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Water soluble sulconazole-β-cyclodextrin complex: physico-chemical characterization and preliminary pharmacological studies

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Abstract Sulconazole-β-cyclodextrin water soluble inclusion complex was prepared by freeze drying method in distilled water. The formation of inclusion complex between β-cyclodextrin and sulconazole has been studied in a previous work. Preliminary pharmacological studies concerning the antifungal activity showed that the minimal inhibitory concentrations for 90% of the tested strains decreased. Also, the acute toxicity of the sulconazole-β-cyclodextrin complex is smaller comparing with the pure drug, analyzed alone. These results recommend the described conjugates as future promising therapeutic agents.

Keywords Sulconazole nitrate · Cyclodextrin · Antifungal activity · Acute toxicity

Introduction

Sulconazole nitrate ((+)-1-[2.4-dichloro-*b*-[(*p*-chlorobenzyl)-thio]-phenethyl] imidazole mononitrate) (SULC) is an imidazole derivative with in vitro antifungal activity. It is a white to off-white crystalline powder, freely soluble in pyridine, slightly soluble in ethanol, acetone, chloroform, and very slightly soluble in water (1.9 mg/mL) [1]. SULC has a broad-spectrum antifungal activity, it inhibits the in vitro growth of the common pathogenic dermatophytes including *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis*. It also inhibits (in vitro) the organism responsible for *Tinea versicolor*, *Malassezia furfur*. Sulconazole nitrate has also been shown to be active in vitro against *Candida albicans* and certain gram positive bacteria. SULC is used to treat skin infections such as athlete's foot, jock itch, and ringworm [2]. Like all azole antifungals, it inhibits the fungal cytochrome P-450 3-A dependent enzyme 14-alpha demethylase, thereby interrupting the synthesis of ergosterol. Inhibition of this critical enzyme in the ergosterol synthesis pathway leads to the depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth [1]. Azole antifungals can also inhibit many mammalian cytochrome P450-dependent enzymes involved in hormone synthesis or drug metabolism [3].

A modified Draize test showed some cutaneous adverse side effects, due to SULC use including: itching, rash, burning, irritation or stinging, redness [4]. Also SULC has been shown to be embryotoxic, given orally to rats resulted in prolonged gestation and dystocia. Several females died during the prenatal period, most likely due to labor complications [5].

Complexation of sulconazole with cyclodextrin offers the possibility to improve the aqueous solubility of the antifungal drug without modification of its original structure. This may allow a homogeneous delivery system of

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SULC increasing its bioavailability. We synthesized and characterized β-cyclodextrin-sulconazole nitrate (β-CD-SULC) inclusion complexes [6], in order to make it more available for the yeast metabolism, and to reduce consequently the dosage, the treatment period and the gravity of all possible side effects.

Materials and methods

Materials

Sulconazole nitrate (SULC) (Fluka) and β-CD (Aldrich) were used as received. Double distilled water was used throughout the study.

Methods

Preparation of the solid complex

The inclusion complex (C) was prepared by freeze drying method. An aqueous solution containing SULC and β-CD, in a 1:1 molar ratio was frozen by immersion in liquid nitrogen and freeze-dried in a Martin Christ, ALPHA 1-2LD Freeze-Dryer. The aqueous solution was obtained by dissolving 4.34×10^{-4} mol SULC and 4.34×10^{-4} mol cyclodextrin in 25 mL distilled water and stirring it at room temperature for 48 h.

Pharmacological studies

Anti-fungal activity studies

Antifungal activity studies were performed on 32 yeast strains belonging to *Candida* genus, isolated from fungemia episodes from patients hospitalized in Iasi, using the testing method M27-A2 recommended by CLSI to evaluate the antifungal susceptibility of the yeasts.

The yeast strain subcultures were obtained by incubating the initial strains at 350 °C for 48 h. Yeast strain inocula were obtained using stock cultures, by the suspension of five colonies of approximately 1 mm in diameter in saline solution. Pure SULC and β-CD-SULC inclusion complex solutions (6.94 (pure SULC) and 2.05 (complexed SULC) micromoles/mL) in DMSO were diluted to the final concentrations (32; 16; 8; 4; 2; 1; 0.5; 0.125; and 0.0625 μg/mL) in DMSO and each solution was inoculated on the yeast strain inoculum. Suspension density adjustment was performed spectrophotometrically, so that the absorbance of each suspension to correspond to that produced by McFarland standard 0.5 at 530 nm. The final density of the suspensions varied from 1×106 to 2.5×106. These suspensions were diluted 1:100 with sterile saline solution and 1:20 in RPMI 1640-MOPS, so that the final cell density to vary from 0.5×103 to 2.5×103. The witness suspension was obtain by adding 900 μL yeast inoculum to 100 μL RPMI 1640-MOPS DMSO 1:10 dilution. The growth was evaluated by comparing the turbidity from each vial containing the antifungal agent to the turbidity of a 1:4 dilution in RPMI of the witness. Minimal inhibitory concentration (MIC) was calculated as the minimal antifungal agent concentration that caused 50% (MIC50) and 90% (MIC90) growth inhibition.

Acute toxicity studies

Acute toxicity studies were performed on laboratory mice, on nulliparous and nonpregnant healthy young females with age between 8 and 12 weeks old, and weight around 20 ± 0.2 g. The animals were housed individually, respecting the same microclimate (temperature around 22 ± 3 °C, the relative humidity 55% and an alternation 12 h artificial light, 12 h darkness) and feeding conditions. The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing, in order to allow the acclimatization to the laboratory conditions. The administration of the pure and complexed SULC was made in a constant volume of 2 mL/100 g body weight in aqueous solutions [7]. The dose volume was administered by gavage using a stomach tube. The experiments were performed on Wistar SD1 NRM1 White/C57Bi6 mice, offered by the Cantacuzino Institute, Bucharest.

The acute toxicity (LD50) was established using the Dixon and Mood method [7–9]. In order to do that each animal tested was sacrificed and the hepatic, hematological and/or renal anomalies were carefully analyzed at the optical microscope on fixed samples taken from the above mentioned organs.

Results and discussion

As already described in a previous paper, the inclusion complex obtained by freeze drying between SULC and β-CD was characterized and it was proved its formation. The authors observed an improving of the dissolution rate of the pure drug after complexation, making the new obtained compound a proper candidate for therapy [6].

In vitro antifungal activity studies

The data presented in Table 1 indicate that SULC is efficient in antifungal treatments, the growth inhibition is 100% for different *Candida* strains tested in our experiment The determined sensibility rate was 93.73% for all

Table 1 The frequency of MIC of SULC for the 32 tested strains

Concentration of the SULC (μg/mL)	0.5	1	2	4	8	16	32	64
Absolute frequency (n)	19	5	2	0	3	1	0	0
Cumulative frequency (%)	59.37	75	81.25	81.25	90.62	100	100	100

Table 2 In vitro susceptibility of the analyzed 32 strains at pure and complexed SULC (the synthesis of the data)

Antifungal agent	Concentration (micromoles/mL)	Geometric average	MIC_{50} (micromoles/mL)	MIC_{90} (micromoles/mL)
SULC	0.0005–0.034	0.82	0.005	0.017
β-CD-SULC	0.00007–0.0025	0.20	0.00007	0.0012

SULC is efficient in the treatment of the *Candida* species in concentrations higher than 16 μg/mL (0.034 micromoles/mL)

The minimal inhibitory concentration of the antifungal agent required for growth inhibition is reduced in the presence of the cyclodextrin

analyzed stains, only two strains (*C. krusei* and *C. norvegensis*) behaving as resistant fungi. As an example we present the microscopic images of the culture of *C. albicans* on the culture media in the absence and presence of the SULC in concentration of 0.017 micromoles/mL.

The percentage variability of the inactivated strains depends in 83.62% proportion on SULC concentration, the multiple correlation coefficient having the value $R2 = 0.8362$. The regression of the inactivated strains depending on the antifungal substance concentration has the following equation: $y = 0.3468\ln(x) + 0.1908$.

From Table 2 one can see that the inclusion complex β-CD-SULC present a higher antifungal activity comparing with the free drug, due to the increase of the hydrosolubility of the complex comparing with the pure SULC. It can be observed that MIC50 is reduced seven times and MIC90 is reduced 14 times for the complexed SULC comparing with the free drug, indicating also the reduction of the treatment dosage and of the gravity of all side effects. These results are also sustained by the microscopic images of the culture of *C. albicans* on the culture media in the absence and presence of the β-CD-SULC in concentration of 0.0012 micromoles SULC/mL. The growth inhibition is clear in the two images, sustained by the reduction of the fungal cells density on the culture media (Figs. 1, 2).

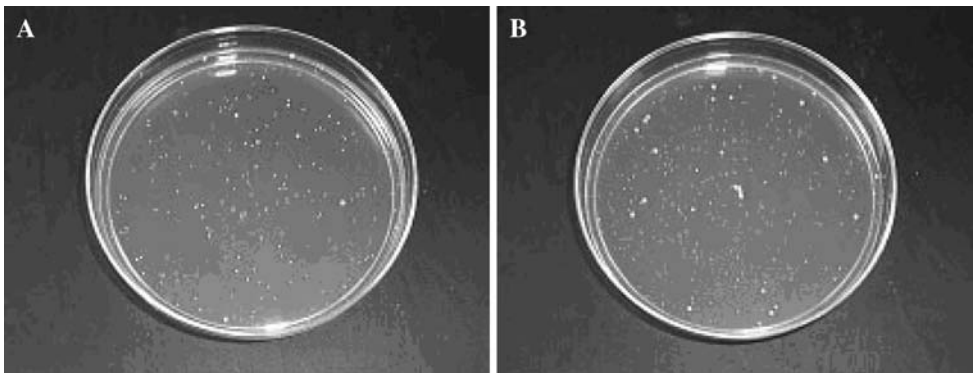

Fig. 1 *C. albicans* culture in Petri dishes: (**a**) Witness; (**b**) Sample in the presence of SULC with a concentration equal to 0.017 micromoles/mL

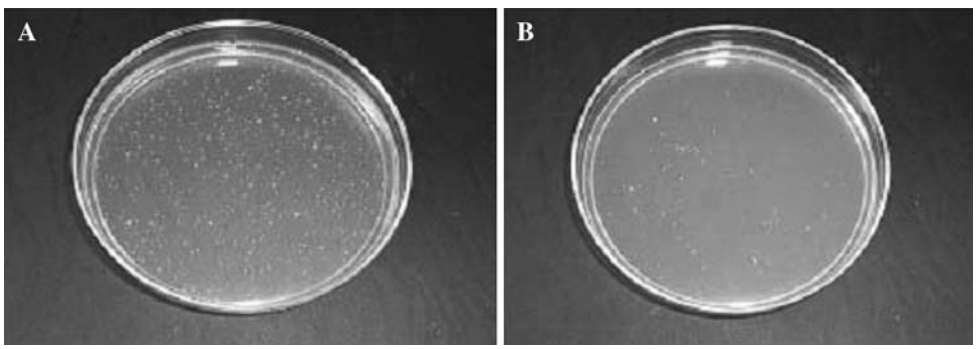

Fig. 2 *C. albicans* culture in Petri dishes: (**a**) Witness; (**b**) Sample in the presence of β-CD-SULC with a concentration equal to 0.0012 micromoles/mL

The performed tests proved that there are significant differences between the antifungal effect of the native substance and of the complexed substance. The improvement of in vitro antifungal effect of complexed SULC, characterized by the decrease of the active concentrations necessary to inactivate the yeasts and also by the decrease of the minimal inhibitory concentrations for 90% of the strains, can be observed.

Acute toxicity studies

The acute toxicity studies data show, that the acute toxicity of the β-CD-SULC inclusion complex is smaller, comparing with the free SULC, analyzed separately. The oral LD50 for the β-CD-SULC lyophilized complex is 0.78 micromole SULC/kg body weight, higher than the value of LD50 obtained for free SULC, 1.62 micromole/kg body weight.

The administration of the β-CD-SULC, on oral route is well tolerated by the organism, due to β-cyclodextrin resistance to α and β-amylase acids hydrolyze processes in the middle segment of the digestive tract (stomach and small intestine) [10]. The inclusion complex showed an improved bioavailability, and a reduction of the toxicity comparing with the pure SULC, aspect sustained by the absence of any hepatic, hematological and/or renal anomalies at 0.87 micromole SULC complexed/kg body weight.

Conclusions

Inclusion complex of sulconazole and β-CD was prepared by freeze-drying method in a molar ratio 1:1. The inclusion efficacy was confirmed by all the data already described in the literature [6]. Complexation by inclusion increases sulconazole solubility and dissolution in water 2.14 times, due to the low crystallinity of the complex. Due to this aspect, cyclodextrin based supramolecular systems represent an interesting formulation platform for delivery of drugs with poor physicochemical and biopharmaceutical properties.

The inclusion complex shows an acute toxicity smaller than the pure drug, oral 0.78 micromole SULC complexed/kg body weight, due to higher solubility and bioavailability of the complexed drug. Also, the in vitro antifungal activity of the complexed drug is higher (MIC50 is half and MIC90 is four time smaller), than of the pure biological active compound.

The increase of the bioavailability of the drug in β-CD-SULC inclusion complexes, combined with the decrease of the active dose and of the toxic effects proves the efficacy of the therapeutic usage of these inclusion complexes.

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