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Development of a Long-Acting Injectable Formulation of Rilpivirine Based On PLGA Microspheres

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Abstract The rilpivirine-loaded spherical and monodisperse PLGA microspheres were produced by microfluidic technology. The technological parameters such as the flow rate ratio and the rilpivirine/PLGA ratio were optimized to ensure desired microspheres size and higher encapsulation efficiency of rilpivirine. The microspheres exhibited a continuous drug release characterized by only a minor burst effect. These results suggest that the PLGA microspheres loaded with rilpivirine is a promising approach to the injectable depot formulation of this drug.

Keywords**:** antiretroviral agent, rilpivirine, PLGA microspheres, long-acting injectable, microfluidic

1. Introduction

Although antiretroviral therapy has made significant progress, the HIV epidemic still poses a serious threat to public health worldwide. One of the important steps towards the improvement of HIV therapy and patients' compliance is the development of long-acting injectable formulations (LAI) of antiretroviral drugs. Thus, recent clinical studies demonstrated high efficacy of the nanocrystal LAI of rilpivirine and cabotegravir [1]. Polymeric microspheres based on poly(lactic-co-glycolic acid) can become an alternative approach.

One of the key parameters that determine the release rate of drug from microspheres is their size, polydispersity and drug content [2]. Traditional methods for microsphere preparation based on emulsification lead to the production of microspheres in a wide range of sizes. In contrast, microfluidic technologies allow precise control of fluid flow at the microscale, overcoming limitations of traditional methods.

The aim of this study was to explore an alternative approach to rilpivirine LAI using PLGA microspheres as drug carriers and to evaluate the effect of microsphere formation conditions on characteristics such as their size and encapsulation efficiency of rilpivirine.

2. Materials and methods

2.1. Chemicals

Rilpivirine (RPV) was synthesized in the Lomonosov Moscow State University, Russia. Poly(lactic-co-glycolic acid) (PLGA, Resomer® RG 756S, LA/GA ratio of 75:25, ester end-capped, η = 0.91 dL/g, Evonik, Germany), poly(vinyl) alcohol (PVA, 9-10 kDa, 80% hydrolysed), ethyl acetate, polysorbate 20 (Tween® 20), and trifluoroacetic acid (TFA, suitable for HPLC) were obtained from Sigma-Aldrich, Germany. Other chemicals were of analytical grade.

2.2. Preparation of rilpivirine-loaded microspheres

The PLGA microspheres loaded with rilpivirine were produced by emulsification technique using a microfluidic system (Dolomite Microfluidics, UK). To generation of oil-in-water droplets the Droplet Junction Chip (100 μm etch depth) with X-junction geometry was used. To prepare an organic phase rilpivirine and PLGA (140 mg) were dissolved in 7 ml of ethyl acetate. To prepare an aqueous phase the PVA was dissolved in purified water at a concentration of 2% (w/v) PVA. The resulting solutions were filtered through a PTFE membrane filter with a pore size of 0.45 μm. The organic and aqueous phase solutions were injected into the channels of the microfluidic chip. The flow rate of organic phase was 8.9 μl/min. The flow rate of aqueous phase was changed depending on the required droplet size in the range from 30 to 100 μl/min. Control of organic and aqueous phases flow rates and droplet size was carried out by a Dolomite Flow Control Centre Advanced software. The emulsion droplets formed inside the chip were collected in a receiver with a 1% (w/v) PVA aqueous solution at room temperature and under constant stirring at 120–150 rpm. The residual organic solvent was removed in vacuo on a rotary evaporator (30 °C, 20 mbar). Then the microparticles were washed with distilled water and freeze-dried.

2.3. Characterization of rilpivirine-loaded microspheres

The size and morphology of PLGA microspheres were examined in a JEOL JSM-6510LV scanning electron microscope. SEM measurements were carried out using the equipment of the D. I. Mendeleev Center for Collective Use. The size distribution was measured by a High Speed Digital Microscope (Dolomite Microfluidics, UK). The obtained data were processed in the ImageJ and Microsoft Excel 2010 analytical programs.

The coefficient of variation (CV) was calculated as [3]:

$$\mathrm{CV}\ (\%) = \frac{\text{standard deviation}}{\text{the average size of microspheres}} \times 100\% \quad (1)$$

The drug loading (DL) and encapsulation efficiency (EE) of rilpivirine were determined after dissolution of freeze-dried PLGA microparticles in acetonitrile (5 mg in 25 ml). The content of rilpivirine was assayed by HPLC. The DL and EE were calculated as [4]:

$$\mathrm{DL}\ (\%) = \frac{\text{Amount of encapsulated rilpivirine}}{\text{Total weight of microspheres}} \times 100\% \quad (2)$$

$$\mathrm{EE}\ (\%) = \frac{\text{Actual DL}}{\text{Theoretical DL}} \times 100\% \quad (3)$$

2.4. Evaluation of rilpivirine release

The release medium consisted of 2% w/v Tween® 20 in 0.15 M PBS at pH 7.4. The release of rilpivirine from PLGA microspheres was evaluated by placing freeze-dried PLGA microparticles into 50 ml of release medium (sink conditions) and further incubation at 37 °C with continuous shaking. Then 600 μL samples were taken at predetermined time intervals (1, 3, 5, 7, and 24 h, and then on daily basis). The concentration of the released rilpivirine was measured by HPLC. All experiments were performed in triplicate.

2.5. High-pressure liquid chromatography (HPLC)

The assay of rilpivirine was performed using a reverse phase HPLC method. The HPLC analysis was carried out with a LC-2030C 3D Plus HPLC system (SHIMADZU, Japan) equipped with a Photodiode Array (PDA) Plus Detector, a C18 pre-column and a Purospher® STAR RP-18 endcapped column (120 × 4 mm, 3 μm). The mobile phase consisted of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) delivered at a flow rate of 1 ml/min. A gradient elution was applied from 10% to 60% solvent B at 25 min. Column oven was set at 35°C, injection volume was 20 μl, and the analysis was carried out at 280 nm. The retention time for rilpivirine was 16.1

min. Data analysis was performed using the LabSolutions software. The linear calibration curve (y = 155894*x; R^2 = 0.9961; standard solution 2–50 μg/ml).

3. Results and Discussion

As shown in our previously study, the sizes of PLGA microspheres depend on the flow rates ratio of the organic phase and the aqueous phase (Qo/Qa) and PLGA concentration in the organic phase, but don't depend on the type and concentration of surfactants in the aqueous phase. Increase of the PLGA concentration in the organic phase and Qo/Qa ratio enabled preparation of the microspheres in the size range of 20-65 μm with a narrow size distribution (CV from 6% to 9%). The optimal parameters for preparation of the rilpivirine-loading PLGA microspheres using a microfluidic technique included employment of a 2% solution of PLGA in ethyl acetate as an organic phase and a 2% solution of PVA as an aqueous phase.

In the present study, the influence of rilpivirine concentration in the organic phase, rilpivirine/PLGA ratio, and Qo/Qa ratio on the microsphere size and encapsulation efficiency of rilpivirine were evaluated.

3.1. Effect of rilpivirine concentration in organic phase

The influence of the concentration of rilpivirine in the organic phase on the size of microspheres and the encapsulation efficiency of rilpivirine into microspheres was studied for microspheres obtained at a flow rate ratio Qo/Qa of 0.15. As shown in Table 1, a change in the concentration of rilpivirine from 1 to 3 mg/ml (corresponding to a change in the mass ratio of RPV/PLGA from 1/20 to 1/6) practically did not affect the size of the particles formed, and their average diameter was 42-45 μm. Differences in the coefficient of variation relates to the flow fluctuations caused by the appearance of air bubbles in a microchannel.

Table 1: Comparison of the microsphere characteristics, depending on the concentration of RPV in organic phase (n=3).

№	Concentration of RPV, mg/ml EA	RPV/PLGA, w/w	DL_{theor}, %	DL_{actual}, %	EE, %	D_{cp}, μm	CV, %
MS_1	1.0	1/20	4	0.4±0.1	9.5±1.1	45.1±2.1	4.7
MS_2	1.9	1/11	8	7.8±0.4	96.9±1.5	45.4±3.9	8.7
MS_3	3.2	1/6	13	12.7±0.1	97.5±1.2	42.7±0.6	1.4

At the same time, the encapsulation efficiency of rilpivirine significantly increases with an increase of rilpivirine concentration from 1 to 1.9 mg/ml. The low encapsulation efficiency of rilpivirine (0.4%) at low concentrations is probably due to diffusion of rilpivirine into the receiving medium simultaneously with ethyl acetate during the formation of the outer polymeric shell of the microspheres. This fact may indicate a low affinity of rilpivirine to the PLGA and a higher affinity to the solvent. The increase of rilpivirine concentration in the organic phase to more than 3.2 mg/ml led to the saturation of the ethyl acetate solution and the formation of a solid-in-oil suspension. This phenomenon could further hamper the supply of the organic phase into microchannels. Thus, a decrease in the weight ratio of RPV/PLGA led to an increase in the encapsulation efficiency. However, it is limited by the solubility of the drug in the used organic solvent.

3.2. Effect of the flow rate ratio

Size, shape and surface morphology are often considered as key microsphere characteristics that have a significant impact on the formulation injectability, polymer degradation, and drug release rate. Generally, the microparticle sizes of 10 to 50 μm are desirable for injections to achieve zero-order linear release kinetic [5], [6]. Smaller and more porous microspheres exhibit faster release kinetics due to increased specific surface area for drug diffusion.

To study the influence of the flow rate ratio on the size and encapsulation efficiency of rilpivirine the PLGA microspheres of various sizes were obtained: MS_4 with an average size of 66.8±3.3 μm, MS_5 – 42.7±0.6 μm, MS_6 – 33.6±0.9 μm. Microspheres were obtained under the same conditions by variation of the flow rate of the organic and aqueous phases (Table 2)

Table 2: Comparison of the microsphere characteristics, depending on the flow rate ratio (n=3).

№	Flow rate of organic phase, µl/min	Flow rate of aqueous phase, µl/min	Flow rates ratio (Qo/Qa)	DL_{theor}, %	DL_{actual}, %	EE, %	D_{cp}, µm	CV, %
MS_4	8.9	30	0.30	13.8	12.9±0.3	93.8±2.0	66.8±3.3	4.9
MS_5	8.9	60	0.15	13.8	13.4±0.2	97.5±1.8	42.7±0.6	1.4
MS_6	8.9	100	0.09	13.8	13.5±0.3	98.5±1.9	33.6±0.9	2.7

As shown in Table 2, decrease of the flow rate ratio from 0.30 to 0.09 resulted in a decrease of the average microsphere size from 67 to 33 µm, as the same result described in our previous study of placebo PLGA microspheres. It should be noted that an increase in the size of the microspheres led to a slight decrease in the encapsulation efficiency of rilpivirine from 98.5% for MC_6 (33 µm) to 93.8% for MC_4 (67 µm). One possible reason for this is that smaller microspheres have a larger surface to volume ratio, which tends to accelerate the diffusion of the solvent into the receiving medium [7]. As a result, a MS_6 hard shell forms faster compared to other microspheres; therefore, the MS_6 microparticles are solidified much faster and the drug diffusion time is reduced.

3.3. Characterization of rilpivirine-loaded microspheres

The surface morphology and size distribution of the prepared rilpivirine-loaded PLGA microspheres were analyzed by SEM (Fig. 1) showing a dense, smooth surface without pores and cracks and an ideal spherical shape of the microspheres. In addition, no separate phase of the non-included rilpivirine was observed between the microspheres and on their surface, which may indicate a high encapsulation efficiency of rilpivirine into the microspheres. Indeed, the encapsulation efficiency of rilpivirine into microspheres exceeds 90%. Also, as shown in Figure 1, the microspheres were obtained with a narrow size distribution.

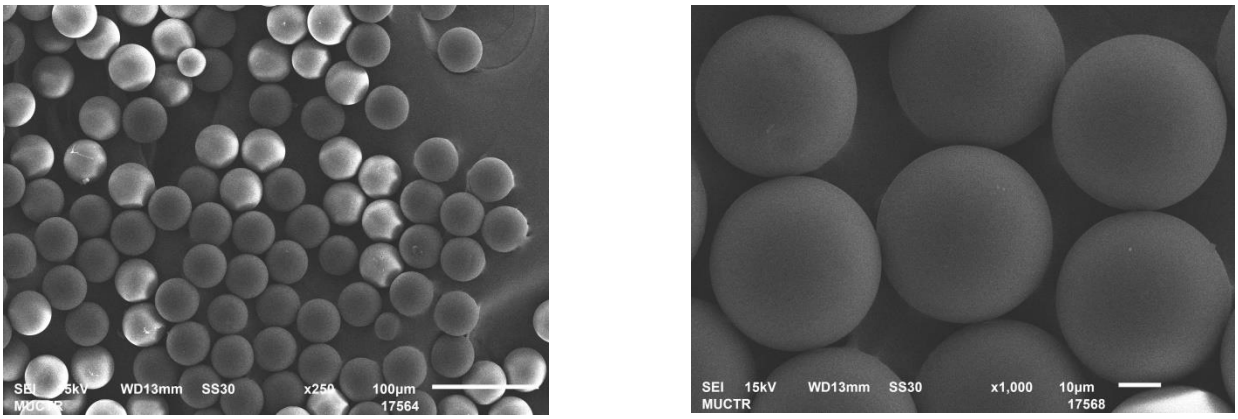

Fig. 1: Morphology and size of the rilpivirine-loaded microspheres MS_5 obtained using a Droplet Junction microfluidic chip.

3.4. In vitro rilpivirine release from PLGA microspheres

The influence of the microsphere size on the rilpivirine release kinetics from the PLGA microsphere was evaluated in the model medium (2% Tween 20 solution in PBS, pH 7.4, 37 °C). Tween 20 was added as a solubilizing agent to prevent sedimentation of rilpivirine in the course of the experiment. In vitro release profiles of rilpivirine from microspheres of various diameters were monitored for 48 days. As shown in Figure 2, all rilpivirine-loaded PLGA microspheres exhibit a biphasic rilpivirine release profile. In the first few days, cumulative release per cent of all microspheres was ~2%, which indicates that there was no initial burst effect. Then slower rilpivirine-release during 18 days for MS_6 and 25 days for MS_4 and MS_5 was observed. In addition, a longer lag time was observed for larger microspheres MS_4 and MS_5. Probably, the lack of a burst effect and the uniform slow release of rilpivirine is due to

the fact that mixing in microfluidic devices is more homogeneous, which leads to a more uniform distribution of the drug in microspheres [8].

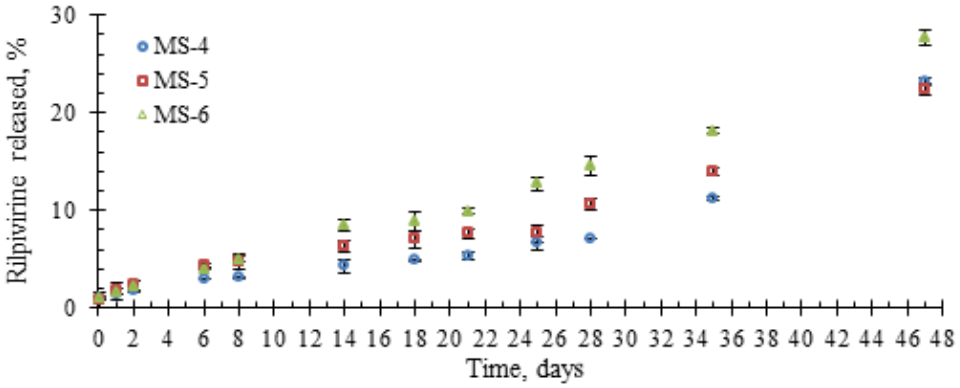

Fig. 2: In vitro release kinetics of rilpivirine during 48 days from PLGA microspheres with different size: MS_4 67 µm, MS_5 43 µm, MS_6 33 µm. Drug release studies were carried out under sink conditions in 0.15 M PBS pH 7.4 containing 2% Tween 20 at 37 °C.

By comparing the three release profiles, we found that there was no significant difference between them during 48 days. However, microspheres with average size of 33 µm exhibited slightly faster release kinetics during the second phase: after 48 days of incubation the MS_6 released 27% of rilpivirine whereas the MS_4 and MS_5 released 22% of rilpivirine. This difference is probably due to a larger surface area to volume ratio of the smaller particles [8]. As well as faster penetration of water into a smaller microparticle due to a reduced diffusion path [7].

4. Conclusion

As shown by the above results, the flow rate ratio significantly affects the size of the microspheres, while the concentration of rilpivirine in the organic phase significantly affects the drug loading and encapsulation efficiency. The release profiles of rilpivirine from PLGA microspheres were characterized by the absence of burst-effects. In addition, the rilpivirine-loaded microspheres exhibited slow release of rilpivirine (less than 30% during 48 days), which is explained by the fact that the RPV release was controlled by diffusion transport. Overall, the results of the study suggest that the PLGA microspheres loaded with rilpivirine is a promising approach to the injectable depot formulation of this drug.

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