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Safety and *in vivo* release of fluconazole-loaded implants in rabbits' eyes

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ABSTRACT

Drug delivery to the eye cavity remains a challenge, owing to the ocular-blood barriers and rapid clearance of the drugs from aqueous humor and choroid. In order to overcome this problem, this study aimed to evaluate the ocular safety of fluconazole-loaded poly-lactide-*co*-glycolide implants (FL-PLGA implants). The concentration of fluconazole in the vitreous of rabbits'eye after FL-PLGA implants (25%w/w) application or intravitreal fluconazole suspension administration (1.8 mg/ml) was compared. At predefined periods, vitreous was collected and the concentration of fluconazole was assayed. *In vitro* tolerance of the implant was performed in ARPE-19 cells. The clinical evaluation of the rabbits'eyes that received FL-PLGA implants was realized during the 6 weeks of the study. Fluconazole was released from implants during 6 weeks, while the drug suspension injected into the eye was detected for 120 min. FL-PLGA implants were well tolerated in the eye, once no ocular abnormalities were clinically detected. The *in vitro* toxicity study revealed that devices did not induce alteration to ARPE-19 cells. Our results suggest that PLGA implants controlled fluconazole release for a prolonged period compared to the fluconazole suspension injected into the vitreous and showed ocular tolerance and *in vitro* biocompatibility.

1. Introduction

Fungal endophthalmitis (FE) is an intraocular fungal infection, which originates either exogenously, due to penetrating traumas or postoperative infections, or endogenously, due to the spreading from hematogenous infections [1]. Fungal pathogens implicated in FE include *Candida* species (spp.) [1,2]. The visual prognosis of FE patients is generally poor [3].

The fluconazole (FL) exhibits antifungal activity against Candida species. This drug is orally administrated regarding its penetration in the eye. However, oral fluconazole doses provide vitreous concentrations of 20–70% of that found in the plasma, leading to treatment failure [4]. Intraocular fluconazole injections are uncomfortable and can cause damages to the eye [5]. The development of targeted delivery systems to cure the FE should be explored.

The poly (lactide-*co*-glycolide acid) (PLGA) is a widely used biodegradable and biocompatible polymer which has many biomedical applications such as controlled release of drugs, tissue engineering, healing of bones defects and in vaccines [6]. There are several PLGA drug delivery systems approved by FDA and European Medicine Agency [7].

In this study, FL-PLGA implants were developed as alternative systems to locally treat the FE induced by *Candida* species. The ocular bioavailability of the fluconazole released from implants and injected into the vitreous cavity of rabbits'eye was compared. Additionally, the *in vivo* and *in vitro* ocular toxicities were evaluated.

2. Materials and methods

2.1. Preparation of FL-PLGA implants

The devices were prepared by the technique of hot molding,

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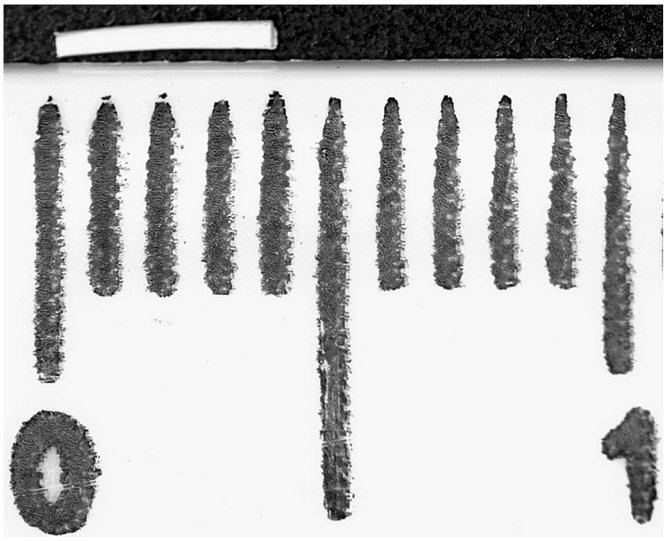

Fig. 1. Photograph of Fluconazole-loaded PLGA implant.

previously described by Fialho and Silva-Cunha [8]. Briefly, the fluconazole (Sigma-Aldrich, Brazil) and PLGA 75:25 (Resomer RG 750S, Evonik Industries, Germany) at a ratio of 1:3 were dissolved in a mixture of acetonitrile and acetone (1:3). Afterwards, the mixture was lyophilized and the powder was molded into rods using a hot plate. Implants without drug were also prepared. FL-PLGA implants had 4.0 ± 0.1 mm, 0.6 ± 0.05 mm and 1.0 ± 0.2 mg (n = 10) of length, diameter and average weight, respectively (Fig. 1). The fluconazole amount was 0.25 ± 0.07 mg corresponding to approximately 25% (w/w).

2.2. Preparation of fluconazole suspension

Fluconazole was dispersed in the vehicle containing carboxymethylcellulose, sodium chloride, polysorbate 80 and ultrapurified water. The mixture was sonicated for 30 min and a final suspension containing 1.8 mg/mL of fluconazole was obtained.

2.3. Animals

Fifty two female New Zealand rabbits weighing 2.5–3.5 kg were divided into two groups: group I (n = 24) received FL-PLGA implants and group II (n = 28) received an intravitreal injection of fluconazole suspension (50 μL). The contralateral eye of both groups was used as control. The experiment was approved by Ezequiel Dias Foundation Ethics Committee in Animal Experimentation (protocol 36/2012).

2.4. Procedures for device implantation and for fluconazole suspension injection

Animals were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (15 mg/kg). The ocular surface was anesthetized with topical 0.5% proxymetacaine hydrochloride. A 25-gauge trocar cannula was placed through the *pars plana*, 2 mm above the limbus in the superotemporal quadrant, where the implant was inserted into the vitreous cavity [9]. A 26-gauge needle and a 1 mL syringe were used to inject the fluconazole suspension through the sclera behind the ciliary body and approximately 7 mm below the limbus.

2.5. Drug level analysis

The vitreous of 4 animals of each group was collected in different times for drug analysis. For group I, the extraction was performed weekly during 6 weeks, whereas in group II animals, the vitreous was collected at 10, 20, 40, 60, 80, 100 and 120 min after injection. After sample retrieval, rabbits were sacrificed with an overdose (100 mg/kg) of intravenous 3% sodium pentobarbital. The concentration of fluconazole in the vitreous was measured by high performance liquid chromatography (HPLC) using a C18 column (125 mm × 4.0 mm × 5 μm, end capped) heated at 30 °C, with a pump set at a constant flow rate of 0.8 mL/min and an ultraviolet detector at a wavelength of 260 nm. The mobile phase was ultrafiltered water and acetonitrile (78:22).

2.6. Clinical examination

Eyes of animals of the group I were observed clinically and photographed during 6 weeks. Clinical evaluations included ocular inspection and binocular indirect ophthalmoscopy preoperatively. Indirect ophthalmoscopy and slit-lamp biomicroscopy were used. This examination was conducted after dilatation of the pupils with one drop of 1% tropicamide.

2.7. ARPE-19 tolerance – morphology

FL-PLGA implants and PLGA implants without drug were used for the evaluation of the tolerance in ARPE-19, an established but non-immortalized human RPE cell line, according to the procedure previously described by Silva et al. [10]. Briefly, ARPE-19 cells

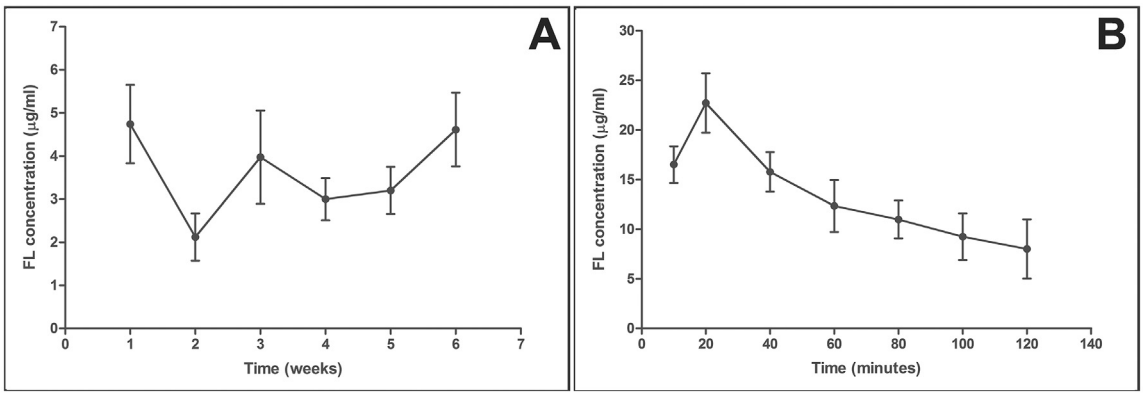

Fig. 2. Concentration of fluconazole (μg/mL) released from implants (A) and after the intravitreal injection of suspension (B) in the vitreous humor (mean ± SD, n = 4).