Evaluation of antifungal activity of miconazole-loaded nanostructured lipid carriers (NLC) formulations for oral mucosal administration

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Abstract Miconazole is a broad-spectrum antifungal agent of the imidazole group [1] that has been widely used for the treatment of oral candidiasis [2], an opportunist fungal infection of the oral cavity caused by an overgrowth of *Candida* species. *C. albicans* is the most prevalent specie isolated from oral cavity [3, 4]. To treat oral candidiasis, miconazole is usually prescribed in the form of oral gel [5]. However, miconazole has poor aqueous solubility requiring the development of alternative drug delivery systems able to improve its antifungal activity [6]. Lipid nanoparticles are suitable for this purpose since they can be used as a carrier system for hydrophobic drugs [7]. Other advantages have been described for these carriers, such as (i) good biocompatibility, since they are composed of lipids similar to the physiological ones [8]; (ii) low risk of acute and chronic toxicity [8]; (iii) improvement of bioavailability [9]; (iv) physical and chemical protection of drugs due to the presence of a solid matrix at body and room temperature [10]; (v) site-specific drug targeting [9] and (vi) controlled drug release [10]. Solid lipid nanoparticles (SLN) were the first generation of lipid nanoparticles, being created in the nineties [11]. However, since their lipid matrix is only composed by a solid lipid, SLN have limited drug loading capacity and can lead to drug expulsion during storage due to the occurrence of lipid polymorphic transitions into perfect crystal lattices [12]. To overcome these limitations, it was developed the second generation of lipid nanoparticles, the nanostructured lipid carriers (NLC). NLC dispersions are made of a blend of a solid lipid with a liquid lipid which provides a higher payload and prevents drug expulsion [11]. Attending to the typical low viscosity of lipid nanoparticles dispersions, the incorporation of these dispersions into semi-solid carriers seems to be crucial for application in the oral mucosa [13]. Hydrogels present a great semi-solid vehicle for nanoparticles intended for drug delivery [14], and are commonly prepared with synthetic polymers (e.g. polyacrylates) that allow a closed contact with the oral mucosa by hydrogen bonds, providing adhesiveness and prolonging the residence time of the dosage form [13, 15]. Thus, hydrogels may contribute to improve the therapeutic performance of the drug delivery system [16].

In this work, the potential of NLC dispersions for the encapsulation of miconazole intending the oral mucosa delivery was investigated. Two placebo NLC dispersions were prepared by ultrasound technique and characterized regarding the particle size, polidispersity index (PI) and crystallinity, in order to selected the most promising formulation for miconazole encapsulation. The composition of these dispersions is presented in Table 1. Based on the lipid matrix of the selected formulation, placebo and drug-loaded NLC dispersions ($NLC_{2b}P$ and $NLC_{2b}M$, respectively) were prepared and stabilized by an aqueous solution containing 2% (w/w) of Tween® 80 and 0.5% (w/w) of benzalkonium chloride as surfactants. Additionally, $NLC_{2b}M$ dispersion, containing 3% (w/w) of miconazole regarding the lipid matrix, was characterized with respect to encapsulation parameters and long term-stability. Afterwards, a semi-solid formulation was prepared, incorporating $NLC_{2b}M$ dispersion in a hydrogel. The antifungal activity of this hydrogel was further compared with a commercial oral gel formulation, containing 2% (w/w) of miconazole. The results of physicochemical characterization revealed that the lipid matrix composed of Gelucire® 43/01 and Miglyol® 812 led to stable NLC dispersions with lower particle size and size distribution (data not shown). Both $NLC_{2b}P$ and $NLC_{2b}M$ dispersions showed a narrow size distribution (PI <0.3) and particles in the nanometric range (about 200 nm). In addition, dispersion of $NLC_{2b}M$ demonstrated an efficient encapsulation of miconazole, which was estimated as higher than 87%, and long-term stability, since no significant differences were observed in particle size and PI after 3 months of storage at 25ºC (data not shown). Thus, as evidenced by Hou et al. [17] and Silva et al. [18], ultrasound technique proved to be effective to prepare lipid nanoparticles dispersions.

Furthermore, the antifungal activity studies showed that miconazole activity was improved when encapsulating in NLC. The minimum inhibition concentration (MIC) of $NLC_{2b}M$ dispersion against *C. albicans* ATCC 10231 was determined through broth microdilution method [19] and was lower than free miconazole (Table 2). In addition, it was observed that the antifungal activity of $NLC_{2b}M$ dispersion was independent of a possible activity of the excipients of the formulation. Further, through agar-well diffusion method [20], it was evaluated the antifungal activity of hydrogel incorporating miconazole-loaded NLC (HG-$NLC_{2b}M$) and compared with the activity of commercial oral gel formulation, by measuring the inhibition zone (Table 3). The encapsulation of miconazole allows us to obtain the same therapeutic effect of commercial gel, using a 17-fold lower dose of miconazole. As a result, hydrogel containing NLC encapsulating miconazole enhance the therapeutic efficacy, emphasizing the potential of the NLC to reduce the amount of drug administrated and, consequently, minimize the undesirable side effects. Therefore, we concluded that the developed NLC-based hydrogel is able to improve the antifungal activity of miconazole and, therefore, it is a suitable vehicle for miconazole delivery.

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Tables

Table 1 - Composition of placebo NLC dispersions.

Dispersion % (w/w)		
Composition	**NLC_1-P**	**NLC_2-P**
Gelucire® 43/01	7,00	7,00
Capryol™ PGMC	3,00	-
Miglyol® 812	-	3,00
Miconazole	-	-
Tween® 80	2,00	2,00
Water	88,00	88,00

Table 2 - MIC (µg/ml) of miconazol and $NLC_{2b}M$ dispersion against *C. albicans* ATCC 10231.

Formulation	**MIC (µg/ml)**
Miconazole	3,125
$NLC_{2b}M$	0,781

Table 3 - Inhibition zones (halo) of HG-$NLC_{2b}M$, commercial oral gel, HG-$NLC_{2b}P$ (incorporating $NLC_{2b}P$ dispersion) and HG (hydrogel without NLC dispersion) against *C. albicans* ATCC 10231.

Formulation	**Miconazole (mg/g)**	**Inhibition zone (mm)**
HG-$NLC_{2b}M$	1,2	25,5 ± 0,9
Commercial oral gel	20,0	24,3 ± 1,4
HG-$NLC_{2b}P$	0,0	11,1 ± 0,6
HG	0,0	0,0 ± 0,0

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