In Vitro Antimicrobial Activity of Hypochlorous Acid (Hocl) on Some Selected *Candida* Species and Oral Microflora

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Hypochlorous acid (HOCl) is used as an antiseptic and a disinfectant. However, antimicrobial activity (AMA) of HOCl on *Candida* species (*CS*) and oral microflora (OM) is poorly investigated. This study aimed to evaluate the AMA of HOCl on some selected *CS* and OM in vitro. AMA of HOCl was tested against standard isolates of *Candida*; *C. albicans* (ATCC 90028), *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 90030) and *C. tropicalis* (ATCC 13803). Further, the effect of HOCl against OM harvested from healthy individuals was also assessed as follows. HOCl produced by Steripower® unit (Japan) was adjusted to different concentrations (200, 100 and 50 ppm). Phosphate buffered saline (PBS) and 0.2% chlorhexidine were used as negative and positive controls. *CS* were cultured in Tryptic Soy Broth (TSB) at 37℃ for 24 h and were harvested by centrifugation, washed and suspended in PBS (10^8 cell/ml; 0.5 McFarland). OM was harvested from 5 healthy volunteers after rinsing the mouth with sterile PBS (10 ml) for 30 seconds. Resultant oral rinses were concentrated into 1ml PBS with centrifugation. Afterwards, 0.1 ml of the *Candida* suspension or concentrated oral rinse was mixed with 0.9 ml of HOCl or control solutions for 1 min. Subsequently, 0.1 ml of the resultant solutions of *Candida* or OM exposed to HOCl were inoculated on to Sabourauds dextrose agar or blood agar plates respectively. *Candida* cultures were incubated at 37℃ aerobically. OM was incubated at 37℃ both aerobic and anaerobic conditions. All cultures were observed up to 48 h for microbial growth. Each experiment was quadruplicated. *CS* (*C. albicans*, *C. glabrata, C. krusei*, *C. parapsilosis*, and *C. tropicalis*) and OM of healthy volunteers exposed to HOCl (200, 100, 50 ppm) and 0.2% chlorhexidine for 1min resulted no growth on solid agar after 48 h incubation at 37℃. Exposure to HOCl (200, 100, 50 ppm) and 0.2% chlorhexidine for 1 min completely inhibited all the tested *CS* (*C. albicans*, *C. glabrata, C. krusei*, *C. parapsilosis*, and *C. tropicalis*) and the OM of healthy volunteers suggesting a remarkable AMA. This could be attributed to the fact that HOCl dissociates into H^+ and OCl^- that are capable of denaturing and aggregating microbial proteins resulting in killing a broad spectrum of microorganisms in a brief exposure. HOCl (200, 100, 50 ppm) may be used as an effective antiseptic/disinfectant similar to 0.2% chlorhexidine against *C. albicans*, *C. glabrata, C. krusei, C. parapsilosis*, *C. tropicalis* and OM. Further studies are recommended to assess the in vivo effects of HOCl to explore its application for oral hygiene.

Keywords: AMA, *candida* species, HOCl, oral hygiene, oral microflora