NANO- SIZED DELIVERY SYSTEMS FOR 1-METHYL-DL-TRYPTOPHAN

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The state of tolerance observed in certain tumours towards their own antigens has been associated with an elevation of the immunomodulatory enzyme IDO in tumor cells and the recruitment of IDO-expressing antigen presenting cells (APC) to tumor-draining lymph nodes. IDO is a enzyme that catabolizes tryptophan and then supresses T cell proliferation [1]. Therefore, it seems feasible to enhance anti-tumor immunity in cancer patients using pharmacologic IDO inhibitors, such as 1-methyl-DL-tryptophan (1-MT). Because of its extreme insolubility in water, 1-MT is actually administered in mice through the subcutaneous chirurgic implantation of pellets that release massive amounts of 1-MT over two weeks [2]. Toxic effects have been frequently observed.
Our objective in this work was the development of 1-MT nano-scale formulations in order to improve their specific and passive capture by IDO-expressing APC. We have developed two types of nanosystems: 1-MT-loaded PLGA nanoparticles (NPs) and cristalline nanometer-sized drug particles or nanocrystals.

A simple emulsion solvent evaporation technique was used to formulate 1 MT-loaded NPs. Briefly, a suspension of the drug and polymer in dichlorometane was emulsified with an aqueous solution of PVA using a sonicator. Different parameters, such as the quantity of 1-MT and the PVA percentage were modified in order to optimize the particle size and encapsulation efficiency.
The preparation of nanocrystals was carried out using a precipitacion technique, the solvent change method by instantaneously mixing two liquids in the presence of a stabilizing agent. An organic solvent (ethanol) loaded with 1-MT and 1% HCL was dispersed in an aqueous phase (PBS) containing a suitable quantity of surfactants. The organic phase was then evaporated under reduced pressure so that the drug particles precipitated instantaneously after pH neutralization. In this case the percentage and type of surfactant was the variable modified in order to obtain nanocrystals with optimal size and dispersion. The size of PLGA NPs and nanocrystals was measured by photon-correlation spectroscopy (PCS). Particles of raw 1-MT were sized by laser diffractometry (Malvern Instruments). The method used to determine the encapsulation efficiency and in vitro release of 1-MT into the NPs, the in vitro release from the polymeric systems and its solubilization from nanocrystals was derivatisation with ortho-phthaldialdehyde (OPA).

As shown in Table 1, it was possible to load appreciable amounts of 1-MT into PLGA NPs, although the trend of the drug to microcrystallize limited its encapsulation efficiency. We found that drug loading into NPs did not increase proportionally with the amount of drug initially incorporated into the organic phase. Moreover, when formulations were prepared with initial drug concentrations upper than 200 mg, we obtained particles of two different sizes. The smallests ones (around 300 nm) are 1 MT loaded NP and the biggest correspond to microcrystals of the drug (around 16 µm). We also evaluated the influence of the PVA concentration. As the PVA% increases the NPs size decreases. On the other hand, its influence on the drug loading was not appreciable (Table 1). A rapid diffusion of the drug, molecularly dispersed into the polymeric matrix, could explain the rapid release of 1-MT from these small polymeric carriers (Figure 1).

Among surfactants and solvents used for the preparation of nanocrystals by the precipitation method, Pluronic® F68 and ethanol were respectively the best choice in terms of optimal size and polydispersity. Nanocrystals thus obtained had an average size of about 300 nm and PDI lower than 0.5 (Table 2). The formulation of 1-MT in nanocrystals accelerated the dissolution rate when compared with both, 1-MT nanoparticles and the raw drug. After 5 days, about a 75% of the drug had been released whereas raw material reached just a 20%. Besides, the amount of drug encapsulated into NPs was completely released within 1 hour (Figure 1). The dissolution velocity increases due to the increase in the surface area among 1-MT nanocrystals and can lead to a sustained release over the time.

Both types of formulations (nanoparticles and nanocrystals) could be directly phagocyted by IDO-expressing DCs having a local and specific inhibitory effect on the activity of the enzyme and avoiding the toxicity produced by a more systemic release of massive doses. However, nanocrystals could be preferable as they are able to release a higher amount of drug with a much more sustained release and their production it is not only cheaper but also much easier when compared to NPs.

References:

[1] David H. Munn et al., Trends in Molecular Medecine. Vol. 10 No.1 January 2004.
[2] Alexander J Muller et al., Nature Medecine. February 2005.

Table1: Characteristics of 1-MT Nanoparticles

Formulation		Size (nm)	E.E (%)	µg 1-MT/mg PLGA
[1-MT] (mg)	[PVA] (%)			
50	5	191.2 ± 1.9	73.1	146.3
	2	311.6 ± 8.3	83.0	119.6
	1	398.0 ± 68.7	90.6	134.7
100	5	260.4 ± 4.1	60.3	148.3
	2	335.2 ± 9.5	62.9	175.7
200	5	255.2 ± 2.1	41.6	177.8
	2	313.5 ± 5.6	53.4	191.8
500	2	300.8 ± 7.0 14500 ± 205.3	31.4	189.8
1000	2	327.1 ± 19.0 16100 ± 314.9	29.6	211.1

Table 2. Characteristics of 1-MT nanocrystals

Formulation		Size (nm)	PDI
Solvent	Surfactant (%)		
EtOH	Plur. 1%	275.3 ± 34.0	0.5
	Plur. 2%	317.7 ± 56.2	0.41
	Plur. 5%	330.6 ± 9.1	0.48
	Tw80+Span20(2%)	299.3 ± 143.1	0.49
	Cremophor 2%	82.1 ± 105.5	0.46
MeOH	Tw 80 1%	-	-
	Plur. 1%	1733.3 ± 724	1.0
Raw drug		16100 ± 314.9	-

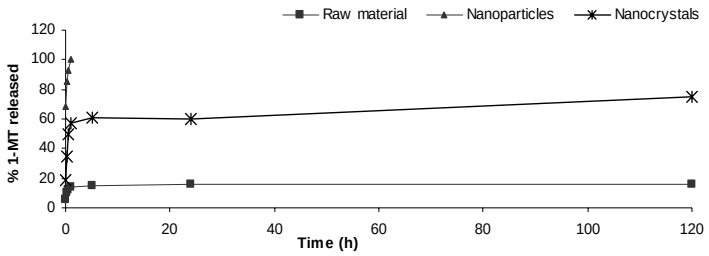

Figure 1: Release profile of 1-MT from the formulations