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Target selective micelles for bombesin receptors incorporating Au(III)-dithiocarbamato complexes

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ABSTRACT

Pure sterically stabilized micelles (SSM) of DSPE-PEG2000, and sterically stabilized mixed micelles (SSMM) containing PC or DOPC phospholipids (5, 10 or 20% mol/mol with respect to DSPE-PEG2000) are developed as delivery systems for the gold based cytotoxic drug Au(III)-dithiocarbamato complex AuL12. In particular, SSMM containing 5% of PC at 5 mM of lipid concentration encapsulates 61.0 μg of AuL12 with a DL% of 1.13. The gold complex remains stable up to 72 h when incorporated in the aggregate, as indicated by UV–vis measurements. Incorporation in micelle composition of a low amount of the peptide derivative MonY-BN-AA1, containing a bombesin peptide analogue does not influence structural parameters of the micelles (diameter around 20 nm) neither the AuL12 loading parameters. Target selective properties of the peptide containing full aggregate on PC-3 cells overexpressing the GRP/bombesin receptors are observed by in vitro cytotoxic studies: a decrease of cell viability, ~50%, is obtained in cells treated with AuL12-targeted micelles at 10 μM drug concentration for 48 h with respect to untargeted micelles.

1. Introduction

Anticancer drugs such as anthracyclines, taxols or platinum derivatives are currently used in the treatment of a large number of solid tumors (Lippert, 1999; Drbohlavova et al., 2013). On the other hand, their high toxicity (in particular, myelosuppression, cardiotoxicity, nephrotoxicity and neurotoxicity), their rapid inactivation and the frequent occurrence of resistance restrict their clinical use and efficacy (Kelland, 2007; Kaur et al., 2012), pushing researchers in the development of sophisticated delivery systems able to target the tumor tissues and so to reduce the adverse side effects (Guarnieri et al., 2013; Tarallo et al., 2011).

Effective delivery systems are usually obtained by entrapping the active drug in supramolecular aggregates; for example, hydrophobic drugs are loaded in micelle cores while more hydrophilic compounds are confined in the inner water compartment of liposomes (Lim et al., 2012; Deshpande et al., 2013; Accardo et al., 2007). Moreover, neoplasia-selective delivery systems are under development by modifying the nanocarriers with a bioactive molecule on the hydrophilic corona, which is able to selectively target specific receptors, for example peptide receptors overexpressed by cancer cells (Accardo et al., 2013a; Falciani et al., 2011; Yu et al., 2012; He et al., 2011).

In the last decade, several metal-based anticancer agents, their archetype being the drug cisplatin, reported in Fig. 1A (compound 1) have been developed (Nagy et al., 2011, 2012; Nardon et al., 2014; Dalla Via et al., 2012; Ronconi et al., 2013). Among them, gold(III) dithiocarbamate complexes have proved to be very promising for their biological behavior, in fact the toxicological profile of these potential anticancer drugs is so far encouraging in animal models, likely resulting from a good selectivity toward cancerous cells (Nagy et al., 2012; Nardon et al., 2014; Casini et al.,

Abbreviations: CDDP, cisplatin; DL%, drug loading coefficient; DLS, dynamic light scattering; DMSO, dimethyl sulfoxide; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DSPE-PEG2000, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000]; DTPA(OtBu)$_4$-OH, diethylenetriaminepentaacetate tetra-tertbutyl ester; ER%, encapsulation ratio; FBS, fetal bovine serum; Fmoc-Ahoh-OH, Fmoc-21-amino-4,7,10,13,16,19-hexaoxaheneicosanoic acid; Fmoc-NH-Peg27-COOH, α-(9-fluorenylmethyloxycarbonyl)amino-ω-carboxy polyethylene glycole; GRP, gastrin-releasing peptide; ICP-AES, inductively coupled plasma atomic emission spectrometer; MBHA, *p*-methylbenzhydrylamine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PC, egg L-α-phosphatidylcholine; SSM, sterically stabilized micelles; SSMM, sterically stabilized mixed micelles.

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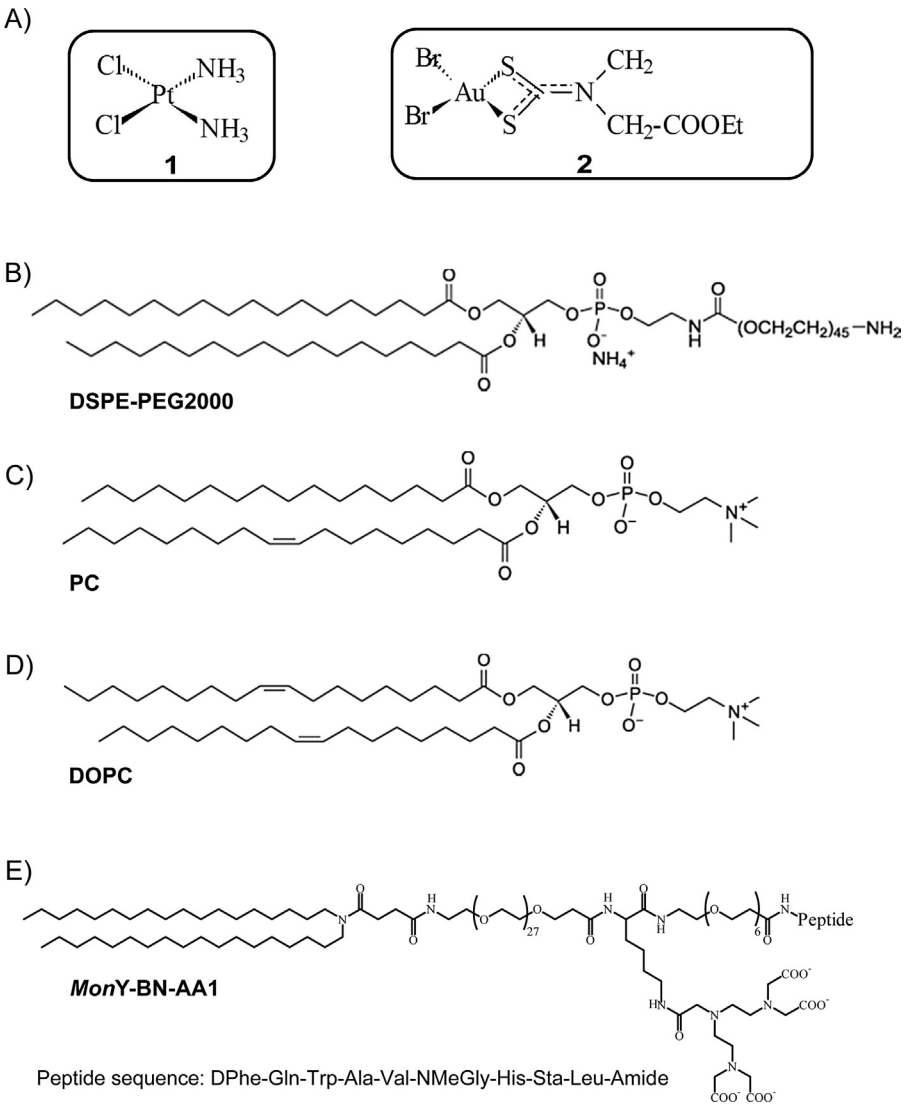

Fig. 1. Chemical drawing of cis-diaminodichloroplatinum (II) (cisplatin) (A, compound 1), dibromo[ethyl *N*-(dithiocarboxy-kS,kS′)-*N*-methylglycinato] gold(III) (AuL12) (A, compound 2), DSPE-PEG2000 (B) PC (C) and DOPC (D) phospholipids; MonY-BN-AA1 amphiphilic peptide (E). Peptide sequence of BN-AA1 analogue is indicated using three letter code.

2009; Marzano et al., 2011). In any case, the low water solubility of these gold complexes pushes toward the development of appropriate delivery systems able to increase their therapeutic index.

Micelles could represent the more suitable vector for this kind of hydrophobic drugs. Sterically stabilized micelles (SSM) and sterically stabilized mixed micelles (SSMM) based on 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000] (DSPE-PEG2000) phospholipid 100% or in combination with egg L-α-phosphatidylcholine (PC) at several molar ratios have been previously described (Krishnadas et al., 2003; Ashok et al., 2004). Both of them are also extensively investigated as drug nanovectors encapsulating hydrophobic drugs such as camptothecin, diazepam and paclitaxel (Koo et al., 2005; Dagar et al., 2012).

In order to increase drug solubility and thus bioavailability, here we study the incorporation of the active gold(III) dithiocarbamato complex AuL12 (Fig. 1A, compound 2) in mixed micelles based on DSPE-PEG2000 (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000] Fig. 1B). The effect of growing percentages of PC or DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) phospholipids (Fig. 1C and D) on AuL12 loading and on the physicochemical properties of resulting micelles is also investigated. Target selective micelles are also prepared by adding a small amount of the amphiphilic peptide derivative MonY-BN-AA1 in micelle composition (Accardo et al., 2013b). MonY-BN-AA1, schematized in Fig. 1E, is a synthetic monomer that contains different functions in the same molecule:

(1) the BN-AA1 peptide sequence, an analogue of [7–14]bombesin peptide fragment; it acts as driver of the monomer or of the full aggregate on gastrin-releasing peptide (GRP) receptors overexpressed by several cancer cells such as prostate and ovarian (Accardo et al., 2012). BN-AA1 peptide sequence presents several modifications with respect to [7–14]bombesin wild-type sequence: (i) Leu^{13}–Met^{14} residues at the C-terminus of the native sequence have been replaced by Sta^{13}–Leu^{14} for stabilization against aminopeptidases; (ii) replacement of Leu^{13} with the Sta residue provides with antagonist properties to the peptide; (iii) glycine has been replaced with *N*-methyl-glycine derivative in order to avoid in vivo enzymatic cleavage of Val–Gly bond; (iv) finally, D-Phe residue on the N-terminal end of the peptide is reported to increase receptor