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Short Communication

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit growth of yeast pathogens

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Studies on yeasts exposed non-steroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid (aspirin) as potential anti-mitochondrial antifungals. In this study, various NSAIDs were tested for antifungal activity on the human yeast pathogens *Candida albicans* and *Cryptococcus neoformans*. Our results suggest a dual action for these drugs, that is, antifungal as well as anti-inflammatory. These results could be useful in the treatment of fungal infections.

Key words: Acetylsalicylic acid, antifungal, anti-inflammatory drugs, anti-mitochondrial, *Candida albicans*, *Cryptococcus neoformans*, *Eremothecium ashbyi.*

INTRODUCTION

Extensive studies conducted by the Kock group in South Africa showed that non-steroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid (aspirin), target yeast growth and especially structures with elevated mitochondrial activity (Kock et al., 2003, 2007). This NSAID also inhibits mitochondrial activity in mammalian cells (Somasundaram et al., 2000; Norman et al., 2004). However, apart from aspirin, the inhibitory effect of other NSAIDs on the human pathogens *Candida albicans* and *Cryptococcus neoformans* has not been reported before.

MATERIALS AND METHODS

Strains used and cultivation

C. albicans UOFS Y-0198 (isolated from a patient with interdigital mycosis) and *C. neoformans* var. *neoformans* UOFS Y-1378 (isolated from a human bone lesion) were used in the study and are preserved at the UNESCO Mircen Culture Collection, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa. All yeasts were streaked out on yeast-malt (YM) agar (Wickerham, 1951) and cultivated at 37°C in Petri dishes for 48 h.

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Bio-assay preparation

Bio-assays of *C. albicans* and *C. neoformans* were separately prepared as described by Kock et al. (2009). Cells were scraped from YM-agar grown cultures and suspended in sterilized distilled water (dH_2O) from where 0.2 ml were streaked out on YM-agar (0.5% m/v agar) to produce a uniform lawn completely covering the agar surface. A well of 0.5 cm in diameter and depth was constructed aseptically in the middle of the agar plate followed by the addition (46 µl) of the following anti-inflammatory compound solutions: Aspirin, Diclofenac, Diflunisal, Fenoprofen, Flufenamic acid, Ketorolac, Meclofenamic acid, Methyl salicylate, Naproxen, Salicylamide, Sulindac (Sigma-Aldrich, Steinheim, Germany), Benzoic acid (The British Drug Houses Ltd., Poole, England) – compound concentration: 8% m/v in ethanol (dissolved in 96% ethanol; Merck, Gauteng, South Africa). In addition, controls were constructed by the addition of similar amounts of only 96% ethanol to wells. All plates were incubated at 37°C for 48 h. Since the bio-assay has been evaluated as a qualitative screen for compounds with specialized antifungal activity, no attempts were made at this stage to determine minimum inhibitory concentrations (MICs).

RESULTS AND DISCUSSION

Previous studies revealed that *C. albicans* and *C. neoformans* produce aspirin-sensitive oxylipins which is indicative of mitochondrial activity (Kock et al., 2007). In these studies, treatment of strains with Aspirin inhibited not only oxylipin production but also growth.

Table 1. The effects of non- steroidal anti-inflammatory drugs (NSAIDs) on growth of the human pathogens, *Candida albicans* UOFS Y-0198 and *Cryptococcus neoformans* var. *neoformans* UOFS Y-1378.

Compounds Tested	*C. albicans*	*C. neoformans*
Anti-inflammatory (8% m/v)		
Aspirin	+	+
Benzoic acid	+	+
Diclofenac	+	+
Diflunisal	+	+
Fenoprofen	+	+
Flufenamic acid	+	+
Ketorolac	-	-
Meclofenamic acid	-	-
Methyl salicylate	+	+
Naproxen	-	+
Salicylamide	+	+
Sulindac	-	-
Control		
Ethanol (solvent)	W	w

+, growth inhibited, **-**, growth not inhibited, w, weak growth inhibition.

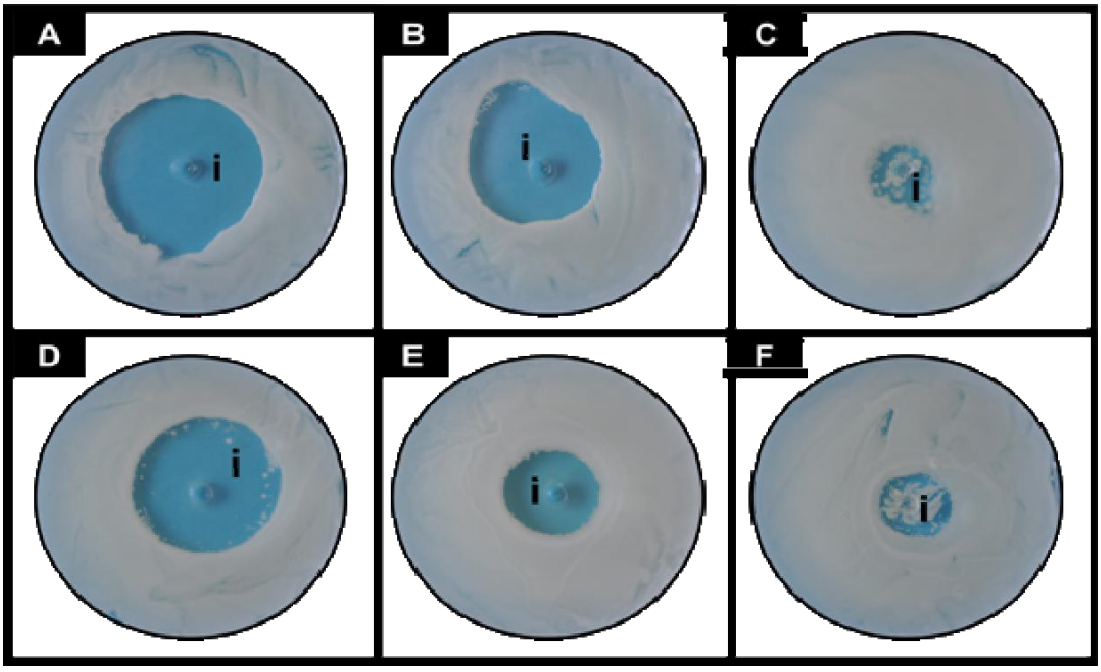

Figure 1. Bio-assays of *Cryptococcus neoformans* var. *neoformans* UOFS Y-1378 (A to C) and *Candida albicans* UOFS Y-0198 (D to F) displaying the effects of different non-steroidal anti-inflammatory drugs (NSAIDs) on growth. (A, D) Diclofenac (8% m/v), (B, E) Aspirin (8% m/v) and (C, F) Ethanol control. i, inhibition zone.

Immunofluorescence microscopy revealed that these oxylipins were selectively located in hyphae as well as vegetative cells of *C. albicans* and *C. neoformans* respectively (Deva et al., 2001; Sebolai et al., 2008).

When the NSAIDs, Aspirin and Diclofenac all dissolved in ethanol (Table 1) were applied to the bio-assays of *C. albicans* and *C. neoformans*, growth was inhibited in both yeasts (Figures 1A, B, D and E). Here Diclofenac was

shown to be more potent than Aspirin in both *C. albicans* (Figures 1A and B) and *C. neoformans* (Figures 1D and E). Similar results were reported for the plant pathogen *Eremothecium ashbyi* which suggests that these NSAIDs are also anti-mitochondrial (Kock et al., 2009; Ncango, 2011). In the latter yeast, NSAIDs have been demonstrated to inhibit mitochondrial activity probably -oxidation and consequently growth (Kock et al., 2009). Here the sexual stage is more susceptible to anti-mitochondrial compounds since sufficient energy is needed to affect sporulation.

Strikingly, most NSAIDs tested inhibited growth of the yeast pathogens *C. albicans* and *C. neoformans* with the exception of Ketorolac, Meclofenamic acid and Sulindac while Naproxen inhibited growth of *C. neoformans* but not that of *C. albicans* (Table 1). Interestingly, all of these NSAIDs inhibited growth as well as sexual reproduction of *E. ashbyi* thereby implicating anti-mitochondrial activity for these dual purpose anti-inflammatory drugs (Kock et al., 2009; Ncango, 2011).

The antifungal action and MICs of NSAIDs in general should now be determined on eukaryotic pathogens to evaluate the potential of these drugs for use as antifungals. Especially since it has been shown that the primary active metabolite of Aspirin, salicylate, inhibits mitochondrial -oxidation through (i) the enzyme 3-hydroxy (OH) acyl- CoA dehydrogenase (Glasgow et al., 1999) and (ii) by inducing changes in mitochondrial energy production through the uncoupling of oxidative phosphorylation (Somasundaram et al., 2000; Norman et al., 2004). However, the prolonged use of NSAIDs as antifungals should be cautioned against since some may result in gastrotoxicity (Wolfe et al., 1999). The application of NSAIDs to prevent human fungal disease is at present a subject of intense investigation. Here the daily continuous use of non-toxic, low dose Aspirin and Diclofenac in the prevention of opportunistic fungal infections in immune compromised patients, is studied (Davis et al., 2009).

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