frequency within a short period. This has initiated interest in research on novel and combination antibacterial therapies. Different antibiotics behave differently in combination when acting against various bacterial strains. This review summarizes the current knowledge of interactions between bioactive compounds from plants with commercially available antibiotics.

Index terms: Antibiotics, Bioactive compounds, Synergism, Additive effect, antagonistic effect

1. INTRODUCTION

Mankind were crippled by a wide variety of pathogens since earliest recordings of human history causing significant morbidity and mortality. In a continuous struggle for existence, a plethora of new drugs were discovered one after another. The historical evolution of drug discovery began with primitive man who by the process of trial and error method revealed the healing power of plants and metal salts. People on all continents have depended on thousands of indigenous plants, dating back to prehistory (Cowan, 1999a). Ayurveda, the traditional Indian system of medicine, developed in the vedic era is believed to be the oldest Indian indigenous medicinal system, probably with its roots in the Indus Civilisation. The Vedic hymns of the migrant Aryan tribes are the earliest literary source of information about healing practices in the sub-continent.

In the history of drug development, the discovery of antibiotic drugs was a boon to mankind. Almost all the antibiotics which are currently available were discovered during the period of 1950-70. Sir Alexander Fleming, a Scottish biologist, paved the way for the development of modern antibiotics with his serendipitous discoveries of enzyme lysozyme in 1921 and the antibiotic substance, penicillin in 1928. The discovery of penicillin opened the door to a new era in the treatment of bacterial infections. Despite these advancements in the therapeutic field, what we are facing as a new challenge is the development of resistance against most of the effective drugs in use and the alarming spread of the drug resistance among microbial populations.

Since evolution is an inevitable phenomenon, antimicrobial resistance can only be considered as a natural process. This natural process of evolution has been accelerated and amplified by a number of human practices and policy failures (While, 2016). Indiscriminate and over use of antibiotics causes selective pressure, allowing only the fittest genotype to survive (Yap et al., 2014a) and hence multidrug resistant organisms flourish which results in recalcitrant and uncontrollable infections.

In such circumstances, owing to the inevitable development of resistance that follows the introduction of antibiotics to the clinic, there is a constant need for newer antibacterial drugs (Yap et al., 2014b). The number of new antibacterial agents has decreased steadily in the United States over the last several decades (Fair & Tor, 2014). The deceleration in the discovery rate of newer antibiotics together with the limited lifespan of each drug once introduced to the clinic urged researchers around the world to turn to alternative approaches for fighting bacterial pathogens. When single antibiotics turned to be inefficient, synergistic antibiotic combinations were used in the treatment of many resistant pathogens.

Another approach to combat ever increasing resistance of pathogens is to use combinations of antibiotics and phytochemicals. Several studies have demonstrated the effectiveness of combining antibiotics with various phytochemicals which were already proven to have significant antimicrobial activity and lesser side effects (Bezerra dos Santos et al., 2015; Sudano Roccaro et al., 2004). Combinations of phytochemicals were also found to exert synergistic antimicrobial effect against various pathogenic microorganisms.

2.1 Terminology of drug combinations

Two antimicrobials are said to have synergistic effect when the combination display an effect greater than the sum of the effects of the corresponding individual antimicrobials. When the combined effect is equal to the sum of the single antimicrobial effects the relationship is said to be additive (Chait et al., 2007; Yeh et al., 2006). A drug combination can be considered antagonistic when the effect of the combination treatment is less than the effect of the respective single-drug treatments(Keith et

al., 2005). At times synergy can be such a powerful force that even molecules too weakly active on their own to be considered for monotherapies can be administered in combination to achieve greater therapeutic effect (Ejim et al., 2011). Synergy between two antimicrobial agents can also be defined as a 2-log increase in bactericidal activity *in vitro* compared with the bactericidal activity of each agent when used alone (Giamarellou, 1986; Klastersky et al., 1977).

2.2 Possible mechanisms of synergistic effect

Understanding the mechanism of action involved in synergy of drug combinations could lead to the development of patentable combinations making research in the field of phytosynergy, commercially relevant(Lambert et al., 2003). The mechanism of action by which two drugs enhance the activity of each other to obtain a synergistic effect are complex. It varies with the drug pair involved in synergistic combination. Sometimes a second compound prevents degradation or modification of the primary drug. This approach is the basis of famous 'augmentin' combination in which the beta lactam drug amoxycillin is combined with clavulanate which is a beta lactamase inhibitor (Ball, 2007). In some cases, one compound promotes the retention of the primary drug by blocking efflux pump extrusion of the primary drug. The antihypertensive plant alkaloid reserpine isolated from the roots of *Rauwolfia vomitoria* Afz. was proved to act as an efflux pump inhibitor against the Bmr efflux pump, which mediates tetracycline efflux in *Bacillus subtilis*(Klyachko et al., 1997). In some instances, one of the drugs used in combination inhibits repair pathway or tolerance mechanism of the bacterial cells to the primary drug. A second compound may also target a similar or different pathway that is inhibited by the primary drug. For instance, in the synergistic relationship that exists between trimethoprim and sufamethoxazole, each of the drug inhibits a different step in the folic acid metabolism pathway(Cottarel & Wierzbowski, 2007). There are cases where one drug facilitates the entry of the other drug and thereby work synergistically when these are combined together. An example is the alteration in cell-membrane permeability by cell wall inhibiting antibiotics that enhances penetration of protein synthesis inhibiting antibiotics into bacterial cells, thereby increasing its bioavailability (Jia et al., 2009).

Development of synergistic combinations of antimicrobial agents, whether they are antibiotics, disinfectants or preservatives, is a much sought after strategy. The use of various drug combinations consisting of two or more individual compounds is commonly practiced in many cases like cancer chemotherapy, in the treatment of HIV and hypertension. In the clinical area, synergistic drug combinations help to prevent the emergence of resistant strains, expand spectrum of antimicrobial activity, reduce severe damages caused due to the cytotoxicity of a single drug used at a high concentration (Chong & Pagano, 1997; Ryan et al., 1981). In the past, drug resistant infections were often controlled successfully using synergistic combinations of various antibiotics. Combinations of antimicrobials which displayed *in vitro* synergism against various pathogenic strains generally exhibited successful therapeutic results (Aiyegoro & Okoh, 2009a).

2.3 Antimicrobial activity of bioactive components from plants

Bioactive compounds of plants can be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals. The antimicrobial activity which has been exhibited by a wide variety of plant secondary metabolites can be attributed both to direct action on bacterial cell components as well as to the suppression of major virulence factors. Antibacterial properties of several plants and their bioactive components are commercially exploited in various commercial products such as dental root sealers (Bal et al., 1990), antiseptics (Tyski et al., 2013), food preservatives (Fatoki & Onifade, 2013) and feed supplements. Bioactive compounds are chemically diverse group of compounds which include flavonoids, phenolic compounds, glycosides, terpenoids, alkaloids, tannins, quinones, lignins, proteins etc.

Flavonoids are known to be synthesized by plants in response to microbial infection. Flavonoids have been reported to possess both antioxidant(Heim et al., 2002; Rice-Evans et al., 1996) and antibacterial activity. Apigenin (Basile et al., 1999), naringenin(Tsuchiya et al., 1996), sophoraflavanone G and its derivatives (Sakagami et al., 1998), epigallocatechin gallate and its derivatives (Ikigai et al., 1993a), quercetin, 3-O-methylquercetin and various quercetin glycosides (Arima & Danno, 2002) are few of the flavonoids that are reported to have significant antimicrobial activity. Investigation into the mechanism of action of flavonoids suggests that flavonoids differ in their action on bacterial cells. Inhibition of nucleic acid synthesis (Mori et al., 1987; Ohemeng et al., 1993), inhibition of cytoplasmic membrane function(Haraguchi et al., 1998a; Ikigai et al., 1993b), inhibition of energy metabolism (Haraguchi et al., 1998b; Salvatore et al., 1998) are some of the suggested mechanisms of antimicrobial action of flavonoids.

Alkaloids have inspired the development of several antimicrobial drugs. The alkaloid quinine, a component of the bark of the Cinchona (quina-quina) tree, formed the basis for the synthesis of the commonly used antimalarial drugs (Bawa et al., 2010). Synthesis of quinine serendipitously yielding a new class of antibacterial drug quinolones (Lesher et al., 1962) and structural alteration of azomycin yielding metronidazole (Cushnie et al., 2014) are few examples in which alkaloid scaffolds are utilized in antibacterial drug development. Inhibition of nucleic acid synthesis (Rao & Venkatachalam, 2000), inhibition of respiration (Tominaga et al., 2002), disruption of membrane integrity (Alhanout et al., 2010) are some of the proposed mechanisms of antimicrobial action of alkaloids.

Many terpenes are known to be active against a wide variety of microorganisms, including gram-positive and gram-negative bacteria and fungi (Cowan, 1999b). Investigations into the effects of terpenoids upon isolated bacterial membranes suggest that their activity is a function of the lipophilic properties of the constituent terpenes (Knobloch et al., 1989a). The biochemical mechanisms underlying the disruption of membrane function include the inhibition of electron transport, protein translocation, phosphorylation steps and other enzyme-dependent reactions (Knobloch et al., 1989b).

2.4 Synergistic effect of plant extracts with antibiotics

Emergence of drug resistant strains can be prevented to an extent by decreasing the dose of antibiotics used in the treatment. This can be achieved by combining different antibiotics or combining antibiotics with other bioactive molecules which can work synergistically to achieve a greater antimicrobial effect by making them more potent at low dosage (Hemaiswarya et al., 2008). Combination antibiotic therapy is most commonly used in critically ill patients due to severity of infections caused by the emergence of multidrug-resistant organisms (MDR). Conditions treated with combination therapy include tuberculosis, leprosy, cancer, malaria, and HIV/AIDS. One major advantage of combination therapies is that they reduce development of drug resistance, since a pathogen or tumor is unlikely to possess resistance to multiple drugs simultaneously (Rendon et al., 2016).

Even though bioactive molecules present in plants are less potent antimicrobials, plants fight infections successfully probably working synergistically. These secondary metabolites from plants which are structurally and functionally diverse can also be exploited and modified as combinatorial drugs with antibiotics. Bioactive components from plants have been found to act as synergistic enhancers of standard antibiotics even in the absence of any antimicrobial activity on their own (Aiyegoro & Okoh, 2009b). Synergistic effect of the association of antibiotics and plant components normally has fewer side effects, since, plant antimicrobials have less toxic effects compared to the antibiotics. This effect also enables to use antibiotics which are no longer effective by themselves during therapeutic treatment (Nascimento et al., 2000a). Various plant extracts and phytochemicals like eugenol, farnesol, benzoic acid and cinnamic acid were studied for possible synergistic antimicrobial effects with various antibiotics and found that association of antibiotics and plant extracts showed synergistic antibacterial activity against antibiotic-resistant bacteria (Nascimento et al., 2000b).

Specific mechanisms of action of the combination of antibiotics and plant bioactive components have only been elucidated for limited number of combinations. Several plant derived compounds have been identified with inhibitory activity towards *β*-lactamases. There are reports available listing the synergistic interactions of β-lactam antibiotics with natural compounds like catechins and epigallocatechin gallate to overcome resistant microorganisms. Polyphenols (epicatechin gallate and catechin gallate) have also been reported to reverse beta-lactam resistance in methicillin-resistant *S. aureus* (MRSA) when used in combination with oxacillin (Stapleton et al., 2004). The essential oil from *Salvia sclarea* was shown to increase the susceptibility of methicillin resistant *Staphylococcus epidermidis* (MRSE) isolates to oxacillin by inhibiting the expression of the resistant gene mec A (Chovanová et al., 2016).

Certain plant derived products augment the activity of antibiotics by inhibiting multi drug resistant efflux systems in bacteria (Tegos et al., 2002). Essential oils from *Salvia fruticosa*, *Salvia officinalis* and *Salvia sclarea* reduce the minimal inhibition concentration of tetracycline, decrease efflux of antibiotic and decrease the expression of *tet*(K) gene in tetracycline resistant clinical isolates of *Staphylococcus epidermidis* (Chovanová et al., 2015).

Compounds like polyphenols have been shown to exercise their antibacterial action through membrane perturbations. This disruption of the cell membrane coupled with the action of beta-lactams on the transpeptidation of the cell membrane could lead to an enhanced antimicrobial effect of the combination (Esimone et al., 2006). It has also been revealed that some plant derived compounds can improve the *in vitro* activity of some peptidoglycan inhibiting antibiotics by directly attacking the same site (that is, peptidoglycan) in the cell wall (Zhao et al., 2001).

The synergies detected in these studies as enumerated above suggests that plant crude extracts are a blend of compounds that can enhance the activity of different antibiotics. Continued and further exploration of plant antimicrobials needs to occur because plant based antimicrobials have enormous therapeutic potentials. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic drugs.

CONCLUSION

The issue of the emergence of bacterial resistance is a major menace in combating the treatment of drug resistant infections in the medical field. Recent ethnopharmacological studies revealed that plants have potent antibacterial compounds. This activity can be enhanced by the synergism between herbal extracts and known antibiotics. Furthermore, these combinations can achieve effective antimicrobial effects with comparatively low dosages, which is highly recommendable as it may decrease both the risk of side effects and the costs of treatment of infectious diseases. Approaches for medicinal plant-based antimicrobial synergy research are developing but *in vitro* research is not progressing into clinical studies. Indeed, the process of selecting complex medicinal plant bioactive compounds for *in vitro*, *in vivo*, and clinical studies is challenging. Therefore, the design of meticulous and reproducible studies is obligatory to prevent unnecessary effort for studies where natural product selection was not carried out with strict criteria. The reported synergism between herbal extract and antibiotics will also help to design improved treatment strategies against drug resistant infections. Further researches are needed to explore the bioactive compounds in these plant extracts and the mechanisms involved in these synergistic effects.

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