Mesoporous particles for transdermal delivery of the antifungal drug griseofulvin

E V Lengert¹, R A Verkhovskii¹, E A Genina², G S Terentyuk³, Y I Svenskaya¹

¹ Research and Educational Institute of Nanostructures and Biosystems, Saratov State University, Saratov, Russia
² Departments of Optics and Biophotonics, Saratov State University, Saratov, Russia
³ Scientific Research Institute of Fundamental and Clinical Uroneprology, Saratov State Medical University, Saratov, Russia

LengertKatrin@mail.ru

Abstract. Griseofulvin is an antifungal antibiotic applying for the treatment of dermatophyte infections. The most common delivery route of this drug is oral. The dosage of griseofulvin varies depending on whether the drug is administered as a microsized or an ultramicromized preparation. In order to avoid possible incidental systemic toxicity associated with the oral delivery route and reduce the therapeutic dose, a novel approach for the griseofulvin administration is proposed. Immobilization of the drug into mesoporous calcium carbonate particles together with their further ultrasonically-assisted topical application provides the efficient transdermal transportation. Accumulation of griseofulvin-loaded carriers inside hair follicles of the rat is demonstrated in vivo, allowing by this means the targeted delivery of the antifungal drug to the lesion area of skin. No significant toxicity of the system is shown for rat and human fibroblasts cells in vitro.

1. Introduction

Recently, development of the approaches related to immobilization of antifungal drugs in various nano- and submicron-sized containers has become highly attended: liposomes, micelles, nanoemulsions, and various polymer and composite particles. The use of drug-carriers in order to immobilize the active substance contributes to their effective transfollicular delivery [1]. Such particulate systems have been found to enhance the penetration of the encapsulated substance into the hair follicles [2]. Physico-chemical parameters of such carriers have a great influence on the depth and abundance of the hair follicle filling.

Furthermore, the usage of a carrying system allows the drug delivery and accommodation into the area of superficial fungal infection localization, that enhances the therapeutic effectiveness of antifungal drugs and decrease the incidental toxicity associated with their application.

Previously, we have demonstrated the possibility of effective transdermal transportation of the drugs by the means of CaCO₃ carriers [3]. It has been shown, that the proposed particulate delivery system provided intrafollicular storage of bioactive molecules allowing the prolongation of therapeutic intervention over the two-week period. This approach revealed the promising outlook for the treatment...
of skin disorders as well as for systemic drug delivery via skin appendages. The aim of this study was to develop a novel topical griseofulvin formulation based on mesoporous CaCO$_3$ particles. The toxicity of the system was evaluated on rat and human fibroblast cells in vivo. The possibility of its accumulation in the rat skin was checked in vivo.

2. Discussion

The container synthesis was carried out as a crystallization from the solution by direct mixing of calcium chloride (CaCl$_2$) and sodium carbonate (Na$_2$CO$_3$) salts [4]. Griseofulvin (GF) is an antibiotic fungistatic administered orally for the treatment of dermatophytosis (fungal skin diseases) and trichophytosis (head and nail diseases). It was immobilized into the calcium carbonate (CaCO$_3$) carriers using the protocols previously elaborated in our group [5]. The morphology of loaded particles was characterized by scanning electron microscopy (SEM) and the image is represented in figure 1. The SEM-images clearly show that the surface of the loaded CaCO$_3$ particles (figure 1 b) appears smoother compared to pure porous carriers (figure 1a).

Figure 1b also demonstrates that the size of obtained particles was 0.8±0.2 μm. The loading efficiency of griseofulvin into the carriers was estimated by spectrophotometry and chromatography and was found to be 1.2 % (w/w) meaning 120 μg of the drug incorporated into 1 mg of CaCO$_3$ particles. The zeta-potential of unloaded and GF-loaded carrier suspension was found to be -5±0.8 mV and -7±1.2 mV, respectively.

![Figure 1. Scanning electron microscopy images of calcium carbonate particles: empty (a) and griseofulvin-loaded (b). Scale bar is 2 μm](image)

Cytotoxic effect of pure and GF-loaded calcium carbonate particles was evaluated on fibroblast cells. It was shown that the particle application, even at a highest concentration, did not affect cell viability.

The penetration of GF-loaded carriers into the skin was studied in vivo in rats. The synthesized GF-loaded particles were topically applied on the lower back of the rat in vivo.

The protocol on animal experiments was approved by the Ethics Committee of Saratov State Medical University (No 8 from 10.04.2018). Particles delivery was promoted by sonophoresis according to the previously reported protocol [6]. The optical coherent tomography (OCT) monitoring of rat skin in vivo demonstrated the efficient penetration of CaCO$_3$ particles through the skin barrier (Figure 2).
3. Conclusions
Here a novel technique for the synthesis of submicron calcium carbonate particles containing antymycotic drug griseofulvin is reported. Efficient transdermal transportation of the proposed system was demonstrated in vivo in rats. Obtained drug carriers were successfully accumulated inside the hair follicles. As far as hair follicles represent a reservoir extended deep into the skin tissue, the proposed approach holds a great potential in terms of targeted antifungal treatment.

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References
[1] Nimni M E, Ertl D, and Oakes R A (1990) Distribution of griseofulvin in the rat: comparison of the oral and topical route of administration. J. Pharm. Pharmacol., 42 (10), 729–731.
[2] Ong S, Ming L, Lee K, and Yuen K (2016) Influence of the Encapsulation Efficiency and Size of Liposome on the Oral Bioavailability of Griseofulvin-Loaded Liposomes. Pharmaceutics, 8 (3), 25.
[3] Zhou Z, Forbes R T, and D’Emanuele A (2017) Preparation of core-crosslinked linear-dendritic copolymer micelles with enhanced stability and their application for drug solubilisation. Int. J. Pharm., 523 (1), 260–269.
[4] Zili Z, Sfar S, and Fessi H (2005) Preparation and characterization of poly-ɛ-caprolactone nanoparticles containing griseofulvin. Int. J. Pharm., 294 (1–2), 261–267.
[5] Brinkmann-Trettenes U, and Bauer-Brandl A (2014) Solid phospholipid nano-particles: Investigations into formulation and dissolution properties of griseofulvin. Int. J. Pharm., 467 (1–2), 42–47.
[6] Svenskaya Y I et al. (2017) Method of transdermal delivery of biologically active substances. Patent No RU2633928C1, 19 october 2017.