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Original Article

A novel approach to prevent candidiasis associated with long term administration of cephalosporins

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ABSTRACT

Background: Antibiotics play a critical role in the pathogenesis of *Candida* infections by invading the competent indigenous flora. Very little is known about the antifungal activity of third generation cephalosporins, but several new groups of cephalosporins were reported to show *in vitro* antifungal activity. EDTA, a chelating and permeable agent has been found to exhibit the effective antifungal activity. The purpose of the study was to evaluate the *in vitro* antifungal activity of Elores, a novel antibiotic adjuvant entity having combination of ceftriaxone, sulbactam and EDTA against ceftriaxone alone in *Candida albicans* in an attempt to prevent the risk of candidiasis after a prolonged cephalosporin antibiotic treatment.

Methods: *C. albicans* (MTCC-227) procured from Institute of Microbial Technology (IMTECH) Chandigarh was used in the study. Antifungal susceptibility of the drugs was determined by agar well diffusion method. Agar dilution and tube dilution methods were used to assess the anti-proliferative activity of Elores and EDTA.

Results: Elores containing EDTA equivalent to 6.25 and 12.5 μg/ml effectively suppressed the growth and the difference was 1318 CFU/ml between ceftriaxone and test sample, which is more significant in preventing overgrowth of *Candida*.

Conclusion: The study showed that Elores effectively slowdown the *Candida* over growth at serum level EDTA concentrations and believed to prevent candidiasis, associated with prolonged cephalosporin antibiotic treatment therapies.

1. Introduction

Prolonged antibiotic treatment is the main cause of fungal infections, especially candidiasis. *Candida albicans*, causative agent of candidiasis is a yeast and one of the constituents of regular flora of the skin, gastro-intestinal tract, mouth, rectum and vagina. Although *Candida* is an endosymbiont of the human body, it can cause problems if there is an overgrowth, resulting in candidiasis.[1] Candidiasis usually occurs when there is an imbalance in the regular flora of the body, and in people who have compromised immune systems.

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Different factors can lead to Candidal overgrowth such as a person's diet, immune suppression and prolonged antibiotic treatment, however, research findings support that the prolonged use of antibiotics can also play a major role in the development of candidiasis. Prolonged dose of antibiotics can lead to an imbalance in the essential gut flora, an imbalance that wipe out beneficial microflora and allows harmful bacteria, yeasts and parasites to overgrow in the stomach.[2–5] Steroids and some cancer medications also weaken the immune system and can allow yeast to flourish. If this condition is not treated and controlled, the affected person can begin to suffer a slew of negative side effects such as oropharyngeal candidiasis, Intertrigo, *Candida vulvovaginitis* (Vaginitis), Systemic yeast infections (IDSA).

Elores is a novel antibiotic adjuvant entity with combination of third generation cephalosporin with beta lactamase inhibitor and EDTA as non-antibiotic adjuvant. Cephalosporins are a class of β-lactam antibiotics whose spectrum of activity and use are limited to treat bacterial infections. However, cephalosporins containing 2-pyridinethiol 1-oxide grouping in their structure were found to exhibit *in vitro* antifungal activity.[6,7]

EDTA has been established as an antifungal agent in many scientific investigations and proved as an effective oral irrigate against *Candida* sp. EDTA is also recognized as a non-antibiotic agent which disrupts the membrane integrity due to chelation property and acts as a potentiator of other lethal agents.[8,9] EDTA antifungal activities were mainly tested on yeasts, being nevertheless reported its synergistic effect with other antifungal or antibacterial agents on the reduction of oral candidiasis.

The aim of the present study was to evaluate the *in vitro* antifungal activity of Elores on *C. albicans* in preventing the risk of candidiasis associated with prolonged cephalosporin antibiotic treatment regimen.

2. Materials and methods

2.1. Chemical material

Elores (Ceftriaxone:Sulbactam:EDTA:2 g:1 g:74 mg), used in the study was provided by Sponsor Venus Pharma GmbH, Germany and ceftriaxone was procured from Hoffmann-La Roche Pharmaceutical Limited (Basel, Switzerland), ceftriaxone plus sulbactam from Formic-Neo, Elder Pharmaceutical limited (Mumbai, India) and di-sodium EDTA from Himedia (Mumbai, India) on behalf of sponsor for the study. All the test substances Elores, ceftriaxone and EDTA were reconstituted with the water for injection as stock solutions. Working solutions were prepared in RPMI media as per the requirement.

2.2. Preparation of inoculum

C. albicans (MTCC-227) procured from Institute of Microbial Technology (IMTECH), Chandigarh was used in the study. Five colonies of *C. albicans* isolates from 24-h-old Sabouraud's Dextrose Agar (Himedia) subcultures at 35 °C were suspended in sterile 0.9% saline, and the turbidity was measured and adjusted by using a spectrophotometer 1×10^6–5×10^6 CFU/ml as recommended by the CLSI.[10] The suspensions were diluted with the RPMI medium, and used at a final concentration of 0.5–2.5 $\times$ 10^3 CFU/ml.

2.3. Antifungal activity of Elores on C. albicans

2.3.1. Agar well diffusion method

Susceptibility determination was carried out by agar well diffusion method. A 0.5 McFarland suspension of *C. albicans* (prepared as per the M27-A3 protocol) was swabbed in three directions on RPMI 1640 medium% glucose agar plates and left to dry for at least 15 min, after which the wells were made by a cork borer and agar plugs were removed. The test substances were loaded at various concentrations on to the wells to yield best range of zone diameters. Zone diameters (in millimeters) were determined after 24 h of incubation at 35 °C. Zone edges were sharply defined and easily determined.

2.3.2. Agar dilution method

Antifungal effect of Elores and EDTA against *Candida* was also evaluated by agar dilution method using RPMI-1640 medium which was recommended by CLSI M27-A3.[10] The medium was supplemented with filter sterilized ceftriaxone, Elores containing EDTA equivalent to 6.25–30 μg/ml and EDTA alone was also used at the same concentrations. 10 μl of the Candidal suspension with an approximate concentration of 1×10^3 was centrally inoculated in triplicate in each media and incubated at 35 °C. The colonies were observed daily and the growth was visually compared with ceftriaxone treated control.

2.3.3. Tube dilution method

Further to estimate the growth inhibition, the experiment was carried out by macro broth tube dilution method,[10] in a set of tubes containing RPMI medium with different concentrations of ceftriaxone, Elores containing 6.25–30 μg/ml of EDTA. The test was conducted in two parts – one part of the experiment was carried with single treatment and CFU were enumerated and the second part was continued with the replenishment of same concentration of the drug dissolved in RPMI medium to replenish the degraded drug and exhausted nutrients every 24 h. After overnight incubation, the organisms were enumerated by colony count. The sample was diluted and pour plated with Sabouraud's Dextrose Agar to count the colony forming units per ml.

2.4. Statistics

Results were expressed as mean and standard deviation. The bacterial counts in the control and treatment groups were

Table 1 – Antifungal susceptibility values of test solutions against *C. albicans* (MTCC-227).

Drugs	Zone diameter in mm
Ceftriaxone (60 μg)	8.21 ± 0.45
Ceftriaxone + Sulbactam (60 μg)	8.22 ± 0.39
EDTA (60 μg)	13.98 ± 0.25
Elores (Ceftriaxone: Sulbactam: EDTA) (60 μg)	18.29 ± 0.32

Values are mean ± S.D. of 3 values each.