Carbohydrate Derived Fulvic Acid (CHD-FA) is a Novel Antifungal Product

Leighann Sherry, Colin Murray & Gordon Ramage
Infection and Immunity Group, Glasgow Dental School, Faculty of Medicine, University of Glasgow, 378 Sauchiehall Street, Glasgow, G2 3JZ, UK.

Background

The oral cavity contains a diverse and complex microbial ecology, which includes yeasts growing as biofilms. Oral candidiasis is a characteristic infection commonly observed in immunocompromised patients, especially those with an HIV infection and causes oral discomfort, pain and taste distortion [1]. Despite Candida albicans being a commensal of the oral cavity in 30-40% of healthy individuals, it is also the most common fungal pathogen in humans [2]. In addition to infections in the oral cavity, C. albicans is also responsible for infections in the ICU; bloodstream infections caused by this organism are associated with morbidity and mortality worldwide.

Alternative non-toxic products with antifungal activity are desirable particularly if naturally derived. Fulvic acid is the predominant compound of humic acids, and have been historically used as natural remedies. However, the purity of these compounds was limited. Fulhold (www.fulhold.com) have a patented technology to produce fulvic acid from plant material through a bioreactor process, producing purified carbohydrate derived fulvic acid (CHD-FA), which is illustrated in Figure 1. Previous investigations have shown this to be effective against a range of bacterial species [3]. However, no studies have tested CHD-FA against microorganisms associated with oral candidiasis, and none have looked at its efficacy against biofilms.

Aims

The aim of this study was to evaluate the antimicrobial activity of CHD-FA against a range of Candida albicans isolates associated with mucosal and systemic candidiasis. In addition, the toxicological properties of the compound were evaluated.

Methods

Susceptibility testing

• The MIC of CHD-FA was determined by standard CLSI broth microdilution method in RPMI media.

• Isolates were standardised to 1x10^8 and 1x10^9 cells/mL for planktonic and sessile cells, respectively.

• Following the XTT assay, 0.5% w/v of crystal violet was added to the plate. Wells were washed and ethanol was used to remove any remaining crystal violet. Absorbance was read at 570nm.

Ala-Nap uptake assay

• Isolates standardised to 1 x 10^8 and 1 x 10^7 cells for sessile and planktonic respectively, in assay buffer solution, and added to a black flat bottomed microtitre plate.

• Ala-Nap was added at a final concentration of 64mg/L, and the relative fluorescence measured for every 30s for 1 hour.

Toxicity OKF6-TERT cells were used to evaluate the toxicity of CHD-FA.

• Cells were grown to confluence in 96 well plates in defined media prior to exposure (2 and 30 min) to concentrations of CHD-FA.

• Cellular viability was assessed using an Alamar Blue assay.

Scanning Electron Microscopy (SEM)

• C. albicans 3153A biofilms were grown on Thermoxon coverslips and treated overnight with 0.25% CHD-FA.

• Fixed and dried samples were sputter-coated with gold and viewed under a JEOL JSM-6400 scanning electron microscope.

Results

Figure 1: Proposed structure of CHD-FA

Figure 2: Biofilm disruption of 40 C. albicans isolates treated with CHD-FA

Figure 3: Efflux pump activity in planktonic C. albicans cells

Figure 4: Efflux activity after a 2 minute exposure to CHD-FA

Figure 5: Toxicity of CHD-FA on oral epithelial cells (OKF6-TERT)

Table 1: Minimum inhibition concentrations ± efflux pump inhibitor

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<tr>
<th></th>
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<td>CHD-FA</td>
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<td>0.25</td>
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<td>0.016-0.31</td>
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Table 1: Minimum inhibition concentrations ± efflux pump inhibitor

Discussion

CHD-FA not only inhibits planktonic and sessile C. albicans cells, but also has fungicidal activity at a similar concentration. When CHD-FA is used at 0.25%, only a few isolates are not inhibited proving that CHD-FA is effective against a variety of C. albicans isolates. Despite this, CHD-FA has shown to be ineffective at biofilm disruption.

Despite C. albicans planktonic and sessile cells exhibiting efflux pump activity, it has been shown that this resistance mechanism is not important when these cells are treated with CHD-FA, indicated by no change in inhibitory concentration when these pumps are impeded. This is in comparison to other commercial antimicrobials which show a decrease in inhibitory concentration. This data suggests that CHD-FA does not work internally.

CHD-FA toxicity against an oral epithelial cell line was assessed and results show CHD-FA to be non-toxic after a 2 min exposure, however, as exposure time increases the viability of OKF6-TERT cells decreases.

Therefore, it can be concluded that naturally derived CHD-FA has potential as an alternative to commercially available oral mouthwashes for the treatment of oral candidiasis. However, further research is required to determine the mode of action of CHD-FA, and also whether the compound can be used in other situations i.e. candidaemia treatment, denture cleanser, disinfectant etc.

References

