Poster

Engineering of gemcitabine-loaded poly(D,L-lactide-*co*-glycolide) nanoparticles by flow focusing for cancer treatment

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Introduction

Gemcitabine is a nucleoside analogue that despite its efficient antitumor activity, suffers from several drawbacks including a very short plasma half-life, thus generating the need to use high doses, simultaneously leading to severe dose-limiting side effects [1]. Drug delivery systems are intended to protect drugs from biological metabolization and elimination, and to induce the highest therapeutic effect with minimal toxicity [2, 3]. Recently [4, 5], we have developed a flow focusing (FF) method to encapsulate biomolecules into biodegradable poly(D,L-lactide-*co*-glycolide) (PLGA) particles. This technique allows the easy formulation of polymeric colloids with interesting drug carrying properties: more narrow size distribution particles, great drug entrapment efficiency and drug loading values and very slow (biphasic) drug release. In order to obtain gemcitabine-loaded PLGA nanoparticles with a narrow size distribution and the best drug loading properties, we investigated the best formulation conditions: the influence of the liquid flow rates of the FF device and the drug concentration.

Materials and Methods

Synthesis of the gemcitabine-loaded PLGA nanoparticles: a water-in-oil emulsion was prepared by mixing appropriate volumes of a gemcitabine aqueous solution with 1 mL of a 1 % (w/v) PLGA and 0.5 % (w/v) Ethocel solution in ethyl acetate. This emulsion was used as focused fluid in a simple flow focusing nozzle [Avant 2 (D = 100 μm), Ingeniatrics Tecnologías S.L., Spain] fixed at different flow rates (table 1). Distilled water was used as focusing fluid. PLGA nanoparticles were formed into a 0.3 % (w/v) PVA solution. The ethyl acetate content was reduced to a very minimum by using a rotary evaporator. Then, they were freeze-dried and stored at ≈ 4 ºC until use.

Characterization methods: the mean particle size and particle size distributions of gemcitabine-loaded PLGA nanoparticles were measured at ≈ 25 ºC by laser scattering (Partica LA-950V2, Horiba). Gemcitabine loading was determined by reverse phase-high performance liquid chromatography (RP-HPLC) (Hitachi LaChrom® (D-7000) Series HPLC system). Gemcitabine content was expressed in terms of gemcitabine entrapment efficiency (EE, %) [2]. The production performance (%) was also determined [6]. In order to establish the variations in particle size, a statistical analysis was performed by the use of the Student's *t*-test. Values with $p < 0.05$ and $p < 0.01$ were considered as significantly different.

Results and Discussion

Under the best FF conditions, this method allowed the formation of well-stabilized spherical nanoparticles with an average diameter of ≈ 600 nm and a narrow size distribution (table 1). Particle morphology was not influenced by the FF conditions. On the opposite, the flow rates of the focused and focusing fluids clearly determined the particle diameter: respectively, the slower and the faster flow rates determined the formation of much smaller particles. In general, a good linear relationship between theoretical and experimental size values was found and, thus, it could be theoretically calculated the experimental conditions needed to achieve any given size.

In addition, FF allows obtaining suitable gemcitabine entrapment efficiencies (≈ 30 %). Gemcitabine entrapment was not significantly influenced by the drug concentration in solution. However, an increase in the volume of the aqueous drug phase determined a very significant reduction in the entrapment efficiency (table 2). Compared to non-loaded PLGA, particle geometry did not vary significantly when gemcitabine is encapsulated (figure 1), as was previously observed with other biomolecules [4, 5].

We hypothesize that the approximation of the hydrophilic drug molecules from the aqueous phase to the hydrophobic polymeric matrix is not thermodynamically favoured. Hence, only the favourable

electrostatic interaction between this positively charged chemotherapy agent and the negatively charged polymer could be responsible for the gemcitabine loading into the PLGA nanoparticles.

Conclusions

The optimal formulation conditions to obtain gemcitabine-loaded PLGA nanoparticles by FF suitable for parenteral administration have been determined. Further experiments are under development to enhance drug loading. The *in vitro* drug release and the antitumor activity are also under investigation.

Acknowledgements

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References

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Figures and Tables

Figure 1. Scanning electron microscopy (SEM) picture of the gemcitabine-loaded PLGA nanoparticles.

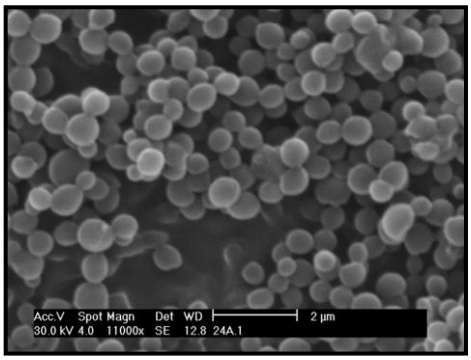

Table 1. Formulations of PLGA particles obtained by flow focusing (FF) under different preparation conditions.

Formulation	Flow rate of the focused fluid (mL/h)	Flow rate of the focusing fluid (mL/min)	Theoretical size (µm)	Experimental size (µm)
1	0.3	1.1	1.8	7
2	0.3	1.2	1.8	4.2
3	0.3	2	1.4	1.1
4	0.3	1.2	1.8	4.3
5	0.1	1.2	1.0	1.0
6	**0.1**	**2**	**0.7**	**0.6**
7	0.05	2	0.5	0.5

Table 2. Size and gemcitabine entrapment efficiency (EE) of gemcitabine-loaded PLGA nanoparticles (flow rate of the focused fluid: 0.1 mL/h; flow rate of the focusing fluid: 2 mL/min).

Formulation	Volume of the aqueous phase (µL)	Gemcitabine concentration (mM)	Production performance (%)	Size (µm)	Gemcitabine EE (%)
6.1	50	50	38.83	0.68	34.44
6.2	50	100	46.50	0.81	23.58
6.3	100	50	69.50	Very high polydispersion	11.43