Bioorganic & Medicinal Chemistry 24 (2016) 1002–1005

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

In vitro interactions between anidulafungin and nonsteroidal anti-inflammatory drugs on biofilms of *Candida* spp.

Antonio Rosato [a], Alessia Catalano [a,*], Alessia Carocci [a,*], Antonio Carrieri [a], Addolorata Carone [a], Giuseppina Caggiano [b], Carlo Franchini [a], Filomena Corbo [a], Maria Teresa Montagna [b]

[a] Department of Pharmacy—Drug Sciences, University of Bari, 'Aldo Moro', Bari, Italy
[b] Department of Biomedical Sciences and Human Oncology, Hygiene Section, University of Bari, 'Aldo Moro', Bari, Italy

ARTICLE INFO

Article history:
Received 16 November 2015
Accepted 14 January 2016
Available online 22 January 2016

Keywords:
Biofilm
Non-steroidal anti-inflammatory drugs (NSAIDs)
Anidulafungin
XTT
Synergism
Candida species

ABSTRACT

Candida spp. are responsible for many biomaterial-related infections; they give rise to infective pathologies typically associated with biofilm formation. We recently reported that the echinocandin anidulafungin (ANF) showed a strong in vitro activity against both planktonic and biofilms cells. Herein, we report the antifungal activities of ANF alone and in association with some non-steroidal anti-inflammatory drugs (NSAIDs) against nine *Candida* strain biofilms: four *Candida albicans*, two *Candida glabrata and* three *Candida guilliermondii*. The activity of ANF was assessed using an in vitro microbiological model relevant for clinical practice. ANF proved oneself to be active against biofilms cells, and a clear-cut synergism was found against *Candida* species biofilms when ANF was used in combination with three NSAIDs: aspirin, diclofenac, ibuprofen. The positive synergism against *Candida* spp. of ANF in association with aspirin or the other NSAIDs proved to be a very effective antifungal treatment (FICI <0.5). These results may provide the starting point for new combination therapies of ANF with NSAIDs against *Candida* biofilm pathologies.

1. Introduction

Some pathogenic *Candida* species cause serious superficial and systemic infections widely recognized in modern clinical practice.[1–3] It is well documented that most *Candida* infections involve biofilm formation[4,5] on implanted devices (indwelling catheters) and tissue surfaces, which facilitates adhesion of the yeast to the host surface or to an associated prosthesis, such as a denture or intravascular catheter.[6] Opportunistic yeast infections such as candidiasis or *Candida* biofilm particularly occur in immunocompromised patients;[7] thus, the treatment of these infections represents a serious problem for contemporary medicine.[8] Nowadays, only a few antifungal agents are available, and their effectiveness is not always optimal as many of them can cause toxicity and resistance.[9–13] Several research works evidenced *Candida* species as planktonic or sessile cells (biofilms) to be strongly resistant to a wide spectrum of conventional antifungal agents, for example azoles. In particular, *Candida* biofilms are reported to be resistant to the new triazoles, voriconazole and ravuconazole, which have an extended spectrum of activity against many azole-resistant organisms as well as fungicidal, rather than fungistatic, activity.[14,15] The failure of antifungal therapy leads to chronic infections that may be cured only by surgery and/or removal of implants. Actually, therapy based on combination of drugs could represent a promising perspective to definitively solve this important aspect.[16–18] Echinocandins (ECs) are antifungal agents that inhibit the synthesis of 1,3-β-D-glucan, a key component of the cell walls of several pathogenic fungi. They act against *Candida* spp. biofilm-associated infections, which are frequently refractory to conventional therapy.[19–21] Several studies have been carried out, mainly on the use of ECs, acting on *Candida albicans* and *C. non-albicans* biofilms.[22,23] A comprehensive report, which compares the in vitro activities of three ECs (anidulafungin, caspofungin and micafungin) against biofilms formed by different non-*Candida* species showed that anidulafungin (ANF), a semi-synthetic

Abbreviations: ANF, anidulafungin; ASA, aspirin; COX, cyclo-oxygenase; DFCN, diclofenac; DMSO, dimethyl sulfoxide; DO, optical density; ECs, echinocandins; FIC, fractional inhibitory concentration; FICI, fractional inhibitory concentration indice; IBF, ibuprofen; MIC, minimum inhibitory concentration; NSAID, non-steroidal anti-inflammatory drug; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide.

* Corresponding authors at present address: Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari 'Aldo Moro', Via E. Orabona 4, 70125 Bari, Italy. Tel.: +39 080 5442745; fax: +39 080 5442724.

E-mail addresses: alessia.catalano@uniba.it (A. Catalano), alessia.carocci@uniba.it (A. Carocci).

http://dx.doi.org/10.1016/j.bmc.2016.01.026
0968-0896/

lipopeptide, had the better efficacy of the three antifungal drugs studied.[24–26] This compound has demonstrated antifungal activity against many amphotericin B-resistant *Candida* spp., too. Recently, we reported that ANF showed a strong in vitro activity against both planktonic and biofilms cells and we confirmed that high ANF concentrations might establish paradoxical growth effect in *C. albicans* and *Candida tropicalis* biofilms.[27] Among the strategies to eradicate fungal biofilms of different *Candida* spp., the use of ECs in combination with other antifungal agents has been proposed.[28] We previously reported the in vitro synergy tests of ANF with other antifungal agents.[29] It has been demonstrated that mammalian cells and pathogenic fungi as *Criptococcus* or *Candida* have the capacity to produce prostaglandins directly or by synthesis from exogenous arachidonic acid.[30–34] Prostaglandin are small lipid molecules with some different activities for the mammalian metabolism such as the modulation of immune response. Thus, drugs able to inhibit prostaglandin synthesis, as the well known non-steroidal anti-inflammatory drugs (NSAIDs), may play an important biochemical role that could affect prostaglandins fungal metabolism. Alem and Douglas clarified the inhibitory effect of some NSAIDs (aspirin, diclofenac, and etodolac) on *Candida* biofilms: diclofenac sodium had the greatest inhibitory impact on the growth of *Candida* biofilms.[35] The significant activity of diclofenac against fungal biofilms was successively confirmed.[36–38] Diclofenac seems also to potentiate the in vivo activity of caspofungin against *C. albicans* biofilm.[39] Moreover, a combination of fluconazole and NSAIDs results in synergistic activity against *C. albicans*.[40,41] On the other hand, in our knowledge, no studies are available about the combination of ANF with currently used NSAIDs against *Candida* spp. biofilms. Our research work was focused on the synergistic in vitro effect of ANF combinations with NSAIDs (aspirin, diclofenac, ibuprofen) against nine *Candida* ATCC strains as biofilms. Since colonization often begins with the appearance of a biofilm, herein we describe a quantitative method for the determination of the inhibitory percentage toward the control and fractional inhibitory index for the synergistic interaction, in order to evaluate this effect. To highlight the effectiveness of associations of NSAIDs with ANF in vitro against different *Candida* strain biofilms, chequerboard method has been used.[42,43] These combinations should improve the management of *Candida* biofilm-associated infections disturb the biofilms and avoid the emergence of resistance.[44]

2. Results and discussion

Three NSAIDs (aspirin, diclofenac, ibuprofen) and the antifungal echinocandin ANF were evaluated on a large panel of yeasts. The most interesting results were those found against nine *Candida* strain biofilms and are reported in Tables 1 and 2. The effect of the combination of ANF with each of the three NSAIDs was also evaluated. MIC value of ANF against all strains was found to be 2 μg/ml in our described experimental conditions, while the strains were susceptible to NSAIDs at concentrations ranging between 0.2 and 100 mM. The inhibition of the biofilms growth by aspirin was more evident at concentrations ranged between 0.2 mM and 1 mM; after 24 h of incubation with 1 mM of aspirin, for considered strains (*C. albicans* ATCC 90028, *C. albicans* ATCC 24433, *C. guilliermondii* ATCC 6260, *C. guilliermondii* ATCC a410), biofilm activity was considerably reduced (44.7%, 48.7%, 54.1%, 43.2%, respectively, Table 2). Ibuprofen showed a lower inhibition activity than aspirin against *C. albicans* strain biofilms (ranging between 16.3% and 25.6% at 1 mM), while its activity against *C. glabrata* and *C. guilliermondii* was more pronounced (ranging between 53.2% and 64.4% at 0.2 mM). The other NSAID diclofenac inhibited the tested biofilms to a lesser level at 100 mM. ANF inhibited biofilms but did not cause a significant decrease in the

Table 1
Non steroidal anti-inflammatory drugs (NSAIDs) and anidulafungin—fractional inhibitory concentration (FIC) and FIC indices (FICI)

	Aspirin				Diclofenac				Ibuprofen			
	MICo	MICc	FIC	FICI	MICo	MICc	FIC	FICI	MICo	MICc	FIC	FICI
Candida albicans ATCC 10231												
NSAID (mM)	0.20	0.01	0.05	0.10	100	5.0	0.05	0.10	1.0	0.05	0.05	0.25
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.10	0.05		2.0	0.4	0.20	
Candida albicans ATCC 90028												
NSAID (mM)	1.0	0.01	0.05	0.10	100	5.0	0.05	0.10	1.0	1.0	0.10	0.30
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.10	0.05		2.0	0.40	0.20	
Candida albicans ATCC 24433												
NSAID (mM)	1.0	0.10	0.10	0.15	100	5.0	0.05	0.10	1.0	1.0	0.10	0.30
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.10	0.05		2.0	0.40	0.20	
Candida albicans 17a18												
NSAID (mM)	0.20	0.01	0.05	0.10	100	5.0	0.05	0.10	1.0	0.05	0.05	0.25
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.10	0.05		2.0	0.4	0.20	
Candida glabrata ATCC 15126												
NSAID (mM)	0.20	0.01	0.05	0.10	100	20	0.20	0.30	0.20	0.04	0.20	0.25
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.20	0.10		2.0	0.10	0.05	
Candida glabrata 18a10												
NSAID (mM)	0.20	0.01	0.05	0.10	100	20	0.20	0.30	0.20	0.04	0.20	0.25
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.20	0.10		2.0	0.10	0.05	
Candida guilliermondii ATCC 6260												
NSAID (mM)	1.0	0.10	0.10	0.15	100	40	0.40	0.45	0.20	0.02	0.10	0.30
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.10	0.05		2.0	0.40	0.20	
Candida guilliermondii a83												
NSAID (mM)	0.20	0.01	0.05	0.10	100	20	0.20	0.30	0.20	0.04	0.20	0.25
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.20	0.10		2.0	0.10	0.05	
Candida guilliermondii a410												
NSAID (mM)	1.0	0.10	0.10	0.15	100	40	0.40	0.45	0.20	0.02	0.10	0.30
Anidulafungin (μg/ml)	2.0	0.10	0.05		2.0	0.10	0.05		2.0	0.40	0.20	

MICo = MIC of an individual sample; MICc = MIC of an individual sample at the most effective combination; FIC = fractional inhibitory concentration (see text); FICI = FIC of NSAID + FIC of anidulafungin.