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A novel approach for skin infections: Controlled release topical mats of poly (lactic acid)/poly(ethylene succinate) blends containing Voriconazole

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ABSTRACT

The oral and injectable formulations of Voriconazole (VRZ), a known antifungal agent with low solubility, seem to cause severe side effects. Consequently, topical application of VRZ could be advantageous for skin fungal infections. In this study, VRZ embedded in a polymeric matrix composed of biocompatible poly(lactic acid) (PLA) and poly(ethylene succinate) (PESu). The mats were prepared via solvent evaporation and fully characterized by Fourier-Transformed Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), *in vitro* hydrolysis and release studies. The prepared blends defined as immiscible by DSC and SEM while FTIR spectroscopy did not disclose noticeable interactions between the polymers. It was found that hydrolysis was improved by increasing PESu content into the blend. VRZ loaded blends spectra exhibit slight differentiation compared to neat blends while the absence of VRZ melting peak, as DSC illustrated, indicated drug amorphization. Lastly, *in vitro* release studies depicted a controlled release pattern dependent on mats' hydrolysis degree. An improved antifungal activity of mats was detected by disc diffusion method against various microorganisms. *Ex vivo* studies of VRZ did not determine high permeation while histopathology results using mice were profitable. The irritation experiments displayed that the mats did not induce any skin irritation.

1. Introduction

Voriconazole (VRZ) [(2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidine-4-yl)-1-(1H-1,2,4-triazole-1-yl)butan-2-ol] is a triazole antifungal agent which is often applied against invasive fungal infections like aspergillosis, candidiasis as well as infections from *Fusarium* species [1–4]. In most cases, fungal infections are found in the skin, the largest human sense organ, whose primary function is to act as a barrier protecting the internal organs from excessive water loss, physical or chemical attack as well as pathogens invasion [5,6]. It has been reported that skin fungal infections such as dermatophytosis and candidiasis, are described as the most common diseases into Asian and African population since they can affect approximately 15% of the population [1,7].

VRZ administration against fungi invasion, either orally or intravenously, is very common due to its favorable safety profile, which is, however, limited by the severe adverse effects such as hepatotoxicity, photophobia, blurred vision as well as photosensitivity etc. In addition, it has been recorded that patients who receive VRZ can present hallucinations and other central nervous system symptoms, such as confusion [8]. Furthermore, it is well reported that azoles during pregnancy are not easily prescribed due to their embryotoxicity and teratogenicity in rodents, rabbits, and rats. In addition, azoles could induce hydronephrosis, reduced ossification as well as fetal mortality. Among others, VRZ possibly can cross the human placenta considering its low molecular weight and so this antifungal agent labeled as category D-fetal risk [9]. In view of such severe toxicity, the topical application of VRZ is required since it could change its limitation and improve its profile.

Topical administration of antifungal agents could have an increased impact on the antifungal therapy, given that current formulations present lack of efficacy due to the rising antifungal drug resistance [10,11]. Although drug administration by skin is very advantageous, it can also be very challenging considering that the skin acts as a barrier to drug transport [12]. Mostly, creams, ointments, oil, and microemulsions are applied such as topical pharmacologic agents because

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they can penetrate the stratum corneum layer and eliminate fungi (fungicidal agents), or at least inhibit their growing or dichotomy (fungistatic agents) [13]. In the literature, several efforts can be found in order to deliver locally VRZ safely, mostly as ocular drug delivery systems [14–18] or skin delivery systems [19,20]. Nonetheless, the application of a polymeric film as VRZ topical carrier has not been attempted yet.

The last decades, aliphatic polyesters, such as poly(ε-caprolactone)-PCL, poly(lactic acid)-PLA, poly(butylene succinate)-PBSu, poly(lactic acid-*co*-glycolic acid)-PLGA, have gained great attention in pharmaceutical technology as a result of their biodegradability, biocompatibility as well as other physical and chemical properties. PLA, owing to its slow hydrolytic degradation, is one of the most widely used polymer for controlled delivery applications [21–23], tissue engineering [24–26] or implants. An easy method to improve PLA hydrophilicity is to blend it with a more hydrophilic polymer. It has been addressed that blending polymers can result in various release patterns [22,27]. Poly (ethylene succinate)-PESu is a biocompatible polymer with chemical resistance and improved mechanical properties [28]. PLA/PESu blends via solvent evaporation method have been already reported in the literature, nevertheless, have never been applied as drug carriers. In the past study, authors dissolved the homopolymers in chloroform [29] in contrast to our work where dichloromethane (DCM) was applied. Herein, DCM was chosen given its lower toxicity compared to chloroform.

The aim of this work was to introduce an alternative option for topical delivery of VRZ by using PLA/PESu blends in different concentrations. The blends were developed via solvent evaporation technique with dichloromethane as a solvent and studied for their miscibility and compatibility via Fourier Transformed–Infrared spectroscopy, Differential Scanning Calorimetry analysis, and Scanning Electron Microscopy observation. VRZ was added and the prepared mats were also evaluated by these means.

2. Materials and methods

2.1. Materials

Poly(ethylene succinate) and poly(lactic acid) were purchased from Aldrich Chemical Co (Steinheim, Germany). VRZ was also purchased by Aldrich Chemical Co (Steinheim, Germany). High performance liquid chromatography (HPLC) grade Acetonitrile (Sigma, Germany) and Methanol (Sigma, Germany) were used for HPLC studies. All other reagents and solvents used were of analytical grade.

2.2. Topical mats neat and loaded with VRZ preparation via solvent evaporation method

In order to prepare PLA/PESu blends, the solvent evaporation method was used; dichloromethane (DCM) applied as the solvent of the polymeric system. PLA/PESu blends presenting weight ratios 100/0, 90/10, 70/30, 50/50 and 0/100 w/w (mg/mg) were prepared. More specifically, 90 mg of PLA and 10 mg of PESu were used for the preparation of 90/10 blends. In such case, the polymers were dissolved in 5 mL of DCM and left to be fully evaporated. After the complete evaporation, films dried in the oven (25 °C) so as to remove any DCM residues. The mixture was dried up to the point where the mats weight was stable. Similarly, PLA/PESu 70/30 and 50/50 were also prepared. In case of VRZ loaded blends, 5% of VRZ drug loading was achieved. For instance, in case of 90/10 blend 85.5 mg of PLA and 9.5 mg of PESu as well as 5 mg of VRZ was dissolved in DCM and left to fully be evaporated. Afterward, the milk opaque films dried in the oven (25 °C) so as to remove any DCM residues. Accordingly, VRZ loaded 70/30 and 50/50 mats were developed.

2.3. Characterization of the topical mats

2.3.1. Fourier-Transformed Infrared Spectroscopy studies

The prepared mats were studied using FTIR–spectrometer FTIR–2000 (Perkin Elmer, Turkey) so as to record their FT-IR spectra. In order to collect the spectra, a thin film of the prepared 50/50, 70/30 and 90/10 neat and VRZ mats was applied to the spectrometer. The spectrum collection area ranged from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1} (64 co–added scans). Herein, the presented spectra are baseline corrected and converted to the absorbance mode.

2.3.2. Differential Scanning Calorimetry (DSC)

For thermal analysis, a differential scanning calorimeter (DSC) (Perkin–Elmer, Pyris Diamond, Turkey) calibrated with Indium and Zinc standards, was used. For the characterization, 8 mg of the mats were used, placed in aluminum pans and heated up to 200 °C using a heating rate of 10 °C/min. The mats were held at that temperature for 5 min and then they were cooled down by 300 °C/min rate.

2.3.3. Scanning Electron Microscopy (SEM)

The morphological examination of the VRZ formulations was carried out using a scanning electron microscope (SEM) (Zeiss EVO, USA). The mats were coated with silver so as to obtain a good conductivity of the electron beam. The accelerating voltage was 20 kV, probe current was 45 nA and counting time was 60 s.

2.4. Chemical stability of VRZ mats

The chemical stability of the VRZ mats was calculated at 5 ± 2 °C and 25 ± 2 °C for 12 months. The amount of VRZ was investigated to examine the monthly storage temperature.

2.5. Water uptake and average thickness of the mats

The water uptake was figured out by immersing the mats in distilled water at 25 °C. After 1 h, the hydrated mats were removed, followed by surface water extraction using filter paper and directly weighed. The water content ($W_{H2O\ \%}$) was calculated using the following type ($W_{H2O\ \%}$) = (W-W_o)/W_o % (W_o and W express the mats weight before and after immersion in water, respectively) [30]. The mats thickness was ruled out by a micrometer at random points on the mat surface area.

2.6. In vitro hydrolysis studies of mats

The hydrolysis degree was estimated accordingly with the mass loss. Circular shaped mats with 0.78 cm^2 area were placed in Petri dishes with simulated body fluid (pH 7.4). Afterward, the samples were incubated at 37 ± 1 °C in an oven for a period of five days. Every 48 h, the mats were removed, washed with distilled water, dried and weighted until constant weight [22]. The hydrolysis was calculated using the following formula: % mass loss = (W_1-W_2)/W_2 × 100 (W_1 and W_2 express the mats weight before and after hydrolysis, respectively). All the experiments were performed in triplicate.

2.7. High performance liquid chromatography analysis

For the determination of the drug loading content and *in vitro* release results, HPLC method was applied. The quantitative analysis of the obtained solutions was assessed by HPLC using HP Agilent 1100 system (Germany) consisted of a gradient pump and a UV detector. The used column was a C18 column (5 μm, 150 × 4.6 mm) (GL Sciences, Japan). The samples were analyzed at 256 nm with 1 mL/min at 25 °C flow rate. The mobile phase consisted of Acetonitrile (ACN): ultrapure water (50:50). The retention time of drug was 4.098 min. The method was validated for linearity, limit of detection (LOD) and limit of quantitation (LOQ), precision, accuracy and specificity, selectivity and