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Solid lipid nanoparticles for the delivery of 1,3,5-triaza-7-phosphaadamantane (PTA) platinum (II) carboxylates

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ABSTRACT

The use of solid lipid nanoparticles (SLN) is a promising route for the delivery of platinum complexes aimed to anticancer activity.
This paper describes the production and characterization of SLN suitable for the loading of Pt complexes containing the biocompatible phosphine 1,3,5-triaza-7-phosphaadamantane (PTA) as neutral ligand.
After a screening of several lipidic phases, stearic acid-based SLN were identified as the most appropriate for the purpose. They were produced by emulsion-dilution method and then characterized in terms of dimension, polydispersity, time stability, pH balance and morphological aspect.
Stearic acid SLN are designed as a system able to coordinate to platinum, acting as anionic carboxylic ligands, replacing the base carbonate of the Pt synthon [$PtCO_3(DMSO)_2$], where also DMSO can subsequently be substituted by phosphinic ligands, namely PTA. SLN functionalised with Pt-PTA were produced and characterized by this synthetic route.
The toxicity of plain SLN and the antiproliferative effect of SLN functionalised with Pt-PTA were evaluated on two human cancer cell lines K562 and A2780. The results indicate that SLN can be exploited as a delivery system for Pt complexes with potential anticancer activity.

1. Introduction

Since the advent of cisplatin [1], platinum complexes have attracted increasing interest as anticancer chemotherapics. Presently, platinum-based anticancer drugs are widely used in medical oncology for the management of tumors of the ovary, testes, head and neck and other cancers [2–4]. Unfortunately, beside a very high rate of success, they present heavy side effects and tend to acquire resistance [5–9]. An extensive search has therefore been developed for platinum-based drugs with improved pharmacological properties as compared to the cisplatin parent compound. It has been progressively clarified that the distribution and the concentration of platinum-based drugs in different body districts are influenced by some physicochemical properties of the ligands such as polarity, hydrophilicity and steric requirements. Thus, many variations of the ligands have been proposed over nearly 40 years, including the possibility of introducing phosphines as neutral ligands, instead of ammonia or amines [10–11], the carrier ligands of Pt drugs of consolidate clinic use.

A very large number of phosphinic platinum complexes have been prepared and exploited for fundamental mechanistic studies because of the great affinity of Pt for phosphorus (hard-soft theory), the stability of Pt-P bonds and the possibility of using ^{31}P NMR as a tool of investigation even in a multi-components environment like biological fluids [12–15]. Nevertheless the choice of phosphine ligands for pharmaceutical purposes is limited on one side by the hydrophobicity of common aromatic phosphines such as PPh_3 or 1,2-bis(diphenylphosphino)ethane, on the other by the instability to oxidation of aliphatic phosphines (e.g. PMe_3), which require to be handled under controlled conditions.

For these reasons, the search of a strategy aimed to the extension of the pharmaceutical application to Pt phosphine complexes has attracted great interest since many years. The first approach was the development of hydrophilic, oxidatively stable and biocompatible phosphines. It is now clear that these three requirements were fulfilled only by 1,3,5-triaza-7-phosphaadamantane (PTA) [16–17]. More recently, nanoscale formulations have been proposed and exploited as carriers for platinum-based anticancer chemotherapeutics [18–19].

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Nanoparticulate-drug delivery systems are able to improve the permeability, retention effect, safety, pharmacokinetics properties and bioavailability of the therapeutic substances in the treatment of tumor cells [19]. The delivery formulations, namely liposomes, micelles or lipid particles, often exploit differences between normal tissues and tumors increasing the selectivity of the drug towards its target [20]. Furthermore, the use of these nanoscale carriers prevents their extravasation in normal tissues and removal by renal clearance [21].

The incorporation of the drug into the carrier can involve different methods, such as encapsulation, conjugation or metal complexation.

As an example of a system designed for metal complexation, in a previous paper we described the preparation and inclusion in SLN of N-alkyl PTA lipophilic derivatives, whose P-donor carrier ligands can coordinate Pt(II) [12–23].

In the present study, to functionalise the solid lipid nanoparticles (SLN) with Pt-PTA groups, a new complexation route based on the use of solid lipid nanoparticles (SLN) of stearic acid is proposed. The carboxylic groups on the surface of the nanoparticles can be deprotonated and then coordinated to platinum-PTA complexes as carboxylates. Considering the evolution of Pt-based drugs, Pt carboxylates, such as carboplatin or oxaliplatin (second generation), represented an improvement in terms of therapeutic index as compared to cisplatin (first generation) [3–4]. This observation allows one to expect that the system here proposed performs efficiently in the cancer therapy.

Indeed, this new way of inclusion in SLN should favour both the delivery and the activity of the Pt-PTA moiety.

2. Materials and methods

2.1. Materials

The phosphine PTA was prepared as described in the literature [24–25]. Polyethylene glycol sorbitan monolaurate (Polyoxyethylenesorbitan monolaurate, Polysorbate 20, Tween 20), Polyethylene glycol sorbitan monooleate (Polyoxyethylenesorbitan monooleate, Polysorbate 80, Tween 80), stearic acid and all other materials and solvents were purchased from Sigma-Aldrich S.r.l. (Milan, Italy).

2.2. SLN preparation

SLN were produced following the emulsion-dilution technique. In particular, as depicted in Fig. 1, stearic acid (2% w/w) and a surfactant (3.34% w/w), namely Polysorbate 20 (Tween® 20, T20) or Polysorbate 80 (Tween® 80, T80), were melted at 80 °C and bidistilled water (21.26% w/w) was heated at the same temperature. Bidistilled water was dispersed in the fused lipid phase in order to form O/W hot emulsion under mechanical agitation at 3000 rpm for 5 min. The hot emulsion was diluted with cold bidistilled water (73.4% w/w) under high-speed homogenizer (Ultra Turrax, Ika-Werk, Sardo, Italy) at 11,000 rpm for 10 min. The dilution of the system leaded to precipitation of the lipid phase forming fine particles that were stored at room temperature [26–27].

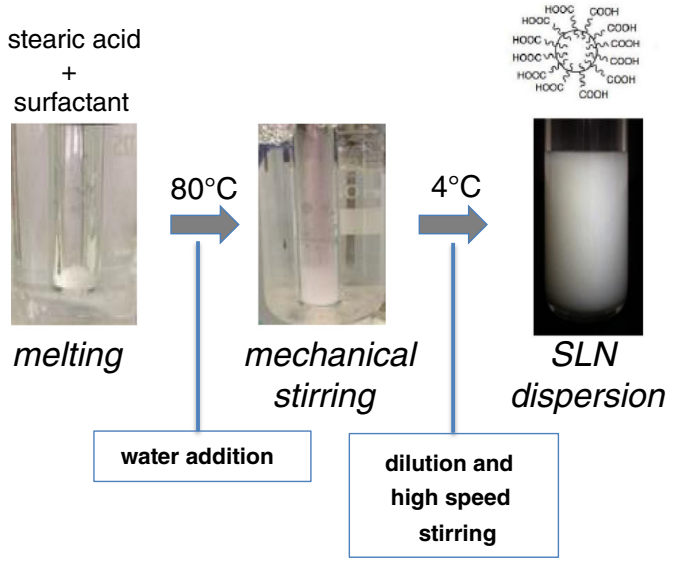

Fig. 1. Schematic representation of the procedure followed for the preparation of ST20 and ST80.

2.3. Syntheses

Recently the synthesis of $[PtCO_3(DMSO)_2]$ from *cis*-$[PtCl_2(DMSO)_2]$ in water with a high yield and its full characterization was reported [28]. In our experience, the observation of the methyl group of coordinated DMSO (singlet at 3.27 ppm with satellites $J_{HPt} = 20.5$ Hz) as the only signal in ^1H NMR in D_2O can be trusted as a purity evidence in every new preparation.

2.4. SLN functionalization with $Pt(PTA)_2$ group

Solid $[PtCO_3(DMSO)_2]$ (8.72 mg, 2.12×10^{-5} mol) was added to a suspension of SLN prepared as above described (1 mL, estimated amount of —COOH functions 8.49×10^{-5} mol, 4 eq), and vigorously stirred at room temperature for 15 min. A solution of PTA (6.66 mg, 4.24×10^{-5} mol, 2 eq with respect to Pt) was then added and the stirring was continued for further 120 min.

The functionalization of SLN with the $Pt(PTA)_2$ group was then observed by ^{31}P NMR analysis (Table 1).

The suspension was characterized as below reported and used for biological assays.

It was possible to obtain complete release of platinum from SLN through the addition of hydrochloric acid in equivalent ratio to PTA (0.1 M, 0.3 mL). After a few minutes, the expected formation of *cis*-$[(PTA)_2PtCl_2]$ was observed by ^{31}P NMR (Table 1).

2.5. Characterization of SLN: size, zeta potential, morphology and X-ray diffraction

Submicron particle size of SLN was determined using a Zetasizer Nano S90 (Malvern Instruments Ltd., UK) equipped with a 4 mW helium neon laser with a wavelength output of 633 nm. Measurements were made at 25 °C at an angle of 90 °C. Samples were diluted 1:10 (v/v) with the aqueous phase of each formulation. Each experimental value results from three independent experiments performed in triplicate [23].

The zeta potential of SLN was determined by the measurement of the electrophoretic mobility using a Zetasizer Nano S90 (Malvern Instruments Ltd., UK). All samples were diluted with ultra-purified water prior to the measurements. Measurements were carried out at 25 °C.

Morphological characterization was performed by Cryo-transmission electron microscopy (Cryo-TEM) analysis. Samples were vitrified as described in a previous study by Esposito and colleagues [29]. A drop of dispersion was placed on a lacey carbon film-coated copper TEM grid (200 mesh, Science Services). Most of the liquid was removed with blotting paper, leaving a thin film stretched over the carbon film

Table 1
^{31}P NMR data in D_2O.

	Chemical shift (ppm)	$^1J_{PtP}$
PTA	−100 ppm	/
cis-$[Pt(PTA)_2NPCOO_2]$, $[Pt(PTA)_2NPCOO_2]$	−63 ppm	3475 Hz
cis-$[PtCl_2\ (PTA)_2]$	−48 ppm	3425 Hz

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