Journal of Drug Delivery Science and Technology 44 (2018) 373–379

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

# Sirolimus-loaded biodegradable implants induce long lasting anti-inflammatory and antiangiogenic effects

Bruna Lopes Costa[a], Renes de Resende Machado[a], Mayara Rodrigues Brandão Paiva[a], Rogéria Serakides[b], Márcio de Matos Coelho[a], Armando Silva-Cunha[a,1], Sílvia Ligório Fialho[c,*,1]

[a] Faculty of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, CEP 31270-901, Brazil
[b] School of Veterinary, Federal University of Minas Gerais, Belo Horizonte, CEP 31270-901, Brazil
[c] Pharmaceutical Research and Development, Ezequiel Dias Foundation, Belo Horizonte, CEP 30510-010, Brazil

ARTICLE INFO

*Keywords:*
Sirolimus
Implant
Inflammation
Angiogenesis
Prolonged release

ABSTRACT

Sirolimus is an immunosuppressive drug with activity in rheumatoid arthritis through inhibition of mTOR and T-lymphocytes activation and proliferation. However, its side effects limit usefulness. In this study, we developed sirolimus-loaded biodegradable implants, evaluated *in vivo* release of the drug and anti-inflammatory and antiangiogenic activities. Implants containing sirolimus were inserted into the subcutaneous tissue of mice and drug released was determined during 42 days. Histology of the skin and subcutaneous tissue close to the implanted site was performed after 7 and 42 days. The antiangiogenic activity of sirolimus implants was evaluated in the chorioalantoic membrane (CAM) from chicken eggs. The mice model of fibrovascular tissue growth induced by implantation of a subcutaneous cotton pellet was used to evaluate anti-inflammatory activity. Sirolimus implant promoted a slow release of the drug during 42 days. Histology did not show signs of intense local inflammatory response at the site of implantation. Sirolimus implants reduced vessels formation in the CAM and proliferation of fibrovascular tissue. Concluding, sirolimus-loaded implants reduced angiogenesis and chronic inflammatory response in the models evaluated. This preliminary study indicates that the implants are safe to the experimental animals and that they may represent a promising alternative for the treatment of chronic inflammation.

## 1. Introduction

Sirolimus, a macrolide compound produced by *Streptomyces hygroscopicus*, is an immunosuppressive agent indicated for the prophylaxis of organ rejection in patients receiving renal transplants. It has also been shown that sirolimus inhibits T-lymphocyte activation and proliferation, as well as antibody production, thus exhibiting antineoplastic properties. Sirolimus binds to FK-binding protein-12 (FKBP-12) to form a complex that inhibits the activation of the mammalian target of rapamycin (mTOR), a regulatory protein kinase. This inhibition suppresses cytokine-driven T-cell proliferation resulting in inhibition of the progression of the cell cycle [1–4].

Generally, mTOR inhibitors may decrease the incidence of comorbidities associated with chronic kidney disease and transplantation including protection from atheroma progression and complications correlated to polycystic kidney disease [5]. Moreover, sirolimus exhibits anti-inflammatory and antiangiogenic activities. Orhan et al. (2013) [6] reported that sirolimus decreases the arthritic lesions and paw edema and exhibits antihyperalgesic and anti-allodynic activities in a model of rat adjuvant-induced arthritis. Futhermore, sirolimus also reduces the endothelial cell response to vascular endothelial growth factor (VEGF), an effect that contributes to its antiangiogenic activity [7,8].

However, treatment with sirolimus has been associated with side effects such as anemia, skin lesions, impaired wound healing, proteinuria, dyslipidemia, insulin resistance and diabetes [9,10]. In order to improve the pharmacokinetic profile of the sirolimus and enhance medication efficacy, some sirolimus delivery systems have been developed [11–15]. Nevertheless, some challenges such as controlled release over long periods of time are still a problem. Considering that implantable polymer systems promote safely deliver doses of drug to required locations over long periods of time, the development of sirolimus-loaded biodegradable implants may be an important alternative. These devices may promote a long lasting release of sirolimus with

* Corresponding author. Pharmaceutical Research and Development, Ezequiel Dias Foundation, Rua Conde Pereira Carneiro, 80 – Gameleira CEP 30510-010, Belo Horizonte, MG, Brazil.
*E-mail address:* silvia.fialho@funed.mg.gov.br (S.L. Fialho).
[1] A. Silva-Cunha and S.L. Fialho are joint senior authors.

https://doi.org/10.1016/j.jddst.2018.01.018
Received 6 June 2017; Received in revised form 24 January 2018; Accepted 24 January 2018
Available online 03 February 2018
1773-2247/ 

maintenance of effective therapeutic levels and may also allow targeted drug delivery thus reducing the risk of systemic side effects [16,17].

In the present study, we prepared sirolimus-loaded biodegradable implants and evaluated their anti-angiogenic and anti-inflammatory activity in two experimental models.

## 2. Materials and methods

### 2.1. Materials

Sirolimus was a gift from Cristália Produtos Químicos Farmacêuticos LTDA (São Paulo, Brazil). Poly-lactide-co-glycolide copolymer (PLGA 50:50, inherent viscosity of 0.4 dl/g; Purac Biomaterials, São Paulo, Brazil), dexamethasone 21-phosphate disodium salt (Sigma-Aldrich, São Paulo, Brazil), methanol and acetonitrile HPLC grade (Merck Brazil, São Paulo, Brazil) were used. Ultrapure water was produce by a Milli-Q System (Millipore, Massachusets, USA). Other chemicals were of analytical grade.

### 2.2. Preparation of biodegradable sirolimus-loaded PLGA implants

The implants were prepared according to the technique described by Fialho and Silva Cunha (2005) [18]. Firstly, 100 mg of the mixture of sirolimus and the polymer (PLGA 50:50) at a ratio of 1:4, were dissolved in 2 ml of acetonitrile and the resulting solution was lyophilized. The powder obtained was molded into rods using a hot plate heated at 100-120 °C. The sirolimus implants, weighing on average 6.0 ± 0.1 mg, had 1.5 ± 0.1 mm in diameter and 4.5 ± 0.1 mm in length (Fig. 1A). The final concentration of sirolimus homogeneously dispersed in the polymeric matrix was 25% w/w.

### 2.3. Animals

Female Swiss mice (25–30 g) with free access to food and water were used. The animals were kept in an acclimatized room with a 12-h light-dark cycle during the whole experiment. The study was approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais (Protocol n° 172/2016, Belo Horizonte, Brazil) and carried out according to the ethical guidelines for investigation of experimental pain in conscious animals [19].

### 2.4. In vivo sirolimus release study

Before insertion of the implants, mice were anesthetized with a mixture of xylazine (10 mg/kg) and ketamine (80 mg/kg) by the intraperitoneal (i.p.) route. The dorsum was shaved and the skin wiped with 70% v/v ethanol. The implants were inserted through a 1 cm incision (Fig. 1B). The sirolimus-loaded implant was inserted into the subcutaneous tissue of 20 mice. At days 3, 7, 14, 28 and 42 after the insertion of the implants, groups of four mice were euthanized and the implants were removed and gently washed with ultrapure water to discard biological residues. The amount of sirolimus released *in vivo* was calculated indirectly from the retrieved implants. For that, each collected implant was dissolved in 5 ml of acetonitrile so that the remained drug could be analyzed. The analysis were realized by high performance liquid chromatography (LabChrom Elite UV/VIS, Merck Hitachi, Malta, NY; L-2130 pump, L-2200 autoinjector, L-2300 oven, L-2400 UV detector) equipped with a C18 column (250 mm × 4.6 mm, 5 µm particle size; Phenomenex), thermostated at 50 °C and a UV detector set at 278 nm. The mobile phase was composed of acetonitrile:methanol (20:80) at a flow rate of 1.0 mL/min. In these conditions, the retention time of sirolimus was about 3.0 min. The area of the sirolimus peak was reported to a calibration curve for the determination of drug concentration.

### 2.5. Evaluation of the morphological changes of the implants

In another protocol, we evaluated the morphological changes of the implants surfaces after their insertion into the subcutaneous tissue. The sirolimus-loaded implant was inserted into the subcutaneous tissue of nine mice. At days 7, 14 and 28 after the insertion of the implants, groups of three mice were euthanized and the implants were removed for stereomicroscopy and scanning electron microscopy analysis. Stereomicroscopy analysis was performed using an Olympus microscope (SZ61TR, Olympus, Brazil) at a magnification of 35× without any special pre-treatment. Scanning electron microscopy (SEM) analysis was performed using a FEG-Quanta 200 FEI microscope (FEI, USA) operating at 5 kV. Before visualization, the implants were gently washed with distilled water, blotted with wipes to dry off excess water and then dried for 72 h in a vacuum desiccator at room temperature. After drying, they were mounted on aluminum stubs. Prior to microscopic examination, the samples were sputtercoated with a gold layer under an argon atmosphere for 1 min (BALTEC MED020 Coating System, BALTEC AG, Germany). The implant surfaces were viewed at 150× magnification. Implants not inserted into the animal dorse were also analyzed for comparison using the same protocol as described above.

### 2.6. Histological analysis

Two groups of eight mice each were used. Group 1 received sirolimus-loaded implants and group 2 received implants without the drug. The implants were inserted using the same protocol as described in the *in vivo* release study. At days 7 and 42 after the insertion of the implants, four mice of each group were euthanized and the surrounding

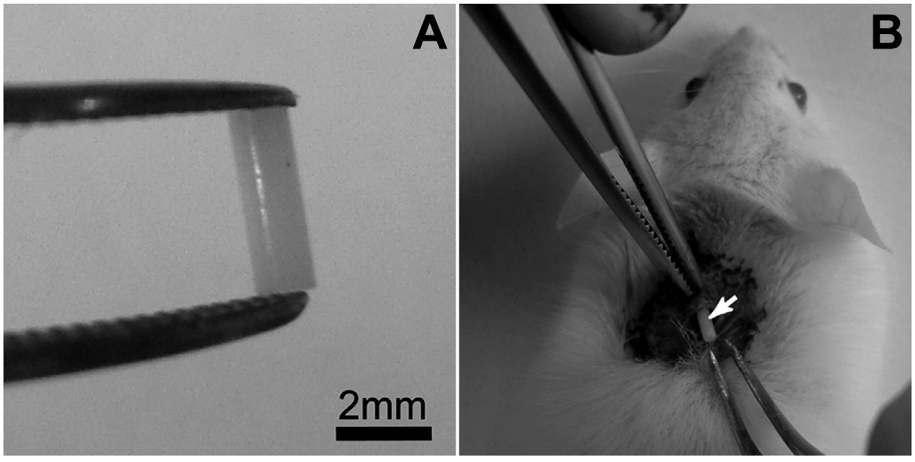


**Fig. 1.** (A) Sirolimus-loaded implant (weight: 6.0 ± 0.1 mg; length: 4.5 ± 0.1 mm; diameter: 1.5 ± 0.1 mm). (B) Insertion of the sirolimus-loaded implant into the dorsum of a mouse (Arrow: sirolimus-loaded implant).