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Review

Hybrid combinations containing natural products and antimicrobial drugs that interfere with bacterial and fungal biofilms

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ABSTRACT

Background: Biofilms contribute to the pathogenesis of many chronic and difficult-to eradicate infections whose treatment is complicated due to the intrinsic resistance to conventional antibiotics. As a consequence, there is an urgent need for strategies that can be used for the prevention and treatment of biofilm-associated infections. The combination therapy comprising an antimicrobial drug with a low molecular weight (MW) natural product and an antimicrobial drug (antifungal or antibacterial) appeared as a good alternative to eradicate biofilms.

Purpose: The aims of this review were to perform a literature search on the different natural products that have showed the ability of potentiating the antibiofilm capacity of antimicrobial drugs, to analyze which are the antimicrobial drugs most used in combination, and to have a look on the microbial species most used to prepare biofilms.

Results: Seventeen papers, nine on combinations against antifungal biofilms and eight against antibacterial biofilms were collected. Within the text, the following topics have been developed: breaf history of the discovery of biofilms; stages in the development of a biofilm; the most used methodologies to assess antibiofilm-activity; the natural products with capacity of eradicating biofilms when acting alone; the combinations of low MW natural products with antibiotics or antifungal drugs as a strategy for eradicating microbial biofilms and a list of the low MW natural products that potentiate the inhibition capacity of antifungal and antibacterial drugs against biofilms.

Conclusions and perspectives: Regarding combinations against antifungal biofilms, eight over the nine collected works were carried out with *in vitro* studies while only one was performed with *in vivo* assays by using *Caenorhabditis elegans* nematode. All studies use biofilms of the *Candida* genus. A 67% of the potentiators were monoterpenes and sesquiterpenes and six over the nine works used FCZ as the antifungal drug. The activity of AmpB and Caspo was enhanced in one and two works respectively. Regarding combinations against bacterial biofilms, *in vitro* studies were performed in all works by using several different methods of higher variety than the used against fungal biofilms. Biofilms of both the gram (+) and gram (-) bacteria were prepared, although biofilm of *Staphylococcus* spp. were the most used in the collected works. Among the discovered potentiators of antibacterial drugs, 75% were terpenes, including mono, di- and triterpenes, and, among the atibacterial drugs, several structurally diverse types were used in the combinations: aminoglycosides, β-lactams, glucopeptides and fluoroquinolones. The potentiating capacity of natural products, mainly terpenes, on the antibiofilm effect of antimicrobial drugs opens a wide range of possibilities for the combination antimicrobial therapy. More *in vivo* studies on combinations of natural products with antimicrobial drugs acting against biofilms are highly required to cope the difficult to treat biofilm-associated infections.

Abbreviations: AA, asiatic acid; Aeth, aethiopinone; AmpB, Amphotericin B; BBR, Berberine; BEC, biofilm eradication concentration; CA, corosolic acid; Carv, carvacrol; CAS, caspofungin; Cin, cinnamaldehyde; Cip, ciprofloxacin; CLSM, confocal laser scanning microscopy; CTC, 5-cyano-2,3-ditolyl tetrazolium chloride; CV, crystal violet; DAPI, 4',6'-Diamidino-2-phenylindole; EGCg, epigallocatechingallate; EPS, exopolysaccharide; Eug, eugenol; Farn, farnesol; FCZ, fluconazole; FICI, Fractional Inhibitory Concentration Index; Gen, gentamicin; MCF, micafungin; MCZ, miconazole; MTP, microtiter plate; Naf, Nafcillin; Oxa, oxacillin; PTs, pentacyclic triterpenes; Salv, salvipisone; SBF, Specific Biofilm Formation; SEM, Scanning Electron Microscopy; SMIC, sessile minimum inhibitory concentration; Str, streptomycin; Thy, thymol; Tobra, Tobramycin; Trc, Tyrocidines; TTC, Triphenyltetrazolium chloride; UA, ursolic acid; Van, Vancomycin; XTT, 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide

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Introduction

Several microorganisms form biofilms on living surfaces or medical devices, which constitute their mode of growth in a hostile environment (Costerton et al., 1978, 1999; Coenye and Nelis, 2010; Donlan, 2002).

First structural studies of microbial biofilms

Lawrence et al. (1991) performed structural studies on microbial biofilms and found that they were highly organized hydrated structures that possess distinctive arrangements depending on the microbial species involved. They also noted that many times the biofilm was formed by more than one microbial species that aggregate one each other forming dense mats that stick to surfaces enclosed in a exopolysaccharide matrix (EPS), thus explaining the mechanisms by which microorganisms form biofilms.

Stages in the development of biofilms

The development of a biofilm involves 5 stages that were clearly explained and graphed by Stoodley et al. (2002) (Fig. 1). In stage 1 an initial attachment of microbial cells to the surface is observed; in stage 2 the EPS matrix is produced resulting in a firmly adhered "irreversible" attachment; in stage 3, an early biofilm architecture is developed and in stage 4 the biofilm reaches maturation; in stage 5, single planktonic cells are dispersed from the mature biofilm leading to the formation of a new biofilm.

Resistance of biofilms to antibiotics and antifungals

A characteristic of microbial biofilms is the markedly enhanced resistance to antimicrobial agents (Ahmad Khan and Ahmad, 2012; Costerton et al, 1999; Nickel et al., 1985; Stewart, 2002; Stewart and Costerton, 2001) possessing about 100–1000 times less susceptibility to antifungals and antibacterials than equivalent populations of planktonic cells (Gilbert et al., 2002; Seneviratne et al., 2008; Simões et al., 2009)

The mechanisms of biofilm resistance have been reviewed by Lewis (2007), who clearly explained that although most of the cells in a biofilm can show susceptibility to antimicrobial agents, a small subpopulation of cells (called persisters) stay alive, irrespective of the concentration of the antibiotic. The immune system can kill the remaining planktonic, but not the biofilm persister cells that are protected by the EPS. So, persisters cells that are contained in the biofilm can survive to both the antibiotic treatment and the immune system. When the concentration of antibiotic reduces, persister cells can grow again and repopulate the biofilm (Fig. 2).

Chronic diseases such as cystic fibrosis, native valve endocarditis, otitis media, periodontitis, and prostatitis appear to be caused by biofilm-associated microorganisms (Monroe 2007; Donlan, 2002) and thus they are considered very difficult to eradicate diseases.

The development of new antimicrobial agents with capacity of eradicating biofilms is urgently needed as alternative therapeutic options for microbial biofilm-related diseases.

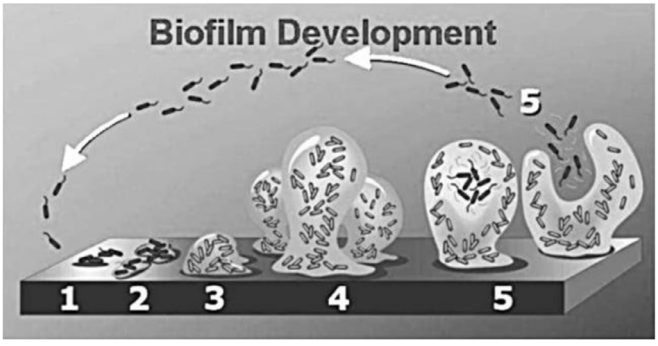

Fig. 1. The five-stage process involved in the development of a biofilm (reproduced from Stoodley et al. (2002). Ann. Rev. Microbiol. 56,187–209 (image credit: D. Davies), with permission of Prof. David Davies.

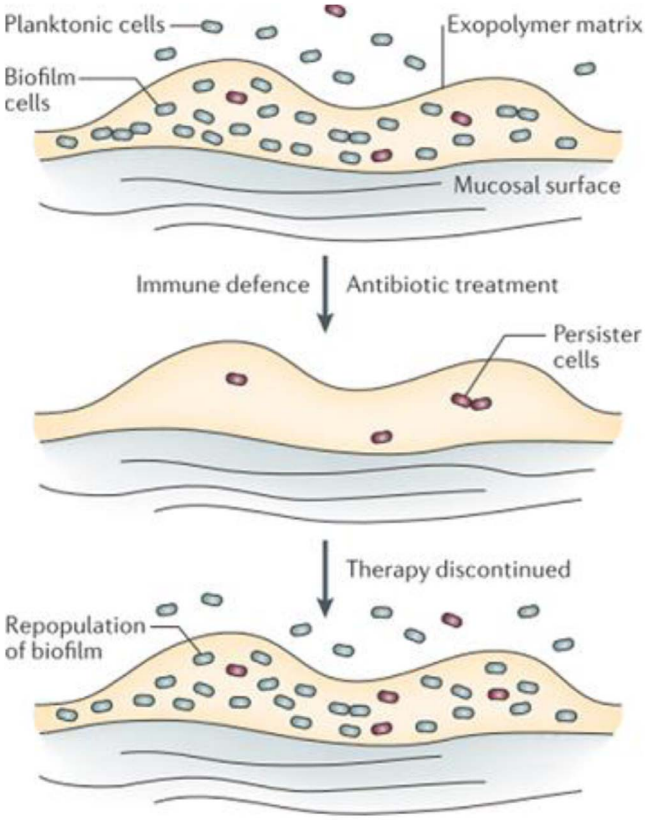

Fig. 2. Model of biofilm resistance to killing based on persister survival. Initial treatment with antibiotic kills normal cells (coloured green, please see the on line version) in both planktonic and biofilm populations. The immune system kills planktonic persisters (coloured pink), but the biofilm persister cells (coloured pink) are protected from the host defences by the exopolymer matrix. After the antibiotic concentration is reduced, persisters resuscitate and repopulate the biofilm and the infection relapses. Reproduced from Lewis (2007), Nature Publishing Group license # 4063660297428 which was a modification of a Fig. appeared in Lewis, 2001. American Society for Microbiology license # 4063670074620. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Natural products with capacity of eradicating biofilms when acting alone

In the last years, many efforts have been made in the exploration of new and effective natural compounds with antibiofilm effects on their own (Bink et al., 2011). So, the sesquiterpene *tt*-farnesol (Farn) showed a modest effect against *Streptococcus mutans* and *Streptococcus sobrinus* biofilms (Koo et al., 2002); the polyphenols epigallocatechingallate (EGCg) and ellagic acid reduced in 30 and 50% respectively the *Burkholderia cepacia* biofilm formation (Huber et al., 2003); the phenylpropanoid cinnamaldehyde (Cin) decreased the *Escherichia coli* biofilm formation in the Specific Biofilm Formation (SBF) assay (Niu and Gilbert, 2004) and the monoterpenephenol carvacrol (Carv) inhibited the biofilm development of *S. aureus* and *S.* Typhimurium (Knowles et al., 2005). Also EGCg at sub-MIC concentrations decreased the EPS production and thus inhibited the biofilm formation of 20 ocular isolated *Staphylococcus* spp including *S. aureus* and *S. epidermidis* (Blanco et al., 2005); the sesquiterpenephenol xanthorrhizol reduced 60% of adherence of *S. mutans* cells (Rukayadi and Hwang, 2006) and the diterpenoide salvipisone (Salv) prevents *S. aureus* and *S. epidermidis*