Particulates of Calcium Phosphate/Cisplatin Overcome Drug Resistance of A2780cis Cells

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Statement of Purpose: Localized, sustained drug release of a variety of hormones and chemotherapy agents has been achieved using calcium phosphate (CaP) disks, pellets or particulates as the drug-delivering biomaterial. The CaP provides a means to minimize unnecessary systemic exposure and associated toxicity and reduces the need for repeated dosing. Polymer-based particulate drug delivery formulations have been shown to overcome chemotherapy drug resistance through intracellular drug/particle uptake (Minko, 1998). This led to our interest in investigating if CaP particulates made of hydroxyapatite (HA) carrying cisplatin (CDDP) could also overcome drug resistance. We tested this hypothesis *in vitro* in a cisplatin resistant cell line: A2780cis.

Methods:

Materials: HA crystals were synthesized through precipitation from the mixture of two solutions. The first solution contained calcium nitrate and magnesium chloride hexahydrate in deionized distilled water. The second solution contained dibasic sodium phosphate, sodium bicarbonate and decahydrate sodium pyrophosphate in deionized distilled water. The first solution was rapidly poured into the second and allowed to mature for 10 minutes. The formed precipitate was collected by filtration, rinsed thoroughly with water, lyophilized, sieved to obtain particles less than 125 μm and calcined for 5 hrs at 200°C. CaP/CDDP conjugates were prepared by incubating the preformed crystals of HA with an aquated CDDP solution for 4 hrs at 37°C. The CDDP attaches through electrostatic adsorption and is released in the presence of chloride ions. After rinsing and lyophilization, the drug loading rate was 35 μg CDDP/mg CaP as determined by platinum (Pt) atomic absorption spectroscopy (AAS) analysis.

Methods: For *in vitro* cytotoxicity activity studies, the CDDP-resistant cell line was used: A2780cis human ovarian carcinoma cell line (Sigma, 93112517) and cultured according to supplier's descriptions. The CellTiter96® AQueous One (Promega) colorimetric proliferation assay was used to determine the IC50 value (50% inhibitory concentration) evaluated from 12 two-fold dilutions of: (a) CDDP (200 μg CDDP/mL) in 0.9% saline (free drug), (b) 1.5 mg of conjugates in 1.75 ml PBS, (c) 1.5 mg of HA in 1.75 ml of CDDP solution. Control wells containing the HA only were required since the HA interferes with the CellTiter96 assay. Absorbance values from the HA only were subtracted from the conjugate data in order to determine the IC50 values. The cytotoxicity assay was conducted as follows: twenty-four hours after seeding 2000 A2780cis cells in 50 ml of media on 96 well plates, 50 ml PBS, or PBS with drug or conjugates was added to the wells.

Results/Discussion:

The IC50 values obtained were: (a) free drug 6.07 ± 0.226, (b) CaP/CDDP 2.6 ± 0.42, (c) CaP and free drug 5.75. The IC50 value of the conjugates was significantly lower than the free drug indicating that the CaP particulates conjugated with cisplatin overcome drug resistance. The IC50 value obtained when testing CaP and free drug without prior conjugation was not different than the free drug indicating that there is no cytotoxicity associated with the CaP particulates. Cisplatin will not adsorb to calcium phosphate unless it is in the positively charged aquated state, therefore it was not possible for the drug to adsorb during the *in vitro* testing.

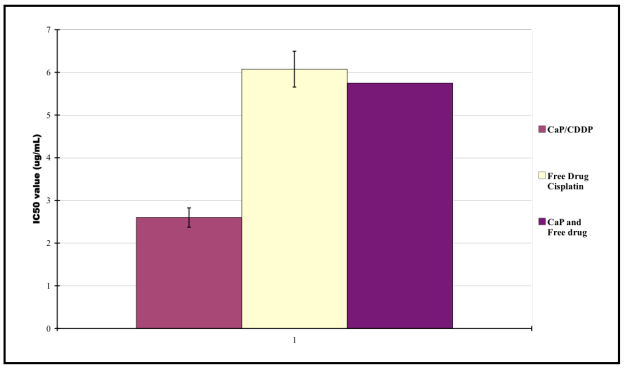

Figure 1. IC50 values of the CaP/CDDP are lower than the free drug cisplatin indicating particulates of CaP carrying cisplatin can overcome drug resistance.

Conclusions: Particulates of hydroxyapatite can assist in overcoming drug resistance to chemotherapy when the drug is conjugated to particle surfaces. Transmission electron microscopy studies will be conducted to determine extent of particle endocytosis that may occur in this system. Calcium phosphate-based particulate delivery systems may have potential for intratumoral chemotherapy delivery to drug resistant tumors.

References:

Minko T et al. J Control Release. 1998;54(2):223-33.