Effective antimicrobial activity of HICA and alpha-mangostin against the multi-species bacterial-fungal biofilms.

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Background: Multi-species bacterial-fungal biofilms are common in a spectrum of infectious diseases. Such biofilms resist antimicrobial treatment and alternative approaches are warranted. Disinfecting agents such as chlorhexidine (CHX) and sodium hypochlorite (NaOCl) are used as adjunctive or alternative therapy, but they can be toxic to human tissue. D,L-2-hydroxyisocaproic acid (HICA) is a highly biocompatible, broad-spectrum bactericidal and fungicidal agent. It has also been shown to have activity against candidal biofilms. 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one (alpha-mangostin) has also been shown to possess antimicrobial activity against a wide range of microorganisms, but its efficacy against biofilms has not been studied. The aim of this study was to determine the activity of HICA and alpha-mangostin on preformed bacterial-fungal multi-species biofilms in vitro, and to visualise their impact on biofilm structure.

Material/methods: Single species of Candida albicans, Enterococcus faecalis, and Lactobacillus rhamnosus, Streptococcus gordonii, dual species (C. albicans and E. faecalis 1:100, respectively) and multi-species (all species 1:1:1:1) biofilms were grown on polystyrene coverslips in RPMI for 48 hours. Following this, the biofilms were exposed to 5% HICA or 0.2% alpha-mangostin for 24 hours. 2% CHX and 2.5% NaOCl were used as positive controls and RPMI as the negative control. 10-fold concentrations of planktonic MIC₅₀ determined by using a standard microdilution method were used.
The metabolic activity of the biofilms after exposure was measured using the XTT assay, and biofilms were visualised using fluorescent BacLight® LIVE/DEAD staining.

**Results:** 50 g/mL of HICA was cidal against planktonic bacteria and *Candida*. 0.008 g/mL of alpha-mangostin was cidal against planktonic bacteria and 1 g/mL for *Candida*. Both alpha-mangostin and HICA were most active against *L. rhamonosus* biofilms (99% and 98% inhibition of metabolism, respectively) and least active against *Candida* biofilms (78% and 42% inhibition, respectively). Alpha-mangostin and HICA also had good activity against dual species biofilms (97% and 80%, respectively). Alpha-mangostin had better activity against multi-species biofilms than HICA (93% inhibition vs 46% inhibition). NaOCl inhibited the metabolic activity of single and multi-species biofilms by at least 98%. HICA and alpha-mangostin exposure reduced the number of cells in the *C. albicans* biofilms and no hyphae were observed. Exposure to HICA or alpha-mangostin reduced the number of viable cells in the biofilms as assessed by the BacLight® LIVE/DEAD staining.

**Conclusions:** Both HICA and alpha-mangostin inhibited the metabolic activity of bacterial-fungal biofilms effectively. HICA is less active against the bacterial biofilms than alpha-mangostin but it is highly biocompatible. The anti-biofilm activity of alpha-mangostin is comparable to that of highly active but toxic NaOCl and thus has potential as a novel antimicrobial agent. Alpha-mangostin could be used to treat superficial infections or as an irrigant in root canal treatment.