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MINIREVIEW

Synergy Between Novel Antimicrobials and Conventional Antibiotics or Bacteriocins

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Abstract

Due to the alarming spread of resistance to classic antimicrobial agents, innovative therapeutic methods to combat antibiotic-resistant bacterial pathogens are urgently required. This minireview examines the enhancement of antibiotic efficacy by their combination with new antimicrobials, such as plant-derived compounds, metal ions and nanoparticles and bacteriophage lytic enzymes. The mechanisms of the observed synergy are also described. The promising results of basic research indicate that in future, combined therapy may be applied in human and veterinary medicine, agriculture and the food industry to combat bacterial pathogens.

Key words: antibiotics, bacteriophages, nanoparticles, plant compounds, synergy

Introduction

The extensive use of antibiotics has led to growing resistance and the spread of many bacterial pathogens, which now constitutes a serious medical problem. For this reason, the number of studies aimed at developing new analogs of known antibiotics, *e.g.* oxazolidinones, glycopeptides, quinolones, aminoglycosides, tetracyclines and ketolides (Theuretzbacher, 2011), and at identifying novel antibacterial therapeutics and strategies, is growing exponentially. Positive validation of new antimicrobial agents is often connected with the discovery of novel targets. To illustrate this point, the so-called switch region of bacterial RNA polymerase, that does not overlap the rifamycin binding site, has been confirmed as the target of mycopyronin, corallopyronin, ripostatin and lipiarmycin – antibiotics which are not cross-resistant with rifamycins (Srivastava *et al.*, 2011). It was also recently postulated that NF-κB (nuclear transcription factor-κB), which is crucial for the cellular response to stress and inflammation caused by *e.g.* microbial infection, could represent a target for antimicrobial and antiviral therapies (Vitiello *et al.*, 2012). In addition, there is current controversy over whether fatty acid biosynthesis pathways may constitute a novel promising target for antibiotics to control bacterial pathogens, particularly *Staphylococcus aureus* (Parson and Rock, 2011). Potential antibacterial treatments include the use of antimicrobial peptides, antivirulence strategies and therapeutic antibodies (Fernebro, 2011), as well as plant-derived compounds, metal nanoparticles and bacteriophage lytic enzymes.

Plant-derived compounds, metal nanoparticles and bacteriophage lysins, which are the subject of this review, may be considered new antimicrobials due to their proven and substantial antibacterial effect, which is, however, weaker than that of common antibiotics produced by bacteria and fungi (Hemaiswarya *et al.*, 2008). In the last decade, the first steps in elucidating the mechanisms of antibacterial activity and the cellular targets of plant-derived compounds have been made, with phenolics, and especially flavones, being the subject of the majority of studies. Flavones cause disruption of the bacterial cytoplasmic membrane and inhibit energy metabolism (Tsuchiya and Iinuma, 2000; Plaper *et al.*, 2003). These compounds can also attenuate the pathogenicity of various bacteria by their ability to inhibit quorum-sensing signal receptors, sortase and urease activity, listeriolysin O, coagulase and α-toxin secretion, and by neutralizing bacterial toxins (for review see Cushnie and Lamb, 2011). There have also been a considerable number of reports describing the antibacterial effect of another group of plant-derived

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compounds, the terpenes, but only a few discuss the molecular basis of their antibacterial activity (for review see Kurek *et al*., 2011; Wolska *et al*., 2010). For example, pentacyclic triterpenoids can inhibit insoluble glycan synthesis by *Streptococcus mutans* (Kozai *et al*., 1999) and peptidoglycan metabolism in *Listeria monocytogenes* (Kurek *et al*., 2010), and also influence *Escherichia coli* gene expression and biofilm formation (Ren *et al*., 2005; Grudniak *et al*., 2011). It was also shown that diterpenoids have potent anti-biofilm activity against staphylococci (Walencka *et al*., 2007). In addition, the sesquiterpene farnesol was found to inhibit *S. aureus* growth by affecting the mevalonate pathway of isoprenoid synthesis (Kaneko *et al*., 2011).

Metal ions and nanoparticles, especially silver compounds, constitute another group of potent antimicrobials that have already been applied in medicine and pharmacology (Chopra, 2007; Li *et al*., 2006; Monteiro *et al*., 2009). They are used as antibacterial surface coatings on medical devices, such as venous and urinary catheters, implants and megaprostheses (Hamill *et al*., 2007; Hardes *et al*., 2010). Silver nanoparticles (AgNPs) display remarkable antimicrobial activity against *Pseudomonas aeruginosa*, *E. coli* and *Enterobacter cloacae*, being more active against Gram-negative than against Gram-positive bacteria (Im *et al*., 2011). Metal ions and nanoparticles are safer *in vitro* and have a greater antibacterial effect when stabilized by various polymer surfactants (Lin *et al*., 2012). Silver nanoparticles can disrupt bacterial membranes (Singh *et al*., 2008), cause membrane lipid peroxidation (Neal, 2008), alter gene expression (Lok *et al*., 2006), and when inside the cell, they may also damage DNA and impair the respiratory chain and cell division (Rai *et al*., 2009).

The lytic enzymes of bacteriophages may also be considered as novel antimicrobials. Bacteriophage therapy to treat bacterial infections has been well studied, but the extensive literature on this subject will not be considered here (for review see Chibani-Chennoufi *et al*., 2004; Górski *et al*., 2009). An alternative to whole phage particles is the application of phage lysins: enzymes that are active against Gram-positive bacteria. It was shown that the lytic enzyme PhyPH is active against *Bacillus anthracis* strains (Yoong *et al*., 2006), and the *S. aureus* bacteriophage ϕMR11 lysin, designated MV-L, can inhibit a number of *S. aureus* strains, including those that are methicillin-resistant (MRSA) or vancomycin-intermediate (VISA) (Rashel *et al*., 2007). Using a mouse model, Grandgigard and coworkers (2008) demonstrated that the recombinant phage lysine Clp-1 may be useful in therapy for pneumococcal meningitis, and Nelson *et al.* (2001) found that the lysin isolated from a virulent C_1 phage, specific for group C streptococci, can prevent and eliminate upper respiratory tract colonization by group A streptococci.

The compounds listed above display antibacterial activity when used alone, but there is also the possibility of using them in combination with conventional antibiotics in order to improve their efficacy. Combination therapy, *i.e*. the simultaneous treatment of infections with more than one drug, may constitute an efficient strategy to combat antibacterial resistance. This minireview summarizes current data on the synergistic activity of antibiotics in combination with plant-derived compounds, metal ions and nanoparticles, and bacteriophage lytic enzymes. The examples of synergy between novel antimicrobials and antibiotics / bacteriocins are also listed in Table I. The determination of synergy between two compounds is based on calculation of the FICI (fractional inhibitory concentration index), where a value of ≤ 0.5 indicates a synergistic interaction (EUCAST, 2000). The possible mechanisms underlying these interactions are described in a separate chapter.

Synergy between plant-derived compounds and antibiotics or bacteriocins

There have been a substantial number of reports on synergistic antibacterial activity between various purified plant-derived compounds and plant oils, and antibiotics (mainly β-lactams) against *Staphylococcus aureus* including (MRSA). The most relevant findings from these studies will be presented in chronological order. Brehm-Stecher and Johnson (2003) observed that treatment with low concentrations of the sesquiterpenoids nerolidol, bisabolol and apritone enhanced bacterial susceptibility to ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline and vancomycin. Synergism was demonstrated between ampicillin and ethanolic extracts from 10 Indian medicinal plants, including *Camelia sinensis* (Chinese tea), that are rich in alkaloids, glycosides, flavanoids, phenols and saponins (Aqil *et al*., 2005), and quinic acid gallates from *Caesalpina spinosa* could intensify the susceptibility of MRSA to oxacillin (Kondo *et al*., 2006). Grande *et al*. (2007) found that the antimicrobial activity of bacteriocin produced by *Enterococcus faecalis,* enterocin AS-48, against *S. aureus* was potentiated when applied in combination with phenolic compounds such as carvacrol. Synergy was demonstrated between the diterpenoids salvipisone and aethiopinone, and antibiotics from the β-lactam, glycopeptide and oxazolidinone groups (Walencka *et al*., 2007). Nascimento *et al.* (2007) observed synergistic activity between ampicillin and the Brazilian plant *Eremanthus erythropappus* oil and β-bisakolene. The hop (*Humulus lupulus*)-derived compounds, lupulone and xanthohumol, showed synergy with tobramycin and ciprofloxacin (Natarajan *et al*., 2008). A synergistic effect of kaempferol glycosides

Table I
Examples of synergy between novel antimicrobials and antibiotics/bacteriocins

Novel antimicrobial	Antibiotic/bacteriocin	Bacterial species	Reference
Plant compound			
Thymol	Nisin	*L. monocytogenes*	Ettayebi *et al*., 2000
EGCg	Ampicillin + sulbactam	MRSA	Hu *et al*., 2001
EGCg	Penicillin	*S. aureus*	Zhao *et al*., 2002
Baicalein	β-lactams, tetracycline	MRSA	Fujita *et al*., 2005
7-methyljuglone	Isoniazid	*M. tuberculosis*	Bapela *et al*., 2006
Carnosol	Aminoglycosides	VRE	Horiuchi *et al*., 2007
Asiatic acid, corosolic acid	Tobramycin	*P. aeruginosa*	Garo *et al*., 2007
Ellagic acid, tannic acid	Novobiocin	*A. baumanii*	Chusri *et al*., 2009
Kaempferol glycosides	Fluorochinolones	MRSA	Liu *et al*., 2009
Galangin	Cefazidime	PRSA	Eumkeb *et al*., 2010
Oleanolic acid	Rifampicin	*M. tuberculosis*	Ge *et al*., 2010
Betulic acid	Methicillin, vancomycin	*S. aureus*	Chung *et al*., 2011
Oleanolic acid, ursolic acid	Ampicillin, oxacillin	*S. aureus, S. epidermidis*	Kurek *et al*., 2012
Ag^+ and AgNPs			
Ag^+	Vancomycin, amoxicillin, penicillin G	*S. aureus*	Shahverdi *et al*., 2007
AgNPs	Polymyxin B	$Gram^-$ bacteria	Ruden *et al*., 2009
AgNPs	Ampicillin	$Gram^+$ bacteria	Fayaz *et al*., 2010
Bacteriophages lytic enzymes			
Cpl -1	Cefotaxime, moxifloxacin	*S. pneumoniae*	Rogríguez-Cerrato *et al*., 2007
LysK	Lysostaphin	MRSA	Becker *et al*., 2008
ClyS	Oxacillin, vancomycin	MRSA	Daniel *et al*., 2010
LysH5	Nizin	*S. aureus*	Garcia *et al*., 2010

purified from *Laurus nobilis* and fluorochinolones on MRSA was shown by Liu *et al*. (2009), while the efficacy of galangin, a flavanol isolated from *Alpinia officinarum*, administered with ceftazidime, against penicillin-resistant *S. aureus* (PRSA) was demonstrated by Eumkeb and coworkers (2010). In a study of the synergistic antimicrobial activity of pentacyclic triterpenoids (*e.g.* betulinic acid) combined with methicillin or vancomycin, it was found that various combinations of these compounds could reduce their minimal inhibitory concentrations (MICs) by 0.05–50% (Chung *et al*., 2011). Two other pentacyclic triterpenoids, oleanolic acid and ursolic acid, have recently been shown to act synergistically with ampicillin and oxacillin against *S. aureus* and *Staphylococcus epidermidis* grown in solution or as biofilms (Kurek *et al*., 2012).

In the last decade, there have been an appreciable number of reports describing synergy between plant compounds and antibiotics against bacteria outside the genus *Staphylococcus*. The antibacterial effect of nisin Z against *Listeria monocytogenes* ATCC 7644 and *Bacillus subtilis* ATCC 33712 was found to be greatly enhanced by a subinhibitory concentration of thymol (Ettayebi *et al*., 2000), and the diterpenoid carnosol reduced the MICs of various aminoglycosides against vancomycin-resistant enterococci – VRE (Horiuchi *et al*., 2007). Garo *et al*. (2007) showed that treatment with asiatic acid and corosolic acid enhanced the susceptibility of *Pseudomonas aeruginosa* bioilms to tobramycin. Alcoholic extracts from 15 traditional Indian medicinal plants exhibited synergy with tetracycline and ciprofloxacin to inhibit the growth of ESbetaL (extended spectrum beta-lactamase)-producing *E. coli* and *Shigella* (Ahmad and Aqil, 2007). The combination of kaempferol with clindamycin or quercetin produced a large synergistic effect against antibiotic-resistant *Propionibacterium acnes* (Lim *et al*., 2007). Studies by a Chinese group have confirmed the synergistic activity between a herbal medicine isolated from *Ramulus cinnamoni* and tetracycline, gentamicin and streptomycin against nosocomical antibiotic-resistant strains of *P. aeruginosa* (Liu *et al*., 2007). Using transmission electron microscopy, Sivarooban *et al*. (2008) observed cell damage in *L. monocytogenes* caused by a combination of nisin with either a grape seed or a green tea extract rich in phenolic constituents. Combination with gerianol isolated from *Helichrysum italicum*, a member of the sunflower family, significantly increased the efficacy of β-lactams, quinolones and chloramphenicol towards multidrug resistant

Enterobacter aerogenes, *E. coli*, *P. aeruginosa* and *Acinetobacter baumanii* (Lorenzi *et al*., 2009). The antibacterial activity of novobiocin was shown to be enhanced by the plant phenolics ellagic and tannic acids, which increased its effectiveness against multi-drug resistant *A. baumanii* (Chusri *et al*., 2009). A crude leaf extract of *Helichrysum pedunculatum* enhanced the activity of eight antibiotics from various groups against bacteria implicated in wound infections (Aiyegoro *et al*., 2010). Mulyaningsih *et al*. (2010) elucidated the synergistic properties of two terpenoids from the essential oil of *Eucalyptus globulus*, aromadendrene and 1,8-cineole, against VRE. A notable study by Ge *et al*. (2010) described synergistic *in vitro* interactions between oleanolic acid and isoniazid, rifampicin or ethambutanol against *Mycobacterium tuberculosis*. The potent synergism of ciprofloxacin with extracts of medicinal plants, *e.g. Angelica sinensis* and *Melissa officinalis,* against Enterobacteriaceae and *P. aeruginosa* has also recently been reported (Garvey *et al*., 2011).

Synergistic activity between metal ions or nanoparticles and antibiotics or bacteriocins

The majority of reports on the interaction between antibiotics and various forms of metals describe the effects of silver ions and silver nanoparticles (AgNPs). In an early study, Modak and Fox (1985) identified synergism between silver sulfadazine and piperacillin (an extended-spectrum penicillin antibiotic), both *in vitro* and *in vivo*. The antimicrobial activities of various antibiotics in the presence of Ag^+ ions have since been studied more systematically and the greatest enhancing effect was observed for vancomycin, amoxicillin and penicillin G against *S. aureus* rather than *E. coli* (Shahverdi *et al*., 2007). In contrast, the synergistic activity between silver nanoparticles (AgNPs) and ampicillin, gentamicin, kanamycin, streptomycin and vancomycin was subsequently shown to be greater against *E. coli* and *P. aeruginosa* than against *S. aureus* (Birla *et al*., 2009). It was also confirmed that silver nanoparticles can enhance the antibacterial activity of chloramphenicol, being an active carrier of this antibiotic (Patil *et al*., 2009). It has yet to be determined whether nanoparticles pre-bound to an antibiotic produce a greater antimicrobial effect than simultaneous addition of silver and the antibiotic (Durán *et al*., 2010). A number of studies have shown that silver can act synergistically with compounds other than antibiotics. The activity of AgNPs against an *E. coli* biofilm was increased by the lipopeptide biosurfactant V9T14 (Rivardo *et al*., 2010), and synergy with chitosan against *S. aureus* has also been reported (Potara *et al*., 2011). Ammons *et al.* (2011) recently showed that a silver wound dressing combined with the immune molecule lactoferrin and the rare sugar-alcohol xylitol, reduced biofilm viability more effectively than standard silver hydrogel.

So far there have been no reports of the direct synergistic activity of gold nanoparticles (AuNPs) and antibiotics. Gu *et al*. (2003) found that AuNP-vancomycin conjugates can act as a potent inhibitor of VRE and *E. coli*. It has also been demonstrated that AuNPs function as useful carriers for ciprofloxacin and other fluoroquinolones (Tom *et al*., 2004). In the case of *E. coli*, the AuNP conjugate showed greater antibacterial activity than free ciprofloxacin (Rosemary *et al*., 2006). However, Burygin *et al*. (2009) found no difference between the antibacterial activity of a gentamicin conjugate with AuNPs and the free antibiotic. A single report has described the synergistic interaction between copper and an antibiotic – erythromycin (Sultana *et al*., 2005). Synergism was observed between this antibiotic and several other trace elements besides copper (*e.g*. cobalt, nickel, chromium), against both Gram-negative and Gram-positive bacteria. Synergistic antimicrobial effects between metal ions and compounds other than antibiotics have also been reported, the most well documented of which is enhancement of the antimicrobial activity of pomegranate extracts against clinical isolates of *S. aureus* and *P. aeruginosa* by combination with cupric sulfate (Gould *et al*., 2009 a, b).

Antibiotics or bacteriocins and bacteriophage lytic enzymes

There have been several recent reports describing interactions between bacteriophage-encoded lytic enzymes and classic antibiotics or bacteriocins. A synergistic effect with penicillin and gentamicin was observed for lytic enzyme Cpl-1 encoded by *Streptococcus pneumoniae* lytic phage Cp-1 and also for another endolysin, Pal, encoded by *S. pneumoniae* lytic phage Dp-1 (López and García, 2004), against several penicillin-resistant and -sensitive *S. pneumoniae* strains (Loeffler and Fischetti, 2003; Djurkovic *et al*., 2005). *In vitro* interactions between Cpl-1 and Pal with cefotaxime and moxifloxacin against antibiotic-susceptible and antibiotic-resistant *S. pneumoniae* have also been studied. Synergistic activity was confirmed for the combination of Cpl-1 and cefotaxime or moxifloxacin and the effect was strain-dependent. It is noteworthy that greater synergy was observed for the combination of these antibiotics with LytA, which is the major pneumococcal autolysin (Rodríguez-Cerrato *et al*., 2007). The combined effect of nisin and two *S. aureus* lytic phages, φ35 and φ88, was assessed, and a synergistic effect was observed in short-term experiments. However nisin adaptation and reciprocal resistance to

both phages have prevented the practical application of this combined therapy (Martínez *et al*., 2008). Another two endolysins, LysK, produced by staphylococcal bacteriophage K (O'Flaherty *et al*., 2005) and the anti-staphylococcal bacteriocin, lysostaphin, synthesized by *Staphylococcus simulans* (Dajcs *et al*., 2000), exhibited synergy in killing MRSA (Becker *et al*., 2008). Recently, a novel chimeric lysin, ClyS, was engineered by fusing the N-terminal catalytic domain of *S. aureus* Twort phage lysin with the C-terminal cell-wall targeting domain of the phage φNM3 lysin. The chimeric protein displayed synergistic interactions with both vancomycin and oxacillin *in vitro* and its combination with oxacillin could prevent septic death in MRSA-infected mice (Daniel *et al*., 2010).

Molecular basis of synergistic activities

Three categories of combination therapy can be distinguished (Fischbach, 2011). The most common strategy utilizes the combination of drugs which inhibit different pathways within bacterial cells. An example of such a strategy is treatment of *Mycobacterium tuberculosis* infections with four drugs: 1) isoniazid, an inhibitor of fatty acid synthesis, 2) rifampicin, an inhibitor of RNA polymerase, 3) ethambutanol, an inhibitor of arabinose transferases involved in cell wall biosynthesis, and 4) pyrozinamide, with an as yet unknown mechanism of action (Ginsberg and Spigelman, 2007). The second strategy is based on the inhibition of different targets in the same pathway. The inhibition of folic acid synthesis by a combination of sulfamethoxazole, an inhibitor of dihydropteroate synthetase, and trimethoprim, inhibiting dihydrofolate reductase, is based on this strategy (Wormster *et al*., 1982). The third strategy requires inhibition of the same target in different ways, *e.g*. the application of streptogramin and virginamycin, which both inhibit the peptidyl transferase center on the 50S ribosomal subunit (Tu *et al*., 2005). It should be noted that such a combined antimicrobial effect is utilized in nature by antibiotic producers to compete effectively with other species (Ohnishi *et al*., 2008). Instead of two antibiotics, combination therapy can utilize antibiotics with their "sensitizers": molecules that make the co-applied antibiotic more effective by inhibiting enzymes responsible for antibiotic resistance or those that metabolize the drug. For example, diazabicyclooctanes (DBOs) are novel class A and class C β-lactamase inhibitors that are more potent than current commercially available inhibitors (Coleman, 2011). Similarly, the synergistic antibacterial activity between various plant-derived compounds increases the effectiveness of herbal extracts in comparison with the isolated single constituents. In phytotherapy, this synergy is more difficult to dissect because plant extracts contain many minor agents that may influence the combined effect. In spite of this, many synergistic activities between phytopharmaceuticals have been demonstrated, and in some cases the mechanism of this effect has been elucidated (Wagner and Ulrich-Merzenich, 2009).

The synergistic antimicrobial effect of an antibiotic combined with another agent requires interaction of the latter compound with the bacterial resistance mechanism. The first details of the molecular basis of synergistic interaction between some plant-derived compounds and various classes of antibiotics have recently been revealed. The ability of novel therapeutics to inhibit lactam- or ester-cleaving enzymes can result in synergy with β-lactams. EGCg (epigallocatechin gallate) inhibits penicillinase activity, thus restoring the effectiveness of penicillin against *S. aureus* (Zhao *et al*., 2002) and potentiating the effect of ampicillin and sulbactam against MRSA (Hu *et al*., 2001). The synergy between galangin and methicillin, ampicillin, amoxicillin, cloxacillin, penicillin G and cefazidime against *S. aureus* was found to be based on the marked inhibitory activity of galangin against penicillinase and β-lactamase (Eumkeb *et al*., 2010). The two antimicrobials in a combination may affect the same cellular target. For example, EGCg administered with a β-lactam antibiotic could inhibit peptidoglycan synthesis (Yam *et al*., 1998; Zhao *et al*., 2001). Subsequently, Fujita *et al.* (2005) demonstrated that the flavone baicalein exhibits remarkable synergy with β-lactam antibiotics against MRSA, possibly by inhibiting the activity of PBP 2a or by affecting peptidoglycan structure, and Kuroda *et al.* (2007) demonstrated that the sesquiterpene farnesol inhibits recycling of the C_{55} carrier of the murein monomer precursor, thus contributing to increased bacterial susceptibility to β-lactams. Several plant compounds appear to inhibit defined targets in the bacterial cell. The flavanol myricetin was found to suppress DnaB helicase activity and glycosylated flavones could inhibit topoisomerase IV, so these compounds have the potential to act synergistically with particular antibiotics (Hemaiswarya *et al*., 2008). Another mechanism of synergy is by increasing the intracellular antibiotic concentration, which may be achieved by overcoming cellular barriers that prevent antibiotics from penetrating the cell or by blocking bacterial efflux pumps that extrude such agents from the cell. The majority of reports have described the effect of plant compounds on bacterial efflux pumps. The indole alkaloid reserpine, a modulator of multidrug pumps enhanced tetracycline activity against MRSA containing the *tetK* determinant (Gibbons and Udo, 2000), and baicalein inhibited TetK-dependent efflux of tetracycline (Fujita *et al*., 2005). Certain plant-derived compounds, *e.g*. EGCg, have been shown to act as bacterial efflux pump inhibitors (EPIs) and are able to restore

the antibacterial effect of ineffective antibiotics such as ciprofloxacin, preferentially against Gram-positive, but also against Gram-negative species (Stavri *et al*., 2007). N-caffeoylphenalkylamide derivatives were found to act as EPIs in *S. aureus,* especially in strains overexpressing the multidrug efflux transporter NorA (Michalet *et al*., 2007). Chusri *et al*. (2009) suggested that ellagic and tannic acids act as efflux pump inhibitors in *A. baumanii*. It was recently demonstrated that the aforementioned synergism between medicinal plant extracts and ciprofloxacin is the result of inhibition of the efflux pump in Gram-negative bacteria (Garvey *et al*., 2011). Another recent study showed that caffeoylquinic acids from *Artemisia absinthium* preferentially bind to Major Facilitator Super Family efflux systems, which are key multidrug resistance determinants in Gram-positive bacteria (Fiamegos *et al*., 2011). Plant-derived compounds may also be involved in the transformation of a non-active antimicrobial into its active form. The naphthoquinone 7-methyljuglone was able to potentiate the effect of antituberculous drugs against extracellular and intracellular *Mycobacterium tuberculosis*, possibly due to the elevated synthesis of superoxide, which catalyzes the transformation of isoniazid into its active form (Bapela *et al*., 2006). A recent attempt to elucidate the mechanism of synergy between oleanolic and ursolic acids and ampicillin failed to produce an unequivocal answer. However, the inactivation of ampicillin target, the PBPs (penicillin binding proteins), the inhibition of β-lactamase translocation and increased β-lactam transport mediated by these compounds, were all excluded (Kurek *et al*., 2012).

There have been few studies on the molecular basis of synergy between antibiotics and metal nanoparticles or bacteriophage lytic enzymes. It has been claimed that the synergism between nanoparticles and antibiotics or bacteriocins is based on the ability of the latter to help nanoparticles reach their cellular targets. Synergistic activity between silver nanoparticles and membrane-permeabilizing antimicrobial peptides, such as the lipopeptide polymyxin B has been reported (Ruden *et al*., 2009). Polymyxin B is a cyclic polycationic lipopeptide that disrupts the outer membranes of Gram-negative bacteria by interacting with lipid A (Schindler and Osborn, 1979), and it was postulated that such antimicrobial peptides allow nanoparticles to gain access to their internal target site. Fayaz *et al*. (2010) showed that AgNPs can act synergistically with several antibiotics, preferentially against Gram-negative bacteria, and the greatest effect was observed with ampicillin. These authors proposed a model in which AgNPs associate with ampicillin, these complexes interact with the bacterial cell wall and subsequently inhibit the formation of peptidoglycan cross-links, leading to cell wall lysis. In addition, the AgNPs may prevent DNA unwinding when inside the cell. It has also been established that AuNPs are a very useful tool for drug delivery and serve as a stable and non-toxic platform for pharmaceuticals, enhancing their stability and improving targeting (Pissuwan *et al*., 2010).

The aforementioned synergy between two peptidoglycan hydrolases, endolysin LysK and lysostaphin, may be due to the fact that LysK has two lytic domain (endopeptidase and amidase) and thus is able to enhance the lytic potential of lysostaphin, which has single lytic domain (Becker *et al*., 2008). García *et al*. (2010) reported synergy between phage endolysin LysH5, which is active against a wide range of staphylococci (Obeso *et al*. 2008), and nisin in killing *S. aureus* in pasteurized milk. The MICs of nisin and LysH5 were diminished by 64- and 16-fold, respectively. It was postulated that LysH5 activity might be increased by the permeabilization of the cytoplasmic membrane by nisin, as was previously documented for the endolysin Lys44 (Nascimento *et al*., 2008).

Conclusion

The number and variety of novel antimicrobials which show synergy with classic antibiotics and bacteriocins is substantial. Many of these compounds have already been used as an alternative to conventional treatments in medicine and agriculture. However, their widespread application is restricted, mainly because their mechanisms of action have not been fully characterized and their effect on eukaryotes has yet to be established. The problem of antibiotic resistance among bacteria has received much coverage in the literature (Fernebro, 2011; Defoirdt *et al*., 2011). The proven synergistic activities of novel antimicrobials with well known antibiotics provides some hope that the latter may still be of use to treat diseases caused by antibiotic-resistant bacteria. Due to their narrow action spectrum and toxicity, bacteriocins were replaced by antibiotics in clinical use and are now extensively used in food preservation (Riley and Wertz, 2002; Falagas and Kasiakou, 2005). Bacteriocin-resistance may be countered by the use of these compounds in combination with novel antimicrobials. Such a strategy might restore the potential of bacteriocin (*e.g.* nisin) to eliminate pathogenic bacteria, like *S. aureus*, from food. Currently, novel antimicrobials cannot replace antibiotics, but they may become valuable antibiotic complements. In order to exploit these new antimicrobials effectively in synergistic combination therapy, it will be necessary to determine the optimal ratio and dosing regimen, and to fully characterize the mechanisms of their activities by employing genomic, proteomic and metabolomic technologies.

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