COMPARISON OF TWO METHODS FOR ANTITUMOR ALKYL LYSOPHOSPHOLIPID EDELFOSINE NANOENCAPSULATION

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Phospholipid analogs are unnatural compounds with high metabolic stability which are readily incorporated into cellular membranes [1]. Edelfosine (ET-18-OCH_3, 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine, Figure 1) is the prototype of this group of phospholipid analogs called alkyl lysophospholipids (ALP) which represents a promising class of antitumor agents. Edelfosine has been reported to inhibit tumor cell proliferation, metastasis and angiogenesis [2]. Despite its beneficial effects, one of the major side effects found *in vivo* is the dose depending hemolysis when administered intravenously which hampers its administration at therapeutic concentrations. To reduce the toxicity of edelfosine, we developed polymeric nanocarriers using two different methods for subsequent *in vitro* characterisation and *in vivo* evaluation. PLGA nanoparticles containing antitumor agents can be prepared by different methods such as single emulsion solvent evaporation (O/W) and double emulsion solvent evaporation methods (W/O/W). The aim of this work is to compare these two preparation methods of edelfosine-loaded PLGA nanoparticles in order to optimize drug entrapment. With this aim, different polymers and solvents were tested.

PLGA edelfosine-loaded nanoparticles were prepared using the single and double emulsion solvent evaporation methods [3]. Briefly, edelfosine was dissolved either in the inner water phase (W/O/W) or in the organic phase (O/W). Single emulsion (O/W): the polymer was dissolved in 1 ml of organic solvent (Table 1) and dropped into 2 ml of an aqueous solution of 1% PVA to form an O/W emulsion by sonication (1 minute). Double emulsion (W/O/W): 100 µl of 1% PVA solution were added to 1 ml of organic solvent containing the polymer (Table 2). The aqueous and organic phase were emulsified by sonication (30 seconds) and added to 2 ml of 1% PVA solution.

In both cases, emulsions were stirred for at least 3 hours with 50 ml of a 0.2% PVA solution. The samples were then washed three times by centrifugation, frozen and freeze-dried. All formulations were prepared at least as triplicate.

Nanoparticles' diameter and polydispersity index were determined in triplicate by a photon correlation spectroscopy and zeta potential by electrophoretic laser doppler anemometry, using a Zetamaster Analyzer System. The amount of edelfosine encapsulated per unit weight of nanoparticles was determined by dissolving a weight amount of nanoparticles in methanol and measuring the drug by a liquid chromatography-tandem mass spectrometry method developed by our group [4].

As shown in Table 1, the mean size of particles prepared by O/W method and using chloroform was always higher than 1 µm. When using dichloromethane, the lowest diameter was obtained when the particles were formulated using 502H polymer. When using W/O/W method, the mean size of nanoparticles was around 300 nm (Table 2). Nanoparticles prepared with 502H polymer showed the smallest diameter (280 nm).

The presence of edelfosine in formulations did not affect the size of the nanocarriers. Furthermore, both loaded and unloaded formulations (Tables 1 and 2) showed a negative surface charge.

The amount of edelfosine encapsulated into the particles prepared by O/W method was around 78%. Presently, W/O/W formulations are being assayed for encapsulation efficiency using HPLC-MS.

Tables:

Table 1. Mean size, polydispersity and zeta potential of nanoparticles formulated by simple emulsion solvent evaporation method.

		Size (nm)	Polydispersity	Zeta Potential
502H	Chloroform	1686,67 ± 476,12	0,473 ± 0,27	-18,10 ± 1,13
	Dichloromethane	741,83 ± 64,59	0,327 ± 0,22	-23,9 ± 2,12
503H	Chloroform	1372,17 ± 1,65	0,612 ± 0,41	-17,05 ± 1,48
	Dichloromethane	820,8 ± 108,88	0,420 ± 0,37	-12,1 ± 8,75
752H	Chloroform	1156,78 ± 123,73	0,31 ± 0,11	-17,30 ± 1,41
	Dichloromethane	988,5 ± 171,76	0,506 ± 0,30	-18,05 ± 0,64

Table 2. Mean size, polydispersity and zeta potential of nanoparticles formulated by double emulsion solvent evaporation method.

		Size (nm)	Polydispersity	Zeta potential
Ethyl Acetate	502H	279,93 ± 7,94	0,065 ± 0,03	-20,94 ± 9,88
	503H	298,17 ± 15,60	0,10 ± 0,008	-15,90 ± 4,71
	752H	310,33 ± 2,36	0,06 ± 0,015	-16,80 ± 2,40

Figure:

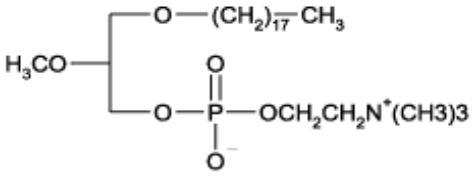

Figure 1. Molecular structure of edelfosine.

References:

[1] Wieder, T., Reutter W., Orfanos, C. E. and Geilen, C. C. (1999). Prog Lipid Res **38**(3): 249-59.

[2] Mollinedo, F., Gajate C., Martin-Santamaria, S. and Gago, F. (2004). Curr Med Chem **11**(24): 3163-84.

[3] Blanco-Prieto, M. J., Lecaroz C., Renedo M. J., Kunkova J. and Gamazo C. (2002) Int J Pharm **242**(1-2): 203-6.

[4] Blanco-Prieto, M. J., Campanero M. A. and Mollinedo F. (2004). J Chromatogr B **810**(1): 85-92.