

ANDREA ARDIZZONI¹, GIORGIA BOARETTO², EVA PERICOLINI¹, ALESSANDRA CAPEZZONE DE JOANNON³, LUCIA DURANDO³, LORELLA RAGNI³, ARIANNA SALA¹, ELISABETTA BLASI¹

Evaluation of benzydamine effects on *Candida albicans* adhesion, biofilm formation and persistence onto abiotic surfaces

¹Department of Surgical, Medical, Dental and Morphological Sciences with interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy

²Graduate School of Microbiology and Virology, University of Modena and Reggio Emilia, Modena, Italy

³Angelini RR&D (Regulatory, Research, & Development), Angelini Pharma S.p.A., Rome, Italy

Introduction. *Candida albicans* is the most abundant yeast colonizing the oral cavity. It behaves as an opportunistic pathogen, causing mucosal infections mainly in immunocompromised individuals; in addition, it is often associated to patients suffering from diabetes, oral cancer and terminally ill conditions. Benzydamine hydrochloride is a non-steroidal and anti-inflammatory agent. It has been included in the formulation of several mouthwashes because endowed with analgesic and anesthetic properties. Since benzydamine exerts antibacterial and antifungal activity *in vitro*, we assessed if this molecule could affect *C. albicans* virulence traits, such as adhesion, biofilm formation and persistence on abiotic surfaces.

Materials and Methods. *C. albicans* CA1398, carrying the bioluminescence ACT1p-gLUC59 fusion product, was employed. Firstly, fungal cells were exposed for 1', 5', or 15' to 4 different benzydamine concentrations (0.075%, 0.15%, 0.3% and 0.6%) and then tested for their capacity to adhere to plastic (90' incubation) or to form a biofilm (24h assay). Secondly, 24 and 48h-old biofilms were exposed to the same concentrations of benzydamine and for the same times in order to assess biofilm persistence and regrowth. Benzydamine effects were quantified by measuring, in parallel, metabolically active fungal cells (bioluminescence assay) and viable cells (Colony Forming Units assay).

Results. Benzydamine impaired ability to adhere to plastic and to form biofilm, in a dose-dependent fashion; such effects could be ascribed to a direct effect of benzydamine on *Candida* viability only when using the highest dosage. Moreover, benzydamine caused a dose-dependent decrement in the viability of *Candida* cells embedded in biofilm, no matter whether a 24h- or a 48h-old sessile community was tested.

Discussion and Conclusions. Benzydamine not only impairs *C. albicans* biofilm formation, profoundly affecting the initial step of fungal cell adhesion to abiotic surfaces, but it is also able to counteract persistence and regrowth of a preformed biofilm. The capacity of benzydamine to affect *C. albicans*, a fungus responsible of oral diseases in several categories of susceptible subjects, makes this molecule a very interesting tool for both prevention and treatment of oral candidiasis. Studies employing benzydamine-containing mouthwashes will be carried out, in order to assess and compare the anti-*Candida* effects of different commercial products.