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New denture adhesive containing miconazole nitrate polymeric microparticles: Antifungal, adhesive force and toxicity properties

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ABSTRACT

Objective. The purpose of this study was to develop a new oral drug delivery system by incorporating polymeric miconazole nitrate (MN) microparticles on an experimental antifungal denture adhesive (DA).

Methods. Spray drying Eudragit L-100 (E) and Gantrez MS-955 (G) MN-microparticles were incorporated in DA. DAE1, DAG1, DAEG1, DAE2, DAG2, DAEG2 groups were obtained from the combination of polymers used in MN-microparticles (E, G and EG) and concentration of MN into DA (1, for 1% and 2, for 2%). DA with 2% pure MN (DAM) and DA without microparticles or drug (DACT) were both control groups. All groups were evaluated to determine microbiological assay, adhesive force and toxicity. Minimum inhibitory concentration (MIC) against *Candida albicans* was performed by broth micro-dilution and agar dilution methods in extract of DAs and conventional gel form (Daktarin®). Adhesive load testing was made between acrylic resin samples on a universal testing machine after immersion in water. The toxicity of several dilutions of DAs was performed with *Artemia salina* bioassay after 24 and 48 h. Data of adhesive force were evaluated with two-way ANOVA and Bonferroni tests ($\alpha = 0.05$).

Results. The concentration required to kill 50% (LC50) was determined using the Provit analysis. DA with polymeric microparticles and pure drug presented MIC between 1.25–5 μg/mL similar to MIC values of DAM. DAEG2, DAEG1, DAG20 showed the most actives against C. *albicans*. The best adhesive properties were exhibited by DAEG2, consisting of high initial adhesive force which was maintained for up to 6 h. The extracts of all DA presented low or not toxicity at 24 and 48 h.

Significance. DA containing 2% of MN loaded in microparticles made by Gantrez MS-955 alone or combined with Eudragit L-100 produce effective antifungal activity, good adhesive force,

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and no toxicity effect being a promising therapeutics for removable denture wearers affected by denture stomatitis.

1. Introduction

Denture stomatitis (DS) is the erythematous inflammatory condition more frequently found in patients with removable dentures [1–3]. When affected patients have symptoms, they often complain of a burning sensation, unpleasant taste and discomfort. In most cases, DS can occur without any symptoms, and individuals without knowledge of the disease are commonly found. Classically, estimation of clinical disease severity has been based on different clinical manifestations categorized according to the severity of inflammation [4]. Independent of the contribution factors like age, systemic disease, smoking, wearing complete dentures during sleep, reduced salivary flow, trauma caused by the lack of retention and stability of the prosthesis, the fungus *Candida albicans* is considered as the primary etiological factor of DS. Despite the prevalence of this microorganism [5,6], non-albicans species (e.g., *Candida glabrata*, *Candida tropicalis*, *Candida guilliermondii*, *Candida dubliniensis*, *Candida parapsilosis* and *Candida krusei*) [6–10] can also be responsible for the disease, among other microorganisms [11].

It has been reported resistance of *Candida* species to conventional anti-fungal agents and ineffective therapies. The strategies to solve this problem, specifically in DS, has focused on oral hygienic education [12], phytotherapy [13], controlling the factors that may cause trauma to the mucosa and searching developing new pharmaceutical formulations [14–16].

The use of denture adhesives (DA) for the improvement on retention and stability of removable dentures it is very common [17]. These materials are available in cream, strip, powder and cushion formulations and can be classified as muco-adhesive compositions. Usually, DA cream formulation is made of a mixture of water-soluble polymers that provide muco-adhesive characteristics and base components that facilitate the placement of it [18,19]. Muco-adhesion is a promising strategy for adequate and prolonging drug release of the conventional oral medications [20,21]. Muco-adhesive formulations can be attached to the mucosa for extended periods of time, allowing an increase of drug bioavailability [22]. They have offered better control of antifungal activity as compared to a marketed gel formulation [15].

Some authors have reported a retentive effect of DA of 6–8 h in denture wearers [17]. This period of use and the muco-adhesive properties of DA could be employed for the drug release of antimicrobial agents such as MN in the therapy of DS. Few studies have investigated this option [23,24], although some DAs have already showed antimicrobial efficacy against oral microorganisms associated to oral malodor [25].

Miconazole nitrate (MN) is one of the first line broad-spectrum triazole antifungal agent used [26] to treat superficial mucosal candidiasis. MN oral gel is the more widely available formulation worldwide. Traditional oral gel has short contact time in the target area [27,28] leading to high initial concentrations of MN, followed by low levels, requiring multiple administrations each day [22]. Furthermore, MN has very low aqueous solubility leading to erratic and unpredictable bioavailability [29]. Drug delivery devices containing MN have been developed for topical treatment of oral mycoses in formulations such as gel [29], chewing gum [30], bioadhesive films [31] and buccal tablets [32].

In order to increase bioavailability and to achieve the therapeutic goals, spray dried polymeric microparticles of MN presenting improvements in drug solubility and antifungal activity against *C. albicans* have been developed [33]. These microparticles were formulated using Eudragit L-100 polymer, which has a favorable release profile on oral pH (pH-sensitive) [34,35] and the muco-adhesive polymer Gantrez MS-955, recognized to extend the residence time of drugs on several membranes [36,37].

The purpose of the present study was to make preliminary in vitro tests (antimicrobial against *C. albicans*, adhesive force calculation and *Artemia salina* toxicity bioassay) of drug delivery systems consisting of antifungal-DA formulations aiming at DS treatment. These systems were obtained by the incorporation of pH-sensitive (Eudragit L-100) and muco-adhesive (Gantrez MS-955) polymeric MN-microparticles on the composition of an experimental DA.

2. Material and methods

2.1. Experimental design

The influence of incorporated spray dried microparticles of MN made with the polymers Eudragit L-100 (E), Gantrez MS-955 (G) or the combination of both (EG) and drug concentrations (10 and 20%) in an experimental cream denture adhesive (DA) was studied. These microparticles were developed and proven adequate about drug loading and encapsulation efficiency [33]. In the present work, the experimental DA was prepared by a mechanical process, mixing the active water-soluble polymers and base components (Table 1). Microbiological activity against *C. albicans*, adhesive force (tensile test) and toxicity (against *A. salina*) were investigated to study the DA added or not with the microparticles. A commonly used MN gel form Daktarin® was used as control when necessary.

2.2. Spray-drying polymeric microparticles of MN

Weighted amounts of polymers (E, G, or EG) and MN was dissolved in ethanol or/and water. The solution obtained were then mixed and magnetic stirred for 6 h. Spray-drying was performed using a MSD-0.5 mini spray dryer (Labmaq, Ribeirão Preto, Brazil) with a standard 0.5 mm nozzle. Outlet tempera-